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Triazolobenzodiazepines and -triazepines as Protein Interaction Inhibitors Targeting Bromodomains of the BET Family

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Screening via DSF / ITC / Co-crystallization

…für Caro

…für meine Familie

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CHAPTER I – INTRODUCTION

1.1 EPIGENETICS

1.1.1 Background information

"There appear to be about 30,000 – 40,000 protein-coding genes in the human genome – only about twice as many as in worm or fly[1]."

After the draft sequence of the human genome was published[1] in *Nature* in 2001 by the International Human Genome Sequencing Consortium (example see Figure 1), this statement, mentioned above, equals a disillusion for science and the result was even revised downwards to 20,000 – 25,000 genes after the publication of the final sequence[2] in 2004. Previous calculations, for example of Randall Scott[3] or John Quackenbush[4], estimated a range between 130,000 and 140,000 genes – approximately the fourfold of the ones actually found. But of what reason the vast complexity of humans does not result in significantly more genes than a worm? One of the answers is that for most gen regulations, and e.g. the following cell differentiations, epigenetic factors are responsible[5].

ATGGGCACCGAGGGACCCCCGCCCCCGGCCGCCTCCCGCGGACGCCAGGGCTGCCTGCTG
GTACCTGCGCGGACGAAAACCACTATTGCTCTGTTGTATGATGAAGAGTCCGAAAATGCC
TATGACATCCGGCTGAAGCTGACGAAAGAGGTGCTGACAATTCAGAAACAAGATGTTGTC
TGTGTGGGCGGAAGCCACCAGGGCAGGAATGCCCCATCGTCACCTTCCTCGCCCATAGCT
AAGGACCCGAGGTATGAGAAGCGCTGGCTGGACACCTTGTCCGTGCCTCTGTCCATGGCT
CGCATCTCAAGGTACAAAGCCGGAACGGAAAAATTAAGGTGGAATGCGTTCGAGGTGCTC
GCCCTGGACGGAGTCAGCTCTGGGATCCTCCGGTTTTACACAGCCCAGGATGGCACCGAC
TGGCTGCGGGCGGTCTCAGCCAACATCAGGGAGCTGACACTTCAGAACATGAAGATGGCG
AACAAATGCTGCTCTCCTTCCGACCAGGTTGTGCATATGGGGTGGGTAAATGAGAAACTC
CAAGGAGCTGACTCCTCTCAAACCTTCAGACCCAAGTTCCTAGCACTGAAGGGCCCGTCC
TTCTACGTTTTCAGCACTCCTCCGGTGAGCACATTCGATTGGGTGCGAGCAGAAAGGACC
TATCACCTCTGTGAGGTGCTATTTAAAGTTCACAAGTTCTGGCTCACAGAGGACTGCTGG
TTGCAAGCAAACTTGTATCTGGGTCTTCAAGATTTTGACTTTGAGGACCAGAGGCCCTAT
TGCTTCAGCATCGTGGCCGGCCATGGGAAGAGCCATGTTTTCAACGTGGAGCTTGGCAGC
GAGCTGGCCATGTGGGAGAAGTCCTTCCAAAGAGCCACGTTCATGGAAGTTCAGAGAACC
GGGTCCAGAACATACATGTGCAGCTGGCAAGGAGAGATGCTGTGTTTCACGGTGGATTTC
GCGTTGGGATTTACCTGTTTTGAGAGTAAGACCAAGAATGTGCTCTGGAGATTTAAATTT
TCCCAGCTTAAGGGATCTTCAGATGATGGGAAAACTCGAGTAAAGCTGCTGTTTCAGAAT
CTGGACACCAAACAGATTGAGACGAAGGAACTCGAGTTCCAGGACCTGAGGGCTGTCCTG
CACTGCATCCACTCCTTCATAGCAGCCAAGGTGGCCTCCGTGGACCCCGGCTTCATGGAC
AGTCAGAGTCTTGCCAGAAAATACATGTACAGCAGCTAA

Figure 1. Protein coding sequence of human gene SNTG2 (splice variant 3) located on chromosome 2[6].

Conrad Hal Waddington wrote in 1942 in his paper “The Epigenotype” about hereditary information concerning the developmental process between genotype and phenotype[7]. For this mechanics of development, Waddington coined the word *epigenetics*[7,8], a combination of *epigenesis* and *genetics*, referring to the term *phenogenetics*, which was predominantly introduced by Haecker[9]. Waddington’s definition could be understood as a further improvement of the well-known theory of epigenesis.

Beside several new definitions for epigenetics along the years, Robin Holliday described it, in 1990, as “the study of the mechanism of temporal and spatial control of gene activity during the development of complex organisms"[10]. A more defined and generally accepted definition was given in 1996 by Arthur Riggs as “the study of mitotically and / or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence"[11].

1.1.2 From DNA to chromosomes

To gain a better insight of how epigenetics affect gene regulations, it is essential to understand the structure and the constitution of chromatin (Figure 2), especially of DNA and histones[12,13].

The double-stranded deoxyribonucleic acid (DNA), built-up of nucleotides, forms a right-handed α-helix, with its two chains running antiparallel to each other[14]. The complementary nucleobase pairs adenine with thymine and guanine with cytosine are located on the inside, while the phosphate-sugar chains of the nucleotides are responsible for the polar surface on the outside of the DNA[14].

The human DNA, with its approximately 6 billion base pairs – divided into 46 chromosomes – and a theoretical length of 2 meters, has to be stored into a cell nucleus of merely 6 µm in diameter[15,16]. Next to supercoiling of DNA, done by topoisomerases[17], histone proteins play an essential role for DNA packing[18].

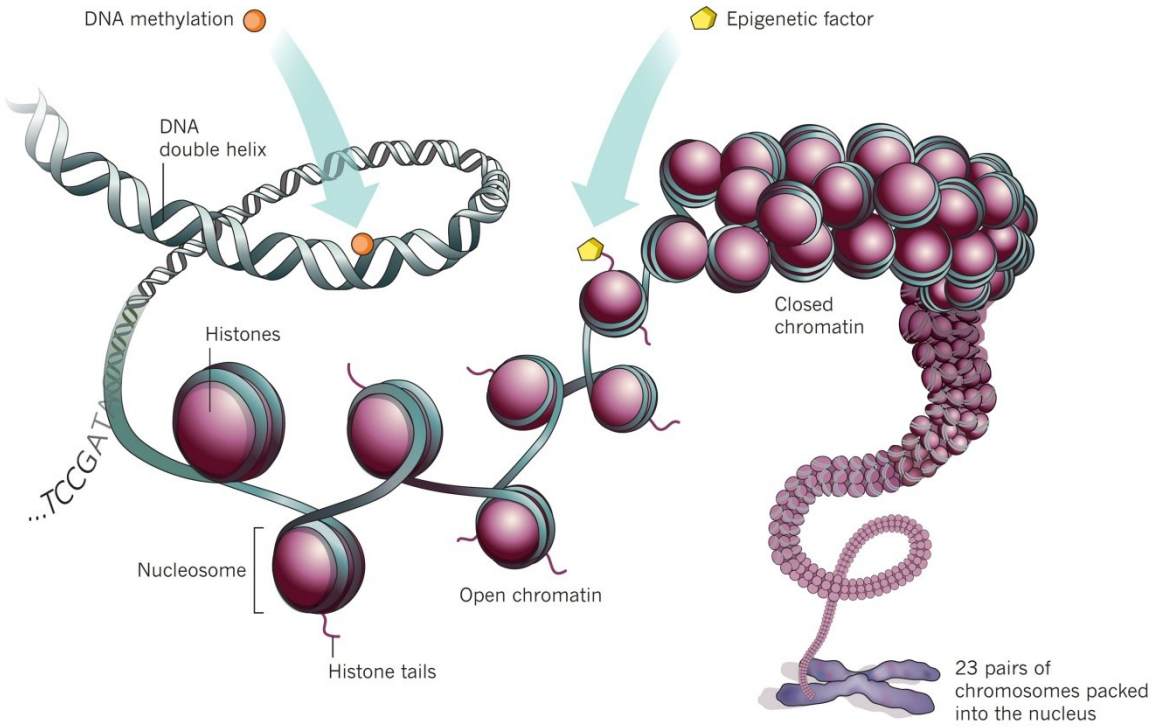


Figure 2. Illustration for the build-up of chromosomes[19].

Histone proteins prefer an octamer as quaternary structure, which is assembled by two of each subunit H2A, H2B, H3 and H4[20]. A high density of amino acids containing basic side chains, like lysine (K) and arginine (R), lead to a surface cluttered with cationic residues[21,22]. With these side chains on the exterior of the histone octamer, DNA – with its negatively charged backbone – gets easily bound to it by salt bridges[23,24] as well as hydrogen bonds and hydrophobic interactions[25]. These histone proteins, each of them wrapped around with an average of 147 base pairs (bp) of DNA, are so called nucleosomes, the central module of chromatin[25]. These nucleosomes are connected consecutively with a 20 to 50 bp long unwrapped linker DNA[26].

Chromatin itself consists of numerous diverse components, including "DNA-binding factors, the basal transcription machinery and its nascent transcripts, replication and repair machineries that copy and maintain DNA, and many other molecules that interact with any of these components"[27]. Furthermore, chromatin has to be divided into two states: (1) heterochromatin, which is highly compacted, e.g. by linker histones H1[28], and (2) euchromatin, a relatively open constitution, where linker DNA is easily accessible[29].

1.1.3 Epigenetic marks

There are two important types of epigenetic modifications, how gene regulation is enabled in chromatin by nature. On the one hand, methylation of DNA occurs, on the other hand, various changes on amino acid side chains of histones take place. These post-translational modifications are regulated by three groups of proteins: writers, erasers and readers (Figure 3)[30,31,32].

(1) Epigenetic writers are responsible for the addition of covalent modifications on histones or DNA, for example histone acetyltransferases (HATs), which are responsible for acetylation of lysine residues in histones.

(2) Epigenetic erasers, like histone deacetylases (HDACs), remove the corresponding epigenetic mark from either the DNA or from histones.

(3) The function of epigenetic readers, represented e.g. by chromodomains or bromodomains (BRDs), is the specific recognition of chemically modified amino acid side chains by protein-protein interactions. Consequently, they are found very often as subunits of eraser or writer proteins, directing and stabilizing those on the right location of the histone or DNA.

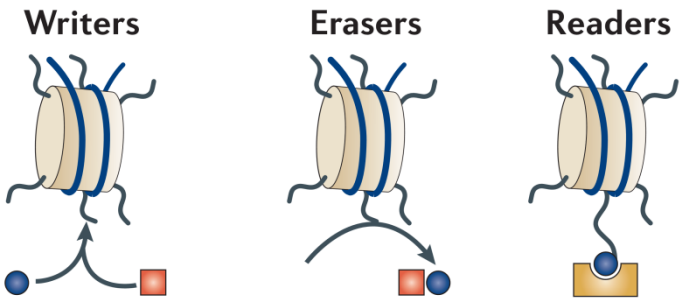


Figure 3. Epigenetic regulators are clustered into three groups: writers, erasers and readers[30].

1.1.3.1 DNA methylation

The most important epigenetic modification of DNA is 5-methylcytosine (5mC), which is mainly observed in 5'-deoxycytidine-3'-phosphate-5'-deoxyguanosine-3' (CpG) dinucleotides[15,33]. However, this heritable methylation has neither an influence on the base pairing with guanine nor an effect on the genetic information of the DNA[34]. Thus, the complementary dinucleotide on the opposite DNA strand shows the same CpG motif and also contains the 5mC (Figure 4)[35].

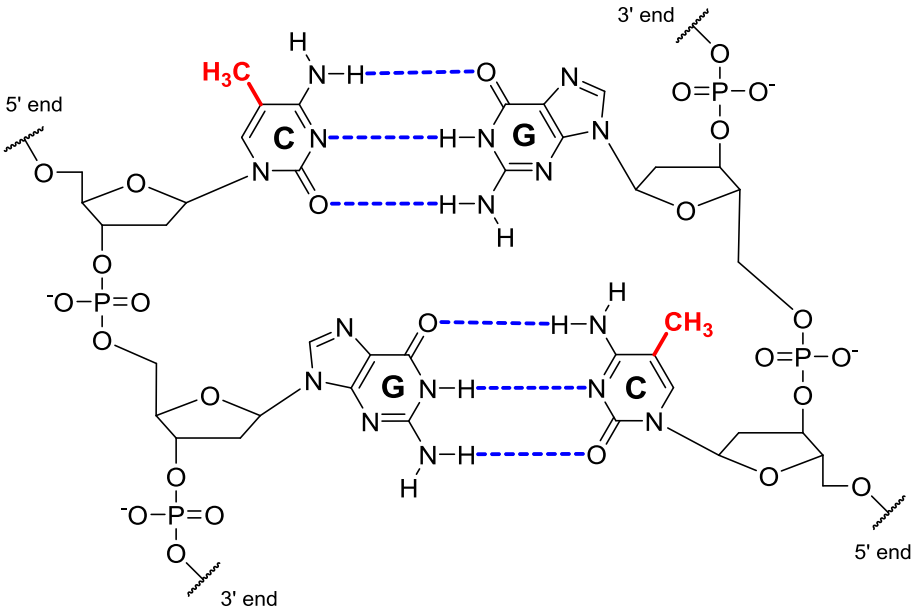


Figure 4. CpG motif of a DNA double strand with both cytosines methylated (red) in 5 position. Hydrogen bonds between cytosine und guanine are colored blue.

DNA methyltransferases (DNMTs) are responsible for transferring methyl groups onto DNA[36]. Family members DNMT3a and DNMT3b are able to methylate cytosine *de novo* on both sides of the DNA strand (Figure 5A), whereas the function of DNMT1 is the complementation of the methyl pattern of a replicated DNA strand (Figure 5B)[35,37]. DNA methylation pattern, for example, can regulate tissue-specific gene transcription and gene silencing[37,38]. Moreover, a lack of DNMTs was observed to lead to lethality of embryonic mice[39].

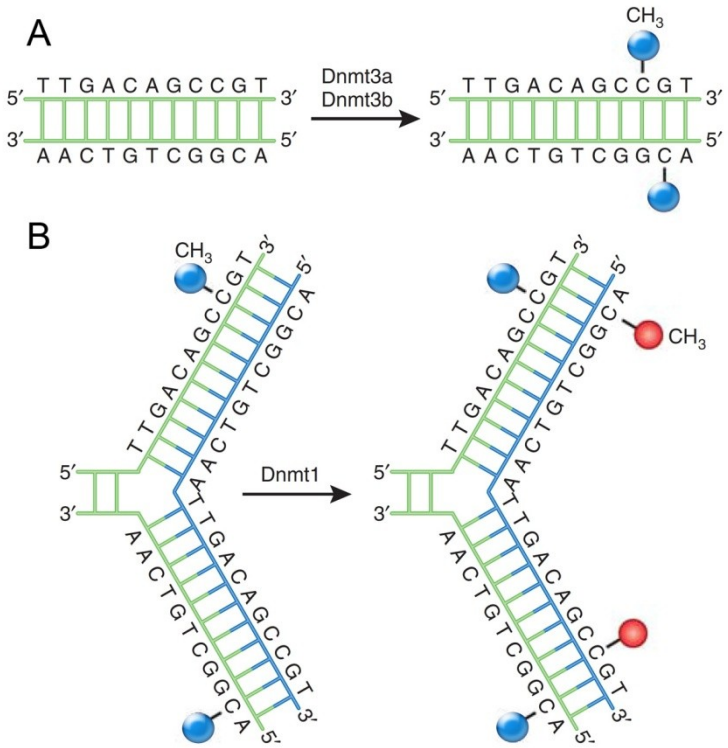
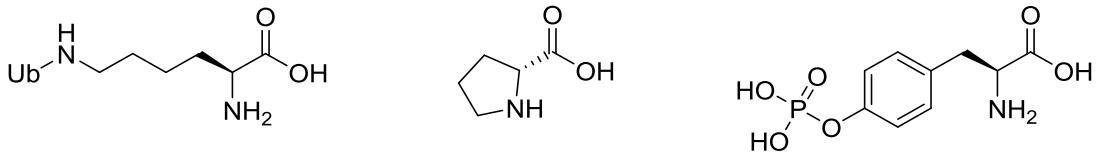


Figure 5. (A) Cytosin *de novo* methylation (blue) of both complementary DNA strands on a CpG motif by DNMT3 a/b. (B) DNMT1 complements the missing methyl groups (red) of the newly synthesized daughter strand[37].

1.1.3.2 Histone modifications

A broader diversity of histone modifications (Figure 7) is given in contrast to simple DNA methylation. This includes ubiquitylation (Ub) of lysines (K), isomerization (Iso) of prolines (P) and phosphorylation (Ph) of hydroxyl group containing amino acids like tyrosine (Y), serine (S) and threonine (T), respectively (Figure 6)[40,41].



ubiquitylated lysine D-proline phosphorylated tyrosine

Figure 6. Epigenetic marks attached to amino acid side chains, like an ε-N-ubiquitylated lysine or a phosphorylated tyrosine. Also the isomerization of L-proline into D-proline plays an important role in epigenetics.

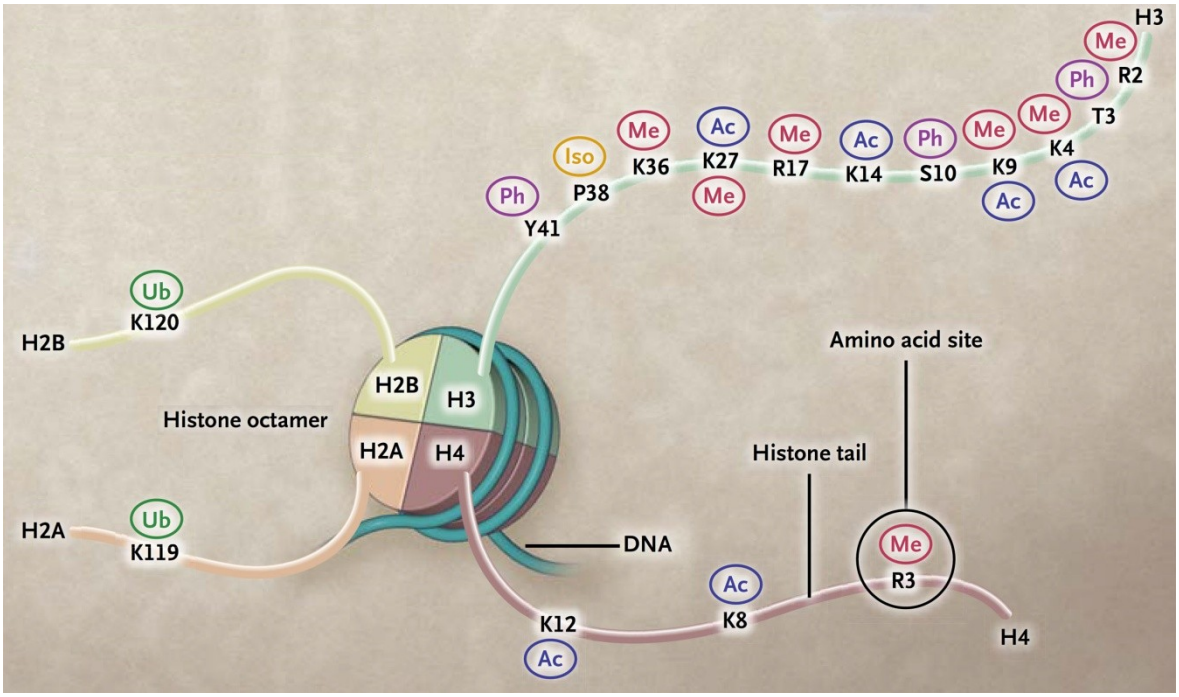
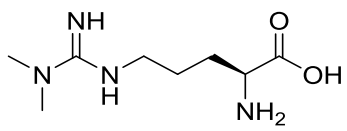
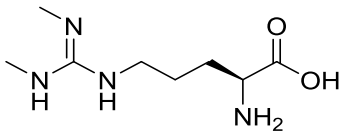


Figure 7. Epigenetic marks on amino acid side chains of histone tails. Methylation (Me), phosphorylation (Ph), acetylation (Ac), ubiquitylation (Ub) and proline isomerization (Iso)[40].

However, these modifications play a secondary role compared to methylation (Me) and acetylation (Ac), e.g. of lysine, which are the most intensively studied ones[42]. In 2002, for example, Tachibana *et al.* showed the necessity of a special lysine methylation in euchromatic histones for early embryogenesis[43]. The terminal ε-amino group of lysine can be monomethylated, dimethylated or trimethylated, whereas arginine (R) has two terminal guanidine nitrogens with different options. Likewise, a monomethylation of one nitrogen, or a dimethylation of either one nitrogen (asymmetric) or of both terminal nitrogens (symmetric)[30].



dimethylated arginine (asymmetric)



dimethylated arginine (symmetric)

Figure 8. Dimethylation of arginine can occur either on the same terminal nitrogen (asymmetric form), or one methyl group on each terminal nitrogen (symmetric form).

Regardless of the respective methylation level, there is no change in the positive charge of the side chain in contrast to acetylation of lysine. By the loss of the cationic amine species in favor of the resulting uncharged amide, the affinity for the DNA phosphate backbone disappears, what allows chromatin to adopt a more relaxed structure[30,44].

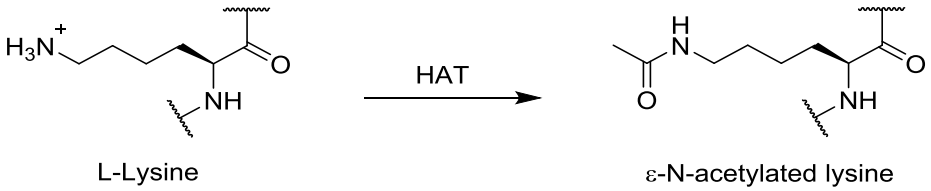


Figure 9. The positive charge of the protonated ε-amino group of lysine disappears after acetylation by a histone acetyltransferase (HAT).

1.2 BROMODOMAINS

1.2.1 An epigenetic recognition module

Besides epigenetic readers and writers, both of which are of enormous interest for drug development research and have already yielded promising targets[45], also the third group – epigenetic readers – extensively gained attention in drug discovery over the last couple of years.

A remarkable class of epigenetic readers are bromodomains, so called because they were first identified in the Drosophila gene *brahma*[46]. They are the main protein interaction modules known for a specifically recognition of ε-N-acetylated lysines (K_{ac})[47]. These evolutionary highly conserved units, with a length of about 120 amino acids, consist of four left-handed α-helices (α_Z, α_A, α_B, α_C), connected by the ZA (between α_Z and α_A) and the BC loop (between α_B and α_C)[48,49]. Both loops have an essential role in determining binding specificity by lining the K_{ac} binding site and marginal variations of their amino acid sequence[50]. Furthermore, in 2000, Owen *et al.* showed[51] by co-crystal structures with proteins, that a asparagine residue, present in most BRDs, which is located in a deep hydrophobic cavity in between both loops, performs a hydrogen bond to the K_{ac} (Figure 10).

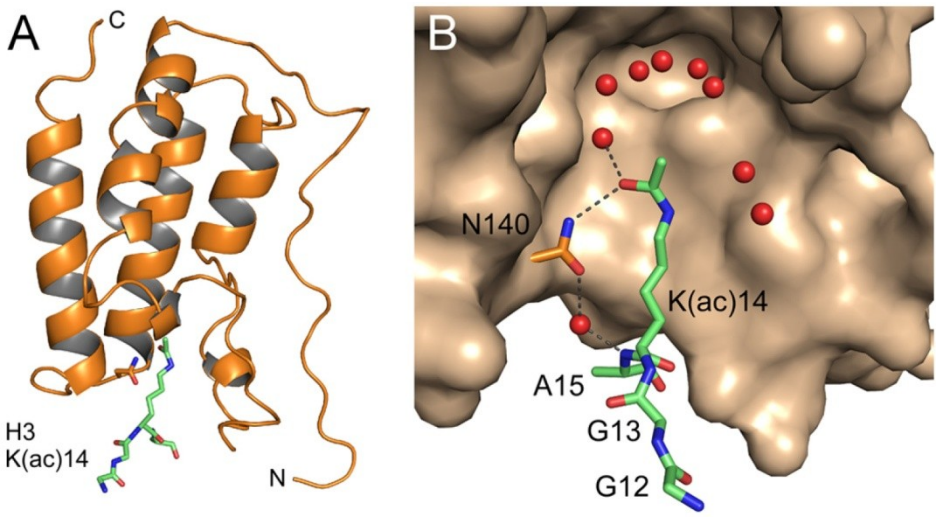


Figure 10. (A) Bromodomain BRD4(1) is shown in complex with a short peptide $GGK_{ac}A$ from H3. (B) Hydrogen bonds between H3 peptide and the asparagine N140 in the hydrophobic cavity of BRD4(1) with several water molecules (red) around[52].

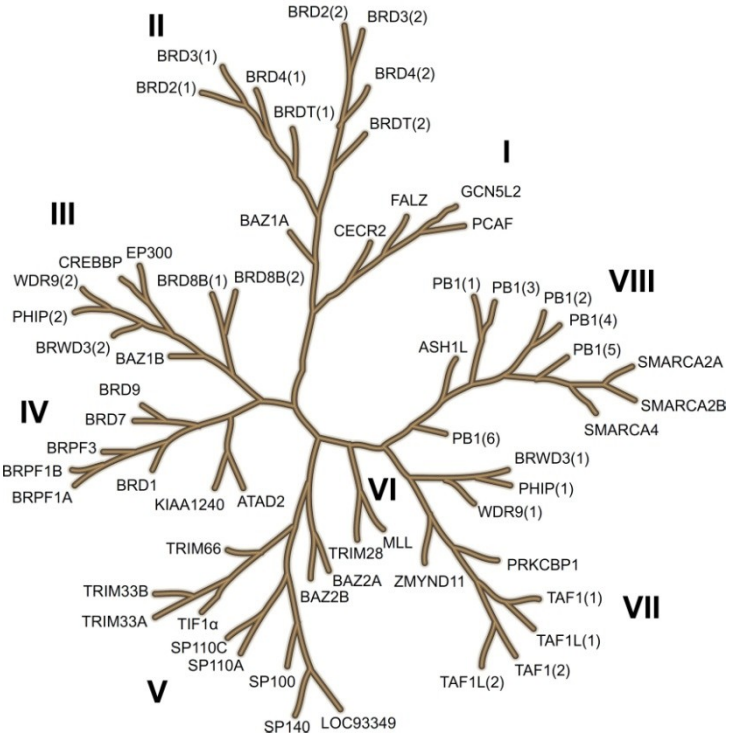


Figure 11. Phylogenetic tree of bromodomains with all 61 modules clustered into eight (using Roman numerals I – VIII) distinct families[48].

46 BRD-containing proteins are encoded by the human genome, comprising one to six modules each protein, leading to a total number of 61 bromodomains[48,53]. They were clustered into eight families (Figure 11), using NMR models, high resolution crystal structures and additionally secondary structure prediction algorithms[48,50]. Müller *et al.* summarized[54] that among these proteins, ATP-dependent chromatin-remodeling complexes[55] and methyltransferases[56,57] were identified as well as nuclear proteins (e.g. HATs)[58] and transcriptional coactivators[59,60,61].

As a consequence of the broad distribution in various classes of proteins, bromodomains represent a highly interesting target for small molecule inhibitors. BRDs are linked to several diverse disease patterns, like HIV transcription, cancer and inflammation[62]. Detailed studies showed a decreased stability and a loss of nuclear localization after deletion of a bromodomain in human HBRM[63,64] (a protein of a remodeling complex), an indispensability of a BRD for the function of GCN5 (a histone acetyltransferase) in yeast[65,66], and the need of BRDs in Bdf1 (protein of *Saccharomyces cerevisiae*) for sporulation and normal mitotic growth[67,68]. In 2011, Dawson *et al.* already described[69] an effective treatment for MLL-fusion leukaemia by the inhibition of bromodomains.

Numerous papers were published dealing with the drugability of this new class of protein-protein interaction modules[70,71]. Bamborough *et al.* improved phenyl-isoxazole sulfonamides[72] after analyzing inhibitor binding modes[73]. 3,5-Dimethyl-isoxazoles[74] and their optimization[75] were published by Hewings *et al.* as potent acetyl-lysine-mimetics. While rhodanines and related heterocycles were discussed[76] to be privileged scaffolds, 2-thiazolidinones were reported[77] as bromodomain inhibitors already one year later by Zhao *et al.*.

1.2.2 The BET family

Subfamily II of the phylogenetic tree of bromodomains (see Figure 11) is the BET (bromodomain and extra-terminal) family, with its members bromodomain-containing protein 2 (BRD2), BRD3, BRD4 and the testis specific species BRDT[48]. They belong to the class of tandem bromodomain proteins, as all of them comprising two N-terminal bromodomains and additionally show highly conserved areas as well as an extra-terminal domain (Figure 12)[52]. BRD2, BRD3 and BRD4 are attributed to be transcriptional regulators[78,79] and BRDT is a chromatin remodeling factor[80]. The chance, that BETs can be addressed selectively by drug-like small molecules, without interfering with other BRDs, raised research efforts and led to promising inhibitors[81,82]. The possibility of using benzotriazepines for BET inhibition, we have already published in 2012[83]. One of the most interesting facts deals with BRDT, which can only be found in testis and is stringently required for spermatogenesis[80]. Endeavors to produce a contraceptive for male, rely on targeting this protein by a potent and selective bromodomain inhibitor[84].

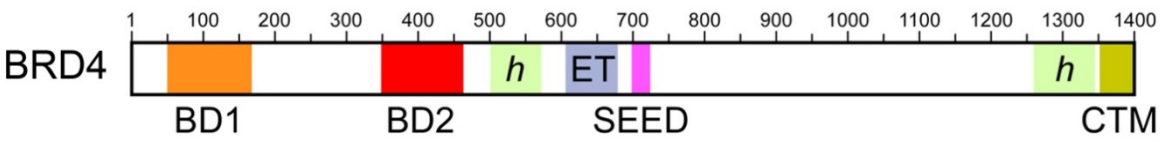


Figure 12. Sequence overview of mouse BRD4, starting with both N-terminal bromodomains BD1 and BD2, followed by an extra-terminal domain ET. Suggested helical areas (*h*) are marked greenish, whereas the magenta SEED region is serine-, glutamate- and aspartate-rich. A conserved C-terminal motif is denoted by CTM[52].

1.3 BENZODIAZEPINE PHARMACEUTICALS – AN IMPORTANT SUBSTANCE CLASS OF DRUGS

The whole benzodiazepine (BzD) success story started in 1960, as Hoffmann-La Roche introduced chlordiazepoxide (Librium®, Figure 13A) in clinical treatment, which was synthesized by Leo H. Sternbach, one of the leading scientists in this research area during that time[85]. He also was responsible for the development of Diazepam, launched under the brand name Valium®, in 1963 by Hoffmann-La Roche (Figure 13B), which is certainly the most famous representative of benzodiazepines[86]. In the following decade from 1965 to 1975, benzodiazepines became the most widely prescribed drugs in the world[87]. Driven by this enormous therapeutic impact, research on benzodiazepines continued and brought worldwide more than 100 pharmaceuticals onto the market over the last 50 years[88].

Figure 13. Chemical structure of (A) chlordiazepoxide (Librium®), the first clinical benzodiazepine drug and (B) diazepam, which was started under the brand name Valium® in 1963.

The mechanism of action of benzodiazepines is described as an allosteric modulation of the function of type A of the GABA (γ-aminobutyric acid) receptor, an ionotropic ligand-gated ion channel[89,90]. Anxiolytic, amnestic, sedative and muscle relaxing properties are ascribed to BzDs, which is why they are successfully used for seizures, anxiety, muscle spasms and sleeping disorders[91,92,93]. Despite these broad fields of application, drugs for different therapeutic use could be developed, as a consequence of unique elimination half-lives and $GABA_A$ subtype selectivity[94].

Midazolam, for example, is used as sedative drug and for treatment of insomnia and acute seizures[95], whereas Verster *et al.* described alprazolam as potent short acting drug for anxiety disorders[96].

The "WHO Model List of Essential Medicines"[97] includes diazepam and midazolam, respectively, which proves the importance of this class of pharmaceuticals.

As a reason of the success of benzotriazepines over decades, the structural closely related benzotriazepines attracted attention for further development of drugs. Research efforts of McDonald *et al.*[98] and Fernandez *et al.*[99] showed new biological activities with benzotriazepines and basic approaches for the future.

CHAPTER II – PROJECT & STRATEGY OF SYNTHESIS

2.1 AIM OF THIS WORK

A cooperation between the group of Prof. Dr. F. Bracher and the group of Prof. Dr. S. Knapp of the University of Oxford led to numerous selective and highly potent kinase inhibitors[100]. A productive chemical synthesis of compounds at the Ludwig-Maximilians University of Munich and a well-organized team for biological assays at the Structural Genomics Consortium (SGC) in Oxford established an efficient collaboration. After years of successful research, the focus – first concentrated exclusively on kinases – became extended by an upcoming class of interesting targets: bromodomains.

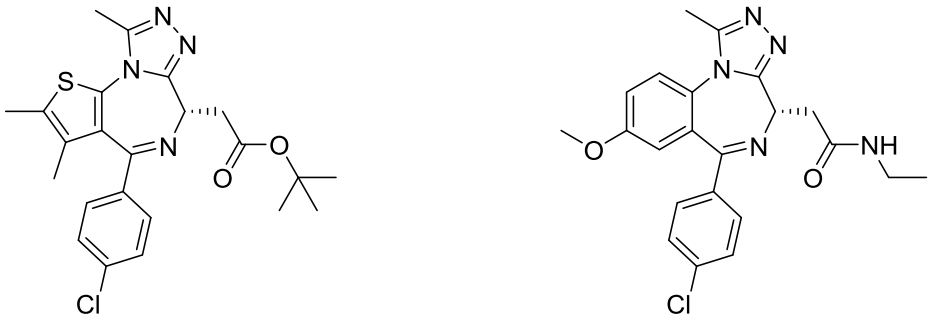


Figure 14. Chemical structures of potent inhibitors of bromodomains.

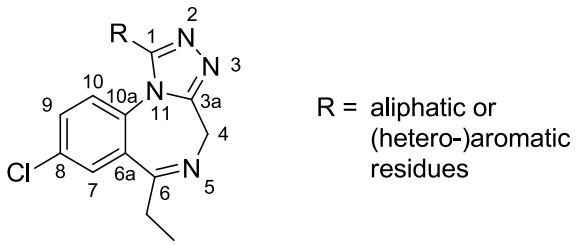
Positive screening results of thienodiazepines against BRD4 by Mitsubishi Pharmaceuticals[101] and the development of potent bromodomain inhibitors like JQ1[102] and iBET762[103] (Figure 14) raised the interest for this class of drug-like small molecules. High-throughput screening (HTS) with commercially available compounds against several diverse bromodomains revealed clinical benzodiazepines (BzDs), e.g. alprazolam and midazolam (Figure 15) as active protein-protein interaction inhibitors selective for BET family members.

Alprazolam Midazolam

Figure 15. Chemical structures of two clinical BzDs.

These results gave the initiation for further investigations towards the development of potent and selective inhibitors targeting bromodomains of the BET family, which was the core issue of this PhD thesis.

To gain better insight of groups relevant or even mandatory for activity of benzodiazepines against BETs, typical structure motifs should be varied. Therefore, molecular modeling based on the benzodiazepine core scaffold was done by the SGC. The resulting target structure (Figure 16) showed significant structural changes.



triazolobenzodiazepine
target structure

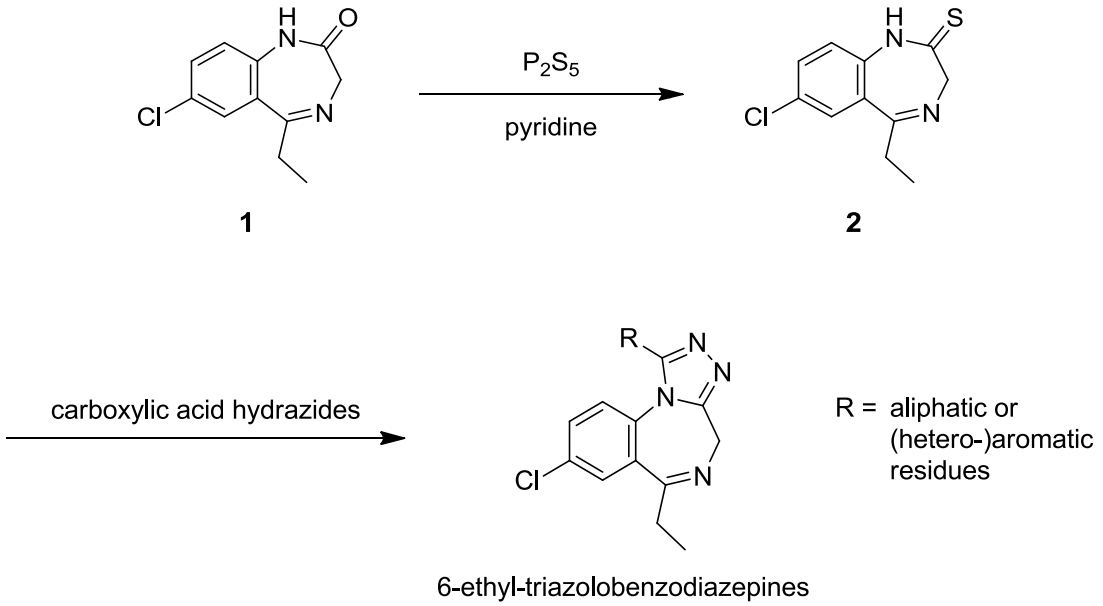
Figure 16. Proposed target structure for triazolobenzodiazepine compounds.

Clinical triazolobenzodiazepines (TBzDs) commonly contain a 6-aryl substituent which was, in our first approach, replaced by an ethyl chain. Furthermore, aliphatic chains or (hetero-)aromatic ring systems instead of a simple methyl group in position 1 were proposed to reach good interactions with the preferred target proteins. These significant changes were supposed to result in either yielding already an active compound or forming the basis for further improvement of inhibitors by meaningful structure-activity releationships (SAR).

2.2 STRATEGY OF SYNTHESIS

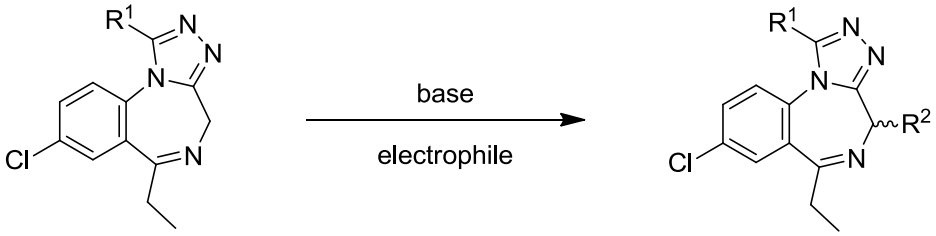
2.2.1 Synthesis development for 6-ethyltriazolobenzodiazepines

Preparation of benzodiazepinone **1** was planned to be achieved according to established procedures[104,105] via a four step synthesis. The following conversion of lactam **1** into thiolactam **2** should be carried out under standard conditions[106] using phosphorus pentasulfide in pyridine. Condensation of carboxylic acid hydrazides with benzodiazepine-thione **2** should give the desired 6-ethyl-triazolo-benzodiazepine compounds (Scheme 1).



Scheme 1. Proposed synthesis for 6-ethyl-triazolobenzodiazepines.

Further modifications were planned to be performed by alkylation or acylation of the triazolobenzodiazepines in position 4. Hester *et al.*[107] synthesized a range of C-4-substituted triazolobenzodiazepines this way. After deprotonation of the slightly acidic methylene group (C-4) by a strong base (e.g. sodium hydride) the addition of an electrophile would lead to a substituted derivative (Scheme 2), considering that both enantiomers would be generated. For first tests in biological assays the racemate would be sufficient.



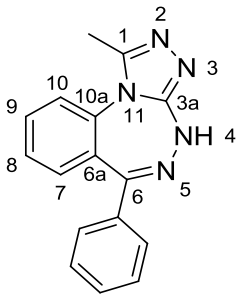
6-ethyl-triazolobenzodiazepines 4-substituted TBzD

R^1 = aliphatic or (hetero-)aromatic residues
R^2 = moieties containing hydrogen bond donors and/or acceptors

Scheme 2. 4-substituted triazolobenzodiazepines.

2.2.2 Synthesis of triazolobenzotriazepines

In consequence of screening results of previously described benzodiazepine compounds the class of target molecules was changed to 1,2,4-benzo**tri**azepines, in the course of our investigations.



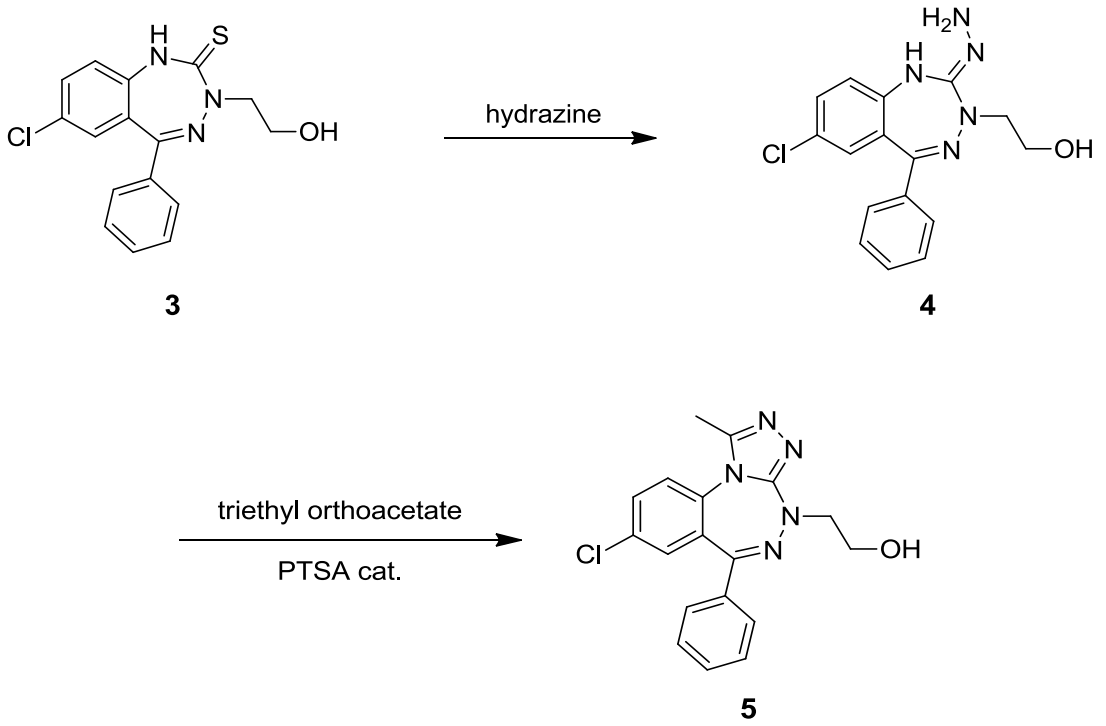
1-methyl-6-phenyl-4*H*-triazolobenzotriazepine

Figure 17. Chemical structure of a simple substituted triazolobenzotriazepine.

The additional nitrogen atom at position 4 required a totally different plan for synthesis in contrast to the one already established for triazolobenzodiazepines. Moreover, the results by differential scanning fluorimetry showed the necessity of the annulated 1-methyltriazole ring as well as the preference of a 6-phenyl instead of a 6-ethyl residue (Figure 17).

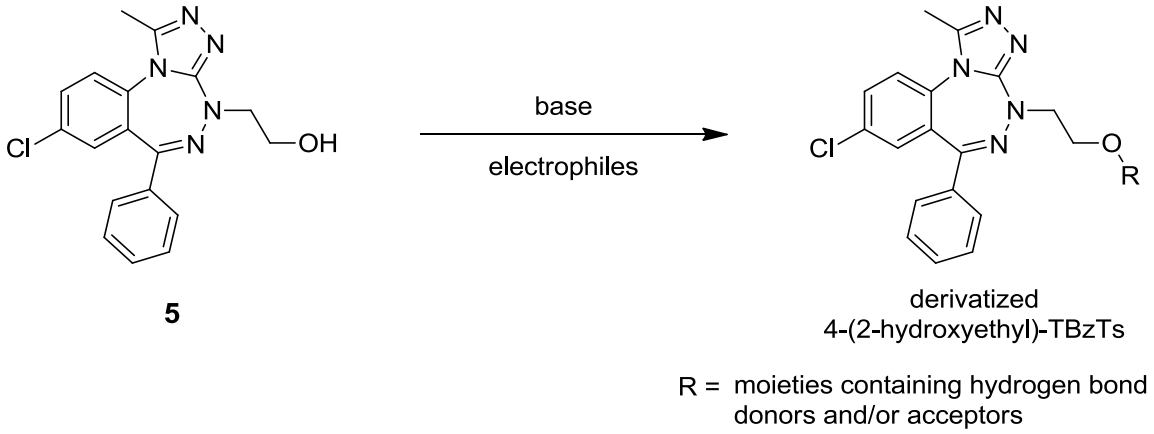
2.2.2.1 4-(2-Hydroxyethyl)triazolobenzotriazepine and derivatives

With regard to further functionalization, synthesis of the triazolobenzotriazepine scaffold was designed already containing a 2-hydroxyethyl chain. Starting from a commercially available 2-aminobenzophenone derivative, known benzotriazepine-thione **3** was synthesized in three steps as described in literature[108,109]. Preparation of the 1-methyl-1,2,4-triazole ring[110] should be realized by treating compound **3** first with hydrazine to generate a hydrazone intermediate. Ring closure should occur by reaction with triethyl orthoacetate and a catalytic amount of para-toluenesulfonic acid (Scheme 3).



Scheme 3. Planned synthesis for 4-(2-hydroxyethyl)triazolobenzotriazepines.

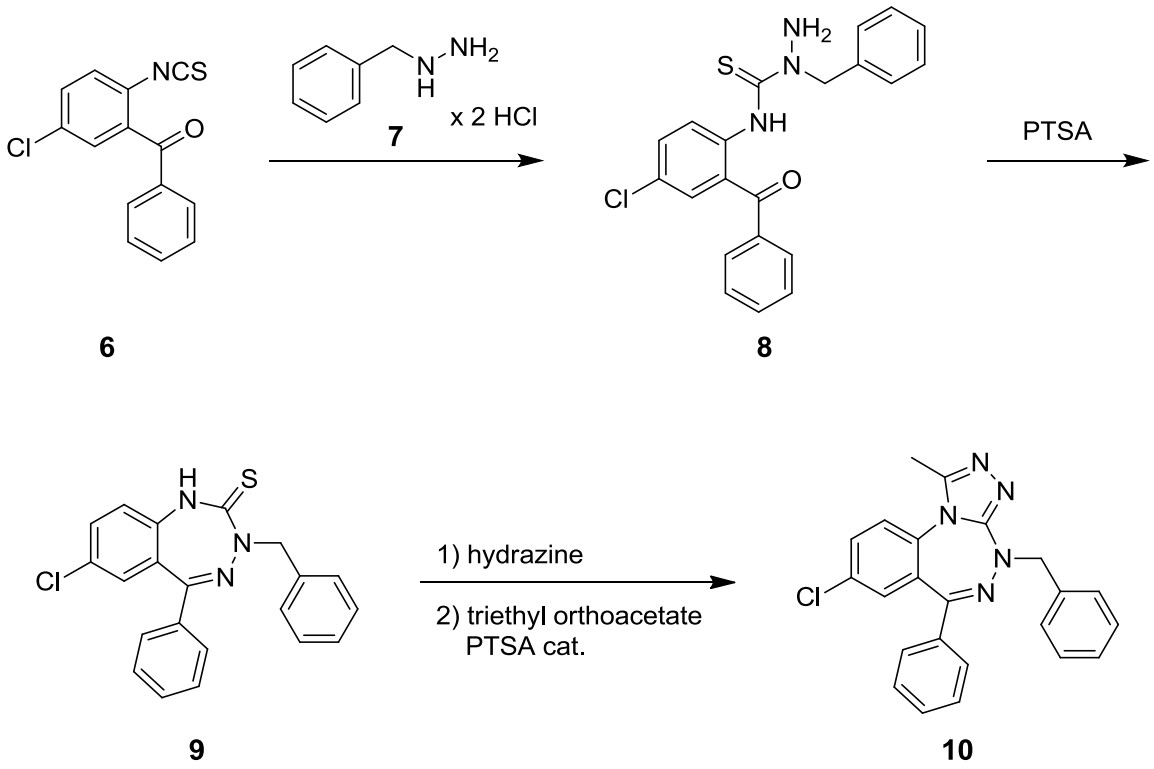
The hydroxyl group in compound **5** makes derivatives easily accessible. The missing steric hindrance of the primary alcohol allows the reaction with a broad variety of electrophiles under basic conditions. Anhydrides or carboxylic acid chlorides, for example, can be used to prepare esters. Isocyanates or isothiocyanates present good building blocks to yield carbamates and thiocarbamates, respectively. Also carbonate esters (e.g. diethyl carbonate) can be used to gain diversity of the attached residues (Scheme 4).



Scheme 4. General procedure for derivatization of 4-(2-hydroxyethyl)triazolobenzotriazepines.

2.2.2.2 4-Benzyltriazolobenzotriazepine

According to the previously described procedure yielding a 2-hydroxyethyl substituent in 4 position, commercially available benzylhydrazine **7** should be used as starting material to realize a 4-benzyltriazolobenzotriazepine **10**.



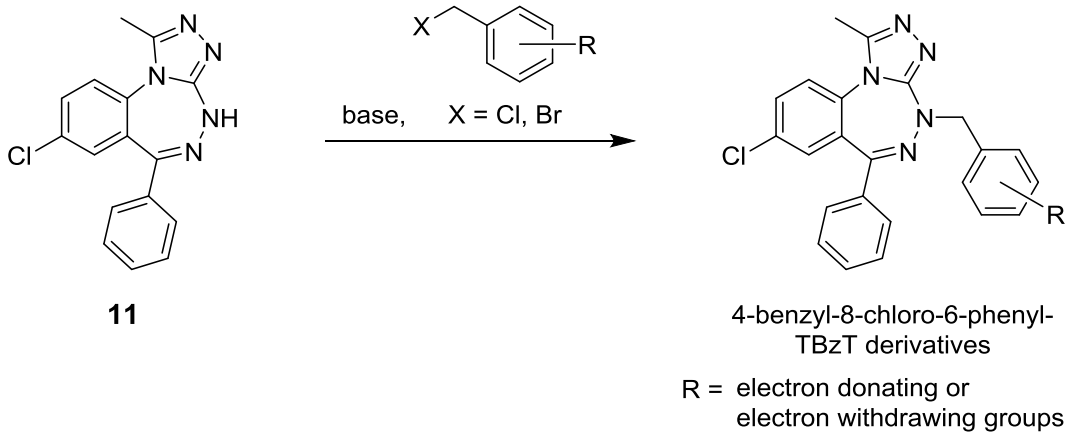
Scheme 5. Proposed synthesis of 4-benzyltriazolobenzotriazepine.

After nucleophilic addition of benzylhydrazine **7** to the isothiocyanate group of known[108] benzophenone derivative **6** the triazepine ring was planned to be formed by treating compound **8** with para-toluenesulfonic acid. The triazole ring of taget compound **10** should be built up in the same manner[110] as explained above by using hydrazine followed by triethyl orthoacetate and a catalytic amount of para-toluenesulfonic acid (Scheme 5).

2.2.2.3 4-Substituted 8-chloro-6-phenyl-4H-triazolobenzotriazepine

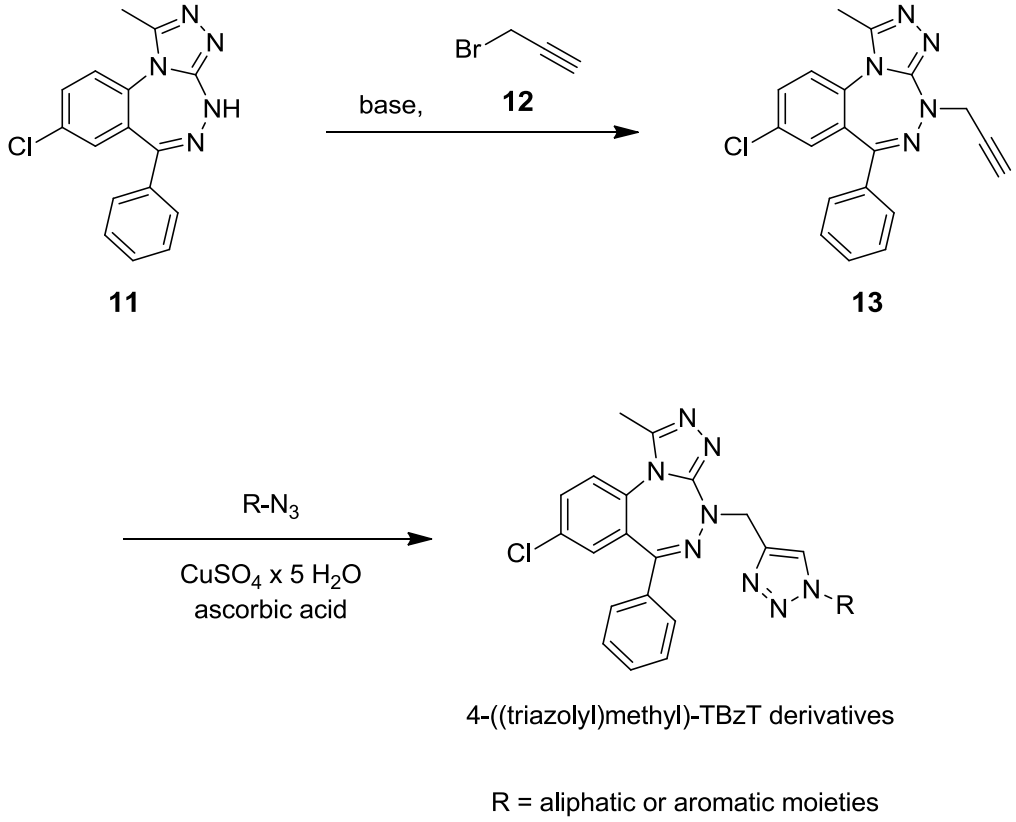
To avoid a permanently repeating synthesis with at least five steps and the poor commercial availability of benzylhydrazines showed the requirement for a central precursor which can be functionalized in a fast and simple way.

Nakamura *et al.* described a synthesis[110] of 8-chloro-1-methyl-6-phenyl-4*H*-triazolobenzotriazepine **11** in a convergent synthesis with overall nine steps. This intermediate can be subjected to N-alkylation by adding a base and various substituted benzyl chlorides or benzyl bromides to generate a series of different 4-benzyltriazolobenzotriazepine derivatives (Scheme 6).



Scheme 6. N-Alkylation of compound **11** using various substituted benzyl chlorides or bromides.

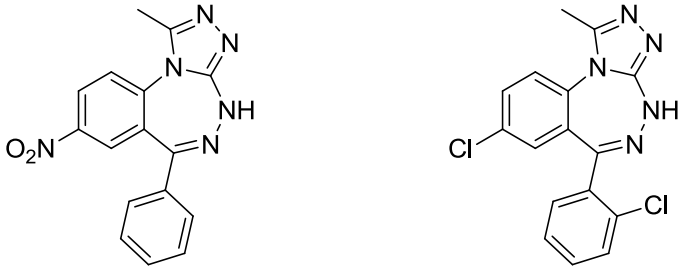
The alkylation of compound **11** with propargyl bromide **12** enables another possibility to gain diversity of target compounds. The terminal alkyne **13** offers the option for copper(I)-catalyzed azide-alkyne cycloaddition[111], which is tolerating a broad variety of functional groups. Primary, secondary and even tertiary aliphatic azides as well as aromatic ones can be used[111]. With additional reagents L-ascorbate, in stoichiometric, and copper(II) sulfate pentahydrate in catalytic amounts the 1,2,3-triazole containing compounds should be obtained (Scheme 7).



Scheme 7. Synthesis of 1,2,3-triazoles via 1,3-dipolar cycloaddition of terminal alkyne **13** and azides.

2.2.2.4 4H-Triazolobenzotriazepines with modified core scaffold

All triazolobenzotriazepines planned to be synthesized above share a common skeletal structure as well as the same substitution pattern: a 1-methyl group, a 6-phenyl residue and chlorine in position 8. For this reason they can be clustered to a group named “chloro series”. As already mentioned the annulated 1-methyltriazole ring revealed as an important structural element, position 8 and the 6-phenyl ring were focused for further variation of substituents to gain a better insight of structure-activity relationship. Again Nakamura *et al.* described the synthesis[110] of two additional 4*H*-triazolobenzotriazepines **14** and **15** (Figure 18).

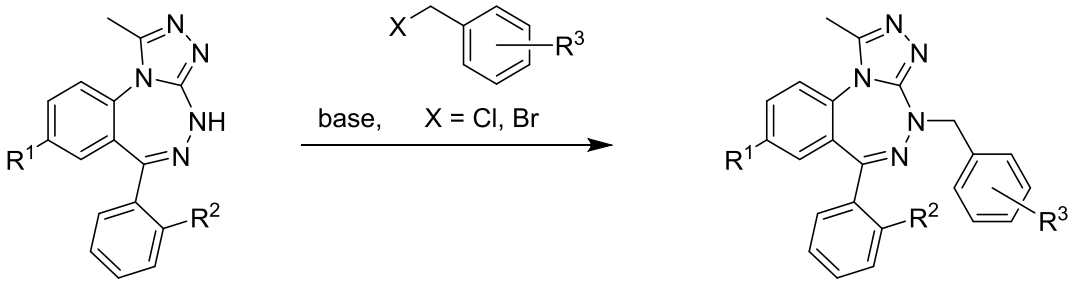


14 **15**

Figure 18. Chemical structures of the 8-nitro-6-phenyl-4*H*-triazolobenzotriazepine **14** and the 8-chloro-6-(2-chlorophenyl)-4*H*-triazolobenzotriazepine **15**.

Starting from the corresponding commercially available 2-aminobenzophenone derivatives, target molecules **14** and **15**, respectively, can be built up in a synthesis of 6 steps. With a similar reaction as shown in Scheme 6, N-alkylation of compounds **14** and **15** with substituted benzyl chlorides or benzyl bromides should be carried out (Scheme 8).

Referring to the term “chloro series” used before and to obtain a better overview of screening results, tables and in discussions the compounds planned to be synthesized were clustered again. All target molecules yielded after alkylation of TBzT **14** were grouped into the “nitro series”, all molecules with the substitution pattern 8-chloro *and* 6-(2-chlorophenyl) according to intermediate **15** were summed up as “dichloro series”.



14 $R^1 = NO_2$, $R^2 = H$
15 $R^1 = Cl$, $R^2 = Cl$

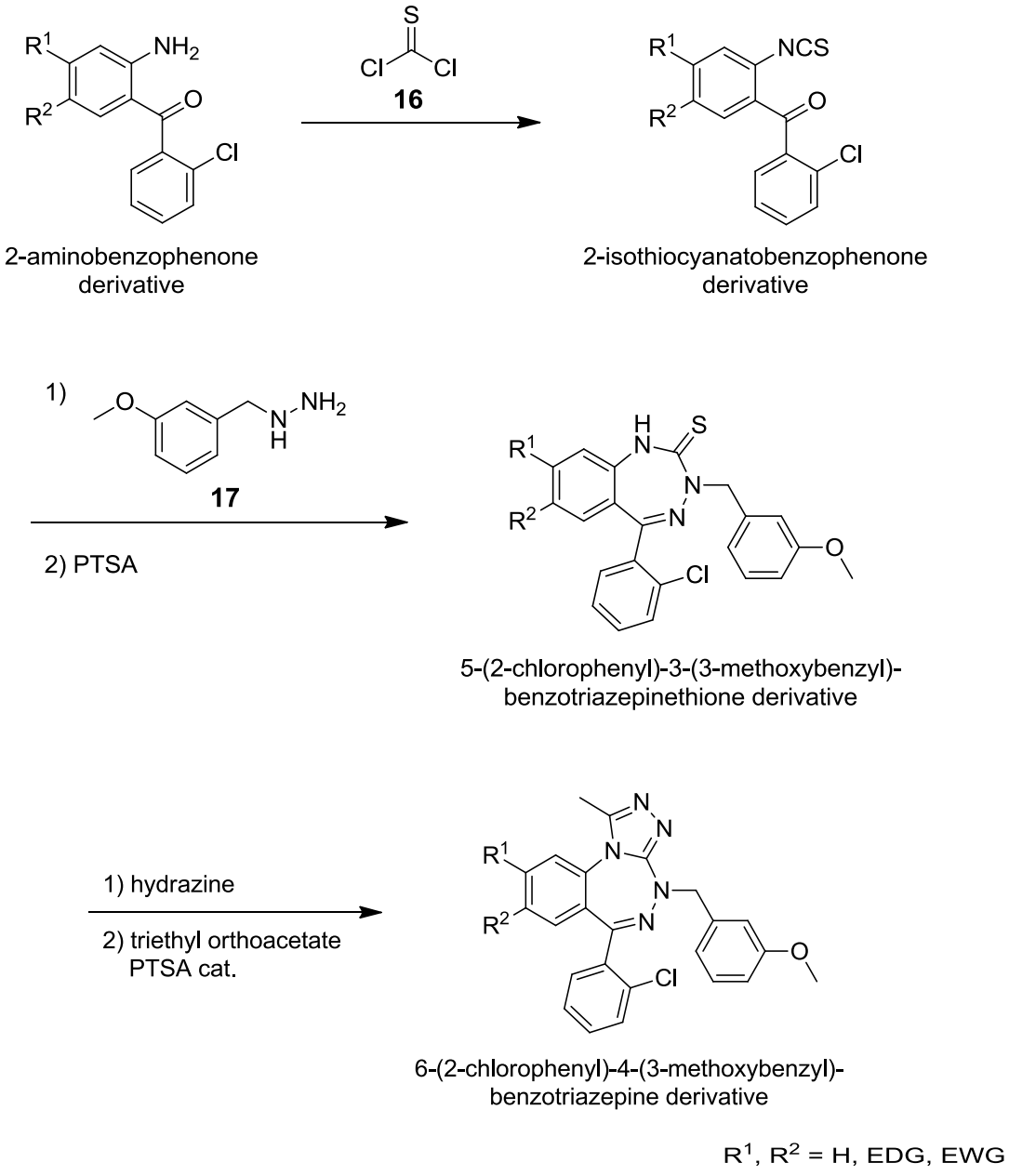
"nitro series"
$R^1 = NO_2$, $R^2 = H$, R^3 = EDG or EWG
"dichloro series"
$R^1 = Cl$, $R^2 = Cl$, R^3 = EDG or EWG

Scheme 8. Planned synthesis for generating the “nitro series” and the “dichloro series”.

2.2.2.5 Further optimization of the annulated benzo ring system

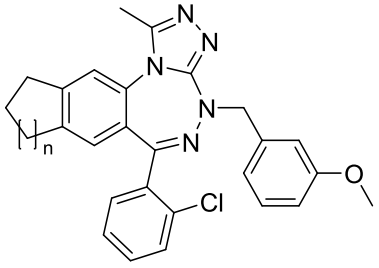
Based on new results further refinement of the bromodomain inhibitors had to be done. Having a 6-(2-chlorophenyl) and a 4-(3-methoxybenzyl) moiety attached to the triazolobenzotriazepine turned out to be the most favorable combination. Therefore, both residues were kept constant during optimization of the benzo ring.

Considering the review[112] of David A. Walsh, the substitution pattern of the 2-aminobenzophenone starting material can almost be chosen freely. The following formation of the 2-isothiocyanatobenzophenones should be done according to Richter *et al.*[108] using thiophosgene **16**. The remaining four steps to obtain the novel substituted triazolobenzotriazepines should be carried out as already described[110] above. Addition of (3-methoxybenzyl)hydrazine **17** and subsequent cyclization – catalyzed with para-toluenesulfonic acid – might yield the corresponding 5-(2-chlorophenyl)-3-(3-methoxybenzyl)benzotriazepinethione derivatives. After treating the benzotriazepinethione derivatives with hydrazine in the first step and with triethyl orthoacetate and para-toluenesulfonic acid in the second step the formation of the annulated 1-methyltriazole ring should be completed (Scheme 9).



Scheme 9. Synthesis outline for structural variations of the annulated benzo ring.

In addition to the described synthesis above using multi substituted 2-aminobenzophenones as starting materials, also compounds containing a further annulated ring system can be chosen. Figure 19 illustrates a possible structure comprised of the triazolobenzotriazepine core scaffold with the preferred substitution pattern and an annulated five- or six-membered carbocycle. However also aromatic and / or heteroatom containing ring systems would be conceivable.



n = 1, 2

annulated triazolotriazepine derivatives

Figure 19. Additional ring annulated to the triazolobenzotriazepine core scaffold to gain diversity within synthesized structures.

CHAPTER III – METHODS FOR STRUCTURE OPTIMIZATION

A broad variety of different substituted compounds has been synthesized during this work. However, we have left nothing to chance. The decision to change the scaffold from benzodiazepines to benzotriazepines, using electron withdrawing groups or electron donating ones as well as adding or removing ring systems and substituents, respectively, was not random. For structure optimization we used three different methods, which are discussed in the following chapters:

A) Differential scanning fluorimetry, DSF (Chapter 3.1)

B) Isothermal titration calorimetry, ITC (Chapter 3.2)

C) Co-crystallization of proteins with ligands (Chapter 3.3)

These experiments were carried out predominantly by Dr. Sarah Picaud under the supervision of Dr. Panagis Filippakopoulos at the Structural Genomics Consortium of the University of Oxford. During two research stays I gained valuable insights into the different techniques and carried out differential scanning fluorimetry of several compounds by myself.

3.1 DIFFERENTIAL SCANNING FLUORIMETRY

The general technique we used for determination of binding interactions between compounds and proteins was differential scanning fluorimetry (DSF). This kind of method, a thermal shift assay, combines two key advantages for broad screenings: it is fast and inexpensive. Both aspects allowed us to screen almost all synthesized target compounds, against up to 13 bromodomains, in a short space of time.

3.1.1 The principle of measurement

The measurement principle of DSF is based on monitoring the melting temperature (T_m) of proteins, which is considered as the point of equal concentrations of folded and unfolded protein.

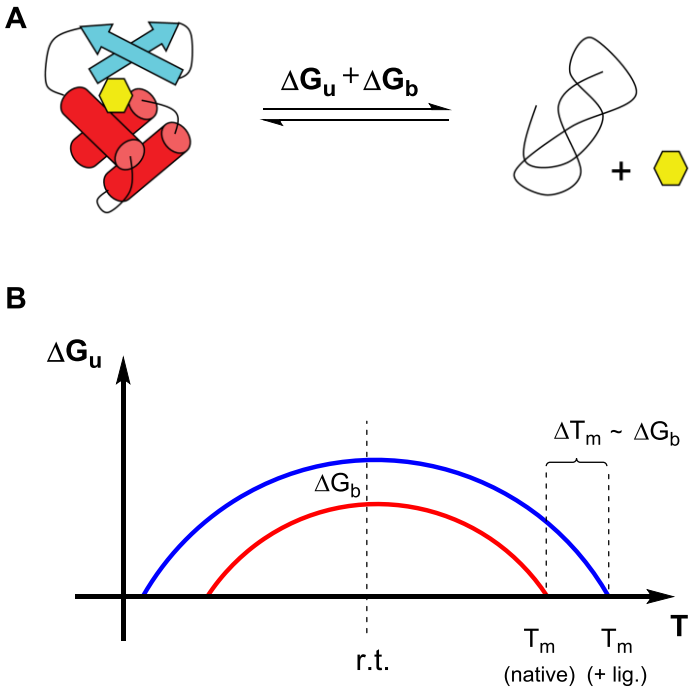


Figure 20. (A) Reversible two-state manner of folded (left) and unfolded (right) protein with ligand binding. (B) Graphical interpretation of the stability of proteins, related to ΔG_u. A protein stabilized by a ligand (blue curve) has a higher stability at room temperature (r.t.) and consequently a higher T_m as the native protein (red curve).

The stability of proteins has its maximum typically close to room temperature and decreases by cooling or heating[113]. If a protein unfolds in a reversible two-state manner, the equilibrium thermodynamics model will apply (Figure 20A)[114]. This thermodynamic interpretation of the stability is given by the temperature dependent Gibbs free energy of unfolding (ΔG_u), which becomes zero at the melting temperature T_m (Figure 20B)[115].

In case of compound interaction with the protein, ΔG_u usually increases by the free energy value of ligand binding (ΔG_b). This stabilization effect leads to a higher T_m value of the protein-ligand complex. Exactly this shift in melting temperature (ΔT_m) is our desired measurement parameter, because it correlates[116] with ΔG_b and consequently with the dissociation constant K_d of the ligand, what leads to the simplified statement: the higher the T_m shift, the better the affinity of a ligand towards a protein (for a more detailed discussion see Chapter 3.1.3).

3.1.2 Measurement and analysis

The sample preparation was done in a standard 96 well microtiter plate by adding the respective compound to each required BRD, mainly represented by the eight domains of our target proteins, the BET family (see Chapter 1.2.2), as well as five more BRDs (PCAF, CREBBP, LOC93349, PB1(5), BAZ2B), to cover the whole phylogenetic tree. The measurements were performed using a real-time PCR instrument by heating the plates from room temperature to about 100 °C. For visualization of protein unfolding, the mixture was treated with a fluorescent dye, named SYPRO® orange.

Analysis of DSF is done with a plot of the fluorescence intensity as a function of temperature (Figure 21). Almost no signal is obtained as long as the protein is present in the native state, because of the quenched fluorescence of the dye in aqueous solution. At higher temperatures, the protein starts to denature and exposes its hydrophobic sites.

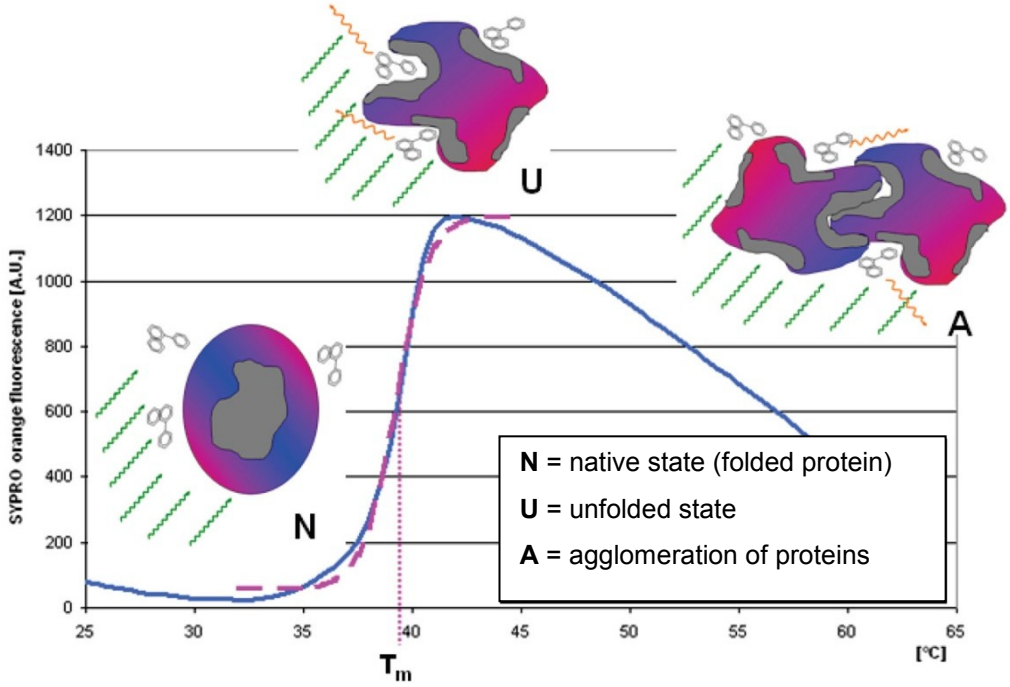


Figure 21. Example graph (blue curve) of a DSF measurement of a protein in presence of SYPRO® orange (symbolized by an aromatic molecule), shows the change in fluorescence during the different states of the protein. After fitting (purple curve) the graph by processing software the T_m value can be determined[117].

In this unpolar environment, the intensity of the fluorescence of SYPRO® orange increases to about 500% (Figure 22), leading to an enormous gain of the measured signal. This two-state transition of folded and unfolded protein results in a sigmoidal curve, which can be analyzed using, for example, the Boltzmann equation to calculate the inflection point T_m. Although SYPRO® orange has a lower gain of fluorescence upon denaturation of the protein, in contrast to e.g. 1-anilino-8-naphthalene sulfonate (1,8-ANS), the advantage is the excitation wavelength of 492 nm. Practically, there is no interference of any small molecule with the optical properties of the dye, which can produce incorrect results[117].

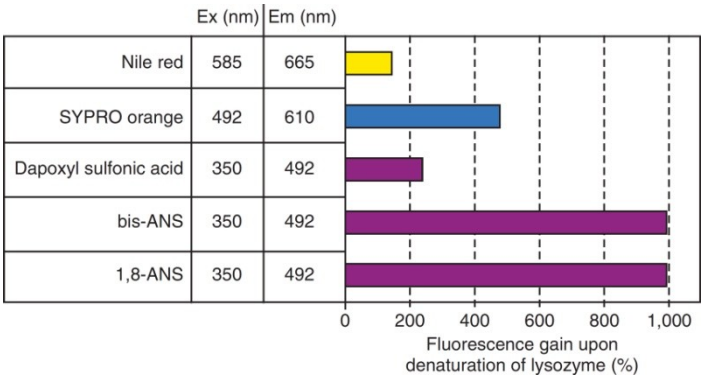


Figure 22. Comparison of the fluorescence intensities of different dyes in the presence of lysozyme. The intensities were measured before and after incubation for five minutes at 100 °C[117].

3.1.3 Thermodynamic view on DSF: entropy vs. enthalpy

The statement mentioned above, "the higher the T_m shift, the better the affinity of a ligand towards a protein", has to be discussed a little bit more in detail. In general, it is right with due regard to two restrictions: one the one hand, you can only compare results of the same BRD and on the other hand, the compounds screened against a certain BRD should share a similar chemical structure to be comparable.

But why these limitations? During our research project, we collected plenty of T_m shifts of diverse compounds with several bromodomains. ITC (see Chapter 3.2) was used subsequently for measuring K_d values of high accuracy. However, we discovered a huge difference of K_d values of compounds with similar ΔT_m. Obviously, all second domains of BDR2, BRD3, BRD4 and BRDT showed higher T_m shifts as the corresponding first domains, but lower affinity of compounds was determined by ITC. The answer to this problem was to factor thermodynamic aspects, more precisely enthalpy and entropy.

As already shown above (Figure 20B), T_m is related to ΔG_u, the Gibbs free energy of unfolding, and in the event of ligand binding, the Gibbs free energy of the system ΔG is given by:

$$\Delta G = \Delta G_u + \Delta G_b = \Delta H - T\Delta S = -RTlnK_a \qquad (i)$$

where ΔG_b is the additional free energy of binding, ΔH the enthalpy change, T the absolute temperature, ΔS the change in entropy, R the gas constant and K_a the association constant.

As a consequence that ΔG is a function of T with T_m being the point of intersection with the x-axis, T_m is also correlated with ΔH and ΔS. Hence, with our T_m shifts, obtained by DSF, it is not possible to decide, whether the major contribution is given by entropy or enthalpy.

The calculation of K_a and K_d values, respectively, is possible only by referring to standard conditions according to equation (ii).

$$\Delta G° = \Delta H° - T\Delta S° = -RTlnK_a = RTlnK_d \qquad (ii)$$

where ΔG°, ΔH° and ΔS° are the corresponding terms under standard conditions. Otherwise, to compare diverse T_m values to affinities of different compounds, long extrapolations to a defined set of relevant standard conditions has to be done, which can produce large errors.

The bond-dissociation energy in chemistry, is defined[118] – with limitations – as the standard enthalpy change ΔH°. Despite reversible binding events of protein-ligand complexes are subjected to different conditions and restrictions, the most important parameters for strong ligand binding are hydrogen and/or halogen bonds as well as electrostatic interactions[119,120]. All these factors are enthalpic, whereas conformational restrictions and the release of water molecules of the binding pocket and the solvated ligand, are associated to entropy[121,122].

These matters of fact led to the assumption, that T_m (and also ΔT_m) is dependent on both, enthalpy and entropy, but K_d is primarily dependent on enthalpy. Holdgate *et al.* published[123], in 2005, a highly interesting paper, dealing with exactly those thermodynamic facts in drug discovery. Our hypothesis is supported by the equation[123] formed by Holdgate *et al.*, which describes the affinity of a ligand at the melting temperature ($K_d^{T_m}$):

$$K_d^{T_m} = \frac{[L]}{exp\left\{\frac{-\Delta H_u}{R}\left(\frac{1}{T_m} - \frac{1}{T_0}\right) + \frac{\Delta C_{p\,u}}{R}\left(ln\left(\frac{T_m}{T_0}\right) + \frac{T_o}{T_m} - 1\right)\right\} - 1} \qquad (iii)$$

where [L] is the free ligand concentration at T_m and T_m is the melting temperature for the protein-ligand complex. ΔH_u is the enthalpy of unfolding, $\Delta C_{p\,u}$ is the heat capacity of unfolding at constant pressure and T_0 is the melting temperature with all three terms referring to the uncomplexed protein.

Holdgate *et al.* also showed[123] theoretical calculations, that entropically driven ligand bindings can result in the same T_m shift by a fifty fold difference in K_d. This is the case in all of the second domains of the BET family, showing high temperature shifts in DSF, but low K_d values by ITC, what correlates to the entropically driven assumption. T_m shifts of first domains, which are more enthalpically driven, resemble very well to results gained e.g. in kinase screenings.

To conclude, DSF can be used as a powerful high-throughput method for screening plenty of compounds against many proteins in a rapid and cost-efficient way. The obtained T_m shifts give a general survey, if compounds show affinity towards any of the proteins or not. For a more detailed consideration of the results, care has to be taken, that ΔT_m can only be compared in between compounds measured with the same protein.

3.2 ISOTHERMAL TITRATION CALORIMETRY

For a very accurate determination of K_d values, isothermal titration calorimetry (ITC) was used. This method combines advantages like direct measurements of binding stoichiometry (n) between ligand and protein, binding affinity (K_a) of the ligand and change in enthalpy (ΔH)[124]. These parameters allow a further calculation of important values like the entropy change (ΔS) and the change in Gibbs free energy (ΔG) by simple thermodynamic laws (see equation (i) and (ii), Chapter 3.1.3)[123]. However, ITC is not a high-throughput technique, due to the need of relatively large amounts of protein and long duration of measurement up to two hours for each sample.

The apparatus (Figure 23A) consists of two cells, located in an adiabatic jacket, and a stirring syringe. The reference cell is connected to a constant power supply and typically heated with <1mW[124]. A feedback power is applied to the sample cell and responsible to maintain the equivalent temperature of the reference cell. The experiment is either be carried out filling the sample cell with the protein and adding the ligand by the syringe or the other way around.

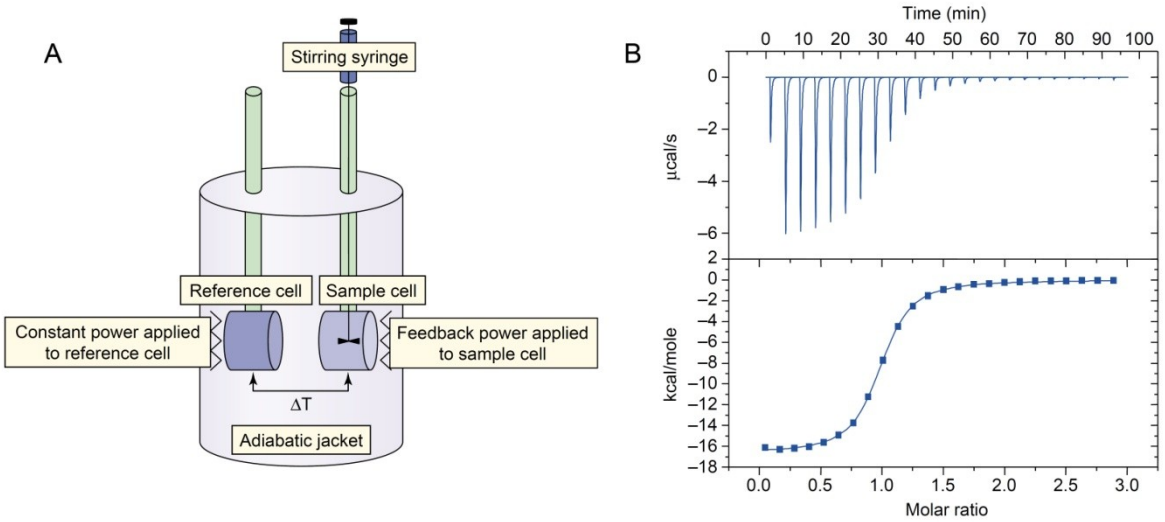


Figure 23. (A) ITC measuring instrument in a schematic representation. (B) Exemplary exothermic ITC diagram with feedback power of the reference cell plotted as a function of time (top) and the integrals of the obtained spikes plotted against the molar ratio and fitted by a sigmoidal curve (bottom)[123].

The inverse experiment is usually favored, because of the lower concentration of the protein in contrast to the ligand. Dilutions always lead to a change in enthalpy, which would be also measured and falsify the results[125]. This effect can be minimized by adding the higher diluted component (protein) by syringe into the sample cell, filled with the more concentrated component (ligand).

As far as the ligand shows affinity towards the protein, ligand binding occurs after every injection, which leads to an exothermic (Figure 23B) or an endothermic reaction. An exothermic binding event, releasing heat, decreases the feedback power to keep the level of the reference cell and thus, generating the negative spikes (peaks in an ITC diagram). In the case of an endothermic reaction, the opposite response would take place. The declining availability of free binding sites for the ligand, resulting in lower heat release and consequently in smaller spikes, which disappear after complete saturation of the binding pockets. The integrals of these spikes can be plotted against the molar ratio between protein and ligand for further analysis, done by processing software, to yield the requested parameters.

3.3 CO-CRYSTALLIZATION OF PROTEINS WITH LIGANDS

DSF and ITC are important tools for measuring affinity and selectivity of ligands for certain proteins, determination of binding constants and magnitudes of thermodynamic parameters, but neither the binding site nor the binding mode can be identified. Therefore, the use of crystallization studies is absolutely essential. Co-crystallization structures of protein and ligand with high resolution (better than 2 Å), give detailed information for further structure refinement (Figure 24).

The question, which part of the protein gets addressed by the ligand and how the ligand co-ordinates into the pocket can unerringly be confirmed. The amino acid sequence of the binding pocket as well as the orientation of their side chains can be ascertained. Moreover, it allows the study of hydrogen bonds, either directly from the ligand to the protein or mediated by water molecules.

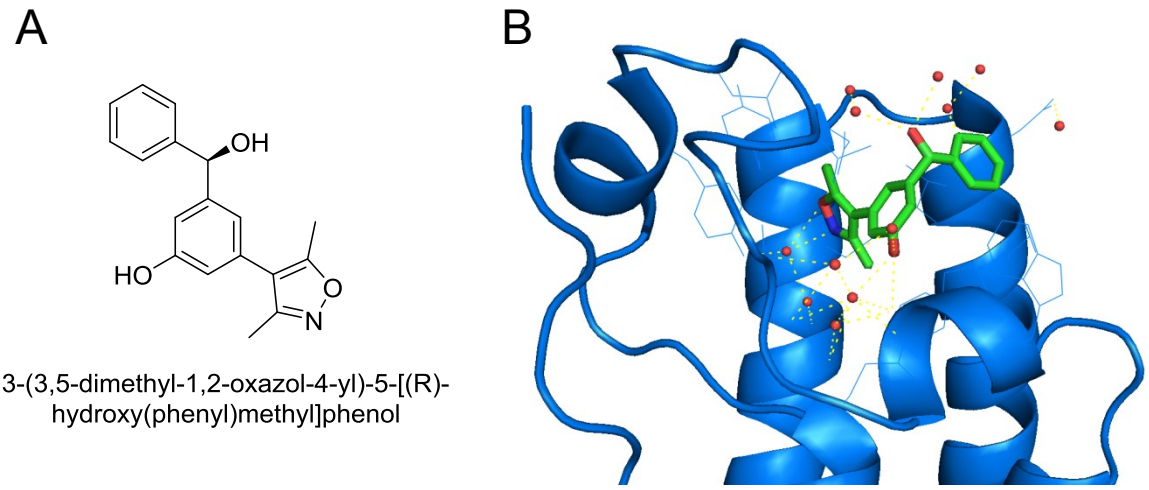


Figure 24. (A) Chemical structure of a 3,5-dimethylisoxazol used as ligand for co-crystallization. (B) Crystal structure (PDB ID = 4J0R) of 3,5-dimethylisoxazol with domain one of human BRD4 in high resolution of 1.72 Å. Water molecules are shown as red spheres[75].

For structure optimization, distances and angles can be calculated by appropriate software (e.g. WinCoot) to estimate chemical modifications of the ligand. The introduction of new (functional) groups might enable the ligand to form further interactions and result in better affinities. Even theoretical molecules can be superimposed with the crystallized ligand to get more illustrative presentations.

These advantages of co-crystallization structures speak for themselves, however, preparation is more characterized by trial and error. Various molar ratios of protein and ligand as well as different concentrations of buffer and cryoprotective agents, to which further reference will not be made here, have to be tested.

CHAPTER IV – SYNTHESIS, RESULTS & DISCUSSION

The following chapter guides through the whole laboratory work in a chronological way. First, detailed syntheses – from commercially available starting materials to target molecules – of each group of compounds are described and specifics get explained. Subsequent to the synthesis part, DSF screening results of the corresponding series are discussed. The analysis of temperature shift results was used for planning further series and whenever available also ITC measurements and X-Ray data (co-crystallizations) were used for their optimization. This cycle (Figure 25) of synthesis, screening and analysis was passed through several times to yield potent and selective target molecules.

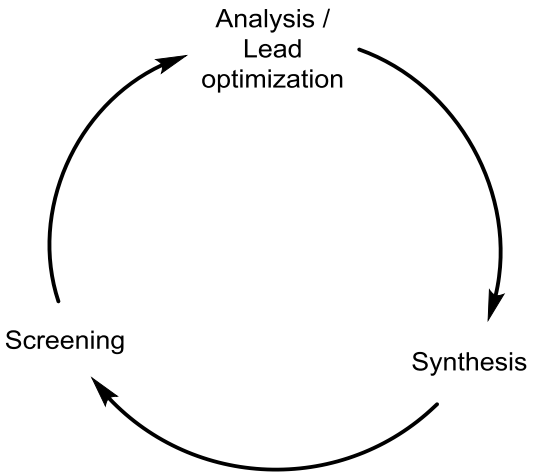


Figure 25. Illustrative model for continuous improvement of compounds.

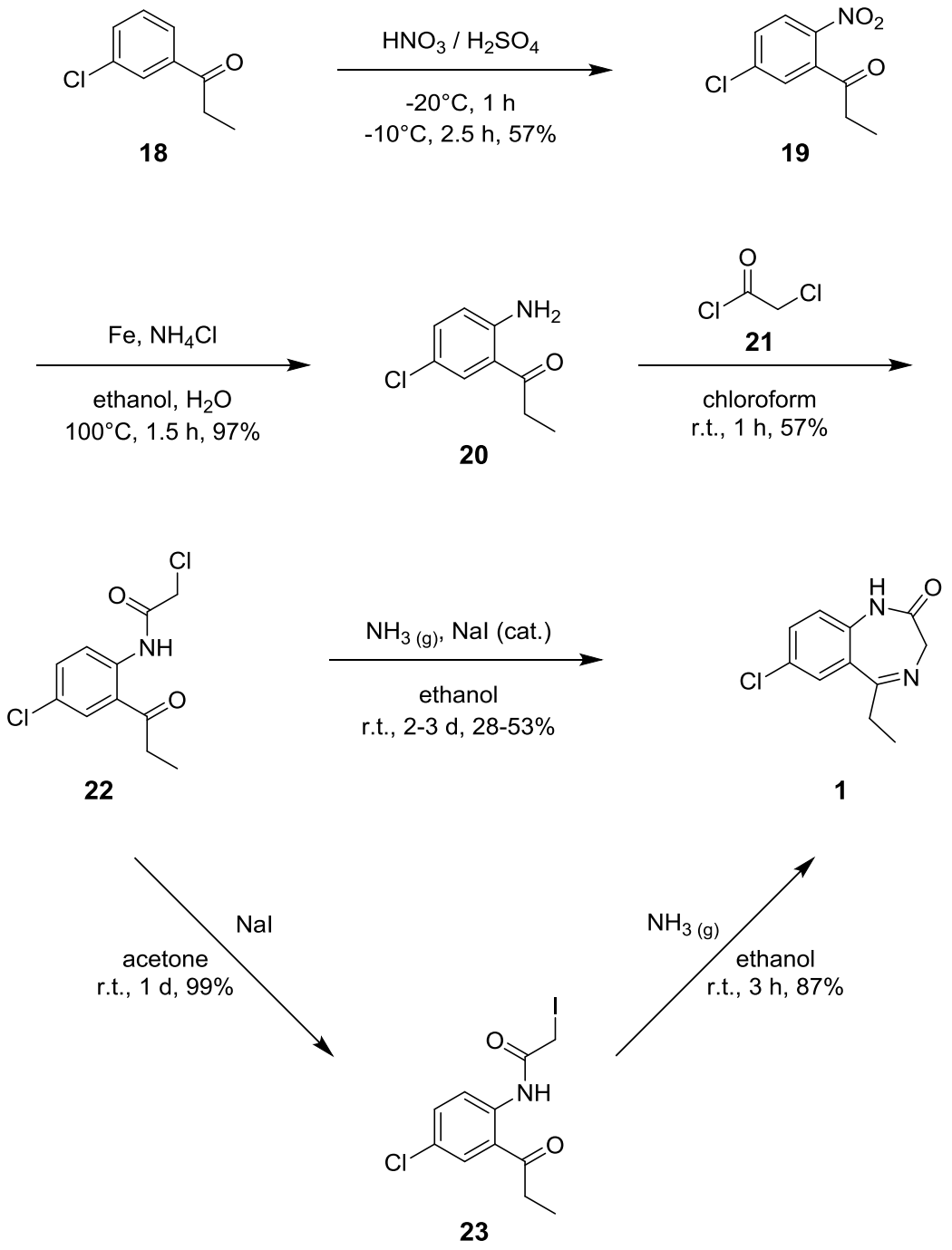
4.1 TRIAZOLOBENZODIAZEPINES:

THE FIRST CLASS OF TARGET COMPOUNDS

The first project was the development of a synthesis for triazolobenzodiazepines. As already mentioned and described in Chapter 2.1 molecular modeling, done by the group of Prof. Knapp at the SGC at the University of Oxford, led to the 8-chloro-6-ethyl-triazolobenzotriazepine target compound with different substitution patterns on the triazole ring in position 1 (Figure 16).

4.1.1 Synthesis of the benzodiazepinone core scaffold

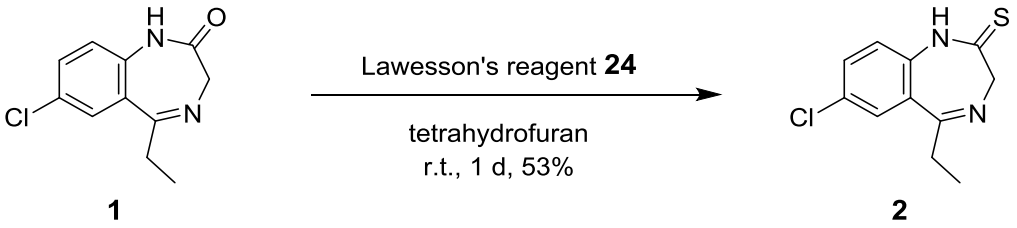
Synthesis of the 2-aminopropiophenone derivative **20** was done according to Mayer *et al.*[104] starting with nitration of commercially available 3-chloro-propiophenone **18** in position 6 using a mixture of fuming nitric acid and concentrated sulfuric acid followed by reduction of the nitro group to an aromatic amine with iron powder and ammonium chloride in an overall yield of 55%. The next steps were planned to follow the U.S. patent procedure of Bell *et al.*[105] published in 1973. The acylation of the prepared amine **20** with chloroacetyl chloride **21** was realized in sufficient yield after stirring for one hour at room temperature. The cyclization of compound **22** into benzodiazepinone **1**, however, was divided into two steps. The described one-pot procedure of Bell *et al.*[105] led to irreproducible and bad to moderate yields. Furthermore a long reaction time was needed of at least two, usually three days. The separation of the Finkelstein reaction[126] – to convert the chloroacetanilide **22** into the iodoacetanilide **23** – from the cyclization step led to a quantitative conversion while stirring at room temperature for one day. The subsequent cyclization in an ammonia saturated ethanol solution offered a practicable way to obtain the desired benzodiazepinone **1** in high yield (86%) over both steps (Scheme 10).



Scheme 10. Preparation of benzodiazepinone core scaffold **1** in a five step synthesis.

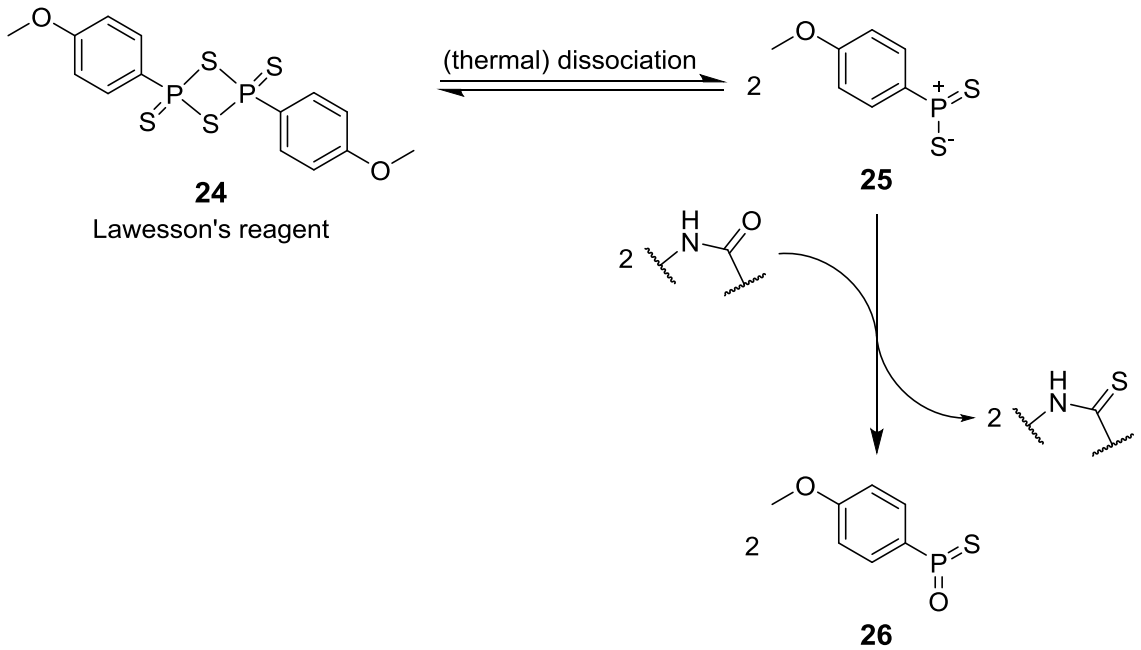
4.1.2 Preparation of 6-ethyltriazolobenzodiazepines

Conversion of benzodiazepinone **1** into thiolactam **2** could not be accomplished under standard conditions[106] using phosphorus pentasulfide in pyridine or other high boiling solvents, but was successfully realized using Lawesson's reagent **24** in anhydrous tetrahydrofuran under nitrogen atmosphere (Scheme 11).



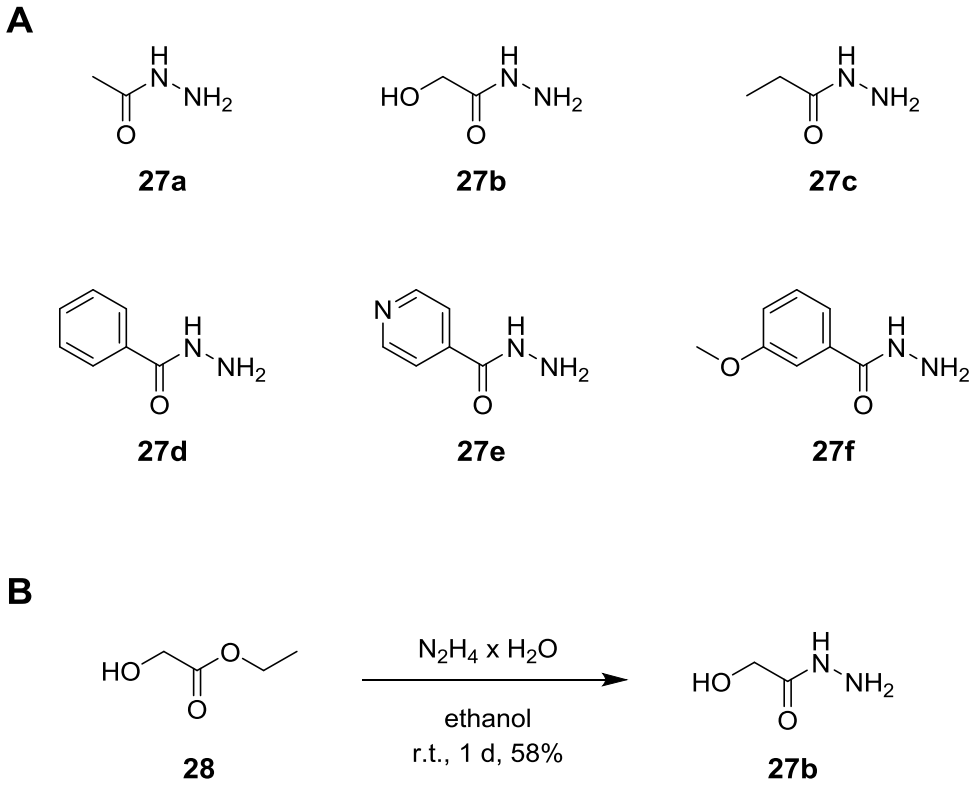
Scheme 11. Conversion of benzodiazepinone **1** into thiolactam **2** using Lawesson's reagent.

The advantage of Lawesson's reagent **24** are the 4-methoxyphenyl substituents, which allow good solubility in organic solvents in contrast to inorganic reagents like phosphorus pentasulfide. However, the dithiophosphine ylide **25** as well as the metathiophosphonate **26** form various oligomeric byproducts[127] in the course of the reaction, which pose a challenge to separate by column chromatography.



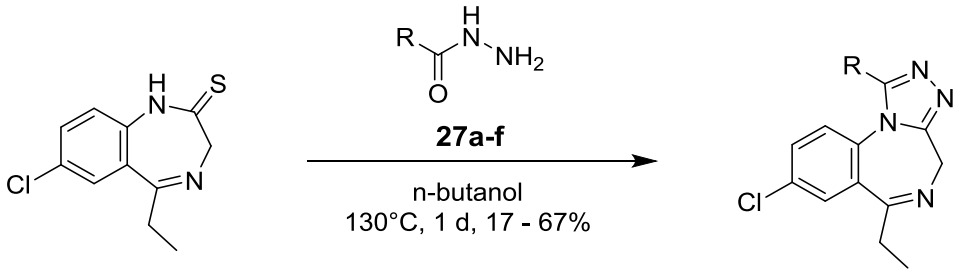
Scheme 12. Dissociation of Lawesson's reagent **24** into the active dithiophosphine ylide **25** followed by the conversion into the final metathiophosphonate **26**.

For preparation of different 6-ethyl-triazolobenzotriazepines a choice of carboxylic acid hydrazides was needed. Therefore, the commercially available building blocks acethydrazide **27a**, propanoic acid hydrazide **27c**, benzhydrazide **27c**, isonicotinic acid hydrazide **27e**, and 3-methoxybenzoic hydrazide **27f** were purchased (Scheme 13A). Starting with ethyl glycolate **28**, 2-hydroxyacethydrazide **27b** was prepared according to literature known procedure[128] (Scheme 13B).



Scheme 13. (A) Used carboxylic acid hydrazides **27a-f** for triazole preparation and (B) conversion of ethyl glycolate **28** into 2-hydroxyacethydrazide **27b**.

The target molecules **29a-f** were obtained by condensation of thiolactam **2** with corresponding carboxylic acid hydrazides **27a-f** (Scheme 14). The reactions took place in a sealed vial under nitrogen atmosphere over one day and gave the desired products in 17% to 67% yields. However an excess of hydrazides were needed for obtaining adequate yields, the residual ones were poorly separable from the product by column chromatography. This problem was solved by converting the hydrazides into water-soluble condensation products upon stirring with glucose solution prior to extraction with a lipophilic organic solvent (dichloromethane).

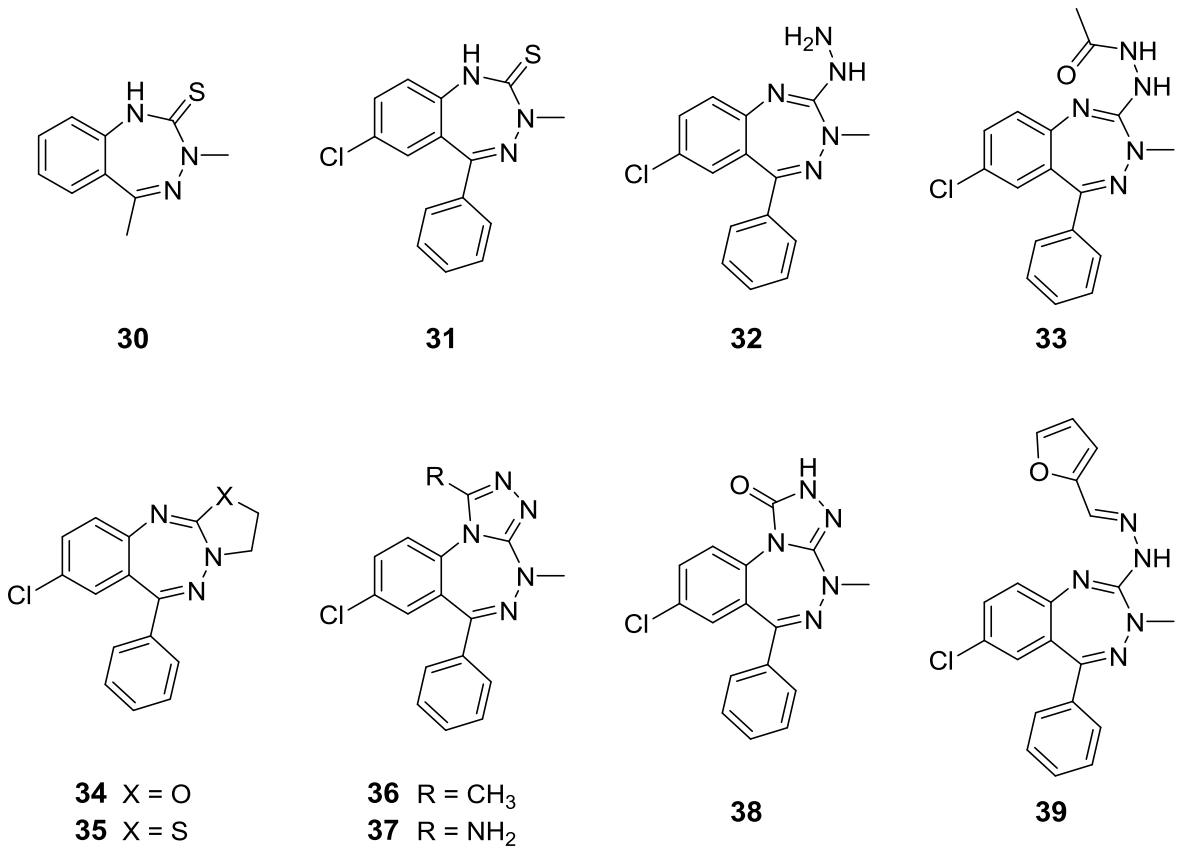


2 **29a-f**

a R = methyl (53%)
b R = hydroxymethyl (28%)
c R = ethyl (45%)
d R = phenyl (61%)
e R = pyridin-4-yl (17%)
f R = 3-methoxyphenyl (67%)

Scheme 14. Condensation of thiolactam **2** with hydrazides **27a-f** yields triazolobenzotriazepine derivatives **29a-f**.

4.1.3 Benzotriazepines provided by the University of Greifswald



30 **31** **32** **33**

34 X = O
35 X = S

36 R = CH_3
37 R = NH_2

38 **39**

Figure 26. Chemical structures of benzotriazepines **30** – **39** provided by the University of Greifswald.

During the work on triazolobenzodiazepines a lot of literature search had to be done for synthesis development. Numerous papers and patents found, were published by Richter *et al.* – a former research group of the University of Greifswald. Their research area deals predominantly with benzotriazepines, a class of compounds structurally closely related to our benzodiazepines. For this reason we asked Dr. O. Morgenstern who kindly sent us a selection of ten diverse compounds **30** – **39** (Figure 26). Those molecules were sent with our synthesized triazolobenzodiazepines **29a-f** as well as their precursors **1** and **2** for screening on bromodomains to the SGC at the University of Oxford.

4.1.4 Screening of the benzodiazepines and -triazepines

The first DSF screening was carried out at a high compound concentration (cc) of 100 µM (except compound **29b** which was screened later at 10 µM cc). Synthesized triazolobenzodiazepines **29a-f**, the precursor benzodiazepinone **1**, thiolactam **2** as well as the benzotriazepines **30** – **39** from the University of Greifswald were screened against seven members of the BET family (BRD2(1/2), BRD3(1/2), BRD4(1/2), BRDT1). Five more domains (BAZ2B, CREBBP, LOC93349, PB1(5), PCAF) of different families were also used in the screening to check against affinity across the phylogenetic tree of bromodomains and selectivity towards the desired BETs.

The analysis of the obtained temperature shift results (Figure 27) was clear. Obviously no compound prepared in our first series, neither the precursors **1** and **2** nor the triazolobenzodiazepines **29a-f**, showed significant (more than 4 °C) shifts. The same effect was monitored with the compounds from Greifswald. Again there was no significant temperature shift detected with the exception of compounds **36** and **37**, which share both a 6-phenyltriazolobenzotriazepine core structure. Especially **36** reached impressive high ΔT_m values, with regard to the high compound concentration of 100 µM. Therefore it had to be re-evaluated at the standard compound concentration of 10 µM.

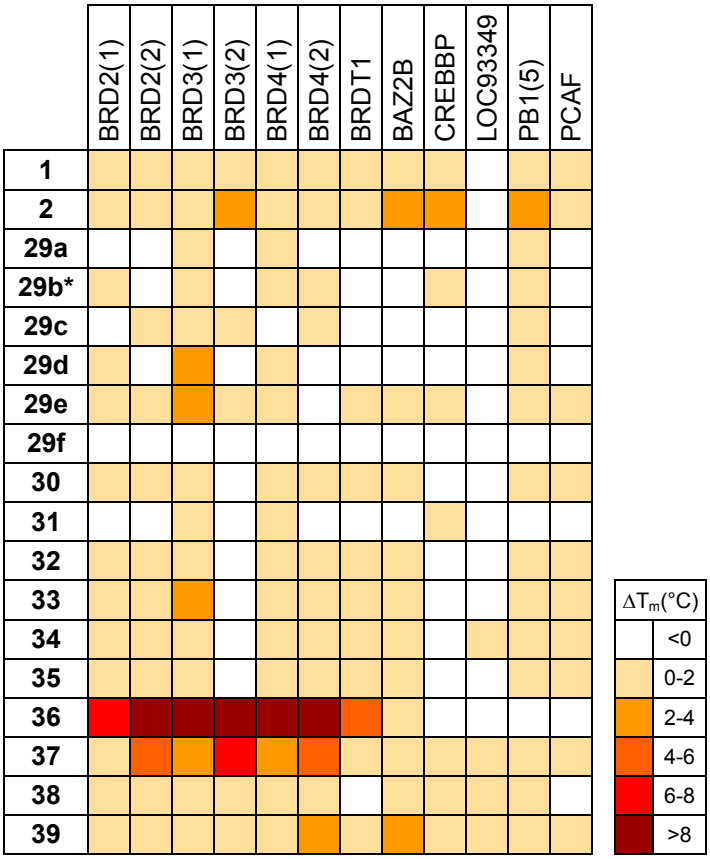


Figure 27. DSF results of triazolobenzodiazepines **29a-f** and their precursors **1** and **2** as well as benzotriazepines **30** – **39** (from the University of Greifswald) screened at 100 µM compound concentration (* screened at 10 µM cc).

For re-evaluation of compounds **36** and **37**, the same bromodomains were used. Additionally, four clinical BzDs (alprazolam, estazolam, midazolam, triazolam) and a structural related compound **GW841819X**[129] (Figure 30) were screened. These results showed, that alprazolam is the only clinical BzD with significant affinity against the BET family. T_m shifts of both triazolobenzotriazepines **36** and **37** decreased, as expected with a ten-fold lower compound concentration, but still gave remarkable values. Also compound **GW841819X** showed high temperature shifts within the BET family but no cross affinity towards any other bromodomain.

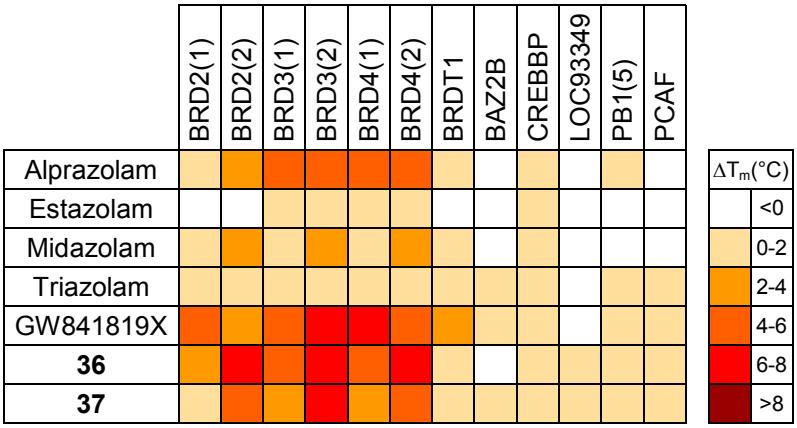


Figure 28. T_m shift results of clinical BzDs (alprazolam, estazolam, midazolam, triazolam), a structural related BzD **GW841819X** and re-evaluation of triazolobenzo-triazepines **36** and **37** screened at 10 µM cc.

These unexpected results initiated the preparation of co-crystallizations of BRD4(1) with the three most promising compounds – alprazolam (Figure 29B), midazolam (Figure 29C) and compound **36** (Figure 29D) by Dr. Filippakopoulos at the SGC (Oxford). Molecule **GW841819X** (Figure 29A) was already available as co-crystallization with BRD4(1) at the protein data bank.

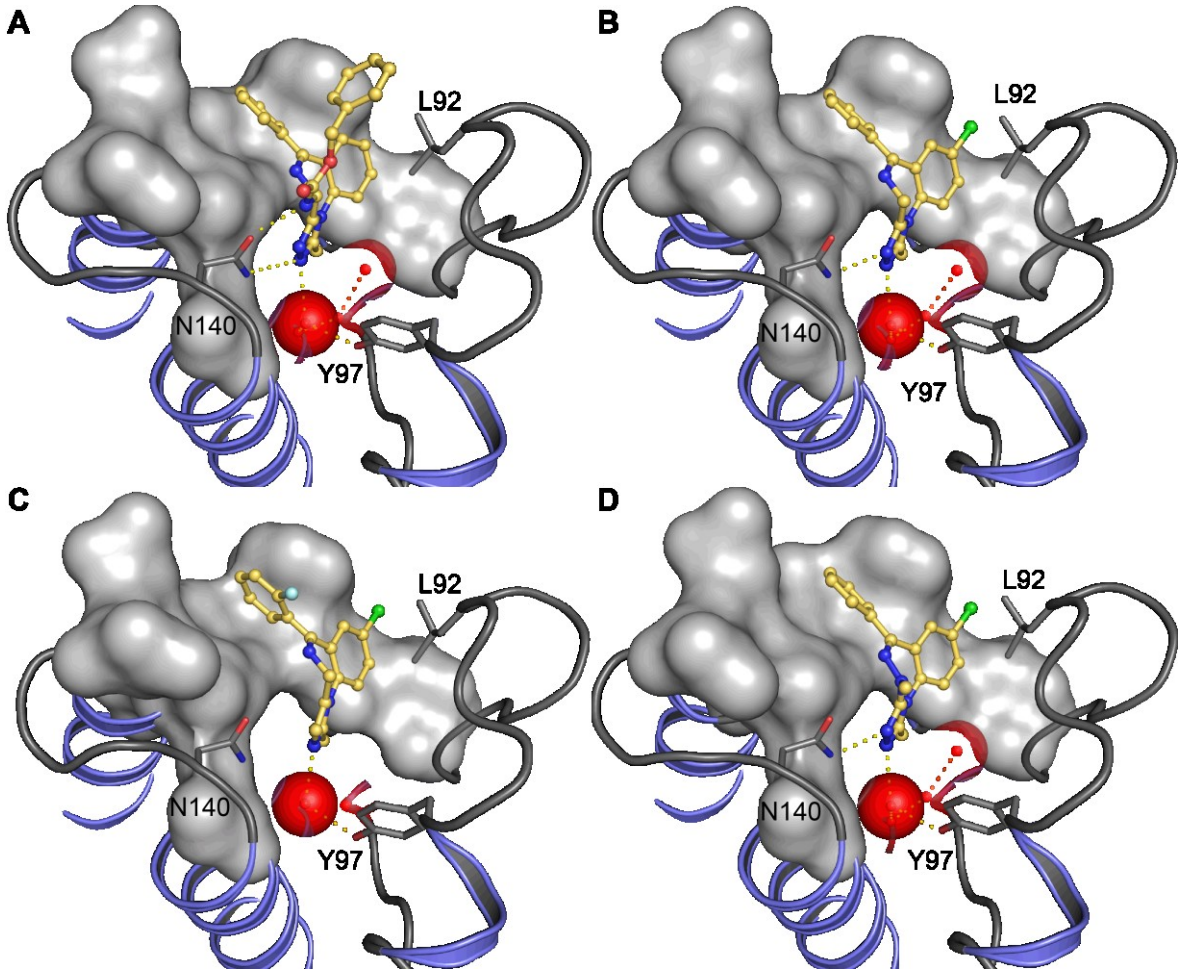


Figure 29. Detailed co-crystallization structures of BRD4(1) with (A) **GW841819X**, (B) alprazolam, (C) midazolam and (D) compound **36**. Conserved water molecules are shown as red spheres[83].

These four crystal structures gave a clear insight into the binding mode of this class of compounds with first domain of BRD4 (cf. Figure 30). Every molecule showed the same orientation into the binding pocket with the five-membered ring ahead which also is responsible for important hydrogen bonds. One nitrogen of this ring always forms a hydrogen bond mediated by a conserved water molecule to the tyrosine 97 side chain.

In case of an annulated imidazole (cf. midazolam) in contrast to an triazole ring the second important hydrogen bond to asparagine 140 is missing. This might explain why midazolam showed much weaker interactions (lower ΔT_m) than alprazolam.

A third interaction is displayed in the structure of **GW841819X**, again with the asparagine 140, which can be a hint for the high T_m value against BRD4(1) in the DSF screening. This time the oxygen of the terminal amide of the amino acid side chain interacts with the NH group of the carbamate residue in position 4.

Structural elements of the molecules can also be explained very well with regard to the crystal structures. The 6-phenyl ring fits to a hydrophobic pocket and consequently shows better interactions than a 6-ethyl group. Furthermore the annulated 1-methyltriazole ring appears to be preferred in contrast to the bulkier substituents as long as all compounds use the same orientation mode.

Due to a long production time for co-crystallizations the outcome was not awaited for development of a new lead structure and are consequently not considered in the following discussion in Chapter 4.1.5. However, the assumption which structural variations and modifications has to be done in the following step turned out to be appropriate.

4.1.5 Discussion of the benzodiazepine screening results

To summarize the first screening results on benzodiazepines, it is obvious that the proposed 8-chloro-6-ethyl-triazolobenzodiazepines have poor effects on bromodomains. Irrespective of which residue is attached to the triazole ring in position 1. Neither short aliphatic moieties like methyl or ethyl nor aromatic or heteroaromatic residues could achieve significant temperature shifts. Hence, no further efforts should be made with this specific class of compounds. Planned derivatizations in position 4 were not longer pressed ahead.

However, other compounds gave unexpected response. With compound **36** and **GW841819X** (Figure 30), two highly interesting molecules could be identified. Both have mainly moderate to high T_m shifts (between 4 and 7 °C) against BETs and good selectivity towards this family.

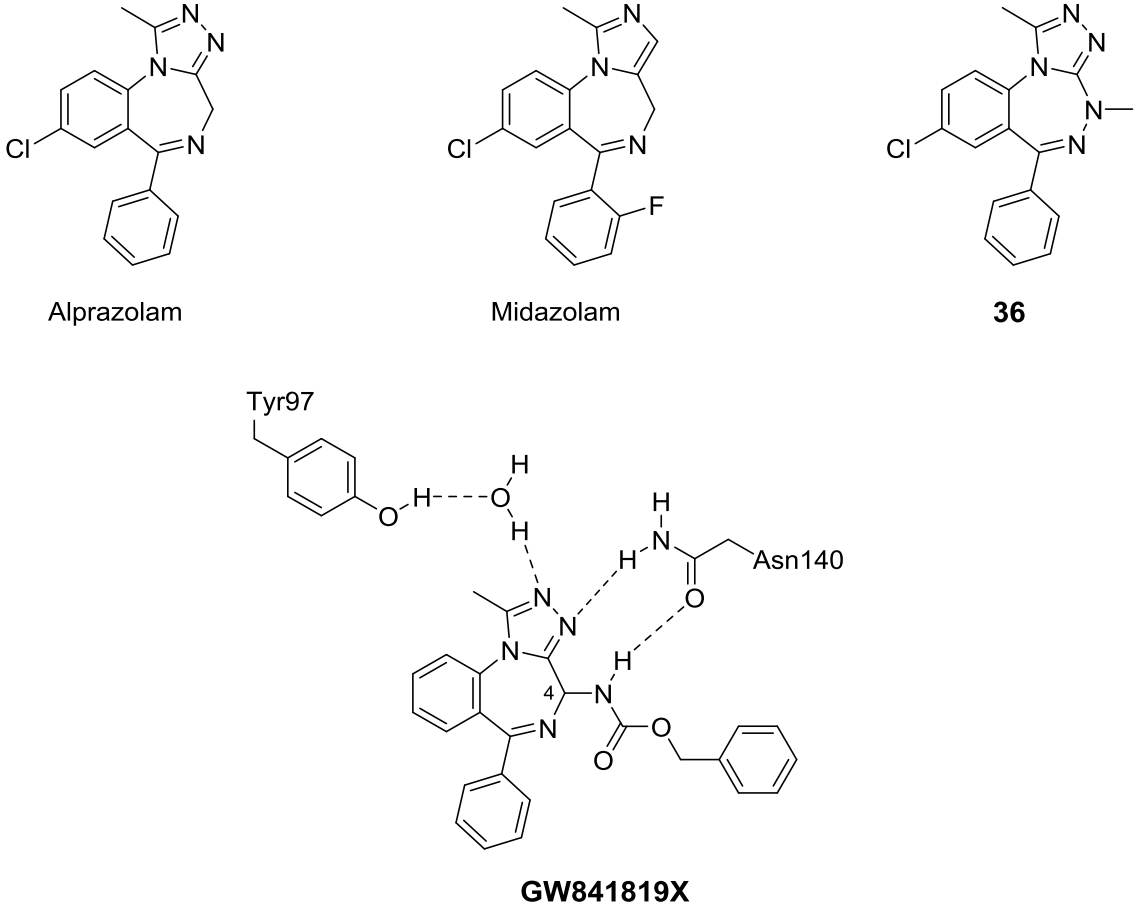


Figure 30. Chemical structures of to co-crystallized compounds and important interactions with the protein using the example of **GW841819X**.

Consequently these two molecules were used as leads for modification of structures. Both compounds share a very similar core scaffold. A seven-membered ring, containing at least two nitrogens, with an annulated benzo-ring system and a 1-methyltriazole ring. In contrast to our synthesized triazolobenzodiazepines both have a 6-phenyl instead of a 6-ethyl residue which is presumably essential for affinity to bromodomains. This leads to the assumption that it occupies a hydrophobic pocket and / or is responsible for pi stacking.

The logical outcome was to adapt the common scaffold and to add unique components of both molecules to assemble a new lead structure (Figure 31):

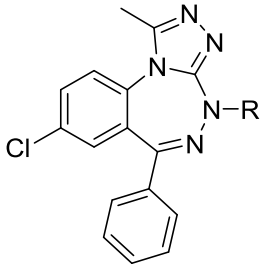
- With regard to the functionalized position 4 of **GW841819X** the decision for triazepines was made. On the one hand the core structure of triazepines requests a different synthesis strategy but on the other hand the functionalization of a NH group is much easier feasible than of a methylene group. Moreover, the attachment of only one group in position 4 in case of benzodiazepines would lead to a stereocenter which could be avoided by using triazepines.

- The annulated (generally chloro substituted) benzo ring – ubiquitous in tested compounds – will be maintained.

- Variations with diverse ring systems, annulated to different sides of the seven-membered ring, as well as open structures reveal the annulated 1,2,4-triazole ring as preferred structural element.

- The methyl group in position 1 seems to be the best choice in comparison of all screened compounds. Even between compound **36** and **37** as well as between alprazolam and estazolam a loss of affinity can be seen with the only difference in the chemical structure is the exchange of the 1-methyl group (in compound **36** and alprazolam) by an 1-amino group (compound **37**) and a hydrogen (estazolam), respectively. Furthermore the broad variations in position 1 of synthesized target compounds **29a-f** also had no effect on temperature shifts of the bromodomain screening.

- However in case of compounds **29a-f** also the 1-methyl substituted compound **29a** showed no effect on target proteins. Because of this reason the ethyl residue in position 6 will be replaced by a phenyl moiety which is the common structural element in this position.



triazolobenzotriazepine
lead structure

R = moieties containing hydrogen bond donors/ acceptors and/or aromatic ring systems

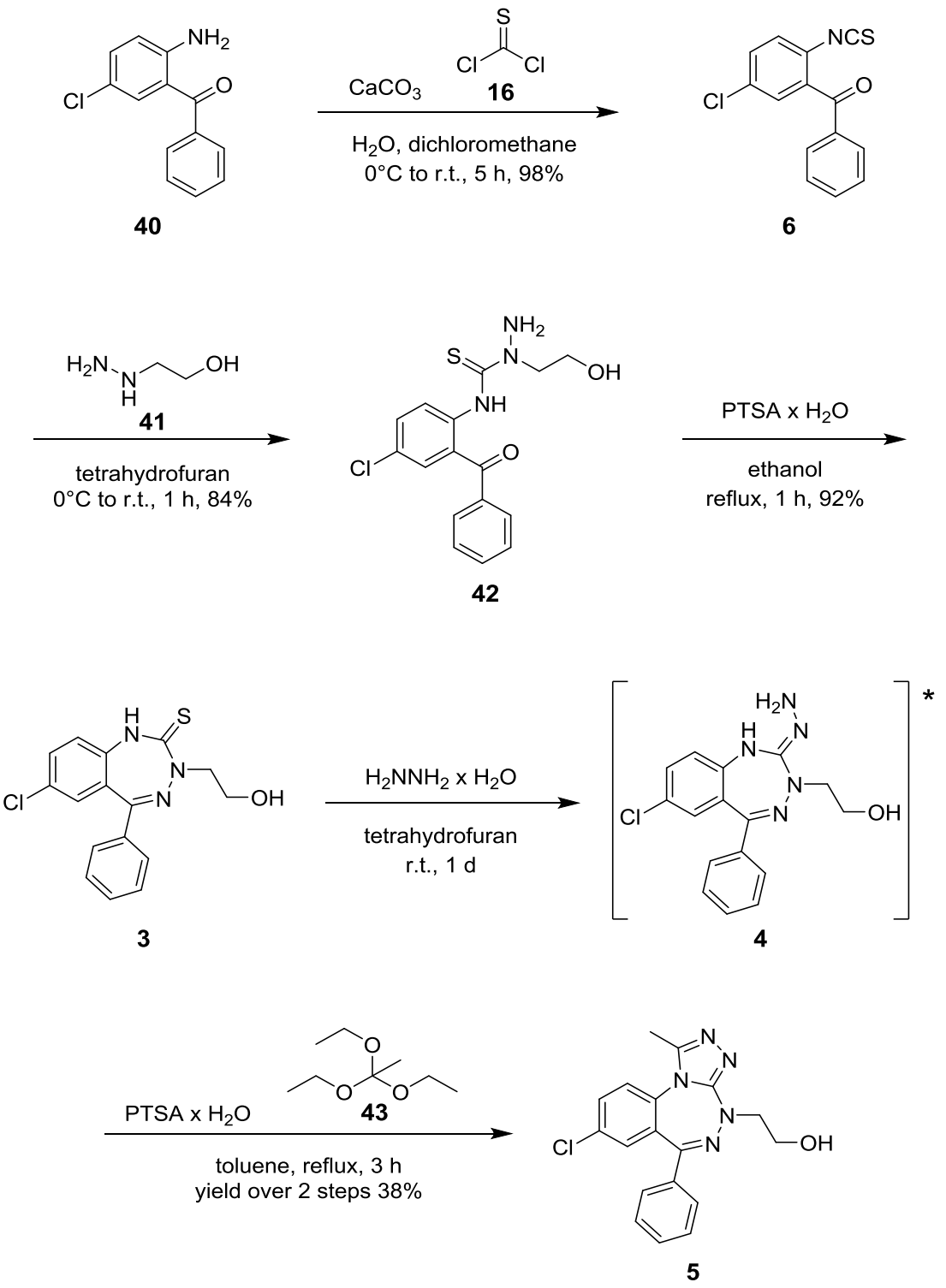
Figure 31. New lead structure development with triazolobenzotriazepine core scaffold.

4.2 FIRST OPTIMIZATION OF TRIAZOLOBENZOTRIAZEPINES

4.2.1 Synthesis and derivatization of 4-(2-hydroxyethyl)triazolobenzo-triazepine

Having generated a new lead structure the synthetic approach had to be changed. On the one hand an additional nitrogen is planned at position 4 in the seven-membered ring, on the other hand hydrogen bond donors and / or acceptors should be attached at this position. Therefore 4-(2-hydroxyethyl)triazolobenzo-triazepine **5** was chosen as a central intermediate containing a primary alcohol which can easily be modified in various reactions.

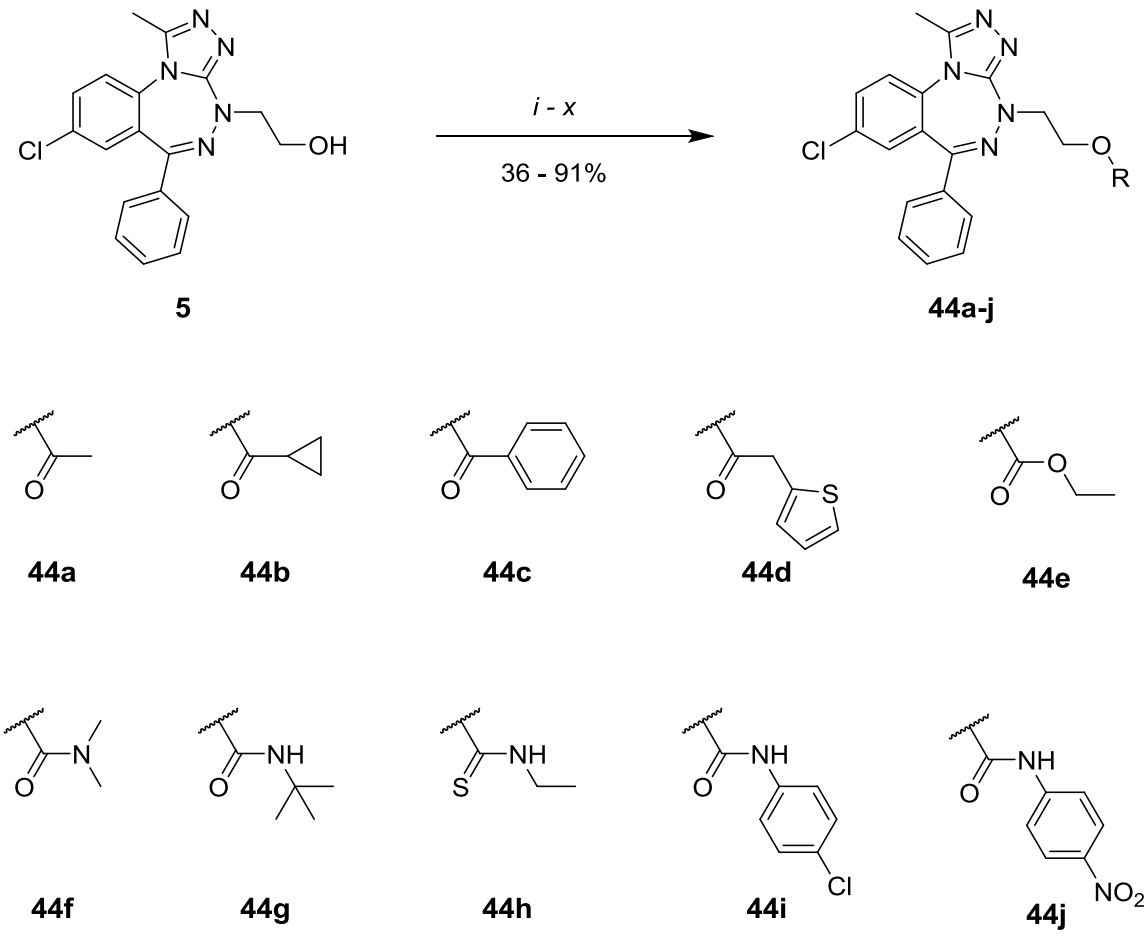
(2-Amino-5-chlorophenyl)(phenyl)methanone **40** was used as starting material for the synthesis of 4-(2-hydroxyethyl)triazolobenzotriazepine **5**. In a similar manner to literature known procedure[108] compound **40** was converted into the isothiocyanate derivative **6** using thiophosgene **16** and calcium carbonate. The highly reactive isothiocyanate derivative **6** was treated subsequently with the commercially available 2-hydroxyethylhydrazine **41**. The obtained thiosemicarbazide derivative **42** was generated by the addition of the alkylated and consequently more nucleophilic hydrazine nitrogen and not by the addition of the less sterical hindered hydrazine nitrogen. As a result of this type of addition the following cyclization step between the free NH_2 group and the keto group can easily occur by heating to reflux in ethanol for one hour with a catalytic amount of para-toluenesulfonic acid (PTSA). The preparation of the annulated 1,2,4-triazole ring was divided into two steps. First a hydrazone like intermediate was generated by treating benzotriazepine-2-thione **3** with hydrazine hydrate. After work-up the crude product of compound **4** was stirred with triethyl orthoacetate **43** and a catalytic amount of PTSA in toluene to obtain compound **5** with the 1-methyltriazole ring in moderate yield of 38% over two steps (Scheme 15).



Scheme 15. Preparation of 4-(2-hydroxyethyl)triazolobenzotriazepine **5** in a five step synthesis starting with commercially available 2-aminobenzophenone derivative **40**.

Derivatization of compound **5** was done with a broad variety of reactants to gain diversity in the resulting target molecules **44a-j** (Scheme 16). To list some examples: anhydrides, carboxylic acid chlorides or iso(thio)cyanates were used. Typical functional groups with hydrogen bond donors and / or acceptors like esters (**44a** – **44d**), a carbonate (**44e**) and (thio)carbamates (**44f** – **44j**) were prepared.

According to the standard protocol[130] to prepare acetylsalicylic acid, acetic acid anhydride with a catalytic amount of sulfuric acid was used to prepare the acetylated compound **44a** in 78% yield. The second ester with an aliphatic moiety was synthesized using cyclopropanecarbonyl chloride after deprotonation of the alcohol with sodium hydride giving compound **44b** in moderate yield (51%). Two additional esters were prepared with an aromatic residue. For the benzoyl derivative again an anhydride (benzoic acid anhydride) was used and treated with a catalytic amount of sulfuric acid to obtain the preferred product **44c** in 37% yield. 2-Thiopheneacetic acid was coupled with the primary alcohol of compound **5** under typical conditions of a Steglich esterification[131] using DCC and a catalytic amount of DMAP to afford product **44d** in 61% yield. The addition of sodium hydride to a solution of molecule **5** in diethyl carbonate gave the carbonate derivative **44e** in high yield (91%). Also carbamates and a thiocarbamate were prepared to offer a broader range of interaction possibilities of the binding pocket with the target molecules. Therefore *N*,*N*-dimethylcarbamoyl chloride was used to prepare compound **44f** in 69% yield. Identical reaction conditions led to the following four compounds **44g-j**. After deprotonation of starting material **5** with sodium hydride, the corresponding iso(thio)cyanate was added to the solution in tetrahydrofuran, followed by stirring for three hours at room temperature. Reaction with *tert*-butyl isocyanate gave compound **44g** in 66% yield, with ethyl isothiocyanate 85% of **44h** and the two aromatic compounds 4-chlorophenyl isocyanate and 4-nitrophenyl isocyanate gave their corresponding target molecules in 64% (**44i**) and 36% (**44j**) yield, respectively.



i	**a**	R = acetyl (78%)
ii	**b**	R = cyclopropanecarbonyl (51%)
iii	**c**	R = benzoyl (37%)
iv	**d**	R = (thiophen-2-yl)acetyl (61%)
v	**e**	R = ethoxycarbonyl (91%)
vi	**f**	R = *N*,*N*-dimethylcarbamoyl (69%)
vii	**g**	R = *tert*-butylcarbamoyl (66%)
viii	**h**	R = ethylcarbamothioyl (85%)
ix	**i**	R = 4-chlorophenylcarbamoyl (64%)
x	**j**	R = 4-nitrophenylcarbamoyl (36%)

Scheme 16. Derivatization of 4-(2-hydroxyethyl)triazolobenzotriazepine **5** to target compounds **44a-j** using various methods. Reagents and conditions: (*i*) acetic acid anhydride, catalytic conc. H_2SO_4, 80 °C, 1 h; (*ii*) cyclopropanecarbonyl chloride (2 equiv), sodium hydride (1.2 equiv), THF, r.t., 3 h; (*iii*) benzoic acid anhydride (3.0 equiv), catalytic conc. H_2SO_4, THF, reflux, 1 h; (*iv*) 2-thiopheneacetic acid (2.0 equiv), DCC (2.2 equiv), catalytic DMAP, DCM, r.t., 1 d; (*v*) diethyl carbonate, sodium hydride (1.2 equiv), reflux, 3 h; (*vi*) *N*,*N*-dimethylcarbamoyl chloride (3.0 equiv), sodium hydride (1.2 equiv), THF, r.t., 1 d; (*vii*) *tert*-butyl isocyanate (2.0 equiv), sodium hydride (1.2 equiv), THF, r.t., 3 h; (*viii*) ethyl isothiocyanate (2.0 equiv), sodium hydride (1.2 equiv), THF, r.t., 3 h; (*ix*) 4-chlorophenyl isocyanate (2.0 equiv), sodium hydride (1.2 equiv), THF, r.t., 3 h; (*x*) 4-nitrophenyl isocyanate (2.0 equiv), sodium hydride (1.2 equiv), THF, r.t., 3 h.

4.2.2 Interesting aspects of NMR analysis of special carbamates

Previously (Chapter 4.2.1) described compounds derived from molecule **5**, with esters and carbonates (**44a-e**) as new functional groups gave standard signals in their NMR spectra. Even the NMR spectra of carbamates **44f** and **44g** with simple aliphatic residues were not out of common. However thiocarbamate **44h** as well as both aromatic substituted carbamates **44i** and **44j** showed a remarkable number of signals in the proton NMR spectra in relation to their molecular formula.

This observation can be explained with the partial double-bond character (Figure 32) in the C-N amide bond, which was extensively analyzed by NMR techniques[132,133,134,135]. Whenever this rotational barrier is high enough, the rotation at room temperature is slower than the NMR time-scale and consequently produces individual NMR signal sets for each of the rotamers (one for the (E) and one for the (Z) isomer). Despite various groups studied[136,137,138,139] the amide resonance in carbamates in detail it has to be proven that both sets of NMR signals arise from the same compound. Therefore NMR spectra of the corresponding probe were performed at five different temperatures with the aim that higher temperatures allow faster interconversion of both rotamers. Thus, the separated signals of the same group of both rotamers should overlap at high temperatures.

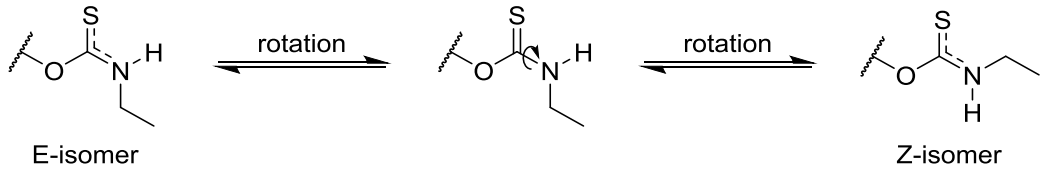


Figure 32. Free rotation around the C-N amide bond can be restricted by a rotational barrier, what leads to a (E) and a (Z) rotamere.

The NMR spectra of thiocarbamate **44h** are illustrated in Figure 33. The measurements were performed in deuterated tetrachloroethane at five different temperatures. Arrows indicate the example signals which are clearly separated at 17 °C but show one defined signal at 100 °C. Also the triplet signals of the 2'''-methyl group (0.89 and 1.11 ppm) converge at higher temperature.

Another interesting fact is given by the difference in the chemical shift. The closer the protons are to the amide bond, the larger the differences of the chemical shifts of the corresponding rotamer signals are. In CD_2Cl_2 at 17 °C the amide proton, for example, shows a difference of 0.23 ppm (peaks at 6.58 and 6.81 ppm), whereas the 1-methyl group has only a shift of 0.01 ppm (peaks at 2.54 and 2.55 ppm). No shift, however, could be detected within the aromatic protons anymore.

The ratio between the isomers can be calculated by correlation of the integrals of the matching rotamer signals. In case of compound **44h** a ratio of 37:63 was obtained. Steric hindrance might prefer the (E)-isomer which should consequently refer to the 63% value in contrast to the (Z)-isomer with 37%. The little disparity between the isomers can be accounted for by the small ethyl group. With regard to the large aromatic substituents in compound **44i** and **44j** the ratio changes enormously to 7:93 (**44i**) and 8:92 (**44j**), respectively, what obviously supports the assumption that (E) and (Z) is controlled by steric hindrance.

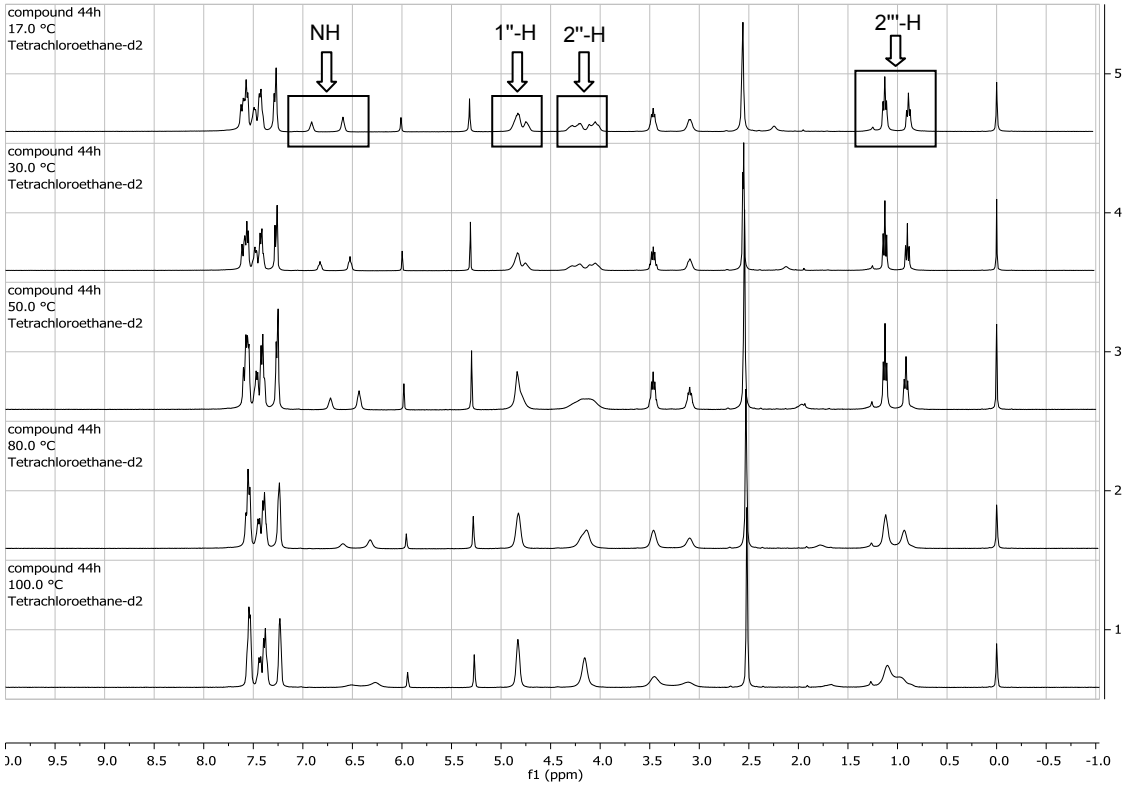
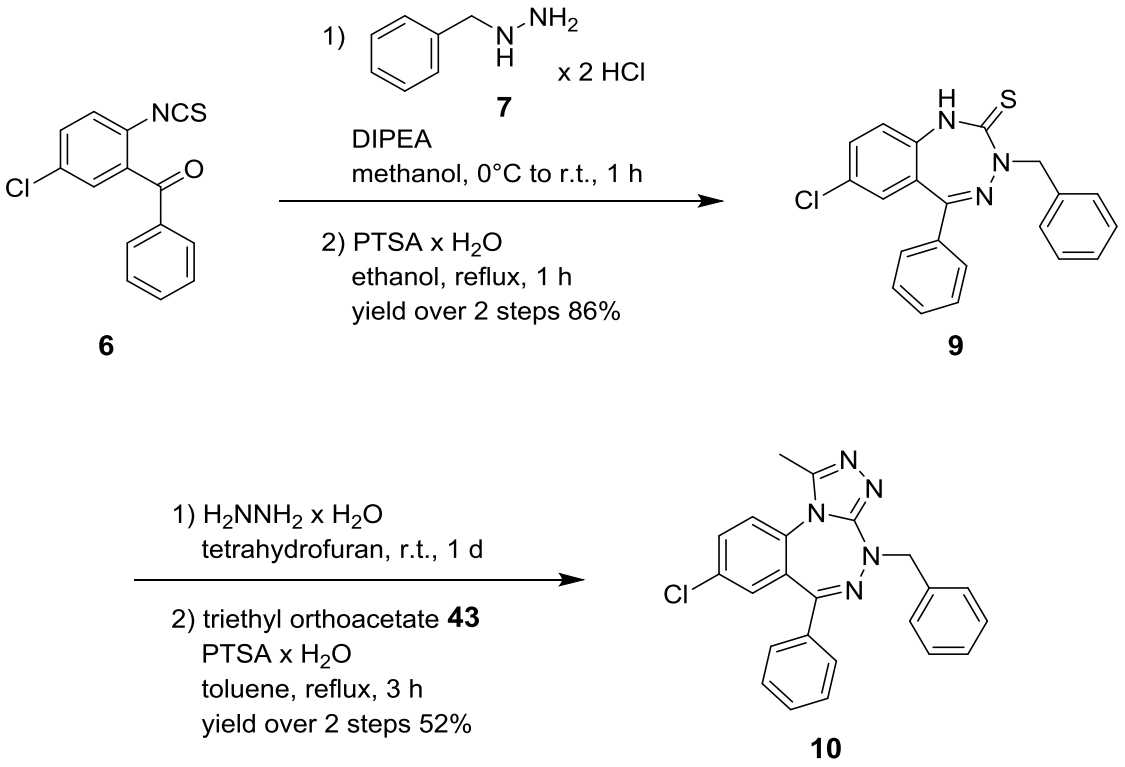


Figure 33. Temperature dependent NMR spectroscopy of thiocarbamate derivative **44h**. Arrows indicate example signals which are clearly separated at 17 °C but become one signal at higher temperature. Measured in tetrachloroethane-d2 at temperatures as indicated in the corresponding spectra (17 °C, 30 °C, 50 °C, 80 °C, 100 °C).

4.2.3 Synthesis of 4-benzyltriazolobenzotriazepine

The developed synthesis of 4-(2-hydroxyethyl)triazolobenzotriazepine **5** facilitates a good access to triazolobenzotriazepines bearing other substituents in 4 position by only varying the alkylated hydrazine derivative. For this reason the commercially available benzylhydrazine dihydrochloride **7** was chosen instead of the 2-hydroxy-ethylhydrazine **41** to prepare a target compound with a completely different substitution.



Scheme 17. Synthesis of 4-benzyltriazolobenzotriazepine **10**.

Deprotonation of benzylhydrazine dihydrochloride **7** with DIPEA was done prior to the stepwise addition of (5-chloro-2-isothiocyanatophenyl)(phenyl)methanone **6** to the cooled solution and the reaction mixture was allowed to warm to room temperature while stirring for one hour. The mixture was heated to reflux for one hour after para-toluenesulfonic acid monohydrate was added to the mixture to complete the cyclization to the benzotriazepine-2-thione **9** in an overall yield of 86%. Preparation of the triazole ring started again with treating compound **9** with hydrazine hydrate in followed by the addition of triethyl orthoacetate **43** and a

catalytic amount of para-toluenesulfonic acid monohydrate. Target compound **10** was thereby prepared with a yield of 52% over the last two steps.

4.2.4 Screening of 4-(2-hydroxyethyl)triazolobenzotriazepine, its derivatives and the 4-benzyl analogon

The prepared compounds with the new triazolobenzotriazepine scaffold were sent to the SGC for DSF screening. This time an additional BET – BRDT2 – was screened to complete the whole family. The temperature shifts were determined at a compound concentration of 10 µM.

Again unexpected results were obtained for the twelve compounds. The not further functionalized 4-(2-hydroxyethyl)triazolobenzotriazepine **5** showed no significant ΔT_m except a low value against the second domain of BRD3. Also the derivatives **44a-j** behaved the same way with tiny T_m shifts between 4 °C and 6 °C against the second domain of BRD4 (**44a**, **44d**, **44f**) and insignificant values against all the other tested bromodomains. However, compound **10** with its unpolar benzyl substituent in position 4 gave significant ΔT_m values against all second domains of the BETs and remarkable signals for BRD2(2) and BRD4(2).

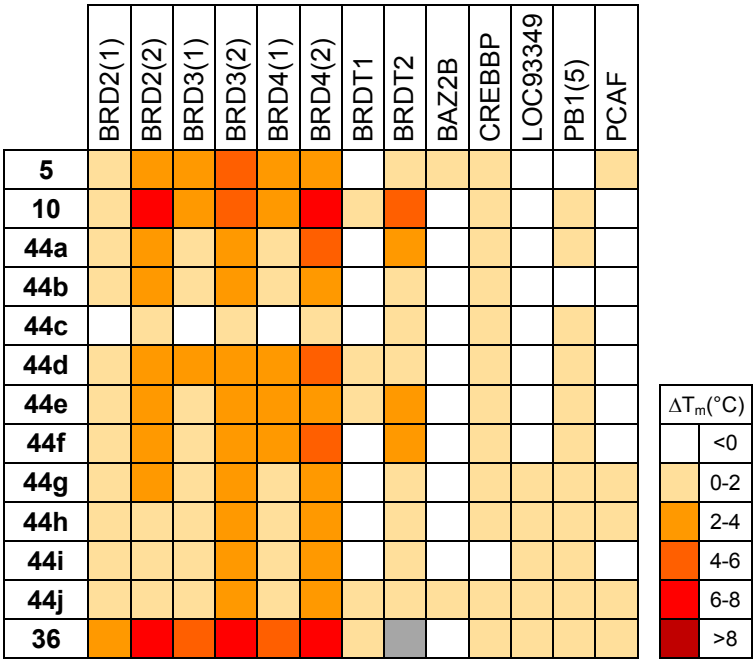
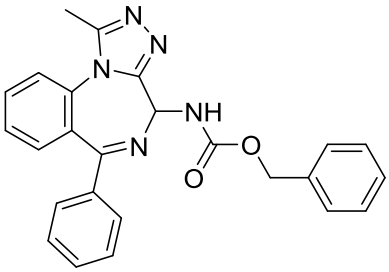


Figure 34. DSF screening results of 4-(2-hydroxyethyl)triazolo-benzotriazepine **5**, its derivatives **44a-j** and the 4-benzyl analogon **10** at a compound concentration of 10 µM. For comparison the values obtained for 4-methyl derivative **36** are presented again.

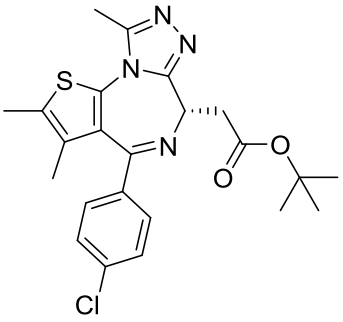
4.2.5 Discussion of temperature shift results of the first triazolobenzotriazepine series

Having generated a new lead structure (Figure 31) according to the obtained results of the benzodiazepine screening, twelve new compounds were synthesized with a benzotriazepine scaffold. All of them shared a common 8-chloro-1-methyl-6-phenyltriazolobenzotriazepine structure with different substitutions in 4 position. Compound **5** was used as starting material to yield derivatives **44a-j**. Surprisingly, those compounds with a variety of hydrogen bond donors and / or acceptors were not able to interact with the screened bromodomains and – as a consequence – to produce high T_m shifts.

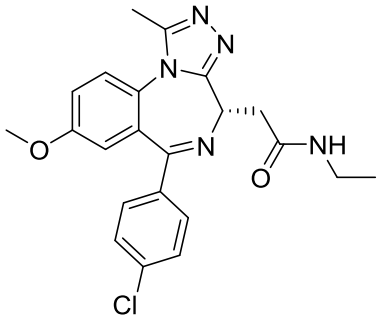
The decision to insert these polar groups was mainly driven by good screening results of **GW841819X**, and the published molecules JQ1 and iBET762 (Figure 35). Nevertheless, our molecules did not show the same effects, what has to be discussed.



GW841819X



(+)-JQ1



iBET762

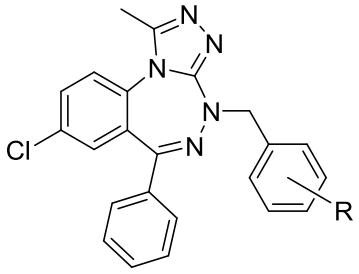
Figure 35. Chemical structures of **GW841819X**, (+)-JQ1 and iBET762.

The 6-phenyl ring shows a para substitution in JQ1 and iBET762, however **GW841819X** contains the unsubstituted phenyl ring alike our molecules. The annulated benzo ring system is again unsubstituted in **GW841819X** , contains a 8-methoxy group in iBET762, and in JQ1 this ring is entirely replaced by a dimethyl-thiophene what led to the assumption that a broad range of groups are tolerated in this position and the 8-chloro substituent is not responsible for the strong loss of affinity.

The most obvious similarity of the three active compounds in contrast to the our synthesized ones is on the one hand their benzodiazepine structure and on the other hand their comparable moiety in position 4. The carbonyl group of the ester (JQ1), the amide (iBET762) and the carbamate (**GW841819X**) is linked by only one atom to the seven-membered ring whereas in compounds **44a-j** the carbonyl group is separated by three atoms. This distinction might be a hint for the poor results in the DSF screening for the polar substituted compounds. The crystal structure of **GW841819X** with BRD4(1) (Figure 29A) also gave another evidence for the need of hydrogen bond donors closer attached to the seven-membered ring. The additional hydrogen bond between the NH group of the carbamate and asparagine 140 definitely increased the interaction strength. However – as already mentioned in Chapter 4.1.4 – the results of the crystal structures had no influence on the optimization of triazolobenzotriazepines from Greifswald to generate a new lead structure.

The most interesting result was reached with compound **10**, a 4-benzyl-triazolobenzotriazepine. Contrary to the other screened molecules the 4-benzyl derivative showed noticeable T_m shifts of 6.2 °C against BRD2(2) and 6.9 °C against BRD4(2). Also the temperature shift values of the second domains of BRD3 (5.2 °C) and BRDT (5.8 °C) implied that good interactions with the corresponding bromodomains occurred.

As a consequence of this results, no longer efforts were made to improve the polar side chains in position 4 but to concentrate on further variations within the benzyl moiety. This decision led to a novel lead structure (Figure 36), containing the well known substitution pattern 1-methyl, 6-phenyl and 8-chloro as well as the new 4-benzyl moiety which had to be optimized. Therefore a broad range of molecules with diverse ortho, meta and para substituents on the 4-benzyl moiety should be prepared to study their effects.



4-benzyl-8-chloro-1-methyl-6-phenyl-
TBzT derivatives

R = electron donating or
electron withdrawing groups

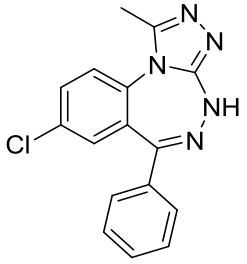
Figure 36. Optimized lead structure for next series of triazolobenzotriazepines.

4.3 8-CHLORO-6-PHENYLTRIAZOLOBENZOTRIAZEPINES WITH AROMATIC SIDE CHAINS IN POSITION 4

4.3.1 Preparation of 4*H*-triazolobenzotriazepine 11

The previously obtained DSF screening results of the second series of triazolobenzotriazepines led to a new lead structure containing a 4-benzyl moiety. Further efforts should be made to improve this scaffold by attaching different substituents to the benzyl residue. Differences should be made within electronic properties by using electron withdrawing or electron donating groups as well as within the size of attached substituents (e.g. small atoms like chlorine or bulky aliphatic groups). To gain a better insight into sterically hindered positions, substituents should be switched between ortho, meta and para and also a chance should be given to multi-substitutions.

To meet the requirements for generating a series of different 4-benzyl substituted triazolobenzotriazepines, a key step was to prepare the 4*H*-triazolobenzo-triazepine (Figure 37), which could easily be further functionalized by N-alkylation. In the syntheses of 4-(2-hydroxyethyl)triazolobenzotriazepine **5** and 4-benzyl-triazolobenzotriazepine **10** the moiety in position 4 of the target compounds was already introduced by using different alkylated hydrazine derivatives. However this synthesis requires each time the preparation of the corresponding hydrazines – whenever they are not commercially available – as well as the following construction of the triazolobenzotriazepine scaffold.

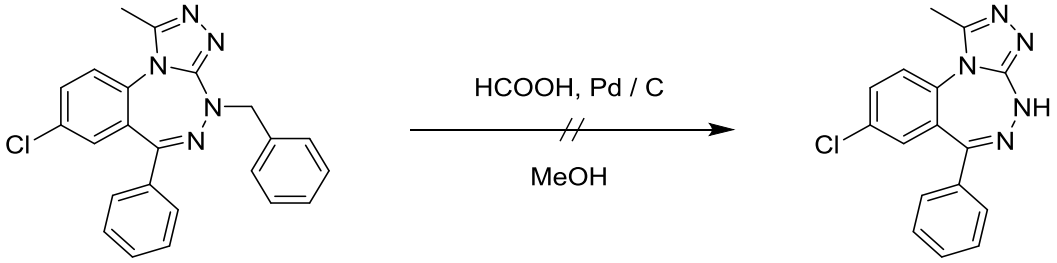


11

Figure 37. Chemical structure of the 8-chloro-1-methyl-6-phenyl-4*H*-triazolobenzotriazepine **11** key compound which can easily be modified by N-alkylation.

*4.3.1.1 Approach starting from synthesized 4-benzyltriazolobenzotriazepine **10***

The already synthesized compound **10** represented an appropriate starting material to obtain the desired 4*H*-triazolobenzotriazepine **11**. The cleavage of the benzyl group was tried using 10% palladium on activated charcoal and formic acid[140] (Scheme 18). Despite several variations of temperature (room temperature to reflux) and time (up to three days) as well as the use of ascending equivalents of formic acid up to an high excess, no conversion could be detected by TLC. As parallel efforts (see the following Chapter 4.3.1.2) yielded in much better perspectives to obtain the target compound **11** this route was no longer pursued.



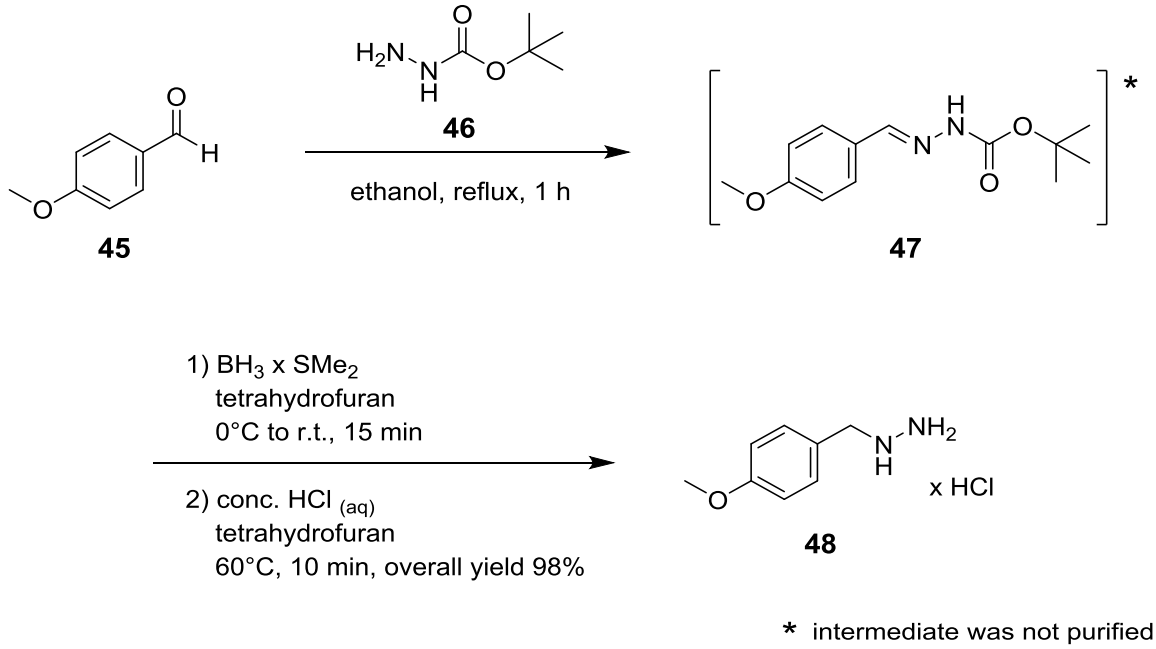
10 **11**

Scheme 18. Attempted cleavage of the benzyl residue in position 4 of molecule **10** to yield the unfunctionalized 4*H*-triazolobenzotriazepine **11**.

*4.3.1.2 Synthesis of compound **11** using 4-methoxybenzyl as protecting group*

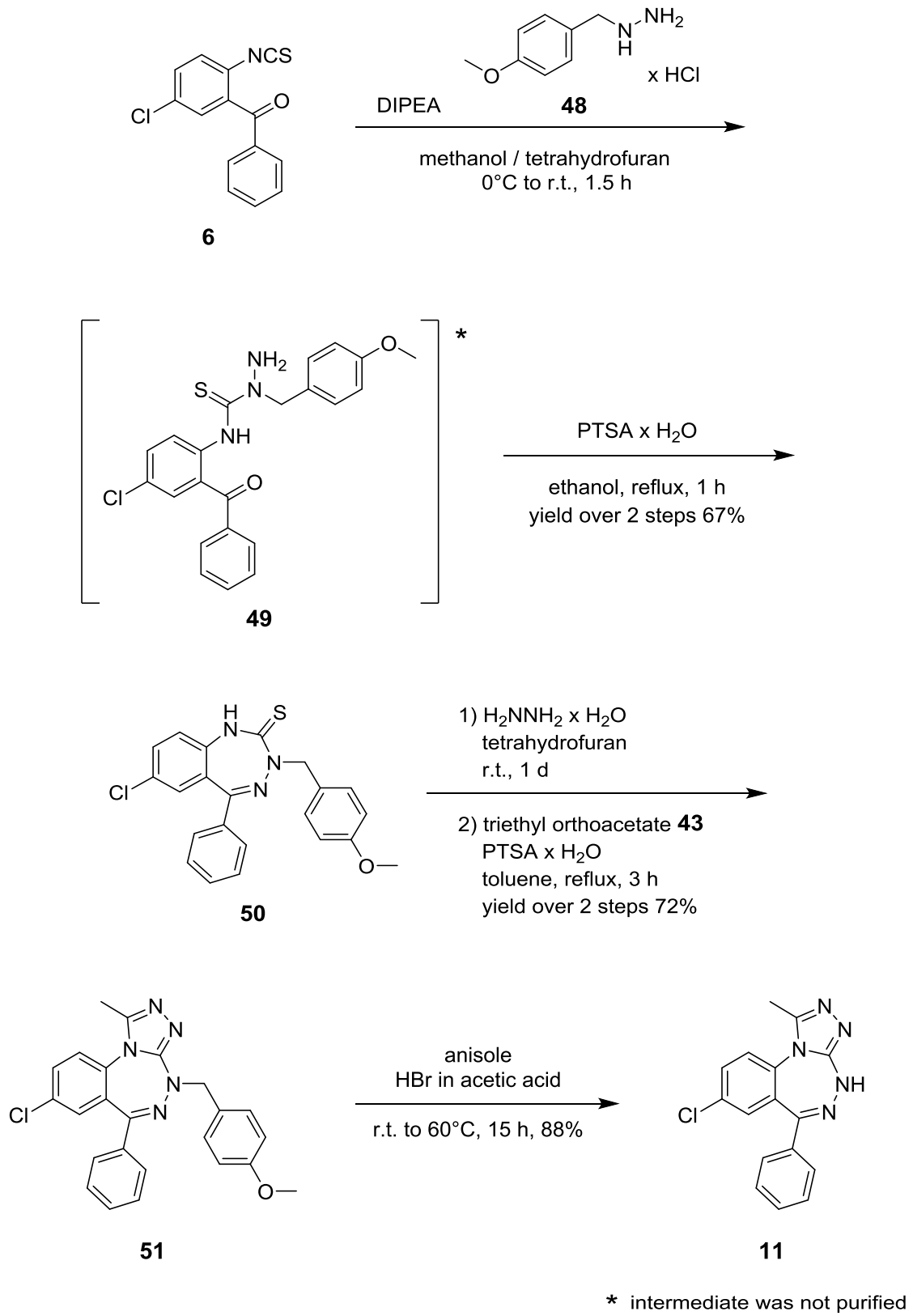
For the preparation of compound **11**, first the alkylated hydrazine derivative **48** has to be synthesized according to single[141] or multi-step[110,142] procedures found in literature. However, product **48** could either not be obtained or only in poor yield and without reproducibility. In 1981, Ghali *et al.* published[143] an interesting way to obtain the hydrochloric salts of related alkylated hydrazines. This way turned out to be the most effective and fastest one and was only slightly modified by using a different borane complex. Starting with 4-methoxybenzaldehyde **45** the hydrazone derivative **47** was obtained by condensation with *tert*-butyl carbazate **46** after heating to reflux in ethanol for one hour. The reduction of compound **47** to the

corresponding hydrazine could not be accomplished using standard reagents like sodium cyanoborohydride[144] or sodium borohydride[145], but was successfully performed with a 2 M dimethylsulfide complex of borane in tetrahydrofuran. The following addition of concentrated hydrochloric acid removed the boc-protecting group selectively with regard to the also acid-cleavable PMB group. Stirring for 10 min at 60 °C gave the colorless solid of (4-methoxybenzyl)hydrazine hydrochloride **48** in 98% yield over three steps.



Scheme 19. Synthesis of the para-methoxybenzyl (PMB) protected hydrazine derivative **48**.

The following synthesis of target compound **11** was already described by Nakamura *et al.*[110] but was mainly modified in isolation and purifications steps as well as in reaction conditions. After the preparation of compound **48** was successfully realized it was subsequently used in the next step. First the deprotonation of the hydrochloric salt **48** was done by stirring in methanol with DIPEA, then the previously synthesized isothiocyanate **6** was added. The generated thiosemicarbazide derivative **49** was not isolated but directly treated with a catalytic amount of para-toluenesulfonic acid monohydrate to build up the seven-membered triazepine ring system of compound **50**.



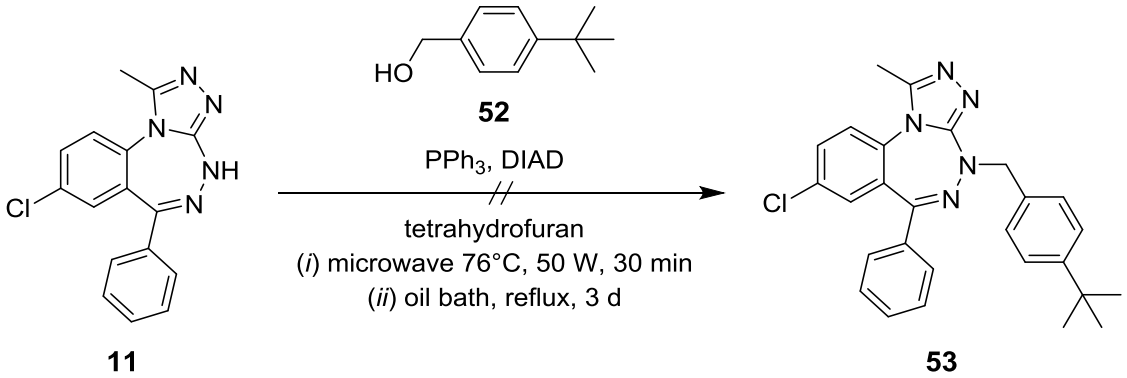
Scheme 20. Synthesis of 4*H*-triazolobenzotriazepine **11**, starting from previously synthesized isothiocyanate derivative **6**.

The conversion of compound **50** in molecule **51** with hydrazine hydrate followed by the treatment with triethyl orthoacetate **43** and para-toluenesulfonic acid monohydrate was carried out in analogous way as already described in the synthesis of 4-(2-hydroxyethyl)triazolobenzotriazepine **5** and 4-benzyltriazolo-benzotriazepine **10**. The last step was the removal of the acid-cleavable para-methoxybenzyl (PMB) protecting group. For this purpose compound **51** was dissolved in a solution of strong hydrogen bromide in acetic acid and was stirred over night. The required 4*H*-triazolobenzotriazepine **11** could be obtained in high yield of 88%.

4.3.2 Derivatization of 4*H*-triazolobenzotriazepine 11 with various substituted 4-benzyl residues

4.3.2.1 Benzylation via Mitsunobu reaction

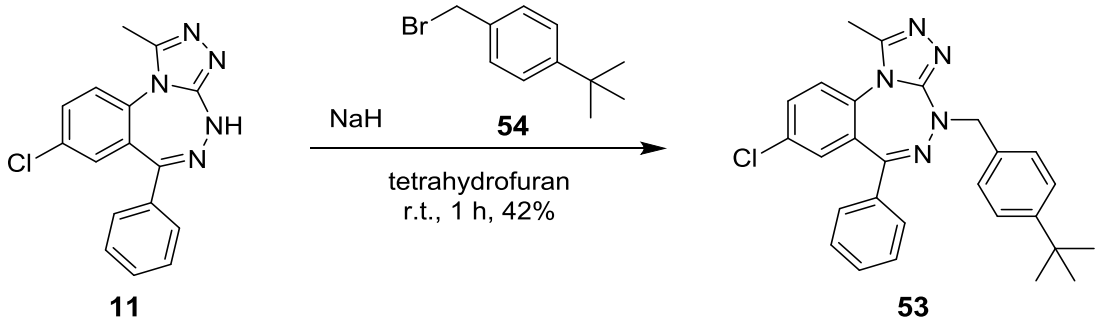
With regard to the fact that benzyl bromide is known as strong lachrymator the original plan (Chapter 2.2.2.3) to synthesize the target compounds by N-alkylation with benzyl bromides was tried to be avoided. As alternative route the reaction of 8-chloro-1-methyl-6-phenyl-TBzT **11** with 4-(*tert*-butyl)benzyl alcohol **52** was attempted under Mitsunobu conditions (Scheme 21).



Scheme 21. Attempted Mitsunobu reaction to attach benzyl residues.

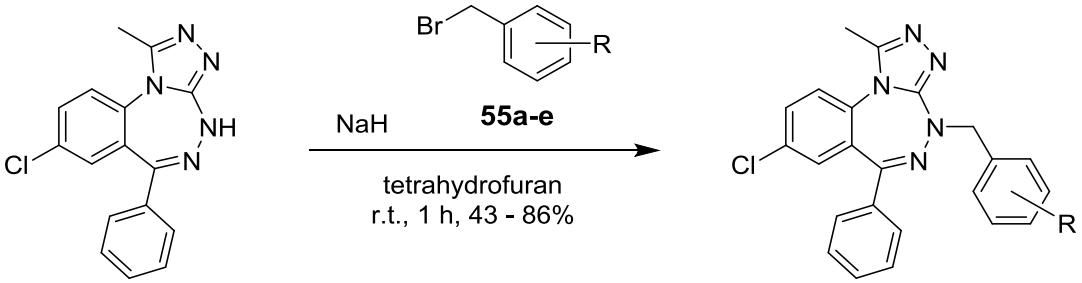
According to a method worked out by Mayer *et al.*[146] for the N-alkylation of imides triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD) were used in tetrahydrofuran as reagents under microwave conditions. However no conversion to product **53** could be detected by TLC and GC/MS. Also another attempt by heating the reaction mixture in the oil bath to reflux for three days did not result in any conversion.

*4.3.2.2 Benzylation via N-alkylation of compound **11** using benzyl bromides or chlorides*



Scheme 22. Successful reaction to convert compound **11** into a 4-benzyl substituted derivative **53**.

With having no success using the Mitsunobu reaction to yield benzylated target compounds, the initial strategy was revisited. In contrast to the 4-(*tert*-butyl)benzyl alcohol **52**, used in the Mitsunobu reaction, the reagent was the closely related 4-(*tert*-butyl)benzyl bromide **54**. Starting with the deprotonation of 4*H*-triazolo-benzotriazepine **11** with sodium hydride the yellowish solution in tetrahydrofuran immediately turned into purple. The benzyl bromide derivative **54** was added and the reaction mixture was stirred for one hour at room temperature. During this time the solution changed its color back again to yellow and the desired product 4-(4-[*tert*-butyl]benzyl)-8-chloro-1-methyl-6-phenyltriazolobenzotriazepine **53** was obtained in moderate yield of 42% (Scheme 22).

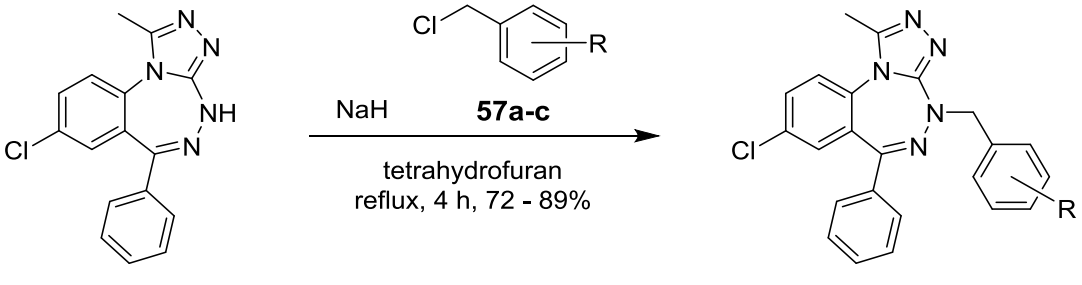


11 **56a-e**

a R = 4-iodo (77%)
b R = 4-nitro (43%)
c R = 4-trifluoromethyl (86%)
d R = 3-chloro (70%)
e R = 2-bromo (61%)

Scheme 23. N-Alkylation of starting molecule **11** with different benzyl bromides **55a-e**.

With the goal to generate a series of compounds with substituted 4-benzyl moieties, the synthesis treating starting compound **11** with sodium hydride followed by benzyl bromides was continued. Three additional para substituted reagents – 4-iodobenzyl (**55a**), 4-nitrobenzyl (**55b**) and 4-(trifluoromethyl)benzyl (**55c**) bromide – were used to prepare the corresponding target molecules **56a-c** in the same manner as compound **53**. Furthermore 3-chlorobenzyl bromide (**55d**) and 2-bromobenzyl bromide (**55e**), respectively, were chosen as representatives of meta and ortho substituted derivatives (Scheme 23).



11 **58a-c**

a R = 2-methyl (74%)
b R = 3,4-dichloro (72%)
c R = 3,4,5-trimethoxy (89%)

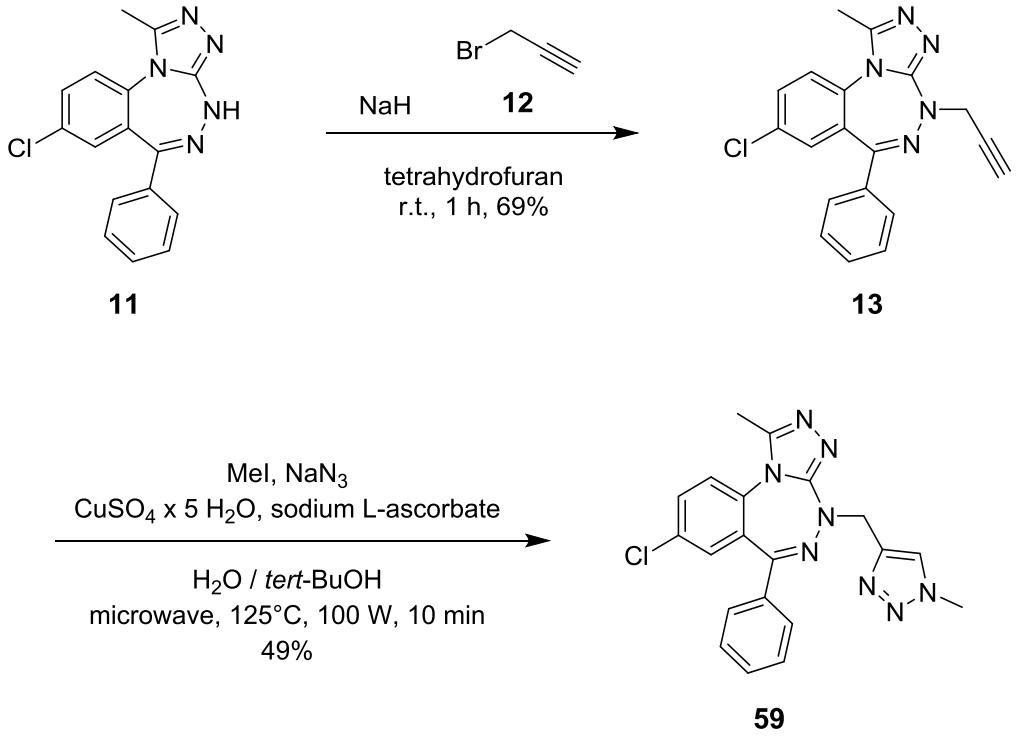
Scheme 24. N-Alkylation of compound **11** carried out with benzyl chlorides **57a-c**.

In addition to the benzyl bromides also benzyl chlorides were converted with compound **11** to benzylated target molecules. However, reaction conditions had to be changed as no or only poor conversion was detected by trying the same conditions used with benzyl bromides. After heating the reaction mixtures to reflux for four hours good yields of target compounds **58a-c** were obtained (Scheme 24). Next to another ortho substituted reagent (**57a**), one disubstituted (3,4-dichloro-benzyl chloride, **57b**) and one trisubstituted (3,4,5-trimethoxybenzyl chloride, **57c**) was used to gain diversity and a more extensive correlation between substitution patterns and activity against bromodomains.

All synthesized compounds were obtained in good to high yields of 61 – 89% (**56a**, **56c-e** and **58a-c**) with the exception of the 4-nitrobenzyl bromide **55b** which gave product **56b** only in moderate yield of 43%.

4.3.3 Preparation of 1,4-substituted 1,2,3-triazole compounds

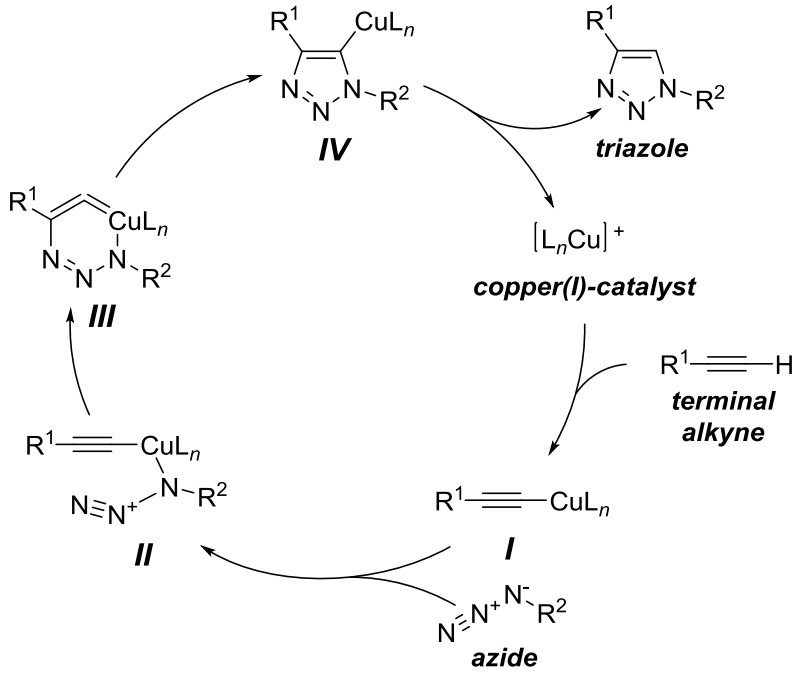
After a broad variety of 4-benzyl substituted compounds were prepared it was considered to replace the phenyl ring by a five-membered aromatic substituent. Therefore 1,2,3-triazoles were chosen as they are not very complex to synthesize. With regard to keep the methylene linker in between the seven-membered triazepine scaffold and the connected aromatic ring, the first step was to attach a propynyl group to starting compound **11**. This was realized by using again sodium hydride to obtain the deprotonated species of 4*H*-triazolobenzotriazepine **11** which was thereupon treated with propargyl bromide **12**. After stirring for one hour in tetrahydrofuran at room temperature the propargyl compound **13** could be isolated in good yield of 69% (Scheme 25). Alkyne **13** complies the requirements to proceed with a 1,3-dipolar cycloaddition, more precisely with the copper(I)-catalyzed azide-alkyne cycloaddition[111]. Only adequate azides had to be chosen to convert the precursor **13** into substituted 1,2,3-triazole containing target compounds.



Scheme 25. Alkylation of compound **11** with propargyl bromide **12** followed by microwave-assisted click chemistry to give compound **59**, containing a 1,2,3-triazole.

On the basis of the work of Prof. Dr. Rolf Huisgen on 1,3-dipolar cycloadditions[147] this type of reaction was constantly further developed. A highly interesting modification of this triazole synthesis was published[148] by Castagnolo *et al.* in 2007. They described a microwave-assisted click chemistry with an in-situ generation of benzyl azide by using sodium azide and benzyl chloride. This one-pot synthesis offers a great economy of time due to a lower number of reaction and purification steps. For the first run, a microwave vial was charged with alkyne derivative **13** and sodium azide as well as copper(II) sulfate pentahydrate in catalytic and L-ascorbate in stoichiometric amounts dissolved in a mixture of water and *tert*-butanol. With the intention to yield a very small substituted triazole ring, iodomethane was added instead of the described benzyl chloride. Using reaction conditions similar to those published by Castagnolo *et al.*, after a short reaction time of 10 min at 125 °C and a maximum power of 100 W the desired product **59** with a 4-(1-methyltriazol-4-yl)methyl residue could be obtained in 49% yield (Scheme 25).

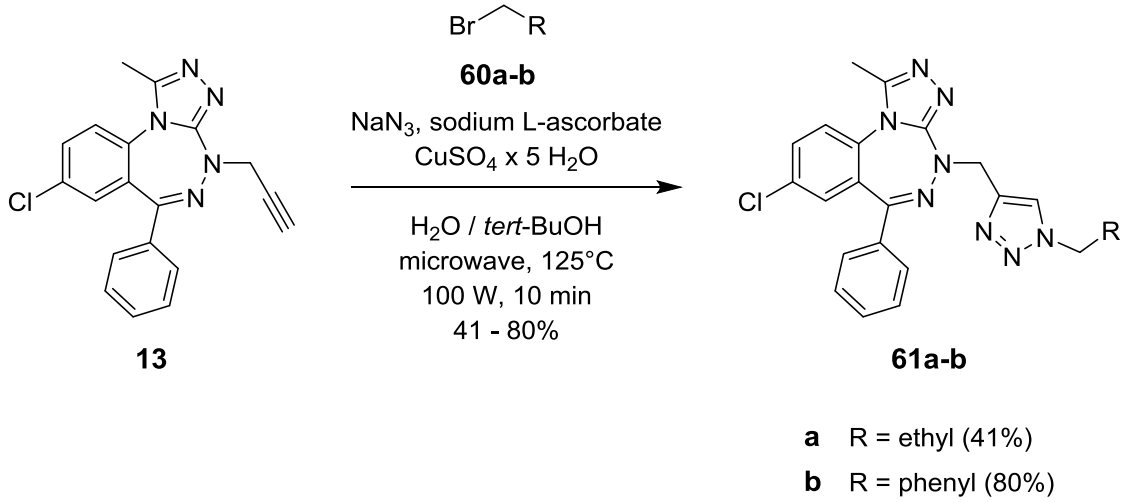
This type of reaction – the copper-catalyzed azide-alkyne cycloaddition (CuAAC) – is the most commonly used 1,3-dipolar cycloaddition in organic chemistry[149]. Despite neither the origin of the selectivity of the cycloaddition nor the complexation of the Cu(I)-species is proven yet, an illustration of a proposed[111] catalytic cycle is shown in Scheme 26.



Scheme 26. Azide-alkyne [3+2] cylcoaddition with copper(I)-catalyst illustrated in an proposed catalytic cycle[111].

The catalytic cycle starts with the formation of the copper(I) acetylide ***I*** out of the copper-catalyst and the terminal alkyne. This first intermediate already explains why only terminal alkynes can be used in CuAACs in contrast to ruthenium catalyzed reactions which also tolerates internal alkynes. Evidence for a non concerted [3+2] cycloaddition directly to ***IV*** was given by DFT calculations of Himo *et. al.*[150]. Instead, over an annealing sequence of Cu(I)-acetylide ***I*** with the terminal azide a six-membered intermediate ***III*** is generated which rearranges to the five-membered triazole ring ***IV***. Regaining of the copper(I)-catalyst by elimination results in the 1,4-disubstituted 1,2,3-triazole ring (Scheme 26). However, recent publications show new suggestions for catalytic cycles including dinuclear[151] or polynuclear[152,153,154] copper(I) intermediates.

To obtain more informative results within the group of 1,2,3-triazole residues two additional derivatives were prepared. This time the corresponding alkyl bromides, 1-bromopropane **60a** and benzyl bromide **60b**, were added to the reaction mixtures to obtain the propylated (**61a**) and the benzylated (**61b**) target compounds, respectively. The reactions were carried out in the same manner as described above (preparation of compound **59**, Scheme 25) using sodium azide, copper(II) sulfate pentahydrate and sodium L-ascorbate in a mixture of water and *tert*-butanol. The microwave-assisted synthesis gave 4-([1-propyltriazol-4-yl]methyl)triazolobenzotriazepine **61a** in moderate yield (41%) and 4-([1-benzyl-triazol-4-yl]methyl)triazolobenzotriazepine **61b** in good yield of 80%.



Scheme 27. Two additional target compounds **61a** and **61b** prepared by click chemistry.

4.3.4 Screening and co-crystallization results of synthesized triazolo-benzotriazepines with 4-benzyl and 4-(triazol-4-yl)methyl moieties

Differential scanning fluorimetry was carried out with 15 compounds again at a concentration of 10 µM against 13 bromodomains, with the exception of compound **58c** which was not screened against LOC93349. The missing data presented not a problem as LOC93349 is not a member of the BET family but only screened to check against any cross interaction with domains of other subfamilies.

The screening (Figure 38) obtained four remarkable compounds (**13**, **51**, **58c**, **61b**) with T_m shifts of more than 6 °C. Especially **51** and **58c** showed high impact on the desired target domains of the BET family without any indication of interactions with other subfamilies. Only three compounds (**53**, **56e**, **58a**) did not show significant ΔT_m values of more than 4 °C. This observation implies that either their substitution is in an unfavorable position or electronic properties are disfavored. In combination with the rest of the screened molecules which cover the not only ortho, meta and para substituted benzyl moieties but also 1,2,3-triazole containing residues first interesting structure-activity relationships could be gained, which are discussed in the following Chapter 4.3.5.

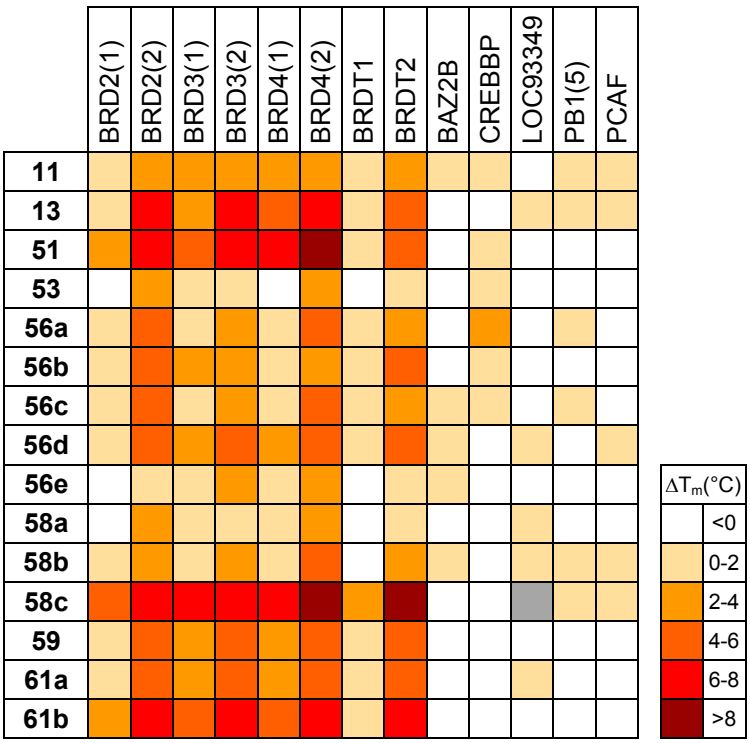


Figure 38. DSF screening results of 4*H*-triazolobenzotriazepine **11**, 4-benzyl substituted compounds **51**, **53**, **56a-e** and **58a-c** as well as propynyl derivative **13** and the 1,2,3-triazolyl moiety containing compounds **59**, **61a** and **61b**. Compound concentration 10 µM. Combination of LOC93349 and **58c** was not screened (grey colored cell).

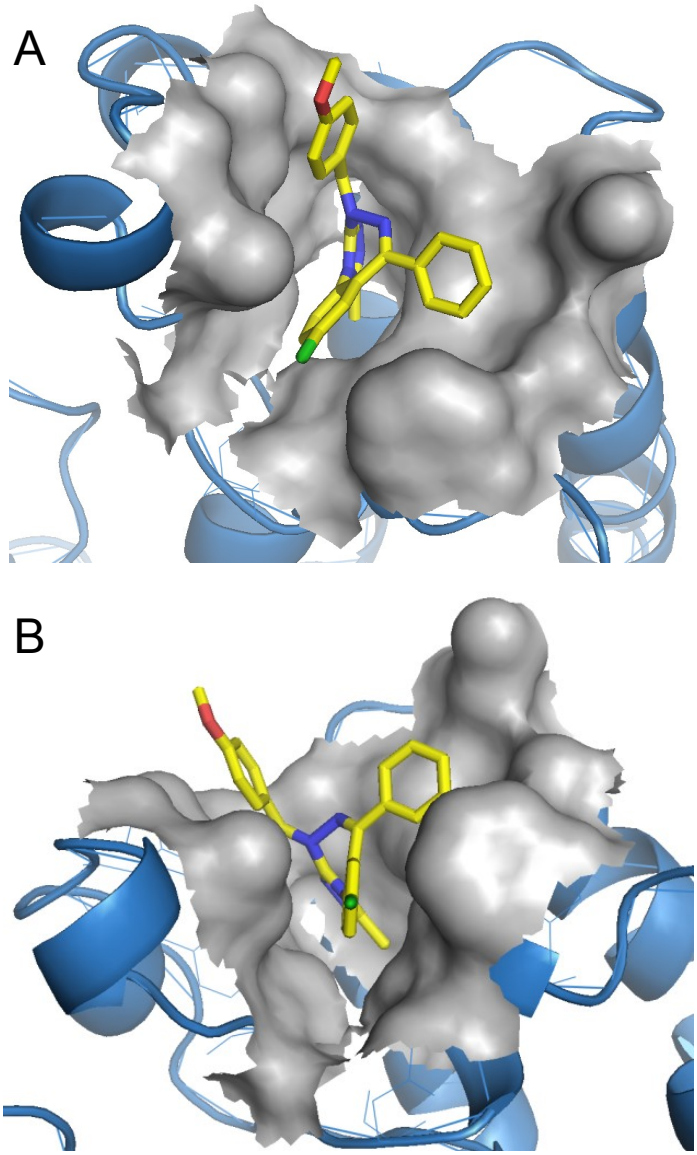


Figure 39. Co-crystallization structure of 4-(4-methoxybenzyl)triazolobenzo-triazepine **51** with first domain of BRD4 shown in two different perspectives (A / B). The amino acids of the bromodomain are colored marine blue and their surface is colored grey. The ligand coloring was done by atom: carbon (yellow), oxygen (red), nitrogen (blue) and chlorine (green).

In addition to the useful screening outcome we tried to receive even more illustrating results by x-ray structures. Molecule **51**, containing the para-methoxybenzyl residue in 4 position, could be successfully co-crystallized with first domain of BRD4 (A/B). This structure displayed the proper fitting of compound **51** into the K_{Ac} binding pocket of BRD4(1). The molecule orientates with the 1-methyltriazole ring towards the inside of the bromodomain pocket while the other ring systems cover groves towards the surface of the binding pocket.

4.3.5 Explanation and discussion of recently screened triazolobenzotriazepines

Temperature shift screening of new synthesized compounds produced positive and interesting results. A progress in research efforts was clearly noticeable as most of the compounds gave significant ΔT_m values. Another important success was the fact that despite of increased affinity to members of the BET family, T_m shifts yielded with bromodomains of other families still were kept quite low. Furthermore incipient stages were made to get information about structure-activity relationships. To get a more expressive information about the obtained T_m shifts, they had to be analyzed more in detail.

Next to both precursors **11** (4*H*-triazolobenzotriazepine) and **13** (4-proynyl-triazolobenzotriazepine) the rest of the screened molecules can be clustered into a small group of 1,2,3-triazolyl containing triazolobenzotriazepines (**59**, **61a-b**) and into the main group of benzylated triazolobenzotriazepines (**51**, **53**, **56a-e**, **58a-c**).

The 4*H*-TBzT precursor **11** was screened for the sake of completeness and showed results as expected with 2.8 °C maximum T_m shift. This insignificant values, however, confirm the assumption that substituents in position 4 are responsible for important interactions to the targeted BETs.

The "click reaction" products **59** and **61a-b** as well as their precursor **13** already gave remarkable T_m shifts. The methyl (**59**) and propyl (**61a**) substituted 1,2,3-triazoles showed similar T_m values, approximately 5 °C for all of the second domains and a lower to nearly no signal for all of the first domains of the BETs. The same trend could be seen with compounds **13** and **61b**. Despite their great diversity of the attached residue, both compounds showed interesting high ΔT_m values (6.3 – 7.0 °C) against second domains but unfortunately also increased affinity towards first domains of the BET family. This effect should be avoided as far as possible to optimize the molecules to site selective BET inhibitors, what means that they only target first domains or second domains of the BETs.

The series of benzylated triazolobenzotriazepines – composed of ten compounds – allowed a much more reasonable discussion about SAR and consequent optimization options. Obviously, ortho substitution decreased affinity for any of the measured domains compared with the unsubstituted benzyl compound **10**. The 2-bromobenzyl (**56e**) and 2-methylbenzyl (**58a**) compound did not show any significant ΔT_m value. A similar observation was made with compound **53**. The very bulky *tert*-butyl moiety in para position destroyed every affinity in comparison with the 4-iodo (**56a**), 4-nitro (**56b**) and 4-trifluoromethyl (**56c**) substituted compounds which showed T_m values of at least 4.5 °C against two bromodomains. The only meta substituted compound **56d**, having a 3-chlorobenzyl residue, also showed a trend to a higher affinity to second domains alike the already discussed compounds of the 4-(triazolo-4-yl)methyl series. The closely related 3,4-dichloro-benzyl substituted compound (**58b**), however, hardly gave significant T_m shifts. Consequently, the additional 4-chloro substitution must be responsible for the decrease in temperature shift values. Outstanding results were obtained with target molecules containing a methoxy substituted residue. Both, the 4-(4-methoxybenzyl)triazolobenzotriazepine (**51**) and the 4-(3,4,5-trimethoxy-benzyl)triazolobenzotriazepine (**58c**) reached generally high T_m shifts (6 – 8 °C) against BETs and very high ΔT_m values against BRD4(2) of 8.3 °C and 8.0 °C, respectively. Even 9.0 °C were achieved against BRDT2 by compound **58c**. Furthermore, these compounds were highly selective for the BET family with no detectable cross activity towards other subfamilies of bromodomains as far as they were screened.

The co-crystallization structure which was obtained of compound **51** with the first domain of BRD4, displayed that the 6-phenyl moiety as well as the annulated benzo ring occupy a groove of the binding pocket. Although the position of the benzyl residue is located more towards the surface of the pocket meaningful SAR studies, resulted from various substitutions of the 4-benzyl moiety, could be obtained. Consequently, modifications of the 6-phenyl and / or of the annulated benzo ring could produce additional interesting SAR results.

To sum up the obtained results, the first goal to synthesize a potent and highly selective compound for bromodomains of the BET family was already achieved, but directly opened a more refined and even more interesting question: Is it possible to optimize the synthesized molecules to site selective inhibitors? Can we address molecules selectively to first OR to second domains of bromodomains of the BET family?

This question gave enormous limitations to the already developed molecules and the direction of the following synthesis sequences. A general view on the last screening results showed that we were able to synthesize molecules which produce good to high shifts in the screening assay. Now the task was to filter out compounds comprising the potential to be optimized to site selectivity or maybe already having a trend to it.

With regard to an improvement of the molecules to a site selective compound and the fact that no clear SAR could be made of the obtained results no further 4-(triazolo-4-yl)methyl) substituted compounds were prepared. The focus was concentrated now towards new benzylated series with different substituted core scaffolds. These efforts should lead to a more informative SAR.

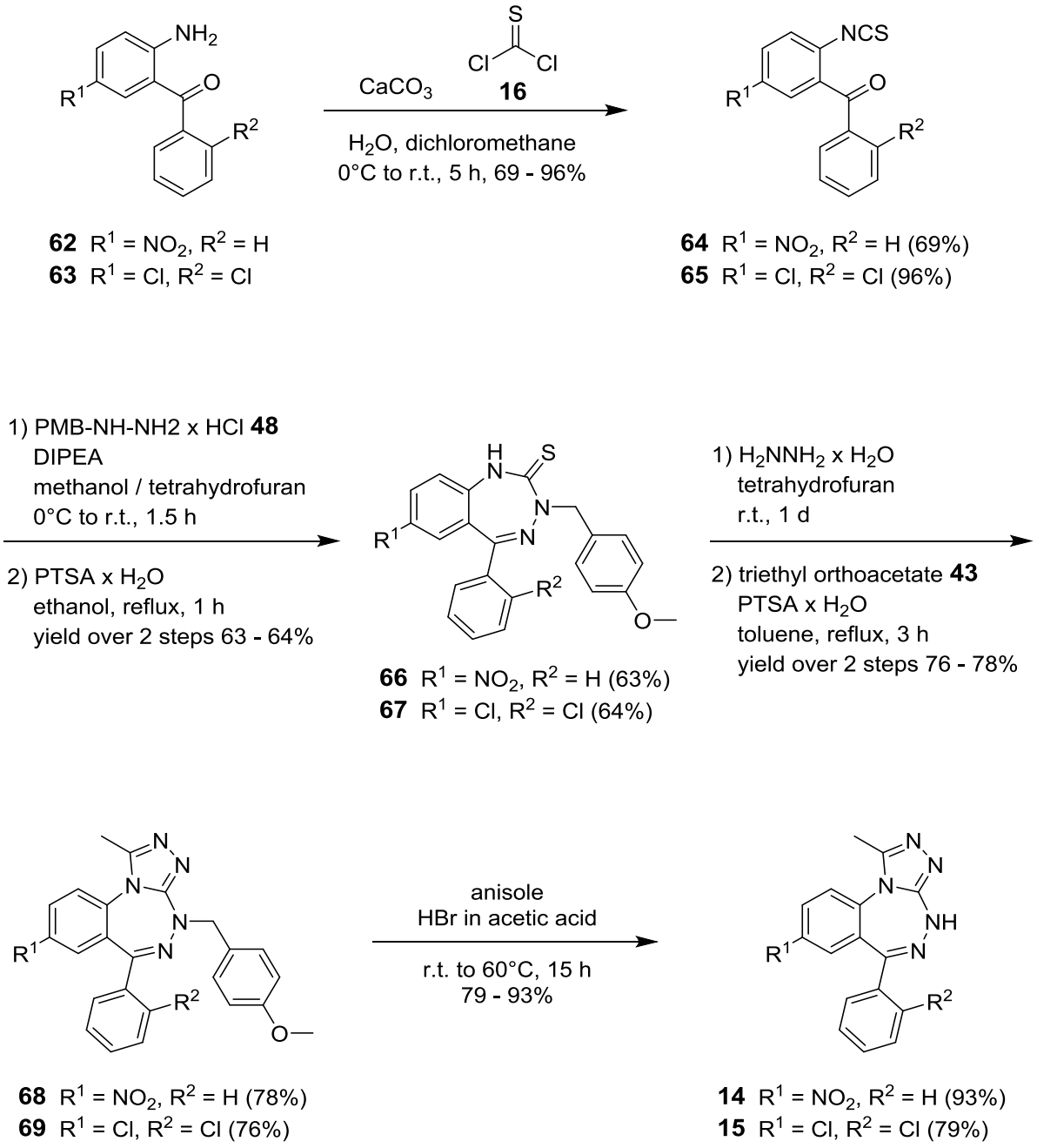
4.4 4-BENZYL SERIES WITH DIFFERENT SUBSTITUTED SCAFFOLDS

4.4.1 Synthesis of a "nitro" and a "dichloro" substituted 4*H*-triazolobenzotriazepine

The first step to improve site selectivity was to get better insight into SAR. Therefore the successfully prepared 4-benzyl series, which already gave good structure to activity relations, should be expanded. The core structure of the hitherto synthesized series contained a 8-chloro and a 6-phenyl substituent. In the patent of Nakamura *et al.* two further 4*H*-triazolobenzotriazepines **14** and **15** as well as their synthesis are described[110] (Scheme 28).

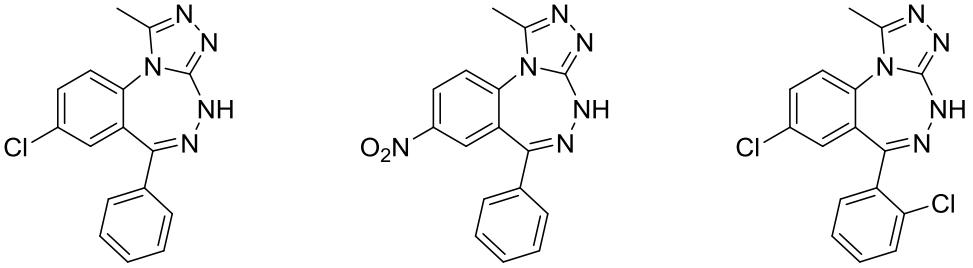
In the same way as described in Chapter 4.3.1.2 for the synthesis of 4*H*-triazolo-benzotriazepine **11**, preparation of the isothiocyanates **64** and **65** as well as the rest of the reaction sequence to the target building blocks **14** and **15** was modified. Mainly work-up and purification steps were improved, e.g. to use flash column chromatography on flash silica gel instead of long crystallization steps.

Conversion of the commercially available 2-aminobenzophenones **62** and **63** into isothiocyanates **64** and **65** followed the same procedure as described for compound **6** using thiophosgene **16** and calcium carbonate to obtain the products in moderate (69%, **64**) to high (96%, **65**) yields. Preparation of the triazepine ring was realized using 4-methoxybenzylhydrazine hydrochloride **48** and DIPEA in the first step, followed by para-toluenesulfonic acid monohydrate in the second step. Both compounds were obtained in similar yields of 63% (**66**) and 64% (**67**) over two steps. The 1-methyltriazole ring was accomplished again by treating the starting materials **66** and **67**, respectively, first with hydrazine hydrate then with triethyl orthoacetate **43** and para-toluenesulfonic acid monohydrate. The 4-methoxybenzyl substituted triazolobenzotriazepines **68** and **69** were obtained in 78% and 76%, respectively. Deprotection of the PMB group to yield the corresponding 4*H*-triazolobenzotriazepines in 93% (**14**) and 79% (**15**) was done in a 45% solution of hydrogen bromide in acetic acid (Scheme 28).



Scheme 28. Synthesis of 4*H*-triazolobenzotriazepines **14** and **15** according to Nakamura *et al.*[110].

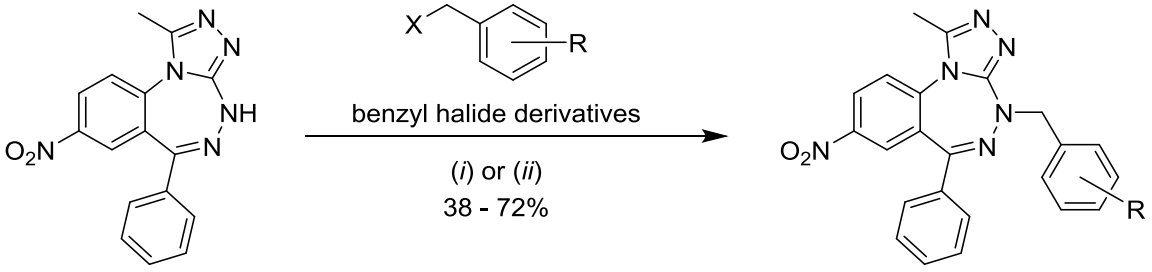
With regard to a more clear and easier discussion of results and comparison of closely related molecules, the synthesized compounds were grouped into series. Comprising the same scaffold with only small variations of the precursors **11**, **14** and **15** (Figure 40), the series were named by their substitution pattern of the corresponding core structure: "chloro series" (**11**), "nitro series" (**14**) and "dichloro series" (**15**).



11 **14** **15**

Figure 40. The substitution pattern of the core scaffold named the corresponding series generated by derivatization in position 4: "chloro series" (**11**), "nitro series" (**14**) and "dichloro series" (**15**).

4.4.2 The nitro series: Derivatization of 1-methyl-8-nitro-6-phenyl-4*H*-triazolobenzotriazepine 14



14 **71a-h**

Benzyl halide derivative No.	X =	Substitution R =	Product No.	Yield
54	Br	4-*tert*-butyl	**71a**	72%
55a	Br	4-iodo	**71b**	68%
55b	Br	4-nitro	**71c**	60%
55d	Br	3-chloro	**71d**	70%
55e	Br	2-bromo	**71e**	63%
57b	Cl	3,4-dichloro	**71f**	48%
57c	Cl	3,4,5-trimethoxy	**71g**	38%
70	Br	3-methoxy	**71h**	62%

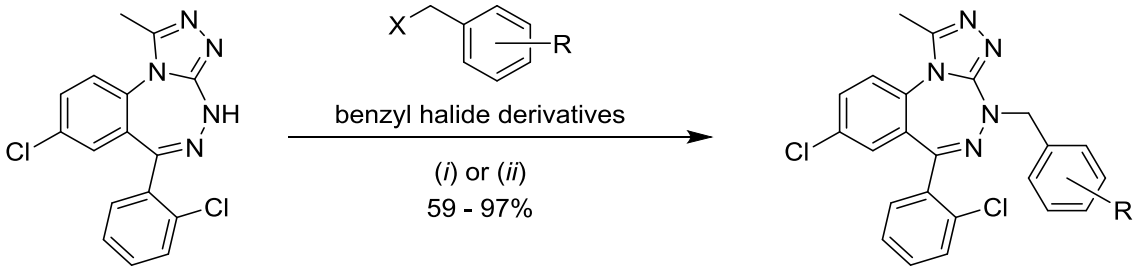
Scheme 29. Derivatization of 4*H*-triazolobenzotriazepine **14** by N-alkylation produced eight target molecules of the nitro series. Reagents and conditions: (*i*) benzyl bromide derivative (3.0 equiv), sodium hydride (1.1 equiv), THF, r.t., 1 h; (*ii*) benzyl chloride derivative (3.0 equiv), sodium hydride (1.1 equiv), THF, reflux, 4 h.

The nitro series was planned to see what effect a change of the substituent in position 8 can have. To be able to compare both series with each other, the replacement of the chloro by a nitro group should be the only modification. The benzyl bromides and benzyl chlorides converted with 4*H*-triazolobenzotriazepine **14** were nearly the same as used in the chloro series. The group of para substituted compounds was reduced by 4-(trifluoromethyl)benzyl compound and one ortho substituted compound (2-methylbenzyl) was also leaved out. 3-Methoxy-benzyl bromide **70** was added to the set of benzyl halides as a consequence of the combination of two aspects. One the one hand the meta substituted 3-chlorobenzyl bromide **55d** gave interesting temperature shift results which showed a trend to site selectivity and on the other hand high T_m values of methoxy substituted compounds (**51**, **58c**) were obtained before.

The synthesis of the target compounds **71a-h** followed the established procedure as already used within the chloro series in Chapter 4.3.2.2. After deprotonation of the 4*H*-triazolobenzotriazepine **14** using 1.1 equivalents of sodium hydride, the corresponding benzyl halide was added. The reaction was carried out in anhydrous tetrahydrofuran for one hour at room temperature when using benzyl bromides. The conversion with benzyl chlorides was done in anhydrous tetrahydrofuran by heating to reflux for four hours. The target molecules **71a-h** could be synthesized in moderate to good yields (38 – 72%) throughout (Scheme 29).

4.4.3 Preparation of the dichloro series by N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-triazolobenzotriazepine 15

The preparation of the "dichloro series" followed the same intention as the development of the nitro series. However, this time the 8-chloro substituent was maintained while an additional chlorine was introduced in ortho position of the 6-phenyl ring. The study of the effect of this labeling in combination with the other two series should allow us to get a comprehensive SAR.



15 **72a-h**

Benzyl halide derivative No.	X =	Substitution R =	Product No.	Yield
54	Br	4-*tert*-butyl	**72a**	97%
55a	Br	4-iodo	**72b**	67%
55b	Br	4-nitro	**72c**	66%
55d	Br	3-chloro	**72d**	77%
55e	Br	2-bromo	**72e**	76%
57b	Cl	3,4-dichloro	**72f**	59%
57c	Cl	3,4,5-trimethoxy	**72g**	68%
70	Br	3-methoxy	**72h**	81%

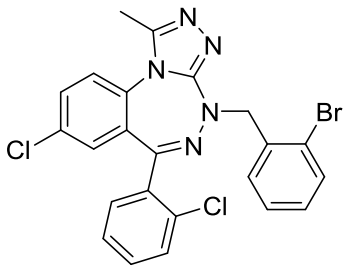
Scheme 30: Conversion of 8-chloro-6-(2-chlorophenyl)triazolobenzotriazepine **15** with a variety of benzyl chlorides and bromides to target compounds **72a-h**. Reagents and conditions: (*i*) benzyl bromide derivative (3.0 equiv), sodium hydride (1.1 equiv), THF, r.t., 1 h; (*ii*) benzyl chloride derivative (3.0 equiv), sodium hydride (1.1 equiv), THF, reflux, 4 h.

Under identical reaction conditions as used preparing the nitro series, the target compounds of the dichloro series **72a-h** were synthesized. The starting material **15** was treated with sodium hydride and the corresponding benzyl halide derivative in anhydrous tetrahydrofuran. Reactions using benzyl bromide derivatives were stirred for one hour at room temperature whereas reactions with benzyl chlorides were heated to reflux for four hours. A good comparability was guaranteed by converting the 4*H*-triazolobenzotriazepine **15** with exactly the same benzyl bromides and benzyl chlorides as used before in the nitro series. Consistently good yields from 59 – 81% were achieved. Even an almost quantitative yield of 97% was reached with 4-(*tert*-butyl)benzyl bromide **54** to target compound **72a**.

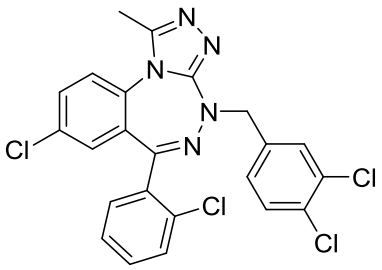
4.4.4 Isotope effects in mass spectra obtained from multi-halide substituted compounds

The distribution of isotopes is an important factor in the analysis of mass spectrometry. In 1922, Francis William Aston was awarded the Nobel Prize in chemistry for his outstanding research of discovering isotopes by mass spectrometry[155]. His research became an area of high interest and his efforts paved the way for numerous scientists who improved his studies especially by more precise mass spectrometers. Despite carbon, hydrogen, oxygen and nitrogen are the main components of organic molecules, their contribution to the isotope peaks is very low. The explanation is their isotope distribution with a very high natural abundance of the main isotope ^{12}C (98.93%), ^{1}H (99.9885%), ^{16}O (99.757%) and ^{14}N (99.632%)[156]. In contrast both chlorine and bromine have a considerable abundance of a second natural isotope, in case of chlorine ^{35}Cl (75.77%) and ^{37}Cl (24.23%) and in case of bromine ^{79}Br (50.69%) and ^{81}Br (49.31%)[157]. Halide substituted compounds consequently show a very obvious and characteristic distribution of their molecule peak (and potential fragments) which increases in complexity with a rising number of halogen atoms. Neglecting the minor isotope effects, in the following discussion only halogens are considered.

Interesting examples for more complex isotope distributions in mass spectrometry are compounds **72e** and **72f**. The synthesized compound of the dichloro series contain three and four halogen atoms, respectively, and thus showed a complex composition of molecule mass peaks in the corresponding mass spectra.



72e



72f

Figure 41. Compound **72e** is substituted by three halogen atoms and compound **72f** even contains four chloro substituents.

For compound **72e** three halogen atoms are present with two abundant isotopes each. Consequently there are 2^3 = 8 feasible combinations of isotopes, resulting in four molecule peaks in the MS spectra (Figure 42). The relative intensities of the molecule peaks can be calculated by the following example.

1) The probability *P* for each isotope pattern over all isotopes *n* has to be calculated by

$$P(isotope\ combination) = \prod_{n}(abundance)$$

In case of the first isotope combination

$$P\left(^{35}Cl, ^{35}Cl, ^{79}Br\right) = 0.7577\ x\ 0.7577\ x\ 0.5069 = 0.2910 \approx 29\%$$

means that with a probability of 29% molecule **72e** comprises the isotope pattern (^{35}Cl, ^{35}Cl, ^{79}Br).

2) In case of multiple combinations for the same mass peak (cf. M + 2, M + 4) the probabilities of each isotope combination have to be summed up. The isotope combinations (^{35}Cl, ^{37}Cl, ^{79}Br) and (^{37}Cl, ^{35}Cl, ^{79}Br) as well as (^{35}Cl, ^{37}Cl, ^{81}Br) and (^{37}Cl, ^{35}Cl, ^{81}Br) are not distinguishable by mass spectrometry and they have the same probabilities. Nevertheless, both possibilities have to be considered by summing up the single probabilities for the corresponding molecule peak. Either they are listed twice and for each combination *P* is calculated or (like in the next example) they are weighted by their number of variations (in this case: two).

3) Now, the peak with the highest probability (in this case: M + 2) will be set to 100%. The other relative intensities are obtained by

$$relative\ intensity\ (X) = \frac{P(X)}{P(M+2)}$$

$$relative\ intensity\ (M) = \frac{P(M)}{P(M+2)} = \frac{P(0.2910)}{P(0.4692)} = 0.6202 \approx 62\%$$

The calculated values match literature[158] as well as practical data very well.

Molecule peak	Isotope combinations	Probability *P* single	summation	Relative intensity
M	^{35}Cl, ^{35}Cl, ^{79}Br	0.2910		62%
	^{35}Cl, ^{37}Cl, ^{79}Br	0.0931		
M + 2	^{37}Cl, ^{35}Cl, ^{79}Br	0.0931	0.4693	100%
	^{35}Cl, ^{35}Cl, ^{81}Br	0.2831		
	^{37}Cl, ^{37}Cl, ^{79}Br	0.0297		
M + 4	^{35}Cl, ^{37}Cl, ^{81}Br	0.0905	0.2107	45%
	^{37}Cl, ^{35}Cl, ^{81}Br	0.0905		
M + 6	^{37}Cl, ^{37}Cl, ^{81}Br	0.0290		6%

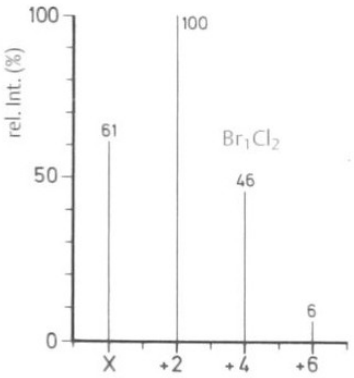


Figure 42. Eight combinations of halogen isotopes are possible for compound **72e**. They produce four molecules peaks in the MS spectra with different intensities[158].

The same method as before can be used calculating the isotope peaks in the mass spectra of compound **72f**. In this case with <u>two</u> different isotopes of <u>four</u> chloro substituents 2^4 = 16 different combinations are possible. However, with only chloro atoms are present in this compound, which are not distinguishable by mass spectroscopy, there are only five different combinations but (M + 2) and (M + 6) can occur in four different variations and (M + 4) can even occur in six different variations. Therefore the single probability of each combination has to be weighted by its number of variations (*V*) to obtain the probability *P* of each molecule peak (Figure 43). The complete calculation led to a distribution of the molecule peak into five isotope peaks of M, (M + 2), (M + 4), (M + 6) and (M + 8) with the corresponding intensities of 78%, 100%, 48%, 10% and 0.8%.

Molecule peak	Isotope combination	*V*	Probability *P*	Relative intensity
M	^{35}Cl, ^{35}Cl, ^{35}Cl, ^{35}Cl	1	0.3296	78%
M + 2	^{37}Cl, ^{35}Cl, ^{35}Cl, ^{35}Cl	4	0.4216	100%
M + 4	^{37}Cl, ^{37}Cl, ^{35}Cl, ^{35}Cl	6	0.2022	48%
M + 6	^{37}Cl, ^{37}Cl, ^{37}Cl, ^{35}Cl	4	0.0431	10%
M + 8	^{37}Cl, ^{37}Cl, ^{37}Cl, ^{37}Cl	1	0.0035	0.8%

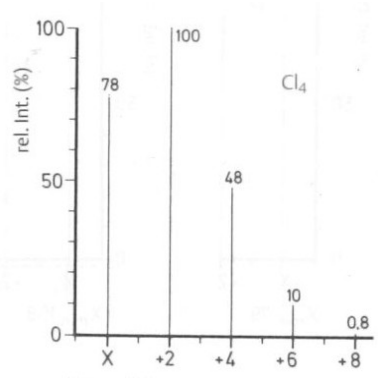


Figure 43. Example for calculation of the molecule peak of compound **72f** which split up into five peaks as a consequence of different isotope combinations[158].

4.4.5 Temperature shift results and discussion of the nitro and the dichloro series

In total 20 compounds, ten of each series, were screened against the standard eight family members of the BETs and the five representatives of other subfamilies of bromodomains. A compound concentration of 10 µM was used again and LOC92249 was left out of both 3,4,5-trimethoxybenzyl substituted compounds **71g** and **72g**.

At a first glance, the compounds of the nitro series looked much more active than those of the dichloro series. On the average the 8-nitro substituted compounds indeed gave higher temperature shift signals, but hardly trends are readable from the chart of results. A much more interesting tendency – a trend to site selectivity – is given by the dichloro series, however most signals are too weak to consider them as significant results (Figure 44).

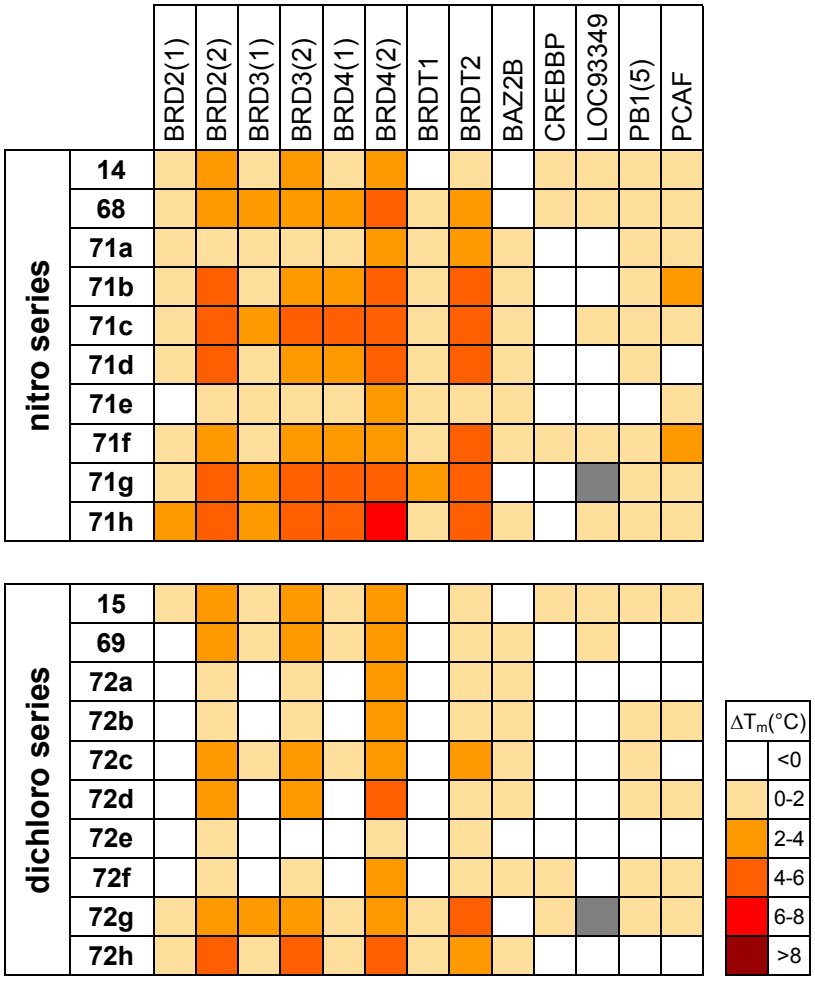


Figure 44. Temperature shift screening results of the nitro series and of the dichloro series. 4*H*-Triazolobenzo-triazepines **14** and **15** were also screened as well as the 4-methoxybenzyl substituted compounds **68** and **69**. Both 3,4,5-trimethoxybenzyl substituted compounds **71g** and **72g** were not screened against LOC93349 (grey colored cells). A compound concentration of 10 µM was used.

The unsubstituted compounds in 4 position **14** and **15**, both gave poor ΔT_m values as expected (cf. chloro series). Compounds **71a** and **72a**, containing the bulky *tert*-butyl group in para position of the N-benzyl moiety, again showed no significant T_m shift, probably as a consequence of steric interference. The same observation was made with both 2-bromobenzyl substituted compounds **71e** and **72e**. As already observed within the chloro series of compound **56e** and **58a**, the ortho substituent obviously is responsible for steric interactions which mismatch the space given by the binding pocket.

The 4-methoxybenzyl substituted compounds **68** and **69** as well as **71g** and **72g** with an attached 3,4,5-trimethoxybenzyl residue showed a enormous decrease in their temperature shifts compared to the analogous compounds of the chloro series **51** and **58c**, respectively. This implies that both modifications, the exchange of the 8-chloro by a 8-nitro group as well as the additional chloro substituent in ortho position of the 6-phenyl rings is responsible for a decrease in affinity towards the targeted domains.

The simple para substituted compounds (**71a**, **71b**, **72a**, **72b**, containing iodo and *tert*-butyl residues) as well as the disubstituted 4-(3,4-dichlorobenzyl)triazolo-benzotriazepines (**71f**, **72f**) either gave only poor response in the DSF screening or showed also no trend to a compound which could be improved to a site specific one.

A very interesting results however was obtained with the newly introduced residue. Compounds **71h** and **72h** containing the 3-methoxybenzyl moiety showed on the one hand the highest T_m shift against the second domain of BRD(4) within the nitro series (**71h**) and on the other hand a distinctive trend towards site selectivity against the second domains of BRD(2), BRD(3) and BRD(4) within the dichloro series (**72h**). Thus, this compound is an evidence of progress and a good example which presents the improvement that can be made by stepwise analysis of structure-activity relationships obtained by DSF.

4.4.6 Lead structure optimization by comparison of the three benzyl series

The complete screening of triazolobenzotriazepines covers 47 compounds clustered into the three series "chloro", "nitro" and dichloro". A lot of information was gained out of the DSF screenings and structural variations could be connected to an increase or a decrease of the corresponding temperature shifts what led to a good prediction of structure-activity relationships.

For the upcoming lead structure optimization the previous screenings results of all three series were used. The screening charts of the series as depicted in Figure 34, Figure 38 and Figure 44 gave first illustrations by their contrast levels starting from colorless for low T_m shifts and turning over five steps into dark red what indicates high T_m shifts. With regard to the development of a site selective compound a second analysis was used. Therefore the difference of the ΔT_m values of both domains (ΔT_m domain 2 – ΔT_m domain 1; $\Delta T_{m(D2-D1)}$) of each BRD was plotted on the ordinate against the residue in position 4 of the corresponding compound on the abscissa (see two examples in Figure 45 and Figure 46).

The chloro series, which was screened in two parts, has to be considered separately. The 4-(2-hydroxyethyl)triazolobenzotriazepine **5** and its derivatives **44a-j** showed weak response in the DSF screening (Figure 34) and consequently no tendency to site selectivity but only spread and low values in the plot of Figure 45. The subsequently prepared compounds, analogs of the screening hit compound **10**, containing substituted 4-benzyl residues or a 4-(triazol-4-yl)methyl moiety showed exceptionally improved T_m values (Figure 38). Even more, a trend to site selectivity against BRD2 and BRDT (Figure 45) is given by several compounds especially of those which contain the 4-(triazol-4-yl)methyl residue (**59**, **61a**, **61b**) as well as their precursor **13** containing a 4-propynyl substituent.

However, both additional series, the nitro and the dichloro one, gave the decisive factor for the next generation of compounds. The greater number of comparable values and the repeating results of SAR confirmed predictions of the chloro series and enabled a further development with profound knowledge.

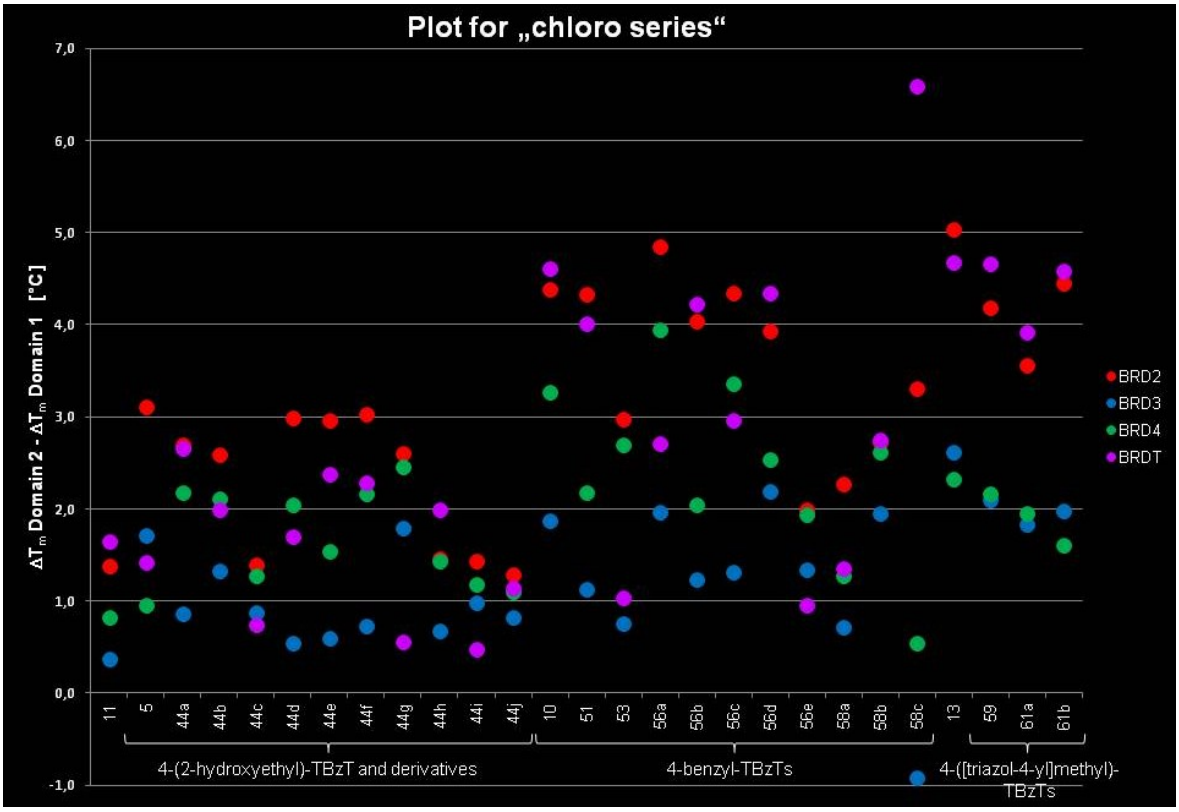


Figure 45. Plot of the whole chloro series with regard to site selectivity.

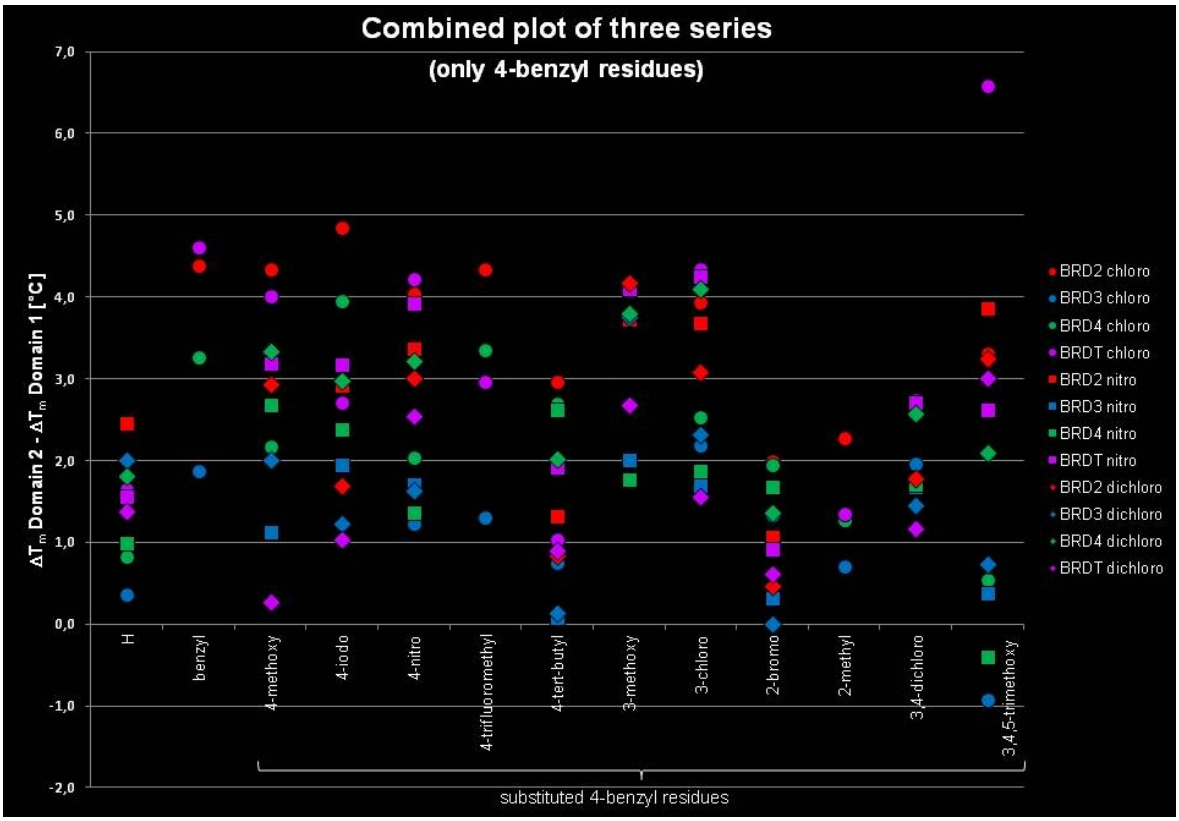


Figure 46. Plot of the benzyl residues of all three series to filter out the moiety in position 4 with the highest site selectivity.

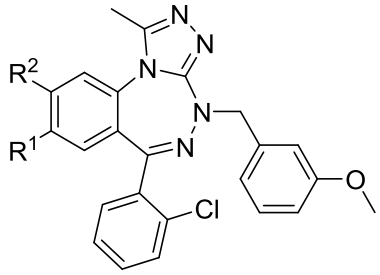
The nitro series as well as the dichloro series showed impressively, by their DSF results in comparison with the chloro series, how a small change of the core scaffold can affect the strength of interaction with the targeted bromodomains.

The replacement of the 8-chloro by a 8-nitro group decreased the T_m values of the best compounds of the chloro series **51** and **58c** but showed an slight increase in T_m shifts of BRD2(2), BRD3(1) and BRD3(2) of the para substituted molecules **71a** and **71b**. For this reason position 8 offered a good starting point for further optimization of the triazolobenzotriazepines. Furthermore, the crystal structure of compound **51** with BRD4(1) (Figure 39) showed a distinctive groove which might also be occupied by an additional substituent in position 9. Thus to both positions a residue should be attached in the next generation of TBzTs (Figure 47).

The great benefit of the dichloro series is the fact that almost exclusively second domains are targeted as illustrated in the chart of results (Figure 44). Despite most of the obtained results are not significant as they gave only poor response in the temperature shift screening the trend is obvious and 3-methoxybenzyl compound **72h** gave an evidence that it is possible to increase affinity towards the second domains. With this observation the new lead structure (Figure 47) was planned to have this 6-(2-chlorophenyl) residue as a constant element of the core structure.

The choice of the residue in position 4 was again tried to filter out by a plot comparing the site selectivity of the corresponding 4-benzyl residues of all three series (Figure 46). Non convincing results were obtained of the three moieties benzyl, (4-trifluoromethyl)benzyl and 2-methylbenzyl as they were only used in the chloro series. The 4*H*-triazolobenzotriazepines as well as those with an ortho substituted benzyl residue in position 4 gave poor site selectivity results in the plot. Similarly weak results were obtained with the (4-*tert*-butyl)benzyl and the 3,4-dichlorobenzyl moieties. The 4-methoxybenzyl and especially the 3,4,5-trimethoxybenzyl residue showed strongly spread results of site selectivity. Consequently both meta substituted benzyl residues and the 4-iodobenzyl as well as the 4-nitrobenzyl were the most interesting ones.

By a more detailed examination, however, the 3-methoxybenzyl residue turned out to be the most promising one. With the lowest distribution and the highest accumulation of data points (five of eight points) at a remarkable $\Delta T_{m(D2-D1)}$ of approximately 4 °C second domains seem to be the preferred target of 3-methoxybenzyl substituted compounds. Hence this 3-methoxybenzyl residue should also be introduced as an invariable structural element of the new lead structure (Figure 47).



R^1, R^2 = electron donating or electron withdrawing groups

6-(2-chlorophenyl)-4-(3-methoxybenzyl)-1-methyl-triazolobenzotriazepine derivatives

Figure 47. New developed lead structure for the next generation of potentially site selective BET inhibitors.

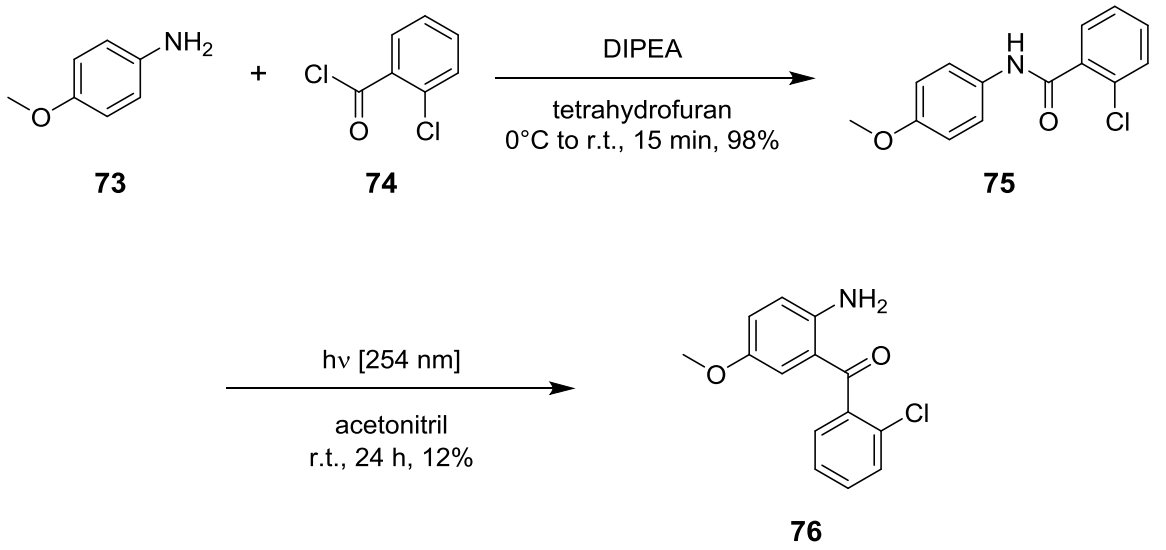
4.5 MODIFICATION OF 6-(2-CHLOROPHENYL)-4-(3-METHOXYBENZYL)-TRIAZOLOBENZOTRIAZEPINE IN POSITIONS 8 AND 9

4.5.1 Preparation of 2-aminobenzophenones

For the desired modifications in positions 8 and 9 of the triazolobenzotriazepines according to the designed lead structure in Chapter 4.4.6 (Figure 47) different 2-aminobenzophenones had to be synthesized as starting materials. Therefore two different approaches were used as described in the following Chapters 4.5.1.1 and 4.5.1.2.

4.5.1.1 Two step synthesis using photochemistry

According to previous optimization steps methoxy groups showed distinct improvement of activity. For this reason, the first approach was to replace the 8-chloro substituent by a 8-methoxy group.

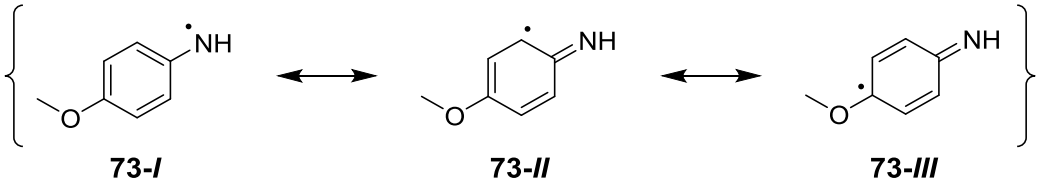


73 **74** **75** **76**

Scheme 31. Photochemical synthesis of 2-aminobenzophenone derivative **76**.

The synthesis of (2-amino-5-methoxyphenyl)(2-chlorophenyl)methanone **76** was successfully accomplished in two steps. Using commercially available 4-methoxy-aniline **73** and 2-chlorobenzoyl chloride **74** known[159] amide **75** was obtained in almost quantitative yield of 98% after stirring for just about 15 min at room temperature in anhydrous tetrahydrofuran. A photo-Fries rearrangement of amide **75**, carried out according to Ferrini *et al.*[160] for 24 h at room temperature in acetonitrile under irradiation with 254 nm led to published[161] product **76** in 12% yield.

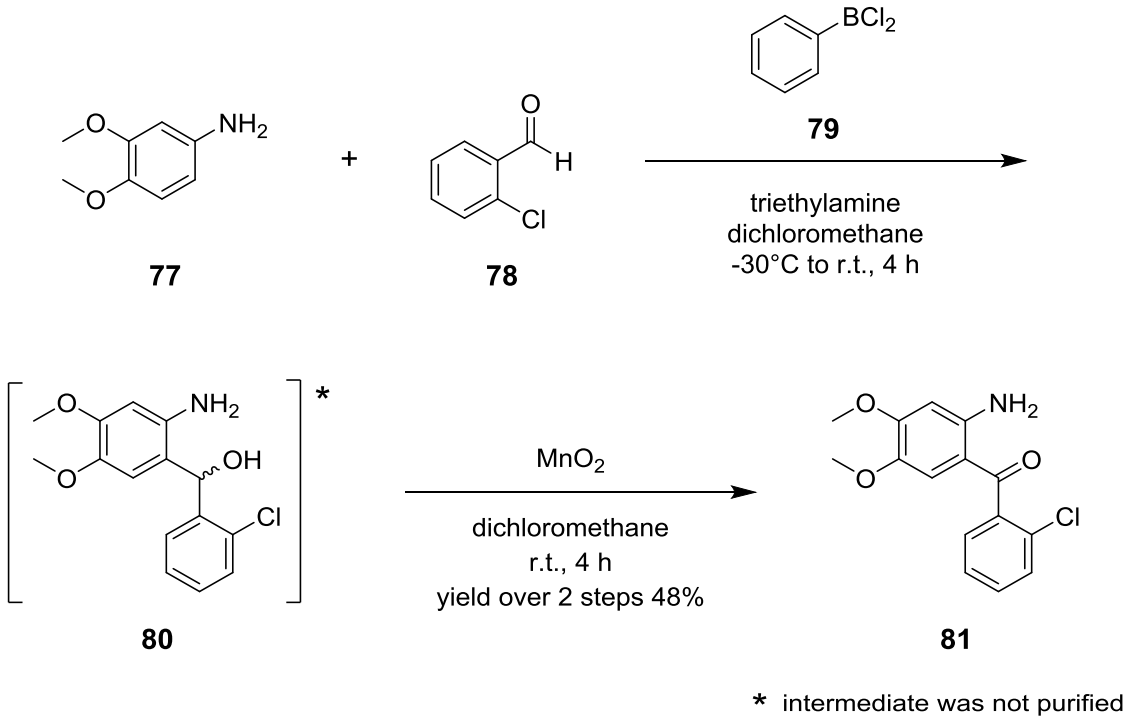
The radical reaction mechanism caused the low yield of product **76**. The homolytic cleavage of the amide bond by UV light of 254 nm produced two monoradical species: 4-methoxyaniline radical and 2-chlorobenzoyl radical. Figure 48 shows the mesomeric structures of the monoradical 4-methoxyaniline species **73** ***I-III***. Whenever reaction occurs with the carbonyl radical, **73-*I*** would lead to the starting material **75** and both ortho radicals **73-*II*** would lead to the desired product **76**. Reaction products with **73-*III*** cannot rearomatize and are consequently disfavored. However the benzoyl radical also comprises different mesomeric structures of radicals next to the carbonyl one what leads to a lot of different other combination possibilities.



73-*I* **73-*II*** **73-*III***

Figure 48. Mesomeric structures of monoradical 4-methoxyaniline species **73** ***I-III*** after homolysis.

4.5.1.2 Synthesis by "ortho selective acylation" of aniline derivatives

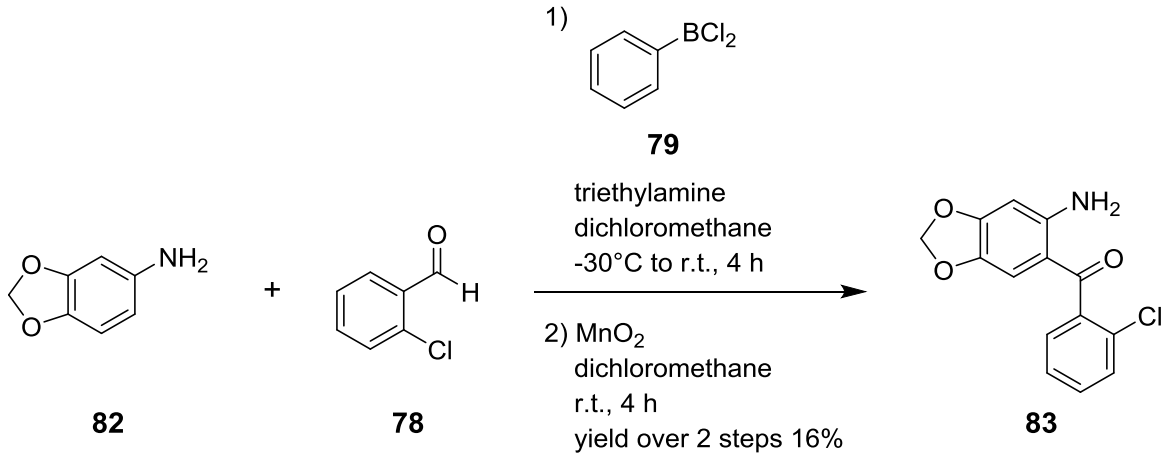


Scheme 32. Borane adduct mediated "ortho selective acylation" of 3,4-dimethoxyaniline **77**.

In contrast to the symmetric 4-methoxyaniline **73** the unsymmetrically substituted 3,4-dimethoxyaniline **77** was even less adapted for photo-Fries rearrangement due to a higher probability of undesired byproduct generation. A different method was found published in 2006 by Liu *et al.*[162]. They described an "ortho selective acylation" method of aniline derivatives with a variety of substitution pattern in position 3 and 4.

The substituted aniline **77** was stirred first with dichlorophenylborane **79** and triethylamine to form a borane adduct followed by the addition of 2-chlorobenzaldehyde **78**. This borane adduct coordinates to the carbonyl oxygen of the aldehyde and directs the carbonyl carbon into ortho position of the aniline derivative. A six-membered transition state provides after basic workup the not purified intermediate **80** which was oxidized to final product **81** using manganese dioxide. According to this patent synthesis of compound **81** (Scheme 32) could successfully be accomplished.

The same type of reaction as described above was carried out again using 3,4-(methylenedioxy)aniline **82** and 2-chlorobenzaldehyde **78** to prepare known[162] compound **83** (Scheme 33).

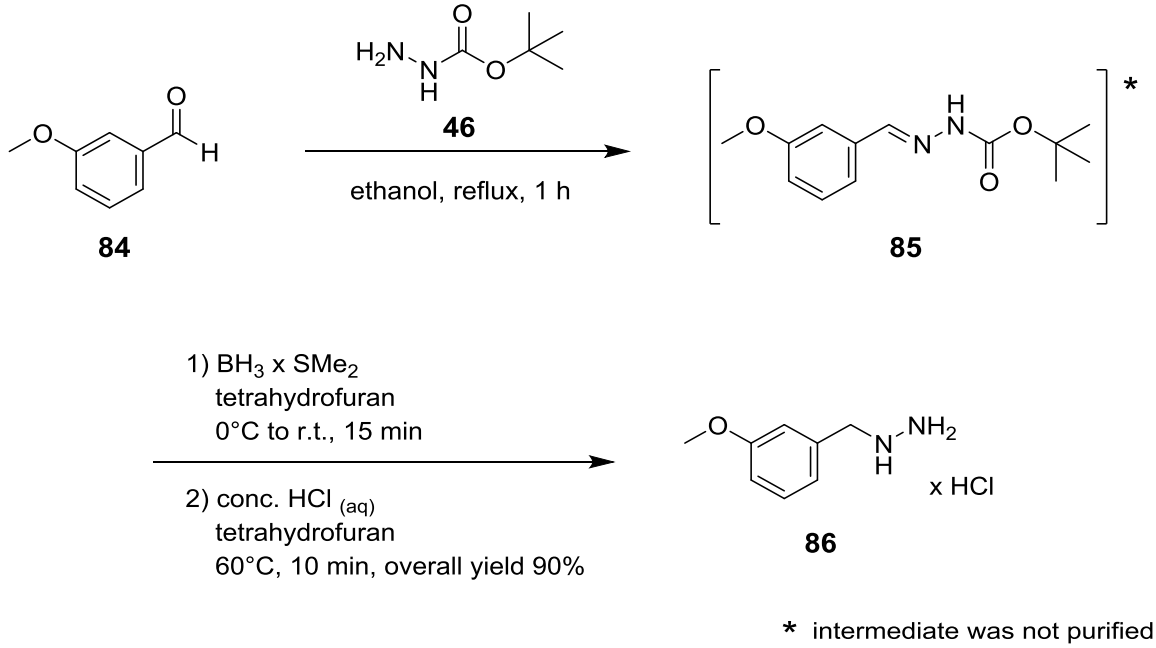


Scheme 33. Preparation of known[162] compound **83** according to Liu *et al.* in two steps.

4.5.2 Preparation of (3-methoxybenzyl)hydrazine hydrochloride 86

For the synthesis of the chloro, nitro and dichloro series a 4-methoxybenzyl residue was used because of its property to act as an acid-cleavable protecting group. With the goal of producing no longer whole series of triazolobenzo-triazepines with different residues in position 4, but maintain the 3-methoxybenzyl group, the detour via the 4-unsubstituted triazolobenzotriazepine was not necessary anymore.

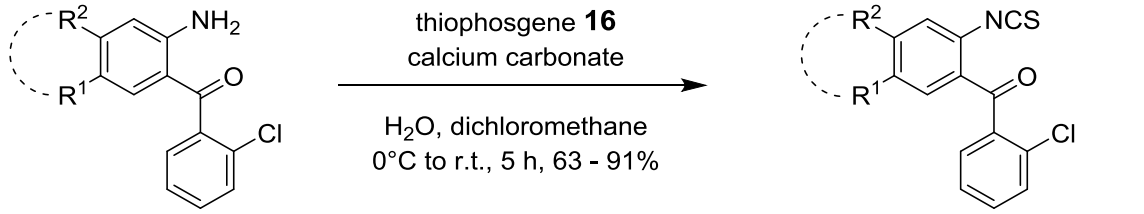
For this reason (3-methoxybenzyl)hydrazine hydrochloride **86** was prepared in the same manner as described for compound **48** in Chapter 4.3.1.2. Condensation of 3-methoxybenzaldehyde **84** and *tert*-butyl carbazate **46** yielded hydrazone **85**. Without purification a 2 M dimethylsulfide complex of borane in tetrahydrofuran was added and after 15 min at room temperature addition of concentrated hydrochloric acid gave product **86** in high yield of 90% (Scheme 34).



Scheme 34. Synthesis of (3-methoxybenzyl)hydrazine hydrochloride **86**.

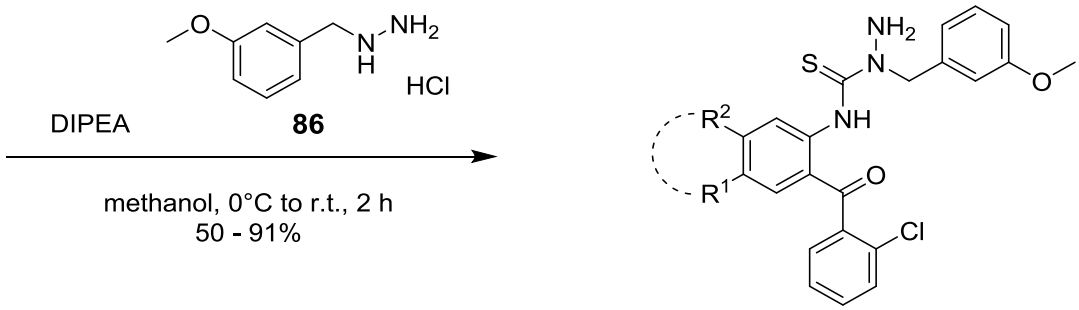
4.5.3 Synthesis of 6-(2-chlorophenyl)-4-(3-methoxybenzyl)triazolobenzo-triazepines

With the successful preparation of the 2-aminobenzophenones (**76**, **81**, **83**) as novel starting materials and (3-methoxybenzyl)hydrazine hydrochloride **86**, which is needed as building block to built up the triazepine ring, the general synthesis of the target compounds followed the well known procedures[108,110] described several times in previous chapters. To obtain the new 6-(2-chlorophenyl)-4-(3-methoxy-benzyl)triazolobenzotriazepines with modifications in the annulated benzo ring, the synthesis started again with conversion of the 2-aminobenzophenones (**76**, **81**, **83**) into the corresponding isothiocyanate derivatives (**87**, **88**, **89**) using thiophosgene **16** and calcium carbonate. The subsequent addition of compound **86** to the isothiocyanates was followed by the para-toluenesulfonic acid catalyzed cyclization to the benzotriazepinethione compounds **93**, **94** and **95**. After treating them with hydrazine hydrate in the first step and triethyl orthoacetate **43** as well as para-toluenesulfonic acid monohydrate in the second step, target compounds **96** (51%), **97** (35%) and **98** (40%) could be prepared in moderate to good yields.

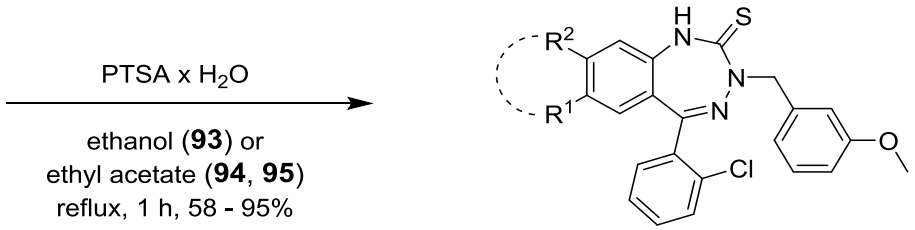


76 $R^1 = OCH_3$, R^2 = H
81 $R^1 = OCH_3$, $R^2 = OCH_3$
83 R^1, R^2 = -O-CH_2-O-

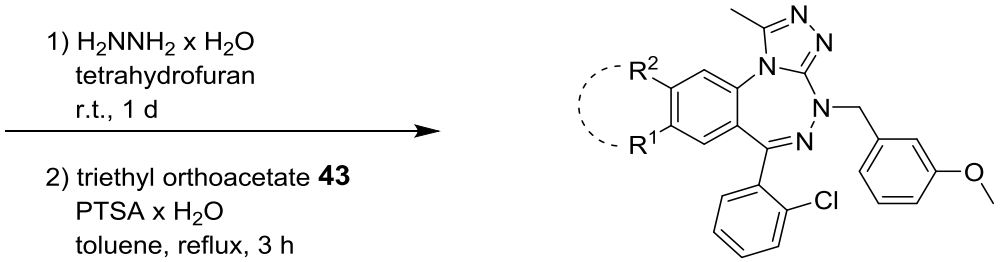
87 $R^1 = OCH_3$, R^2 = H (71%)
88 $R^1 = OCH_3$, $R^2 = OCH_3$ (91%)
89 R^1, R^2 = -O-CH_2-O- (63%)



90 $R^1 = OCH_3$, R^2 = H (not purified)
91 $R^1 = OCH_3$, $R^2 = OCH_3$ (91%)
92 R^1, R^2 = -O-CH_2-O- (50%)



93 $R^1 = OCH_3$, R^2 = H (58%, 2 steps)
94 $R^1 = OCH_3$, $R^2 = OCH_3$ (84%)
95 R^1, R^2 = -O-CH_2-O- (95%)



96 $R^1 = OCH_3$, R^2 = H (51%)
97 $R^1 = OCH_3$, $R^2 = OCH_3$ (35%)
98 R^1, R^2 = -O-CH_2-O- (40%)

Scheme 35. Synthesis of modified 6-(2-chlorophenyl)-4-(3-methoxybenzyl)-TBzTs **96**, **97** and **98**.

4.5.4 Screening results and discussion of novel triazolobenzotriazepines

The differential scanning fluorimetry was carried out the common way with the three novel triazolobenzotriazepines **96**, **97** and **98** (Figure 49A) in a compound concentration of 10 µM. The compounds were screened against all eight members of the BET family as well as against BAZ2B, CREBBP, PB1(5) and PCAF.

The 8-methoxy-TBzT **96** an the 8,9-dimethoxy-TBzT **97** showed highly improved results as they combined high affinity towards specific bromodomains like BRD3(2) and BRDT2 as well as a strong selectivity towards second domains of the corresponding BRDs – at least according to the ΔT_m values. In contrast the 8,9-methylenedioxy-TBzT **98** gave poor T_m values and showed no site selectivity.

A

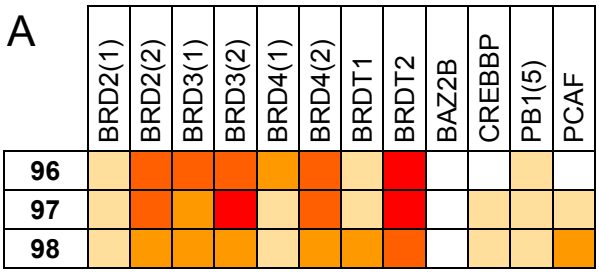
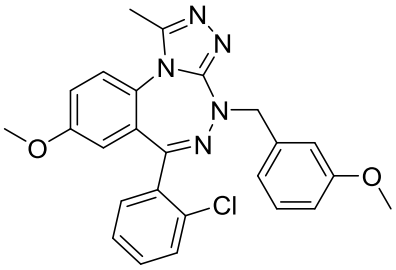
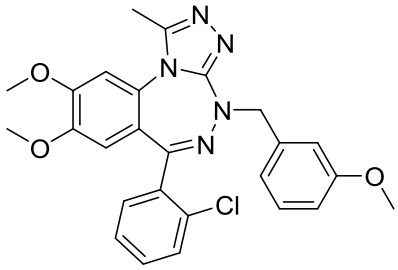


Figure 49. (A) Screening results of 8-methoxy-TBzT **96**, 8,9-dimethoxy-TBzT **97** and 8,9-methylenedioxy-TBzT **98** carried out at a compound concentration of 10 µM.
(B) Compound **96** and **97** in a more detailed presentation with the measured ΔT_m values.

B

	 **96**	 **97**
BRD2(1)	1.1	1.7
BRD2(2)	5.9	5.9
BRD3(1)	4.2	3.7
BRD3(2)	5.6	6.0
BRD4(1)	3.8	1.8
BRD4(2)	4.6	4.7
BRDT1	1.6	1.4
BRDT2	7.5	6.3

ΔT_m(°C)	
	<0
	0-2
	2-4
	4-6
	6-8
	>8

With all three compounds showed the same core structure and even similar substitution pattern in position 4 and 6, the question raised why compound **98** led to comparatively poor temperature shifts. The rigid methylenedioxy structural element obviously is responsible for the decrease of most T_m values and even more a deterioration of selectivity in case of BRDT. The freely rotatable methoxy groups in compound **96** and **97** are consequently much more flexible and adaptable to the given spatial conditions in the binding pocket.

Both compounds **96** and **97** showed highly interesting results (Figure 49). Against BRDT they reached a remarkable $\Delta T_{m(D2-D1)}$ of 5.9 °C (**96**) and 4.9 °C (**97**). Especially the 8,9-dimethoxy substituted triazolobenzotriazepine **97** was able to obtain further good selectivity – according to DSF results – against all the other BRDs with $\Delta T_{m(D2-D1)}$ of 4.2 °C (BRD2), 2.3 °C (BRD3) and 2.9 °C (BRD4). The advantage of the second methoxy group attached to the annulated benzo ring system appeared primarily with the important decrease of ΔT_m (2 °C) of the first domain of BRD4 but maintaining the T_m shift (4.7 °C) of BRD4(2).

As already discussed in Chapter 3.1 the obtained temperature shift results of DSF screenings do not correlate directly with each other. Meaning that a ΔT_m shift of 4 °C of first and of second domain does not consequently results in the same K_d values determined by ITC. Especially second domains mostly result in a higher T_m shift as first domains by tendency.

Previous measurements of K_d values by ITC did not yield any binding constant, although compound **56a** showed a ΔT_m of 4.9 °C against second domain of BRD2 and compound **69** has a ΔT_m of 5.1 °C against second domain of BRDT. However, the measurement was planned to give results in a very low micromolar or even nanomolar range which was obviously not reached.

The following four measurements of binding constants of compound **96** yielded results with remarkable affinity towards the desired proteins BRD4 and BRDT as well as unexpected values.

The first ITC measurements of compound **96** were carried out with the domains of BRD4 and gave the following results:

K_d [BRD4(1)] = 481 nM [ΔT_m = 3.8 °C]

K_d [BRD4(2)] = 92 nM [ΔT_m = 4.6 °C]

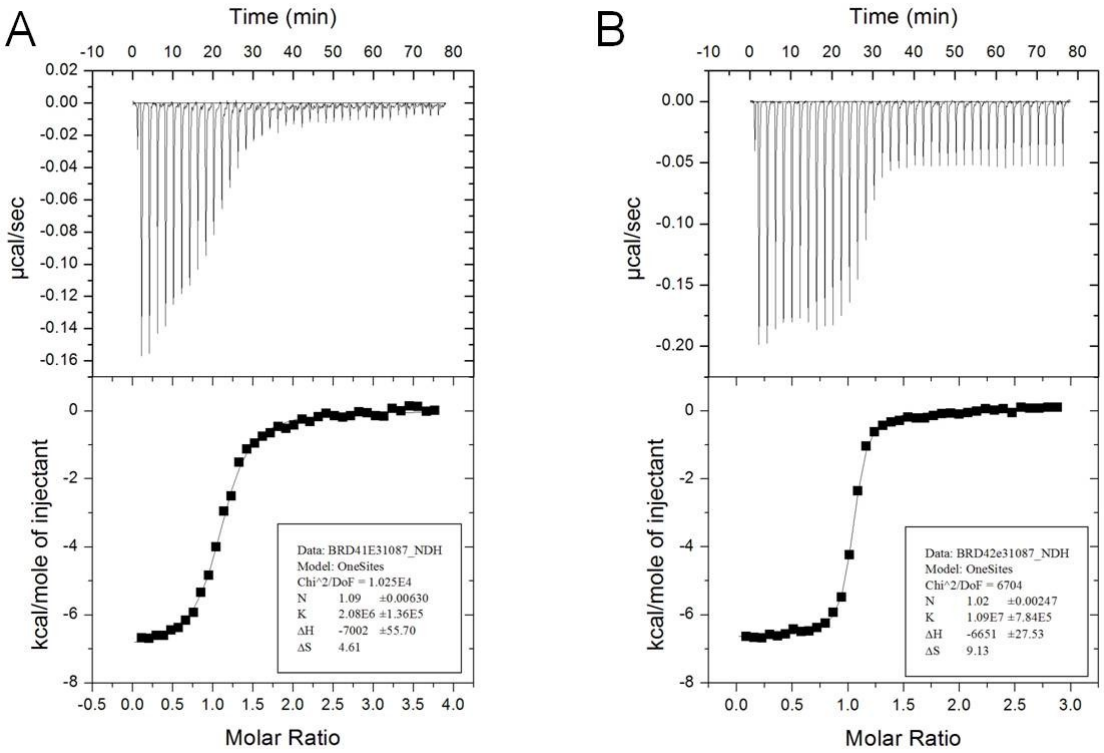


Figure 50. ITC plots and data analysis of binding measurements of compound **96** with (A) BRD4(1) and with (B) BRD4(2).

With a K_d of 481 nM compound **96** showed an expected result for a ΔT_m of 3.8 °C of the first domain of BRD4 (Figure 50A). However a remarkable results was gained with the second domain of BRD4. A temperature shift of 4.6 °C – more an average value than a very high one – obtained a strong binding constant of 92 nM (Figure 50). Consequently a more than fivefold lower K_d was measured for the second domain of BRD4 representing already a good selectivity. With regard to compound **97**, which obtained a ΔT_m value of 1.8 °C (2 °C less than compound **96**) for BRD4(1), the K_d value must be clearly weaker and thus a much higher selectivity must be gained. However, no ITC results of compound **97** were available until completion of the thesis.

The noticeable difference of 5.9 °C ($\Delta T_{m(D2-D1)}$) between first and second domain of BRDT in temperature shift screening with 8-methoxytriazolobenzotriazepine **96** caused additional measurements by isothermal titration calorimetry. The following data were obtained by ITC performance:

K_d [BRDT1] = 1.54 µM [ΔT_m = 1.6 °C]

K_d [BRDT2] = 145 nM [ΔT_m = 7.5 °C]

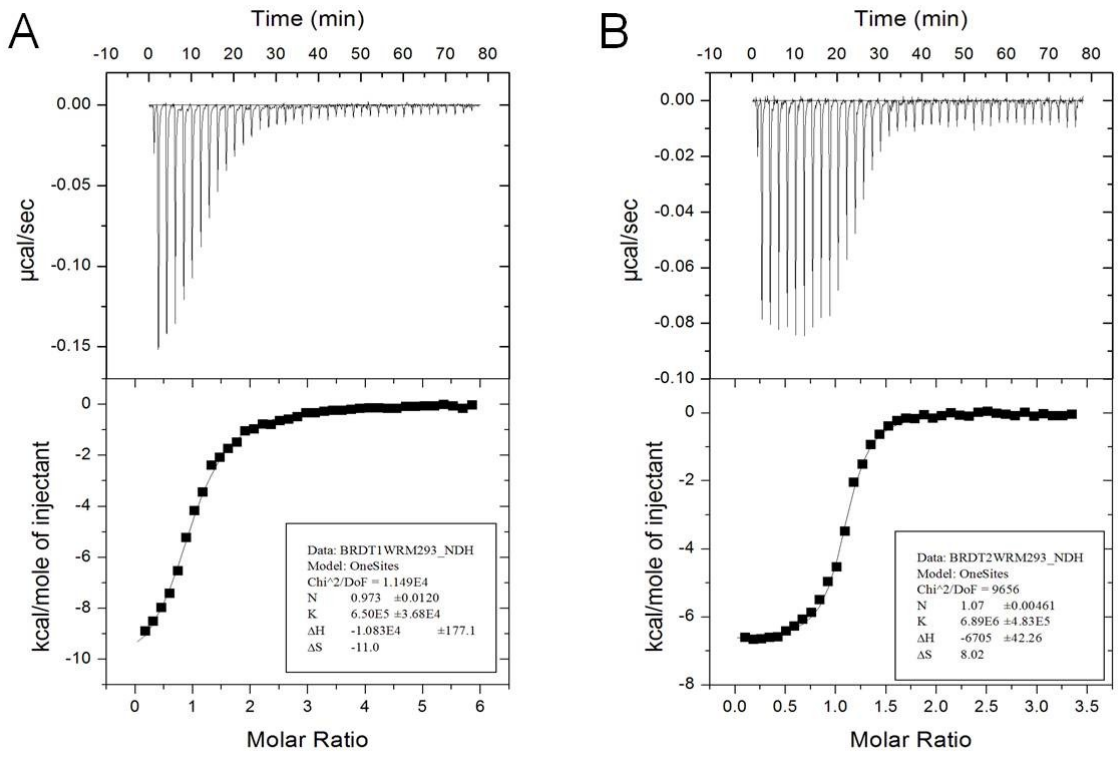


Figure 51. Binding constants of compound **96** with (A) BRDT1 and (B) BRDT2 measured by ITC.

The temperature shift of the first domain with only 1.6 °C was very low and consequently containing relatively high errors in contrast to higher ΔT_m values. Thus the K_d value for the first domain was hard to estimate and with 1.54 µM stronger than expected. However the second domain produced a low K_d value of 145 nM what led to an impressive comparison of the results. 6-(2-Chlorophenyl)-8-methoxy-4-(3-methoxybenzyl)-1-methyltriazolobenzotriazepine **96** showed more than a tenfold difference in K_d against the first and the second domain of BRDT.

4.6 ADDITIONAL PERFORMED BIOASSAYS

Both bioassays, the agar diffusion test and the MTT assay, were performed in our group at the Ludwig-Maximilians University of Munich by Martina Stadler.

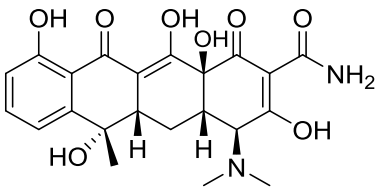
4.6.1 Agar diffusion test (ADT)

4.6.1.1 Background

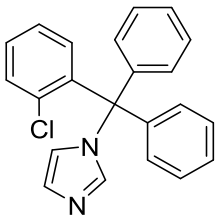
All synthesized compounds were routinely screened for antibacterial and antifungal activity against eight different model germs. These model germs were grown on agar plates and treated with the compound to be examined. Whenever growth inhibition (GI) or a total inhibition (TI) of the corresponding germ takes place a bacteria-free areola can be observed of which the diameter is measured. This zones of inhibition are correlated to the ones obtained with the reference substances tetracycline for antibacterial and clotrimazole for antifungal activity (Figure 52). Nevertheless this experiment gives only a general overview whether a substance is an antimicrobial agent.

The following microorganism were used as model germs:

DSMZ-No. 426	*Escherichia coli*	gram-negative bacterium
DSMZ-No. 8361	*Pseudomonas antimicrobia*	gram-negative bacterium
DSMZ-No. 20675	*Staphylococcus equorum*	gram-positive bacterium
DSMZ-No. 14446	*Streptococcus entericus*	gram-positive bacterium
DSMZ-No. 1345	*Yarrowia lipolytica*	yeast
DSMZ-No. 11226	*Candida glabrata*	yeast
DSMZ-No. 70663	*Aspergillus niger*	dermatophyte
DSMZ-No. 1988	*Hyphopichia burtonii*	mold fungus



Tetracycline



Clotrimazole

Figure 52. Reference compounds for antibacterial (tetracycline) and antifungal (clotrimazole) activity.

4.6.1.2 Screening results and discussion

Compound	*Escherichia coli*	*Pseudomonas antimicrobia*	*Staphylococcus equorum*	*Streptococcus entericus*	*Yarrowia lipolytica*	*Candida glabrata*	*Aspergillus niger*	*Hoyphopichia burtonii*
	used reference: **Tetracycline**				used reference: **Clotrimazole**			
1	---	---	**11 mm** (40 mm)	---	---	---	---	---
20	---	---	---	---	**10 mm GI** (22 mm TI, 25 mm GI)	**8 mm GI** (30 mm)	**8 mm GI** (13 mm TI, 22 mm GI)	**8 mm GI** (30 mm)
23	**10 mm** (28 mm)	---	**12 mm** (40 mm)	**10 mm** (20 mm)	**8 mm** (22 mm TI, 25 mm GI)	**12 mm** (30 mm)	**13 mm GI** (13 mm TI, 22 mm GI)	**11 mm** (30 mm)
64	---	---	**10 mm** (40 mm)	**7 mm** (21 mm)	**7 mm** (20 mm TI, 28 mm GI)	**7 mm** (25 mm)	---	**10 mm GI** (25 mm)
65	---	---	**11 mm** (40 mm)	**7 mm** (21 mm)	**7 mm** (20 mm TI, 28 mm GI)	**7 mm** (25 mm)	---	**11 mm GI** (25 mm)
87	---	---	**10 mm** (40 mm)	**7 mm** (22 mm)	---	---	---	---
88	---	---	**10 mm** (30 mm)	**8 mm** (30 mm)	---	---	---	---
89	---	---	**20 mm** (30 mm)	**12 mm** (30 mm)	---	---	---	---

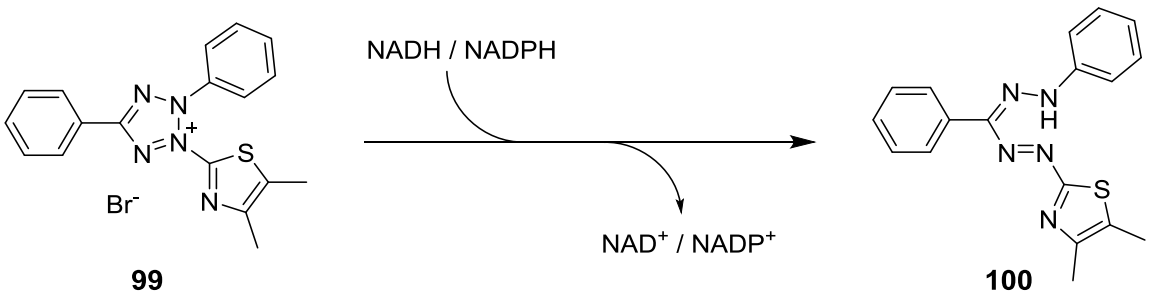
Figure 53. ADT results of compounds which showed total inhibition (TI) against the screened microorganism. Whenever growth inhibition (GI) is (also) observed the results are marked accordingly. The inhibition zone diameters of the corresponding reference substance are given in brackets.

Figure 53 shows compounds with a total or a growth inhibition against the screened model germs. No final target compound, neither a triazolobenzodiazepine nor a triazolobenzotriazepine gave any response in the antibiotic and antifungal test. Only intermediates of the triazolobenzodiazepine reaction sequence showed zones of inhibition. Benzodiazepinone **1** only against *Staphylococcus equorum* as antibiotic agent and 2-aminopropiophenone **20** with an antifungal growth inhibition against all four representatives. Iodoacetanilide **23** showed mainly total inhibition of almost all screened microorganisms except *Pseudomonas antimicrobia*. All isothiocyanates (**64**, **65**, **87**, **88**, **89**) were active against both gram-positive bacteria whereas **64** and **65** additionally showed activity against yeast and the mold fungus.

4.6.2 MTT assay

4.6.2.1 The colorimetric assay principle

The MTT assay was used for measuring cytotoxicity of all synthesized compounds. Therefore the standard protocol according to Mosmann[163] was followed, which described the conversion of a water-soluble yellow tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) **99** into the deep blue formazan product **100** which is insoluble in water (Scheme 36).



Scheme 36. Reaction of the yellow tetrazolium salt **101** into the blue formazan product **102**.

This reaction only occurs in the cytosol of viable cells by mitochondira, peroxisomes and special oxidoreductases. As reducing agent either nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) is used. The level of produced formazan, detected by photometric quantification, consequently gives a direct correlation to the cell viability. For the assay human leukemia cell line LH-60 was used and Triton X-100 as positive control.

4.6.2.2 Screening results and discussion

Compound	IC_{50} [µM]
5	26
6	40
9	21
10	19
11	47
15	29
22	38
23	9
29b	40
29e	46
29f	29
42	32
44b	28
44c	46
44e	33
44f	31
44h	31
44i	15
44j	16

Compound	IC_{50} [µM]
51	17
53	17
56a	15
56b	14
56c	11
56d	13
56e	14
58a	15
58b	11
58c	28
59	21
61b	4
64	34
65	14
66	9
67	18
68	32
69	22
71a	17

Compound	IC_{50} [µM]
71b	13
71c	15
71d	21
71e	17
71f	15
71h	32
72a	12
72b	14
72c	14
72d	23
72e	18
72f	16
72h	31
87	12
88	10
89	6
96	25
98	29

Figure 54. MTT screening results of compounds with a IC_{50} of 50 µM or lower.

The screening results (Figure 54) provide a good insight whether compounds show a trend to cytotoxicity, however, the results have to be treated with caution as they are not measured in triplicates and are consequently uncorrected. Furthermore no statement can be made about the mechanism of action. Below a IC_{50} value of 5 µM a compound is defined as cytotoxic, therefore only compounds are listed in Figure 54 with less than the tenfold IC_{50} value (50 µM). Therefore several compounds are not listed in Figure 54 as they showed an IC_{50} value higher than 50 µM and all except **61b** are above the critical concentration of 5 µM.

The low IC_{50} value of 9 µM of iodoacetanilide **23** might be reasoned due to its capability to react as strong alkylating reagent. The same reason could cause the IC_{50} value of 6 µM of compound **89** with the strong electrophilic isothiocyanate group. In comparison with the other benzotriazepinethiones only compound **66** with the 8-nitro substituent showed a low IC_{50} value probably caused by the nitro group. The only significantly cytotoxic target compound was the 4-([1-benzyl-triazol-4-yl]methyl)triazolobenzotriazepine **61b**.

CHAPTER V –

SUMMARY

Development of new protein-protein interaction inhibitors targeting bromodomains of the BET family was the superior goal of this thesis. The importance of these epigenetic readers modules, which are selective for ε-N-acetylated lysine residues, is their predominately association to nuclear proteins. For example, deletion of BRD2 or BRD4 led to lethality of mice[164,165] and deletion of the first domain of the testis-specific BRDT induced male sterility in mice[166]. Moreover the expression level is of prime importance as studies with BRD4 showed that upregulated as well as downregulated expression caused cell cycle arrest[167,168].

During the work demands increased and the aim was specified to obtain a site selective compound for second domains of bromodomain and extra-terminal domain proteins. To reach this goal, the work in hand combines purposeful organic synthetic chemistry – done at the Ludwig-Maximilians University in Munich – with powerful techniques for screening, structure refinement and analysis carried out at the Structural Genomics Consortium (SGC) of the University of Oxford.

Synthesis efforts yielded 56 target compounds with triazolobenzodiazepine or -triazepine core structure and a number of precursor compounds. More than 70 compounds were screened by differential scanning fluorimetry (DSF) against – at least – twelve domains to find potential inhibitors for bromodomains of the BET family. K_d values of promising compounds were determined by isothermal titration calorimetry (ITC) and even co-crystallization structures of interesting compounds with bromodomains were prepared.

With the clinical benzodiazepine alprazolam (Figure 55A) was identified as a potent inhibitor of bromodomains of the BET family, structure **29c** (Figure 55B), resulting from molecular modeling studies at the SGC provided the basis for first series of compounds.

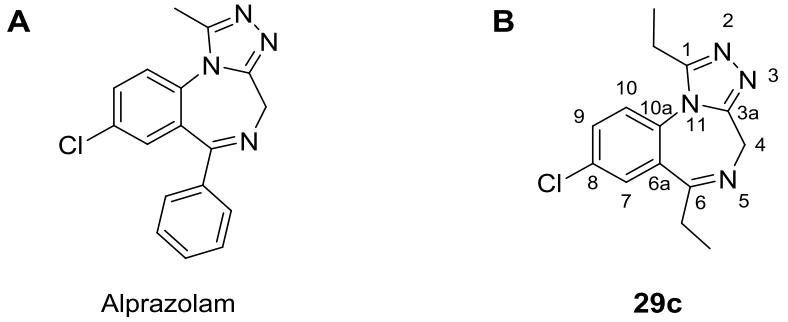
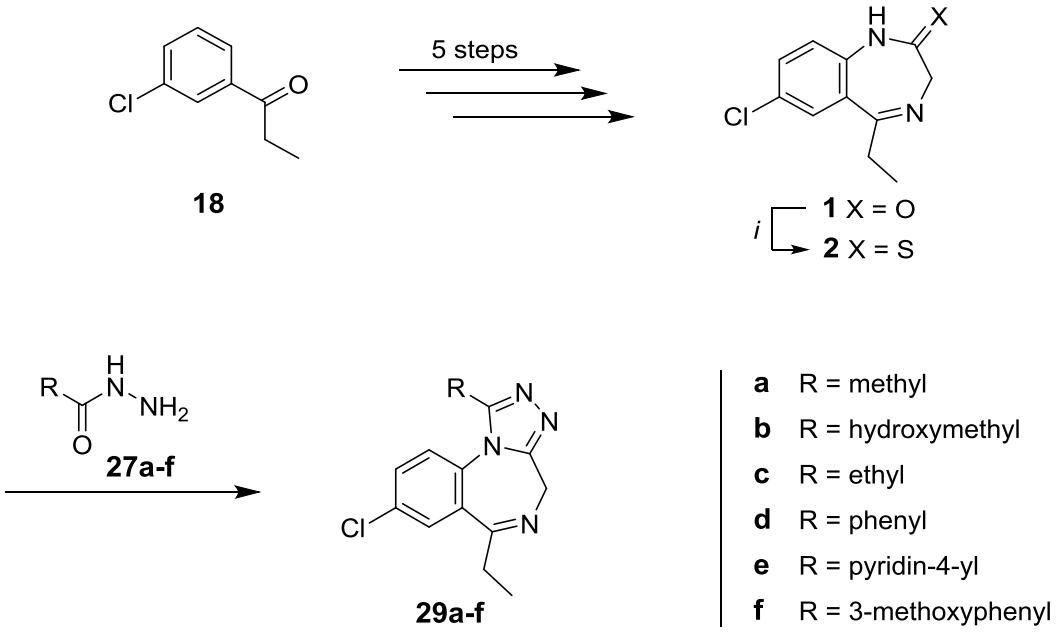


Figure 55. (A) Chemical structure of alprazolam. (B) First TBzD target structure.

Starting with commercially available 3-chloropropiophenone **18** known[105] benzodiazepinone **1** was prepared in a five step synthesis. The conversion into thiolactam **2** was carried out using Lawesson's reagent **24**, followed by a condensation of different carboxylic acid hydrazides **27a-f**. According to this procedure six different substituted triazolobenzodiazepine (TBzD) target compounds **29a-f** were synthesized and screened subsequently via DSF.



Scheme 37. Outline for synthesis of TBzD target compounds. Lawesson's reagent **24** was used for conversion (*i)* of compound **1** into thiolactam **2**.

With the expectation to obtain a convincing SAR already after the first screening we sent additional ten compounds provided by researchers from the University of Greifswald to the SGC. Those compounds shared a closely related benzotriazepine scaffold with a variety of substitutions on the core structure.

Despite screening at a high compound concentration of 100 µM, synthesized benzodiazepines gave no response in DSF. Almost the same result was obtained within the benzotriazepines with two exceptions **36** and **37**. Especially the 1-methyl substituted triazolobenzotriazepine (TBzT) **36** (Figure 56A), showed remarkable ΔT_m values against the BET family. Two core statements were made after the analysis of these screening results. On the one hand the annulated triazole ring was essential for activity but no other groups than methyl in position 1 were tolerated. On the other hand as compound **29a** comprised this structural element of the annulated 1-methyltriazole ring but showed no activity obviously also an aromatic substituent in position 6, instead of the used 6-ethyl moiety, is essential for activity.

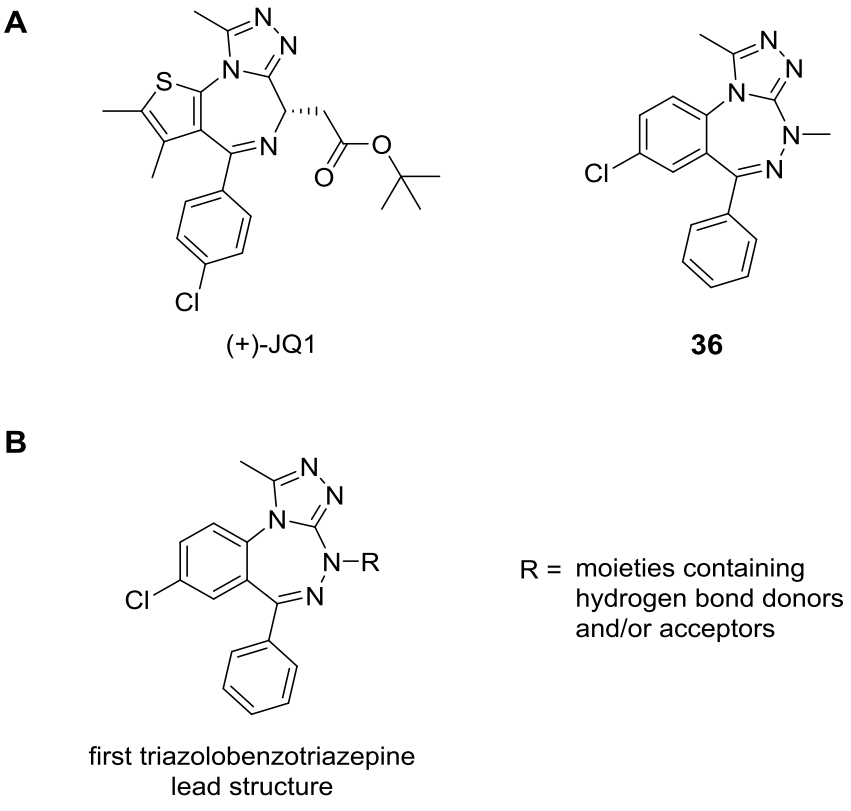
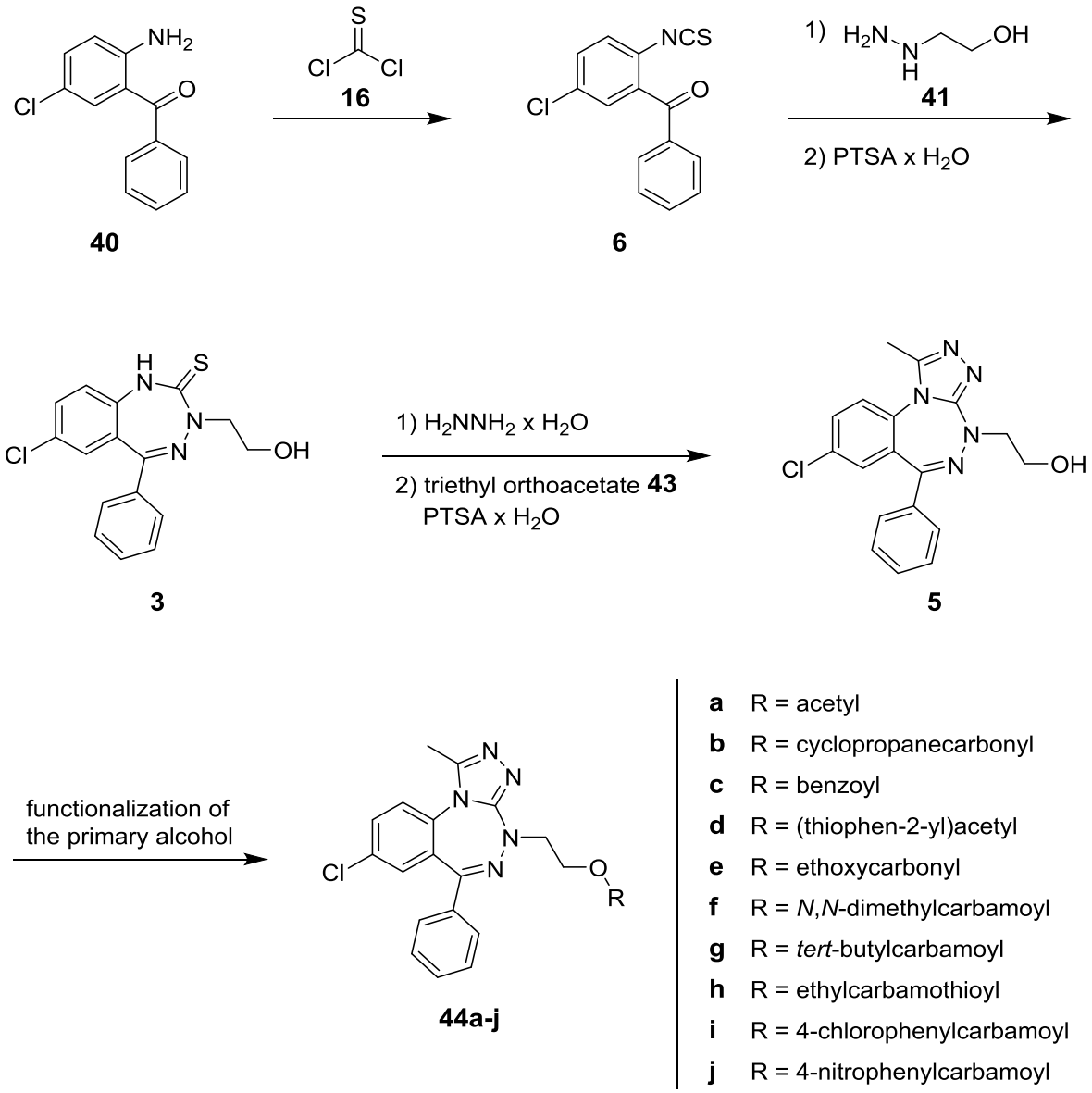


Figure 56. (A) Chemical structures of JQ1 and new screening hit **36**. (B) Lead structure for new triazolobenzotriazepines.

Consequently the triazolobenzotriazepine scaffold was chosen as new lead structure. Furthermore, the knowledge of functional groups like carbamates or amides in residues in position 4 of molecules like JQ1 (Figure 56B) and different iBETs was one reason to plan to attach also moieties with similar groups in position 4 of TBzT screening hit. This led to a modified lead structure with a benzotriazepine core scaffold, bearing a phenyl residue instead of the ethyl group in position 6, an annulated 1-methyltriazole ring and a variety of polar moieties in position 4 (Figure 56B).

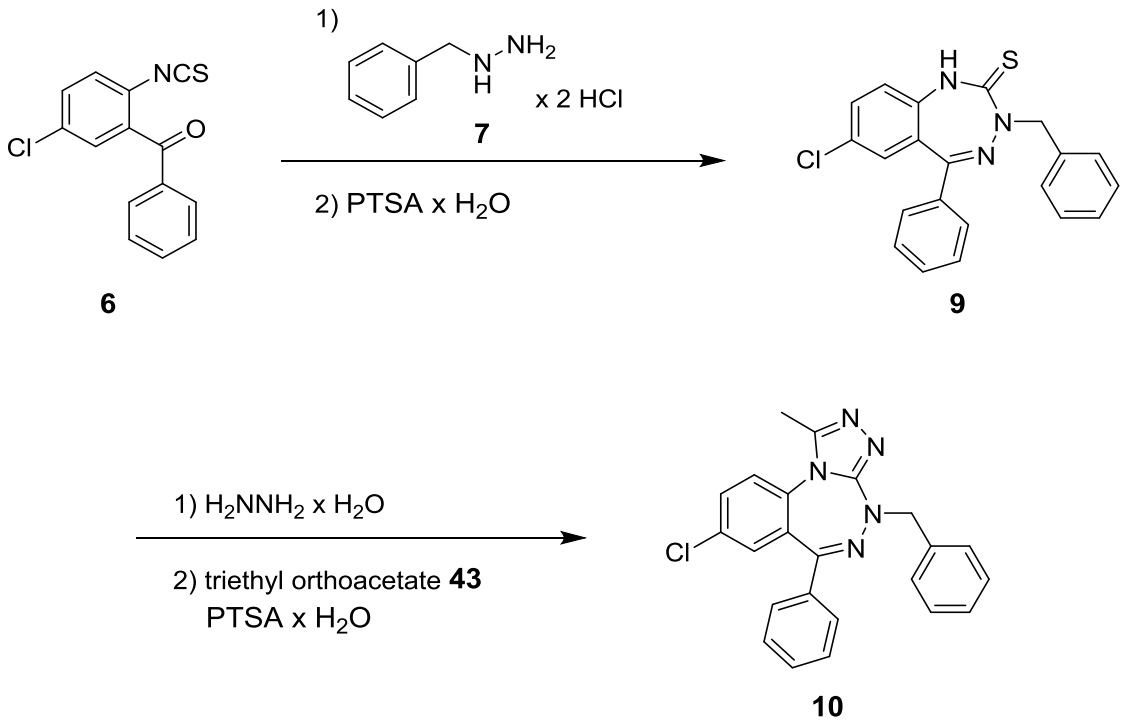


Scheme 38. Preparation of the first TBzT series starting from a commercially available 2-amino-benzophenone derivative **40**.

To improve the first generation of triazolobenzotriazepines from the University of Greifswald by the introduction of polar residues in position 4, central intermediate **5** was synthesized according to Richter *et al.*[108] and Nakamura *et al.*[110] (Scheme 38). Conversion of commercially available 2-aminobenzophenone **40** with thiophosgene gave isothiocyanate **6**, which upon reaction with 2-hydroxyethyl-hydrazine **41** and subsequently para-toluenesulfonic acid gave the triazepine **3**. Treating compound **3** first with hydrazine hydrate, followed by the addition of triethyl orthoacetate **43** led to the desired TBzT bearing a 2-hydroxyethyl residue at C-4.

This primary alcohol could easily be functionalized by various methods using different starting materials to produce target compounds **44a-j** containing esters, a carbonate and (thio)carbamates in the side chain (Scheme 38).

With a working synthesis in hands, it was also tried to introduce a completely different group in position 4. Therefore only the used hydrazine derivative was changed from 2-hydroxyethylhydrazine **41** to benzylhydrazine **7**.



Scheme 39. Preparation of 4-benzyltriazolobenzotriazepine **10**.

In the same manner as used for preparation of compound **5** the 4-benzyl substituted compound **10** was generated starting from isothiocyanate derivative **6**. The addition of the benzylhydrazine **7** to the isothiocyanate group of compound **6** and the subsequent cyclization obtained compound **9**. The triazole ring of compound **10** was prepared using hydrazine hydrate followed by triethyl orthoacetate **43** (Scheme 39).

The screening using DSF of the obtained twelve target compounds showed unexpected results. Low ΔT_m values were detected for compound **5** and its derivatives **44a-j**. Presumably the ethylene spacer between the tricyclic ring system of the core structure and the hydrogen bond donors and / or acceptors of the residue is too long and consequently responsible for poor interaction with the protein. However, the rather unpolar benzyl compound **10** gained remarkable ΔT_m values for second domains of BRD2 and BRD4 as well as significant values for BRD3(2) and BRDT2. For that reason no longer efforts were made to improve the polar side chain in position 4 but to concentrate on SAR of various substituted 4-benzyl residues (Figure 57).

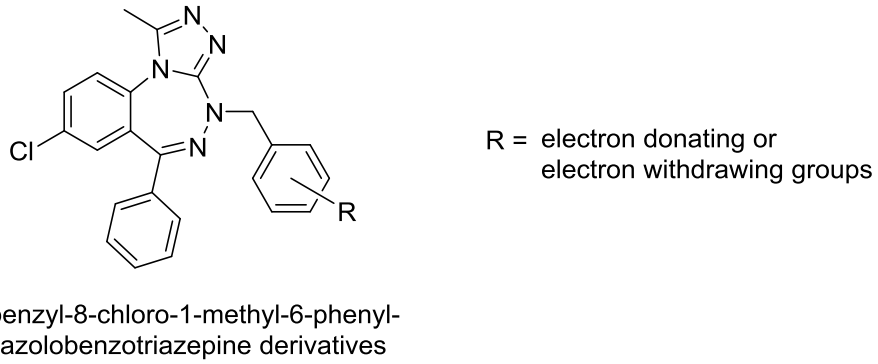
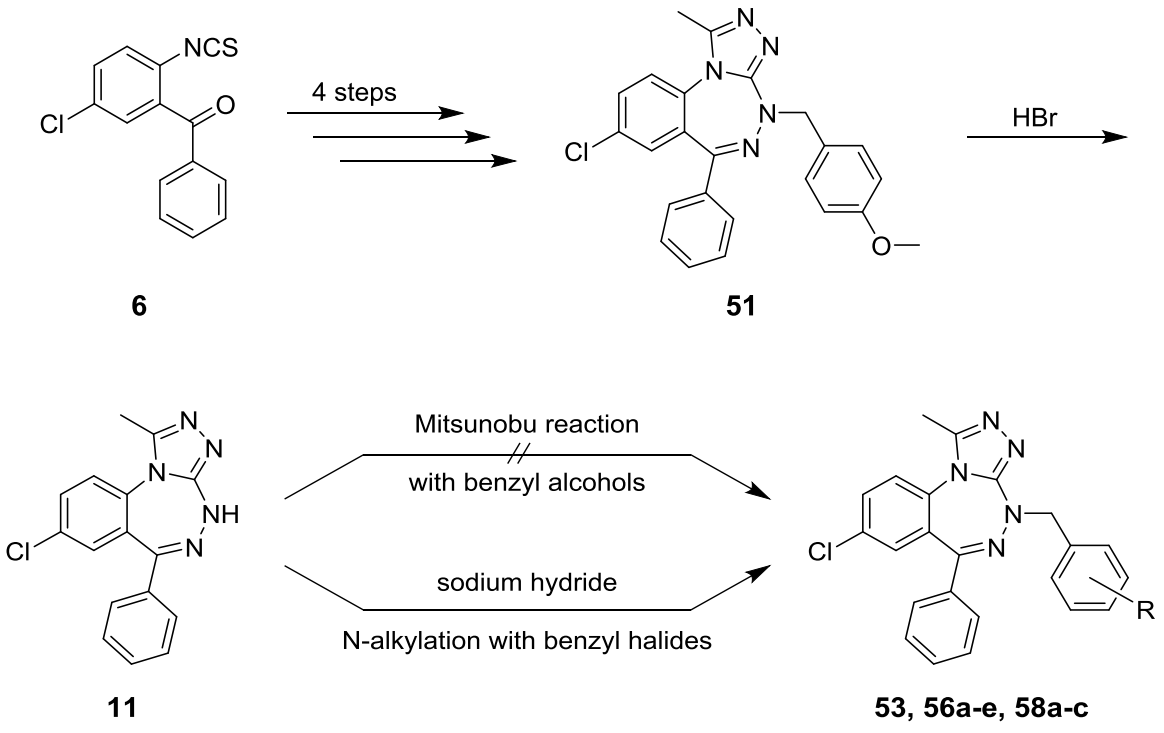


Figure 57. Lead structure with 4-benzyl residues.

To prepare a series of 4-benzylated TBzTs the reaction sequence used before was ineligible. For most of the desired substitution patterns the corresponding benzylhydrazines had to be prepared first as they were not commercially available and moreover, for each target compound the whole reaction sequence would have been to be passed through in a non-convergent manner.

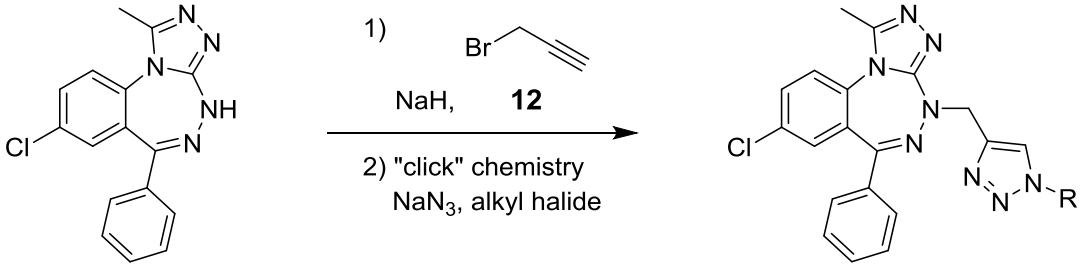
The solution of this problem was using the 4*H*-triazolobenzotriazepine **11**, published[110] by Nakamura *et al.* in 1996, as a central building block which could easily be alkylated at N-4. The 4-(4-methoxybenzyl)triazolobenzotriazepine **51** was prepared the same way as described above in a four step synthesis starting from isothiocyanate **6**. Compound **51** was deprotected by treatment with hydrogen bromide in acetic acid to yield intermediate **11**. The attempted N-alkylation of **11** by Mitsunobu reaction using benzyl alcohols failed while the preparation of nine benzylated compounds (**53**, **56a-e**, **58a-c**) could be accomplished by simple N-alkylation using the corresponding benzyl bromides or chlorides after N-deprotonation (Scheme 40).



53	R = 4-tert-butyl	**56e**	R = 2-bromo
56a	R = 4-iodo	**58a**	R = 2-methyl
56b	R = 4-nitro	**58b**	R = 3,4-dichloro
56c	R = 4-trifluoromethyl	**58c**	R = 3,4,5-trimethoxy
56d	R = 3-chloro		

Scheme 40. Reaction scheme for preparation of 4-benzylated TBzTs.

Additional three compounds (**59**, **61a-b**) with triazolylmethyl side chains as hetero-analogues of the N-benzyl series were prepared by N-alkylation of **11** with propargyl bromide **12** followed by Huisgen [3+2]-cycloaddition reaction with appropriate alkyl azides (Scheme 41).



11 **59, 61a-b**

59 R = methyl
61a R = propyl
61b R = benzyl

Scheme 41. Synthesis of 1,2,3-triazolylmethyl-TBzTs.

With regard to later discussions all hitherto synthesized compounds containing the 8-chloro-6-phenyl substitution pattern were clustered into the "chloro" series. The screening via DSF showed that considerable progress was made according to the previous two screenings with triazolobenzodiazepines and -triazepines.

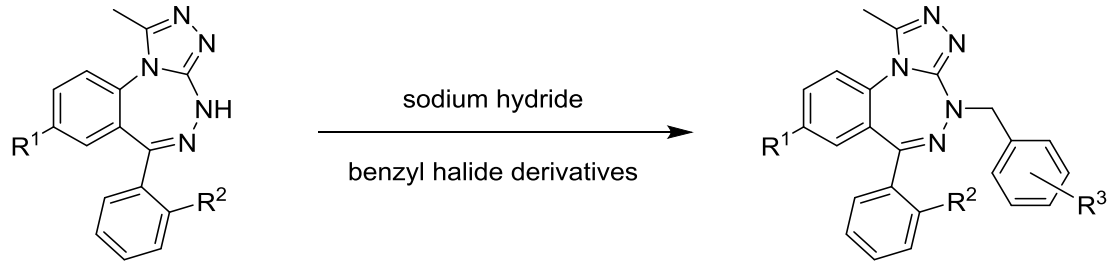
The comparison of ten different substituted benzyl compounds elucidated first meaningful structure-activity relationships and highly active compounds with T_m shifts up to 9 °C were obtained. Interestingly, both compounds (**51**, **58c**) including methoxybenzyl substituents obtained the highest ΔT_m values but still showed no cross activity towards any other bromodomain than the ones of the BET family.

Lower T_m shifts but also interesting information were provided by compounds like **56d**. The 3-chloro substituted benzyl residue, for example, showed weak tendency for site selectivity for second domains. Furthermore ortho (**56e**, **58a**) and very bulky (**53**) substituents at the benzyl residue led to a strong decrease of activity.

The small series of 1,2,3-triazole containing compounds consequently were less expressiveness. Their throughout significant T_m values, however, might still be of high interest for further optimizations. In medicinal chemistry, the exchange of a phenyl ring by a 1,2,3-triazole is a very common tool to introduce diversity into a molecule ("bioisosterism") and the simple chemistry allows a fast and easy way to generate them, but in our experience 1,2,3-triazoles are less active than their phenyl analogues in most cases. Thus, the introduction of the triazoles was a success. These compounds were quite active but their optimization was no longer pursued as the benzylated compounds already offered interesting SAR which were more promising to be followed.

With the decision to go on with benzyl residues in position 4, two additional scaffolds were used to obtain a broader SAR. The known[110] compounds, a 8-nitro as well as a 8-chloro-6-(2-chlorophenyl) substituted 4*H*-triazolobenzotriazepine **14** and **15**, were synthesized following the same reaction sequence as used for the 8-chloro substituted compound **11**.

Compounds **14** and **15** were alkylated in the same manner as compound **11** with various benzyl bromides or benzyl chlorides to obtain eight target compounds in each series which were named "nitro" (**71a-h**) and "dichloro" (**72a-h**) series according to the "chloro" series introduced above (Scheme 42).



14 $R^1 = NO_2$, $R^2 = H$
15 $R^1 = Cl$, $R^2 = Cl$

71a-h $R^1 = NO_2$, $R^2 = H$
72a-h $R^1 = Cl$, $R^2 = Cl$

a R^3 = 4-*tert*-butyl
b R^3 = 4-iodo
c R^3 = 4-nitro
d R^3 = 3-chloro
e R^3 = 2-bromo
f R^3 = 3,4-dichloro
g R^3 = 3,4,5-trimethoxy
h R^3 = 3-methoxy

Scheme 42. Generation of the "nitro" and the "dichloro" series.

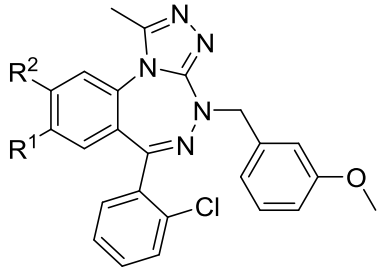
DSF results confirmed most of the already obtained trends with regard to the benzyl residues. In both new series the bulky *tert*-butyl substituent knocked out activity of the corresponding compounds (**71a**, **72a**) against proteins of the BET family. Also the compounds bearing ortho substituted benzyl residues (**71e**, **72e**) were inactive. Comparison of the series by their scaffold variations led to clear messages: the "nitro" series only improved activity in general, the "dichloro" series raised site selectivity for second domains.

The 8-nitro substitution improved most of the compounds and led to higher ΔT_m values in contrast to the "chloro" series. However the increase in affinity was linked to all of the BETs equally and consequently no trend towards site selectivity was obtained.

The "dichloro" series showed lower T_m shifts throughout the chart of results. This was expected as the same observation was already made in the first screening of clinical benzodiazepines. The only difference of alprazolam and triazolam is this chloro substituent in ortho position of the 6-phenyl ring, and triazolam was virtually inactive in the screening. The great advantage of the "dichloro" series, however, was the evident trend to site selectivity for second domains of the BET family.

Another decisive step was made by the introduction of the new 3-methoxybenzyl substituent of compound **72h** which was added as a combination of the best residues of the "chloro" series. Methoxy substituted benzyl moieties showed highest activity and 3-chlorobenzyl showed best trend to site selectivity providing in combination with the dichloro scaffold compound **72h** which only showed significant T_m values for second domains.

The new lead structure (Figure 58) for final triazolobenzotriazepines combined the well known structural element of the tricyclic core scaffold with the best substitution pattern consisting of a 1-methyl, a 4-(3-methoxybenzyl) and a 6-(2-chlorophenyl) moiety. The nitro series already showed that variations in position 8 can lead to improved activities in DSF screening, therefore different substitutions on the annulated benzo ring should be tested.

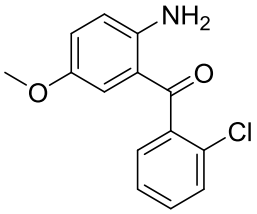


final TBzT lead structure

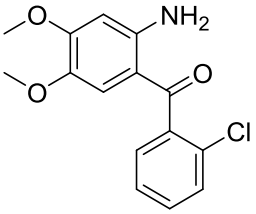
R^1, R^2 = electron donating or electron withdrawing groups

Figure 58. Lead structure for next generation of triazolobenzotriazepines.

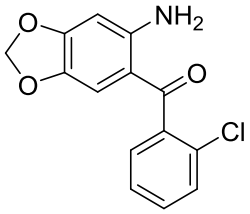
Three different 2-aminobenzophenones (Figure 59) were synthesized as starting materials. Known[161] compound **76** was obtained by using a photochemical approach according to Ferrini *et al.*[160] whereas both literature known[162] compounds **81** and **83** were generated by an ortho selective acylation method using dichlorophenylborane **79**.



76



81

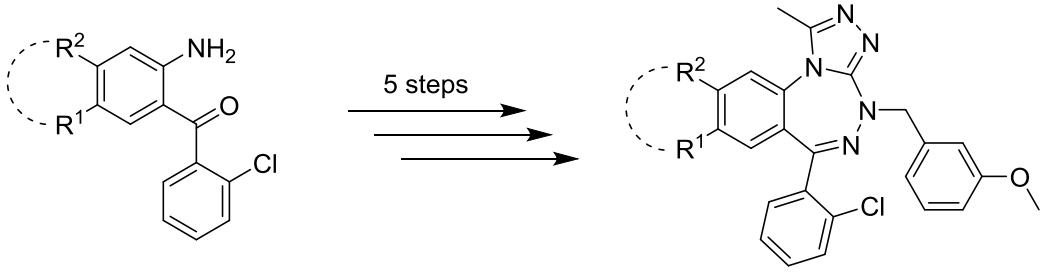


83

Figure 59. Three 2-aminobenzophenones used as starting materials for new target structures.

The 2-aminobenzophenones (**76**, **81**, **83**) were converted according to previously described procedures[108,110] in a five step synthesis to yield desired target compounds **96**, **97** and **98** (Scheme 43). This time the preferred 3-methoxybenzyl substituent was directly inserted by utilization of the corresponding hydrazine derivative. This allowed a shorter reaction sequence to the target compounds as the cleavage of the para-methoxybenzyl protecting group and the following N-alkylation with the matching benzyl bromide was avoided.

Although the methylenedioxy substituted target triazolobenzotriazepine **98** only gave weak ΔT_m values in DSF and showed no trend to site selectivity, remarkable selectivity towards second domains was gained by compounds **96** and **97**, respectively, according to the results of differential scanning fluorimetry.



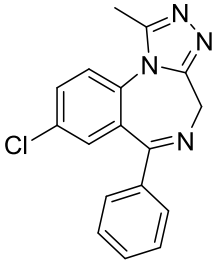
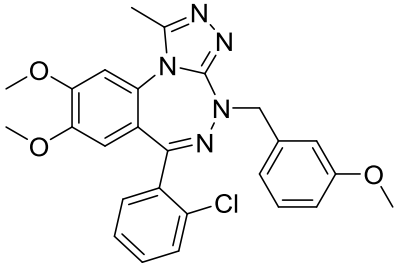
76 $R^1 = OCH_3$, $R^2 = H$
81 $R^1 = OCH_3$, $R^2 = OCH_3$
83 $R^1, R^2 = -O-CH_2-O-$

96 $R^1 = OCH_3$, $R^2 = H$
97 $R^1 = OCH_3$, $R^2 = OCH_3$
98 $R^1, R^2 = -O-CH_2-O-$

Scheme 43. Preparation of final target compounds.

K_d values of compound **96** determined by isothermal titration calorimetry confirmed the results obtained by DSF. More than a fivefold difference in K_d was measured for first (481 nM) and second (92 nM) domain of BRD4. An even stronger divergence of more than a tenfold value was reached within protein BRDT. First domain showed a K_d of 1.54 µM whereas the second domain showed 145 nM. The second methoxy group in position 9 of compound **97** obviously reduces activity only for first domains and keeps it as high for second domains as yielded of compound **96**. Consequently the 8,9-dimethoxy derivative **97** must lead to even more diverging K_d values and thus represents the most site selective compound produced in this thesis, however no ITC data were available until the completion of this work.

This dissertation chronologically describes the development of a new inhibitor starting from the discovery of a clinical benzodiazepine (alprazolam) as screening hit followed by several cycles of lead structure improvement to finally yield a site selective compound. While alprazolam only showed weak T_m shifts against both domains of BRD3 and BRD4 the optimized compound **97** showed distinctly improved ΔT_m values for second domains of BET proteins and consequently remarkable selectivity (Figure 60).

	 Alprazolam	 **97**
BRD2(1)	1.9	1.7
BRD2(2)	3.3	5.9
BRD3(1)	4.1	3.7
BRD3(2)	4.6	6.0
BRD4(1)	4.8	1.8
BRD4(2)	4.1	4.7
BRDT1	0.8	1.4
BRDT2		6.3

ΔT_m(°C)	
	<0
	0-2
	2-4
	4-6
	6-8
	>8

Figure 60. DSF result comparison of screening hit alprazolam and final compounds **97**.

CHAPTER VI – EXPERIMENTAL SECTION

6.1 GENERAL INFORMATION AND INSTRUMENTS

NMR, MS and IR measurements were carried out by the analytical division of the Department of Pharmacy at the LMU Munich under the direction of Dr. Lars Allmendinger. Elemental analysis was done by the analytical division of the Department of Chemistry at the LMU Munich.

6.1.1 Nuclear magnetic resonance spectroscopy

The measurements (^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC) were performed on either a "JNM-Eclipse+400" (^{1}H: 399.8 MHz, ^{13}C: 100.5 MHz) or a "JNM-Eclipse+500" (^{1}H: 500.2 MHz, ^{13}C: 125.8 MHz), (Jeol, USA). The chemical shifts are in δ-values (ppm) relative to the internal standard TMS. The spectra were analyzed by first order and coupling constants *J* are given in Hertz [Hz]. Abbreviations for the characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublets of doublets. Integration is determined as the relative number of protons. Error of reported values: chemical shift 0.01 ppm for ^{1}H NMR, 0.1 ppm for ^{13}C NMR; coupling constant: 0.1 Hz. The used solvent for each spectrum is reported.

6.1.2 Mass spectrometry

Low resolution mass spectrometry was carried out for EI and CI on a “Hewlett Packard 5989A” (Hewlett Packard, USA) and for ESI and APCI on an “AB Sciex API 2000” (AB Sciex, USA). High resolution mass spectrometry was measured for EI on a “Jeol JMS GCmate II” (Jeol, USA) and for ESI on a “Thermo Finnigan LTQ FT” (Thermo Fisher Scientific, USA).

6.1.3 Infrared spectroscopy

Liquid or oily substances were measured neat between NaCl plates, for solids KBr pallets were prepared. A “Perkin Elmer FT-IR Spectrometer Paragon 1000” (PerkinElmer, USA) was used. Abbreviations of the corresponding intensity of the signals: s = strong, m = medium, w = weak.

6.1.4 Melting points

Measurements were done on a "Büchi B-540" apparatus (Büchi, Switzerland). All values are given in °C and are uncorrected.

6.1.5 High pressure liquid chromatography

The analytical HPLC purity was performed on either a “Merck Hitachi LaChrom Elite” (Hitachi-Merck, Germany) or a “HP Agilent system 1100 series” (Agilent Technologies, Germany) with an “Agilent Poroshell 120 EC-C18 3.0 x 100 mm 2.7-Micron” column. For detection a DAD detector was used. The chromatographic separations were monitored at 210 nm or 254 nm using a bandwidth of 4 nm. Column temperature: 40 – 45 °C; Injection volume: 5 µL of a dilution of 100 µg / mL; Flowrate: 0.5 mL / min; Eluent: MeCN / H_2O / THF (400 : 99 : 1).

6.1.6 Elemental analysis

Elemental analysis was measured on either a CHN-Rapin (Heraeus, Germany) or a Vario EL (Elementar, Germany).

6.1.7 Flash column and thin layer chromatography

Flash column chromatography was performed on flash silica gel (60 M, 0.040 – 0.063 mm) purchased from Merck (Merck, Germany). For each compound the eluent is reported in the corresponding experimental part.

Analytical thin layer chromatography (TLC) was performed on unmodified standard silica layers “Polygram polyester sheets” (designation: SIL G/UV_{254}; thickness of layer: 0.20 mm; plate size: 4 x 8 cm; fluorescent indicator: UV_{254}) purchased from Macherey-Nagel (Germany). Visualization was done by UV-light using wavelength λ = 254 or 365 nm.

6.1.8 Microwave assisted synthesis

Reactions were done using a single mode cavity microwave reactor “Discover SP System” (CEM, USA).

6.1.9 Chemicals and solvents

All chemicals were purchased from the Sigma-Aldrich Corporation (Germany), Fisher Scientific (Germany) or Th. Geyer GmbH & Co. KG (Germany). All chemicals were of analytical grade and no further purification was needed. Commercially available solvents were used if not stated otherwise. THF was dried under reflux over sodium sticks with benzophenone as indicator.

6.2 PROTEIN STABILITY SHIFT ASSAY

Experiments were carried out at the University of Oxford.

Thermal melting experiments were carried out using an Mx3005p Real Time PCR machine (Stratagene). Proteins were buffered in 10 mM HEPES pH 7.5, 500 mM NaCl and assayed in a 96-well plate at a final concentration of 2 μM in 20 μL volume. Compounds were added at a final concentration of 10 μM or 100 μM in order to probe weaker interactions. SYPRO® Orange (Molecular Probes) was added as a fluorescence probe at a dilution of 1:1000. Excitation and emission filters for the SYPRO® Orange dye were set to 465 nm and 590 nm, respectively. The temperature was raised with a step of 3 °C per minute from 25 to 96 °C and fluorescence readings were taken at each interval. The temperature dependence of the fluorescence during the protein denaturation process was approximated by the equation:

$$y(T) = y_F + \frac{y_U - y_F}{1 + e^{\Delta uG_T/RT}}$$

where $\Delta uG(T)$ is the difference in unfolding free energy between the folded and unfolded state, R is the gas constant and y_F and y_U are the fluorescence intensity of the probe in the presence of completely folded and unfolded protein, respectively. The baselines of the denatured and native states were approximated by a linear fit. The observed temperature shifts, ΔT_m^{obs}, were recorded as the difference between the transition midpoints of sample and reference wells containing protein without ligand in the same plate and determined by non-linear least squares fit.

6.3 ISOTHERMAL TITRATION CALORIMETRY

Experiments were carried out at the University of Oxford.

Experiments were carried out on a VP-ITC titration microcalorimeter from MicroCal™, LLC (Northampton, MA). All experiments were carried out at 15 °C while stirring at 295 rpm, in ITC buffer (50 mM HEPES pH 7.4 at 25 °C, 150 mM NaCl). The microsyringe was loaded with a solution of the protein sample (150 μM BRD4(1) in ITC buffer). All titrations were conducted using an initial control injection of 2 μL followed by 34 identical injections of 8 μL with a duration of 16 s (per injection) and a spacing of 250 s between injections. The heat of dilution was determined by independent titrations (protein into buffer) and was subtracted from the experimental data. The collected data were evaluated using a single binding site model and the MicroCal™ Origin software. Thermodynamic parameters were calculated ($\Delta G = \Delta H - T\Delta S = - RTlnK_B$, where ΔG, ΔH and ΔS are the changes in free energy, enthalpy and entropy of binding, respectively).

6.4 CRYSTAL STRUCTURES

Experiments were carried out at the University of Oxford.

6.4.1 Crystallization

Aliquots of the purified proteins were set up for crystallization using a mosquito™ crystallization robot (TTP Labtech, Royston, UK). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100 + 50 nL, 75 + 75 nL and 50 + 100 nL). Initial hits were optimized further scaling up the drop sizes. All crystallizations were carried out using the sitting drop vapor diffusion method at 4 °C. BRD4(1) crystals with alprazolam were grown by mixing 200 nL of the protein (9.5 mg/mL and 5 mM final ligand concentration) with 100 nL of reservoir solution containing 0.20 M sodium sulfate, 0.1 M BT-Propane pH 6.5, 20% PEG3350 and 10% ethylene glycol. BRD4(1) crystals with midazolam were grown by mixing 200 nL of protein (9.36 mg/mL and 5 mM final ligand concentration) with 100 nL of reservoir solution containing 0.1 M magnesium chloride, 0.1 M MES pH 6.5, 15% PEG6000 and 10% ethylene glycol. BRD4(1) crystals with **36** were grown by mixing 200 nL of protein (9 mg/mL and 5 mM final ligand concentration) with 200 nL of reservoir solution containing 0.1 M MES pH 6.5, 10% PEG3350 and 10% ethylene glycol. In all cases diffraction quality crystals grew within a few days.

6.4.2 Data collection and structure solution

All crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data were collected in-house on a Rigaku FRE rotating anode system equipped with a RAXIS-IV detector (alprazolam and midazolam complexes) or at the Diamond beamline I04.1 (**36** complex). Indexing and integration was carried out using MOSFLM[169] and scaling was performed with SCALA[170] or XDS[171]. Initial phases

were calculated by molecular replacement with PHASER[172] using the known models of BRD4(1) (PDB ID 2OSS). Initial models were built by ARP/wARP[173] followed by manual building in COOT[174]. Refinement was carried out in REFMAC5[175]. In all cases thermal motions were analyzed using TLSMD[176] and hydrogen atoms were included in late refinement cycles. Data collection and refinement statistics can be compiled in Table 2 of our paper in *Bioorg. Med. Chem.*[83] The models and structure factors have been deposited with PDB accession codes: 3U5J (BRD4(1)/alprazolam), 3U5K (BRD4(1)/midazolam), 3U5L (BRD4(1)/**36**), respectively.

6.5 AGAR DIFFUSION TEST

Bacteria and fungi were purchased from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and cultivated according to supplied procedures. For sterile working conditions the bioassay was carried out using a laminar air flow HERAsafe safety cabinets (Heraeus, Germany).

AC agar, purchased from the Sigma-Aldrich Corporation (Germany), was used as growing medium for *Escherichia coli*, *Pseudomonas antimicrobial*, *Staphylococcus equorum*, *Streptococcus entericus*, *Yarrowia lipolytica*, *Candida glabrata* and *Hyphopichia burtonii*. 35.5 g AC agar and 2 g agar were suspended in 1 L of water and autoclaved. As long as the suspension was still warm and fluid, each Petri dish was filled in sterile conditions with 15 mL and was allowed to stand for 30 min. For *Aspergillus niger* potato dextrose agar (Sigma-Aldrich Corporation, Germany) was used as growing medium. Preparation procedure followed the one of AC agar using 39 g potato dextrose agar and 1 g agar.

A 1% (m/v) DMSO screening solution was prepared of the compounds. 5 µL of the corresponding solution were spread at a filter circle plate (Schleicher & Schuell, Germany). 2.5 µL (m/v) of a 1% solution in DMSO of the reference compounds clotrimazole and tetracycline, respectively, were also spread at a filter circle. A blank value was prepared by spreading 5 µL of pure DMSO on a filter circle. The solvent was evaporated over night.

In sterile working conditions, a Petri dish was prepared by adding the respective germ on a metal insert, four substance filter plates, the corresponding reference filter plate and the blank value plate. Incubation was done in a drying cabinet at a temperature of 32.1 °C (bacteria) and 28.0 °C (fungi), respectively. After 36 h analysis of the screening results was done by manual measuring of the diameter of the zones of inhibition.

6.6 MTT ASSAY

The MTT assay was performed using human leukemia cell line HL-60 purchased from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkurlturen GmbH, Braunschweig, Germany). A 10 mM stock solution in DMSO was prepared of the compounds to be tested. A serial dilution was prepared by a successively 1:1 dilution with DMSO ten times.

For the blank value DMSO was used. For the positive control a solution of the detergent Triton® X-100 in a final concentration of 1 µg/mL was used. Wells containing control cells were neither treated with DMSO nor with a compound solution or with Triton® X-100.

Each well of a microtiter plate was filled with 99 µL of a HL-60 suspension and 1 µL of either the compound solution, the pure DMSO (blank) or the Triton® X-100 (positive control). The plate was incubated for 24 h at 37 °C and 5% CO_2. Subsequently, each well was treated with 10 µL of a MMT stock solution of 5 mg MTT in 1 mL of phosphate buffered saline (PBS) and incubated again for two hours under the same conditions. After the following addition of 190 µL of DMSO the solution was allowed to stand for another 60 min while shaking occasionally.

Photometric quantification of the yielded dye was performed on an ELISA MRX II Microplate reader (Dynex, Germany) at a wavelength of λ = 570 nm. The statistical analysis and the calculation of the corresponding IC_{50} values was done using Prism 4 software (GraphPad, USA).

6.7 GENERAL PROCEDURES

GP 1 – Preparation of triazolobenzodiazepine derivatives

7-Chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (1 equiv) and the respective carboxyhydrazide (2 equiv) were dissolved in n-butanol (volume as stated in procedure) and heated to 130 °C in a sealed vial under nitrogen atmosphere for 1 d. The reaction mixture was cooled to room temperature and stirred with 25 mL of an aqueous glucose solution (0.1 g/mL) at room temperature for 2 h. The mixture was extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with water (25 mL), dried over sodium sulfate, filtered and the solvent was evaporated. Purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 9:1), yielding triazolobenzodiazepines in 17 – 67% yield.

GP 2 – Preparation of the isothiocyanate group using thiophosgene

Calcium carbonate (1.5 equiv) was suspended in cooled (0 °C) water (50 mL/mmol) and thiophosgene **16** (1.1 equiv) was added to the reaction mixture. 2-Aminobenzophenone derivative (1.0 equiv) was dissolved in dichloromethane (2.5 mL/mmol) and added over a period of 30 min to the vigorously stirred suspension. After complete addition, the reaction mixture was stirred for another hour with the ice bath in place, followed by additional 4 h at room temperature. The precipitate was filtered off prior to separation of the organic and the aqueous layer. The organic layer was washed three times with water (1 mL/mmol), dried over sodium sulfate, filtered and evaporated. Where required, purification was done by flash column chromatography on flash silica gel – unless stated otherwise – with dichloromethane, giving the isothiocyanate product in 63 – 98% yield.

GP 3 – Two step annulation of the 1-methyl-1,2,4-triazole ring

The respective benzotriazepine-2-thione derivative (1.0 equiv) was dissolved in tetrahydrofuran (8 mL/mmol) and treated with hydrazine hydrate (5.0 equiv). After stirring at room temperature for 18 h the solvent was evaporated. The residue was dissolved in ethyl acetate (8 mL/mmol), washed three times with water (4 mL/mmol), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in toluene (5 mL/mmol) and after adding triethyl orthoacetate **43** (1.3 equiv) and para-toluenesulfonic acid monohydrate (0.2 equiv), the mixture was heated to reflux for 3 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 19:1), yielding the corresponding triazolobenzo-triazepine compounds in 38 – 78% yield.

GP 4 – 4-N-alkylation of triazolobenzotriazepines with benzyl bromide derivatives

4*H*-Triazolobenzotriazepine derivative (1.0 equiv) was dissolved in anhydrous tetrahydrofuran (9 mL/mmol) and sodium hydride (1.1 equiv) was added portionwise as a 60 % dispersion in mineral oil to the vigorously stirred solution. Subsequently, the corresponding benzyl bromide (3.0 equiv) was added, and after stirring for 1 h at room temperature, the solvent was evaporated and purification was done by flash column chromatography on flash silica gel – unless stated otherwise – with dichloromethane / methanol (9:1), yielding the 4-*N*-benzyl functionalized triazolobenzotriazepine compounds in 42 – 97% yield.

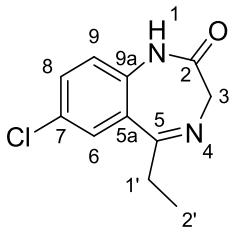
GP 5 – 4-N-alkylation of triazolobenzotriazepines with benzyl chloride derivatives

4*H*-Triazolobenzotriazepine derivative (1.0 equiv) was dissolved in anhydrous tetrahydrofuran (9 mL/mmol) and sodium hydride (1.1 equiv) was added portionwise as a 60 % dispersion in mineral oil to the vigorously stirred solution. Subsequently, the corresponding benzyl chloride (3.0 equiv) was added, and after stirring for 4 h at reflux, the solvent was evaporated and purification was done by flash column chromatography on flash silica gel – unless stated otherwise – with dichloromethane / methanol (9:1), yielding the 4-*N*-benzyl functionalized triazolobenzotriazepine compounds in 48 – 74% yield.

GP 6 – Microwave assisted synthesis of 1,2,3-triazoles via copper(I)-catalyzed 1,3-dipolar cycloadition

A 10 mL microwave vial was charged with 8-chloro-1-methyl-6-phenyl-4-(prop-2-yn-1-yl)-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **13** (1.1 equiv), sodium azide (1.1 equiv) and the respective halogen compound (1.0 equiv). The starting materials were suspended in a 1:1 mixture of *tert*-butanol / water (10 mL/mmol) prior to the addition of sodium L-ascorbate (0.2 equiv) and copper(II) sulfate pentahydrate (0.05 equiv). The tightly sealed vial was stirred for 10 min under microwave irradiation (max. power: 100 W; ramp time: 1 min; max. temperature: 125 °C; max. pressure: 8 bar). After cooling to 60 °C, the reaction mixture was concentrated under reduced pressure and extracted with dichloromethane (2 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and evaporated. Purification was done by flash column chromatography on flash silica gel – unless stated otherwise – with ethyl acetate / methanol (9:1), yielding the 1,4-disubstituted 1,2,3-triazoles in 37 – 73% yield.

6.8 DESCRIPTION OF COMPOUNDS



7-Chloro-5-ethyl-1H-benzo[e][1,4]diazepine-2(3H)-one (1)

(Literature known compound but different procedure[104])

N-(4-Chloro-2-propionylphenyl)-2-iodoacetamide **23** (6.5 g, 18 mmol, 1.0 equiv) was dissolved in 130 mL of ethanol and ammonia was passed through the solution three times (each time for 5 min). The ammonia saturated solution was stirred at room temperature for 3 h. Purification was done by flash column chromatography on flash silica gel (ethyl acetate, R_f = 0.50). Compound **1** (3.6 g, 16 mmol, 87 %) was yielded as pale yellow solid.

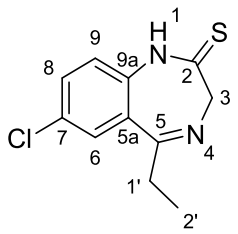
^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 1.11 (t, $^3J_{HH}$ = 7.4 Hz, 3 H, 2'-H), 2.75 (q, $^3J_{HH}$ = 7.4 Hz, 2 H, 1'-H), 4.08 (s, 2 H, 3-H), 7.06 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H), 7.42 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 8-H), 7.56 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 6-H), 9.09 (bs, 1 H, 1-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 11.5 (C-2'), 32.2 (C-1'), 56.2 (C-3), 123.0 (C-9), 128.3 (C-6), 129.6 (C-7), 130.1 (C-5a), 131.5 (C-8), 136.4 (C-9a), 172.1 (C-2), 172.8 (C-5)

MS (CI): *m/z* (%) = 225 (30), 223 (100) $[M + H]^+$

MF: $C_{11}H_{11}ClN_2O$

MW: 222.67 g/mol



7-Chloro-5-ethyl-1H-benzo[e][1,4]diazepine-2(3H)-thione (2)

7-Chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-one **1** (2.0 g, 9.1 mmol, 1.0 equiv) and Lawesson's reagent **24** (4.0 g, 10 mmol, 1.1 equiv) were suspended in 19 mL of anhydrous tetrahydrofuran and stirred over night at room temperature under nitrogen atmosphere. Water (250 mL) was added and the mixture was extracted three times with dichloromethane (each 40 mL). The combined organic layers were washed with water, dried over sodium sulfate, filtered and evaporated. Purification was done by flash column chromatography on flash silica gel (ethyl acetate / isohexane 1:2, R_f = 0.46), yielding product **2** (1.1 g, 4.8 mmol, 53 %) as yellow solid.

mp: 168.7 °C

^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) = 1.13 (t, $^3J_{HH}$ = 7.4 Hz, 3 H, 2'-H), 2.75 (q, $^3J_{HH}$ = 7.3 Hz, 2 H, 1'-H), 4.49 (s, 2 H, 3-H), 7.12 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H), 7.45 (dd, $^4J_{HH}$ = 2.3 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 8-H), 7.58 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 6-H), 10.19 (bs, 1 H, 1-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 11.4 (C-2'), 31.8 (C-1'), 62.9 (C-3), 122.9 (C-9), 128.7 (C-6), 131.1 (C-7), 131.5 (C-5a), 131.6 (C-8), 136.9 (C-9a), 172.4 (C-5), 201.6 (C-2)

IR [cm^{-1}]: $\tilde{\nu}$ = 3113 (w), 3070 (w), 2973 (m), 2899 (m), 2853 (m), 2731 (m), 2675 (w), 1635 (s), 1576 (m), 1520 (m), 1474 (s), 1358 (s), 1163 (s), 1009 (m), 838 (m)

MS (CI): *m/z* (%) = 241 (35), 239 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 240 (35), 238 (100) $[M]^{+\cdot}$, 203 (50)

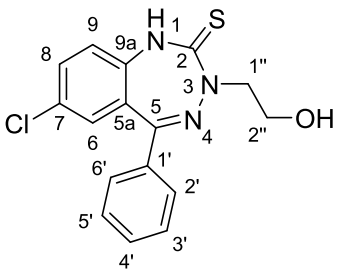
HR-MS (EI): calcd. for $C_{11}H_{11}ClN_2S$ $[M]^{+\cdot}$ 238.0331; found 238.0296

Elemental analysis calcd. (%) for $C_{11}H_{11}ClN_2S$ (238.7): C 55.34, H 4.64, N 11.73; found C 55.21, H 4.62, N 11.36

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{11}H_{11}ClN_2S$

MW: 238.74 g/mol



7-Chloro-3-(2-hydroxyethyl)-5-phenyl-1H-benzo[e][1,2,4]triazepine-2(3H)-thione (3)

(Literature known compound but different procedure[109])

A catalytic amount of para-toluenesulfonic acid monohydrate (3.0 mg, 14 µmol, 2.5 mol%) and *N*-(2-benzoyl-4-chlorophenyl)-1-(2-hydroxyethyl)hydrazinecarbothioamide **42** (200 mg, 0.572 mmol, 1.00 equiv) were dissolved in ethanol (20 mL) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane / ethyl acetate 9:1, R_f = 0.50), yielding product **3** (175 mg, 0.527 mmol, 92 %) as yellow solid.

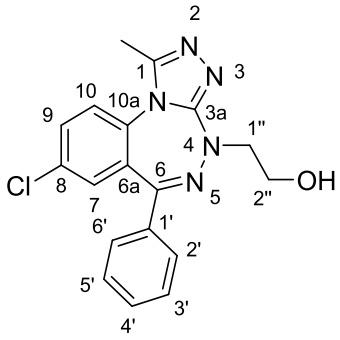
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.91 (s, 1 H, OH), 4.08 – 4.15 (m, 2 H, 2''-H), 4.28 – 4.35 (m, 2 H, 1''-H), 6.92 (d, $^3J_{HH}$ = 8.5 Hz, 1 H, 9-H), 7.04 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 6-H), 7.40 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 8-H), 7.42 – 7.55 (m, 5 H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 7.78 (s, 1 H, 1-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 58.3 (C-2''), 61.3 (C-1''), 122.0 (C-9), 127.3 (C-5a), 129.0 (C-3', C-5'), 129.5 (C-2', C-6'), 130.0 (C-7), 130.6 (C-6), 131.5 (C-4'), 133.2 (C-8), 135.6 (C-1'), 143.0 (C-9a), 167.0 (C-5), 192.6 (C-2)

MS (CI): *m/z* (%) = 334 (35), 332 (100) $[M + H]^+$

MF: $C_{16}H_{14}ClN_3OS$

MW: 331.82 g/mol



8-Chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (5)

Synthesis of the triazole-ring followed **GP3**. 7-Chloro-3-(2-hydroxyethyl)-5-phenyl-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **3** (1.7 g, 5.3 mmol, 1.0 equiv) was treated first with hydrazine hydrate (1.3 mL, 26 mmol, 5.0 equiv). Triethyl orthoacetate **43** (1.3 mL, 6.9 mmol, 1.3 equiv) and para-toluenesulfonic acid monohydrate (200 mg, 1.06 mmol, 0.20 equiv) were used in the second step. Product **5** (710 mg, 3.16 mmol, 38 %) was obtained after purification (R_f = 0.27) as colorless solid.

mp: 226.3 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 3.49 (bs, 1 H, OH), 3.83 – 4.07 (m, 4 H, 1''-H, 2''-H), 7.26 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.46 – 7.50 (m, 1 H, 4'-H), 7.52 – 7.55 (m, 2 H, 2'-H, 6'-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.2 (C-2''), 60.3 (C-1''), 124.4 (C-10), 128.9 (C-3', C-5'), 129.5 (C-2', C-6'), 130.7 (C-6a), 130.9 (C-4'), 131.7 (C-7), 132.2 (C-9), 133.1 (C-8), 133.8 (C-10a), 136.4 (C-1'), 148.7 (C-1), 160.4 (C-3a), 161.5 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3058 (w), 2924 (w), 1630 (w), 1520 (s), 1489 (m), 1431 (m), 1319 (m), 1074 (m), 825 (m), 696 (m), 536 (m)

MS (CI): *m/z* (%) = 356 (30), 354 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 353 (5) $[M]^{+\cdot}$, 295 (100)

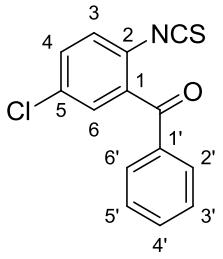
HR-MS (EI): calcd. for $C_{18}H_{16}ClN_5O$ $[M]^{+\cdot}$ 353.1043; found 353.1046

Elemental analysis calcd. (%) for $C_{18}H_{16}ClN_5O$ (353.8): C 61.10, H 4.56, N 19.79; found C 60.98, H 4.49, N 19.74

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{18}H_{16}ClN_5O$

MW: 353.81 g/mol



(5-Chloro-2-isothiocyanatophenyl)(phenyl)methanone (6)

(Literature known compound but different procedure[108])

Compound **6** was prepared according to **GP2** using 2-amino-5-chloro-benzophenone **40** (5.0 g, 22 mmol, 1.0 equiv) as starting material, calcium carbonate (3.3 g, 33 mmol, 1.5 equiv) and thiophosgene **16** (1.8 mL, 24 mmol, 1.1 equiv). No purification was needed after workup procedure, yielding product **6** (5.8 g, 21 mmol, 98 %) as cream-colored solid.

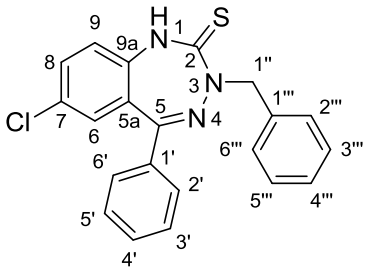
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.29 (d, $^3J_{HH}$ = 8.4 Hz, 1 H, 3-H), 7.46 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 6-H), 7.48 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.4 Hz, 1 H, 4-H), 7.50 – 7.54 (m, 2 H, 3'-H, 5'-H), 7.62 – 7.67 (m, 1 H, 4'-H), 7.78 – 7.83 (m, 2 H, 2'-H, 6'-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 128.5 (C-2), 128.7 (C-3), 129.1 (C-3', C-5'), 130.1 (C-6), 130.3 (C-2', C-6'), 132.1 (C-4), 132.9 (C-5), 134.3 (C-4'), 136.2 (C-1'), 136.5 (C-1), 138.3 (NCS), 193.2 (CO)

MS (CI): *m/z* (%) = 276 (40), 274 (100) $[M + H]^+$

MF: $C_{14}H_8ClNOS$

MW: 273.74 g/mol



3-Benzyl-7-chloro-5-phenyl-1H-benzo[e][1,2,4]triazepine-2(3H)-thione (9)

To a well stirred and cooled (0 °C) solution of benzylhydrazine dihydrochloride **7** (374 mg, 1.92 mmol, 1.05 equiv) in 3 mL of methanol, *N*,*N*-diisopropylethylamine (668 µL, 3.84 mmol, 2.10 equiv) was added dropwise. After stirring for 30 min at room temperature, a solution of (5-chloro-2-isothiocyanatophenyl)(phenyl)-methanone **6** (500 mg, 1.83 mmol, 1.00 equiv) in 10 mL of tetrahydrofuran was added and stirring was continued for 16 h. The mixture was concentrated under reduced pressure, dissolved in ethanol (15 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (9.0 mg, 46 µmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane, R_f = 0.55), giving compound **9** (593 mg, 1.57 mmol, 86 %) as pale yellow solid.

mp: 195.5 °C

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 5.18 (s, 2 H, 1''-H), 6.91 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 6-H), 7.18 – 7.22 (m, 2 H, 2'-H, 6'-H), 7.23 – 7.27 (m, 2 H, 9-H, 4'''-H), 7.27 – 7.34 (m, 4 H, 2'''-H, 3'''-H, 5'''-H, 6'''-H), 7.41 (t, $^3J_{HH}$ = 7.7 Hz, 2 H, 3'-H, 5'-H), 7.48 – 7.53 (m, 1 H, 4'-H), 7.62 (dd, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 8-H), 10.62 (s, 1 H, 1-H)

^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) = 58.6 (C-1''), 123.1 (C-9), 127.2 (C-4'''), 127.6 (C-5a), 127.9 (C-7), 128.3 (C-2''', C-6'''), 128.3 (C-3''', C-5'''), 128.7 (C-3', C-5'), 129.0 (C-2', C-6'), 129.1 (C-6), 131.0 (C-4'), 132.7 (C-8), 135.2 (C-1'), 137.0 (C-1'''), 143.2 (C-9a), 166.5 (C-5), 193.1 (C-2)

IR [cm^{-1}]: $\tilde{\nu}$ = 3217 (m), 3062 (w), 2994 (w), 1605 (m), 1512 (m), 1482 (s), 1371 (m), 1324 (m), 1237 (m), 1191 (s), 1169 (s), 1003 (m), 832 (m), 740 (s), 697 (s)

MS (CI): *m/z* (%) = 380 (40), 378 (100) $[M + H]^+$, 344 (20), 290 (60), 274 (10), 145 (20), 108 (45)

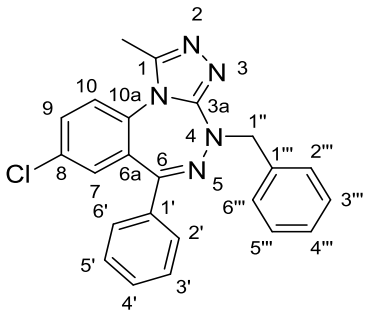
MS (EI): *m/z* (%) = 377 (5) $[M]^{+\cdot}$, 344 (100), 273 (40), 241 (30), 91 (55), 77 (40)

HR-MS (EI): calcd. for $C_{21}H_{16}ClN_3S$ $[M]^{+\cdot}$ 377.0753; found 377.0750

HPLC purity: 91 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{21}H_{16}ClN_3S$

MW: 377.89 g/mol



4-Benzyl-8-chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]-triazepine (10)

Synthesis of the triazole-ring followed **GP3**. 3-Benzyl-7-chloro-5-phenyl-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **9** (320 mg, 0.847 mmol, 1.00 equiv) was treated first with hydrazine hydrate (206 µL, 4.23 mmol, 5.00 equiv). Triethyl orthoacetate **43** (201 µL, 1.10 mmol, 1.30 equiv) and para-toluenesulfonic acid monohydrate (32 mg, 0.17 mmol, 0.2 equiv) were used in the second step. Product **10** (176 mg, 0.440 mmol, 52 %) was obtained after purification (R_f = 0.31) as pale yellow solid.

mp: 105.8 – 108.2 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 4.88 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1''-*H*H), 5.11 (d, $^2J_{HH}$ = 13.6 Hz, 1 H, 1''-H*H*), 7.22 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.24 – 7.28 (m, 1 H, 4'''-H), 7.29 – 7.34 (m, 3 H, 10-H, 3'''-H, 5'''-H), 7.34 – 7.38 (m, 2 H, 3'-H, 5'-H), 7.39 – 7.42 (m, 4 H, 2'-H, 6'-H, 2'''-H, 6'''-H), 7.42 – 7.46 (m, 1 H, 4'-H), 7.60 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 58.1 (C-1''), 124.3 (C-10), 128.6 (C-3''', C-5'''), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 129.6 (C-2''', C-6'''), 127.7 (C-4'''), 130.8 (C-6a), 130.8 (C-4'), 131.5 (C-7), 132.1 (C-9), 132.9 (C-8), 133.9 (C-10a), 136.6 (C-1'), 137.8 (C-1'''), 148.6 (C-1), 160.3 (C-3a), 161.2 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 3029 (w), 2922 (w), 2857 (w), 2363 (w), 2344 (w), 1535 (m), 1518 (s), 1489 (m), 1445 (m), 11430 (m), 1319 (m), 1271 (w), 1171 (w), 1029 (w), 826 (w), 736 (m), 695 (m)

MS (CI): *m/z* (%) = 402 (100), 400 (85) $[M + H]^+$, 149 (30)

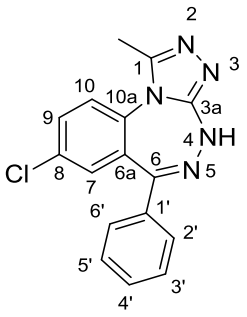
MS (EI): *m/z* (%) = 401 (8), 399 (25) $[M]^{+\cdot}$, 371 (40), 295 (35), 280 (40), 91 (100), 77 (75)

HR-MS (EI): calcd. for $C_{23}H_{18}ClN_5$ $[M]^{+\cdot}$ 399.1251; found 399.1252

HPLC purity: 94 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{18}ClN_5$

MW: 399.88 g/mol



8-Chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (11)

(Literature known compound but different procedure[110])

8-Chloro-4-(4-methoxybenzyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-c[1,2,4]triazepine **51** (1.04 g, 2.42 mmol, 1.0 equiv) was dissolved in a 45 % solution of hydrogen bromide in acetic acid (15 mL) and treated with anisole (526 µL, 4.84 mmol, 2.00 equiv). The mixture was stirred over night at room temperature and further for 30 min at 60 °C yielding the hydrobromic salt of target compound **11**. The mixture was taken to dryness under reduced pressure and re-dissolved in tetrahydrofuran (30 mL). A saturated aqueous sodium hydrogen carbonate solution (35 mL) was added and after stirring for 20 min at room temperature the reaction mixture was concentrated under reduced pressure. Extraction was done with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with water (50 mL). The organic layer was dried over sodium sulfate, filtered and evaporated. Purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 9:1, R_f = 0.66), yielding compound **11** (635 mg, 2.05 mmol, 88 %) as colorless solid.

mp: 139.8 °C (lit. 140 – 142 °C)[110]

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.57 (s, 3 H, 1-CH_3), 7.25 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.33 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.44 – 7.49 (m, 1 H, 4'-H), 7.52 – 7.55 (m, 2 H, 2'-H, 6'-H), 7.60 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 8.38 (s, 1 H, 4-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.9 (1-CH_3), 124.3 (C-10), 128.9 (C-3', C-5'), 129.4 (C-2', C-6'), 130.6 (C-4'), 130.7 (C-6a), 131.9 (C-7), 132.1 (C-9), 133.0 (C-8), 133.8 (C-10a), 136.8 (C-1'), 148.5 (C-1), 158.6 (C-3a), 161.0 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3346 (w), 3242 (m), 3208 (m), 3178 (m), 3065 (m), 3029 (m), 2360 (w), 1713 (w), 1615 (m), 1578 (m), 1548 (s), 1527 (s), 1495 (s), 1489 (s), 1446 (s), 1425 (s), 1376 (m), 1350 (m), 1309 (s), 1270 (m), 1172 (m), 1136 (w), 1105 (m), 1076 (m), 1033 (w), 987 (w), 916 (m), 889 (m), 825 (s), 775 (s), 744 (s), 698 (s)

MS (CI): *m/z* (%) = 312 (50), 310 (100) $[M + H]^+$

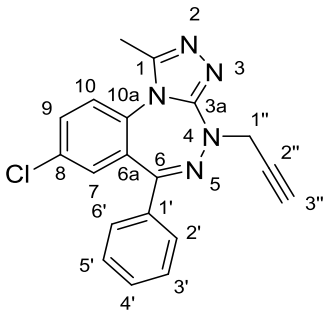
MS (EI): *m/z* (%) = 311 (35), 309 (100) $[M]^{+\cdot}$, 253 (60), 219 (70), 77 (60)

HR-MS (EI): calcd. for $C_{16}H_{12}ClN_5$ $[M]^{+\cdot}$ 309.0781; found 309.0781

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{16}H_{12}ClN_5$

MW: 309.75 g/mol



8-Chloro-1-methyl-6-phenyl-4-(prop-2-yn-1-yl)-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (13)

8-Chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (0.65 g, 2.1 mmol, 1.0 equiv) was dissolved in anhydrous tetrahydrofuran (12 mL) and sodium hydride (92 mg, 2.31 mmol, 1.10 equiv) was added portionwise as a 60 % dispersion in mineral oil to the vigorously stirred solution. Subsequently, propargyl bromide **12** (584 µL, 5.25 mmol, 2.50 equiv) was added as 80wt% solution in toluene and after stirring for 1 h at room temperature the solvent was evaporated. Purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 9:1, R_f = 0.63), yielding product **13** (504 mg, 1.45 mmol, 69 %) as pale yellow solid.

mp: 231.8 – 232.4 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.37 (t, $^4J_{HH}$ = 2.5 Hz, 1 H, 3''-H), 2.56 (s, 3 H, 1-CH_3), 4.46 – 4.64 (m, 2 H, 1''-H), 7.29 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.34 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.40 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.46 – 7.51 (m, 1 H, 4'-H), 7.54 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.62 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 44.3 (C-1''), 72.8 (C-3''), 79.5 (C-2''), 124.4 (C-10), 128.9 (C-3', C-5'), 129.6 (C-2', C-6'), 130.5 (C-6a), 131.0 (C-4'), 131.7 (C-7), 132.3 (C-9), 133.1 (C-8), 133.6 (C-10a), 136.3 (C-1'), 148.9 (C-1), 159.4 (C-3a), 161.6 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3308 (s), 3074 (w), 2949 (w), 2922 (w), 2361 (m), 2342 (w), 1636 (m), 1560 (m), 1541 (s), 1522 (s), 1487 (m), 1444 (m), 1422 (m), 1381 (w), 1348 (w), 1319 (m), 1308 (m), 1272 (m), 1170 (m), 1104 (w), 1064 (w), 1036 (w), 829 (m), 692 (m)

MS (CI): *m/z* (%) = 350 (35), 348 (100) $[M + H]^+$, 255 (10), 210 (20)

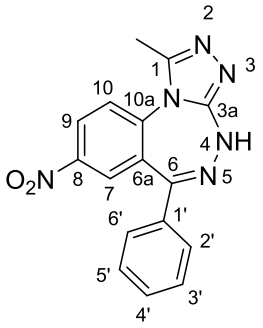
MS (EI): *m/z* (%) = 349 (7), 347 (25) $[M]^{+\cdot}$, 332 (95), 305 (15), 295 (25), 280 (10), 270 (25), 253 (25), 243 (40), 229 (5), 219 (20), 204 (20), 181 (10), 177 (25), 163 (10), 151 (15), 136 (10), 110 (15), 77 (100), 51 (35)

HR-MS (EI): calcd. for $C_{19}H_{14}ClN_5$ $[M]^{+\cdot}$ 347.0938; found 347.0937

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{19}H_{14}ClN_5$

MW: 347.80 g/mol



1-Methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (14)

(Literature known compound but different procedure[110])

4-(4-Methoxybenzyl)-1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-[1,2,4]triazepine **68** (2.2 g, 5.0 mmol, 1.0 equiv) was dissolved in a 45 % solution of hydrogen bromide in acetic acid (20 mL) and treated with anisole (1.1 mL, 10 mmol, 2.0 equiv). The mixture was stirred over night at room temperature and further for 30 min at 60 °C yielding the hydrobromic salt of target compound **14**. The mixture was taken to dryness under reduced pressure and re-dissolved in tetrahydrofuran (40 mL). A saturated sodium hydrogen carbonate solution (40 mL) was added and after stirring for 20 min at room temperature the reaction mixture was concentrated under reduced pressure. Extraction was done with ethyl acetate (3 x 35 mL) and the combined organic layers were washed with water (35 mL). The organic layer was dried over sodium sulfate, filtered and evaporated. Purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 19:1, R_f = 0.32), yielding compound **14** (1.49 g, 4.65 mmol, 93 %) as yellow solid.

mp: 259.4 °C (lit. 258 – 260 °C)[110]

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.62 (s, 3 H, 1-CH_3), 7.40 – 7.46 (m, 2 H, 3'-H, 5'-H), 7.48 – 7.52 (m, 1 H, 4'-H), 7.53 – 7.56 (m, 2 H, 2'-H, 6'-H), 7.56 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.09 (d, $^4J_{HH}$ = 2.5 Hz, 1 H, 7-H), 8.44 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H), 8.83 (s, 1 H, 4-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.1 (1-CH_3), 124.1 (C-10), 126.9 (C-9), 127.4 (C-7), 129.1 (C-3', C-5'), 129.3 (C-2', C-6'), 130.4 (C-6a), 131.0 (C-4'), 136.3 (C-1'), 140.3 (C-10a), 145.9 (C-8), 148.6 (C-1), 158.8 (C-3a), 160.5 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3232 (m), 3172 (m), 3083 (m), 2361 (w), 1616 (m), 1578 (m), 1551 (m), 1523 (s), 1489 (m), 1445 (m), 1427 (m), 1345 (s), 1322 (s), 1280 (m), 1253 (m), 1101 (w), 1078 (w), 1034 (w), 989 (w), 911 (w), 881 (w), 778 (m), 749 (m), 737 (m), 698 (m)

MS (CI): *m/z* (%) = 321 (100) $[M + H]^+$

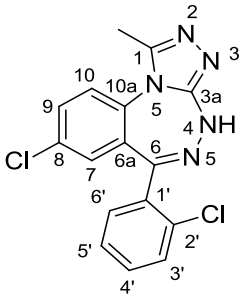
MS (EI): *m/z* (%) = 320 (100) $[M]^{+\cdot}$, 290 (10), 273 (25), 248 (30), 218 (80), 205 (25), 178 (20), 151 (25), 75 (75)

HR-MS (EI): calcd. for $C_{16}H_{12}N_6O_2$ $[M]^{+\cdot}$ 320.1022; found 320.1029

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{16}H_{12}N_6O_2$

MW: 320.31 g/mol



8-Chloro-6-(2-chlorophenyl)-1-methyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]-triazepine (15)

(Literature known compound but different procedure[110])

8-Chloro-6-(2-chlorophenyl)-4-(4-methoxybenzyl)-1-methyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **69** (2.9 g, 6.3 mmol, 1.0 equiv) was dissolved in a 45 % solution of hydrogen bromide in acetic acid (25 mL) and treated with anisole (1.4 mL, 13 mmol, 2.0 equiv). The mixture was stirred over night at room temperature and further for 30 min at 60 °C yielding the hydrobromic salt of target compound **15**. The mixture was taken to dryness under reduced pressure and re-dissolved in tetrahydrofuran (40 mL). A saturated sodium hydrogen carbonate solution (40 mL) was added and after stirring for 20 min at room temperature the reaction mixture was concentrated under reduced pressure. After addition of ethyl acetate (50 mL) precipitation of a colorless solid occurred. The precipitate was filtered off and dried under reduced pressure yielding compound **15** (1.69 g, 4.91 mmol, 79 %) as colorless solid.

mp: 279.9 °C (lit. 282 – 284 °C)[110]

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 6.99 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.38 – 7.46 (m, 3 H, 3'-H, 5'-H, 6'-H), 7.56 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.57 – 7.60 (m, 1 H, 4'-H), 8.56 (s, 1 H, 4-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.9 (1-CH_3), 124.2 (C-10), 127.7 (C-5'), 130.0 (C-7), 130.1 (C-3'), 131.4 (C-6a), 131.5 (C-6'), 131.9 (C-4'), 132.0 (C-9),

132.9 (C-10a), 133.2 (C-2'), 133.4 (C-8), 135.9 (C-1'), 148.6 (C-1), 158.5 (C-3a), 159.3 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3239 (m), 3177 (m), 3131 (m), 3094 (m), 3067 (m), 3030 (m), 2362 (w), 1935 (w), 1817 (w), 1618 (m), 1553 (s), 1529 (m), 1494 (s), 1424 (s), 1350 (m), 1315 (s), 1281 (m), 1178 (m), 1106 (m), 1068 (m), 1014 (m), 917 (m), 892 (m), 847 (m), 828 (m), 762 (s), 711 (m), 579 (s)

MS (CI): *m/z* (%) = 348 (10), 346 (70), 344 (100) $[M + H]^+$

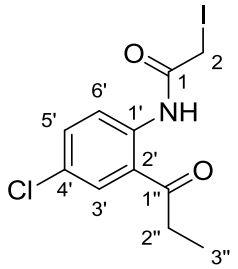
MS (EI): *m/z* (%) = 347 (5), 345 (30), 343 (55) $[M]^{+\cdot}$, 287 (30), 253 (100), 239 (30), 177 (30), 151 (20), 111 (30), 100 (20), 75 (90)

HR-MS (EI): calcd. for $C_{16}H_{11}Cl_2N_5$ $[M]^{+\cdot}$ 343.0392; found 343.0387

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{16}H_{11}Cl_2N_5$

MW: 344.20 g/mol



N-(4-Chloro-2-propionylphenyl)-2-iodoacetamide (23)

(Literature known compound but different procedure[177])

2-Chloro-*N*-(4-chloro-2-propionylphenyl)acetamide[177] **22** (5.5 g, 21 mmol, 1.0 equiv) and sodium iodide (6.3 g, 42 mmol, 2.0 equiv) were dissolved in 140 mL of acetone. The solution was stirred over night at room temperature. The produced sodium chloride precipitated and was filtered off. Acetone was evaporated and the residue was dissolved in dichloromethane and washed with water in a separating funnel. The organic phase was dried over sodium sulfate, filtered and evaporated, yielding product **23** (7.4 g, 21 mmol, 99 %) as colorless solid.

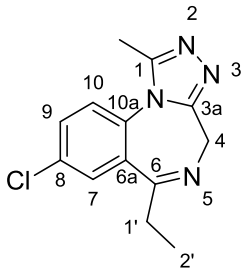
^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 1.20 (t, $^3J_{HH}$ = 7.2 Hz, 3 H, 3''-H), 3.06 (q, $^3J_{HH}$ = 7.1 Hz, 2 H, 2''-H), 3.88 (s, 2 H, 2-H), 7.52 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 5'-H), 7.93 (s, 1 H, 3'-H), 8.54 (d, $^3J_{HH}$ = 9.0 Hz, 1 H, 6'-H), 12.00 (bs, 1 H, NH)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 0.9 (C-2), 8.4 (C-3''), 33.7 (C-2''), 122.4 (C-6'), 123.4 (C-2'), 128.2 (C-4'), 130.7 (C-3'), 134.7 (C-5'), 139.4 (C-1'), 167.1 (C-1), 204.8 (C-1'')

MS (CI): *m/z* (%) = 354 (25), 352 (80) $[M + H]^+$, 288 (20), 264 (30), 226 (80), 208 (20), 186 (15), 184 (100), 154 (15), 146 (20), 121 (60)

MF: $C_{11}H_{11}ClINO_2$

MW: 351.57 g/mol



8-Chloro-6-ethyl-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepine (29a)

According to **GP1**, compound **29a** was synthesized, using 7-chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (66 mg, 0.28 mmol, 1.0 equiv) and acethydrazide **27a** (41 mg, 0.55 mmol, 2.0 equiv) dissolved in 5 mL of n-butanol. After purification (R_f = 0.61), product **29a** (29 mg, 4.8 mmol, 53 %) was obtained as yellow solid.

mp: 173.5 – 174.9 °C

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.09 (t, $^3J_{HH}$ = 7.3 Hz, 3 H, 2'-H), 2.60 (s, 3 H, 1-CH_3), 2.56 – 2.65 (m, 1 H, 1'-*H*H), 2.80 – 2.89 (m, 1 H, 1'-H*H*), 3.93 (d, $^2J_{HH}$ = 13.0 Hz, 1 H, 4-*H*H), 5.28 (d, $^2J_{HH}$ = 13.0 Hz, 1 H, 4-H*H*), 7.35 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 10-H), 7.60 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H), 7.68 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 11.0 (C-2'), 12.4 (1-CH_3), 32.3 (C-1'), 45.7 (C-4), 124.6 (C-10), 128.9 (C-7), 130.7 (C-10a), 131.1 (C-9), 131.9 (C-6a), 133.9 (C-8), 150.2 (C-1), 154.9 (C-3a), 170.9 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3066 (w), 2971 (m), 2933 (m), 2852 (w), 2360 (w), 1635 (s), 1542 (s), 1485 (s), 1426 (s), 1378 (m), 1111 (m), 831 (m)

MS (CI): *m/z* (%) = 263 (30), 261 (100) $[M + H]^+$

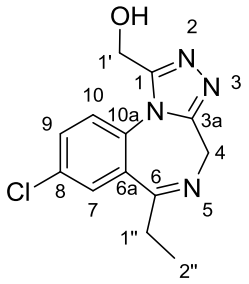
MS (EI): *m/z* (%) = 262 (10), 260 (25) $[M]^{+\cdot}$, 231 (30), 225 (100), 197 (25)

HR-MS (EI): calcd. for $C_{13}H_{13}ClN_4$ $[M]^{+\cdot}$ 260.0829; found 260.0813

HPLC purity: 95 % [λ = 210 nm], 94 % [λ = 254 nm]

MF: $C_{13}H_{13}ClN_4$

MW: 260.72 g/mol



8-Chloro-6-ethyl-1-hydroxymethyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]-diazepine (29b)

According to **GP1**, compound **29b** was synthesized, using 7-chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (300 mg, 1.26 mmol, 1.00 equiv) and 2-hydroxyacethydrazide **27b** (226 mg, 2.51 mmol, 2.00 equiv) dissolved in 7 mL of n-butanol. After purification (R_f = 0.47), product **29b** (98 mg, 0.35 mmol, 28 %) was obtained as pale yellow solid.

mp: 198.0 – 199.6 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.08 (t, $^3J_{HH}$ = 7.3 Hz, 3 H, 2''-H), 2.58 – 2.72 (m, 1 H, 1''-*H*H), 2.80 – 2.92 (m, 1 H, 1''-H*H*), 3.00 (s, 1 H, OH), 3.99 (d, $^2J_{HH}$ = 13.1 Hz, 1 H, 4-*H*H), 4.47 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1'-*H*H), 4.99 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1'-*H*H), 5.24 (d, $^2J_{HH}$ = 13.1 Hz, 1 H, 4-H*H*), 7.66 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H), 7.68 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 8.15 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 10-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 11.1 (C-2''), 32.4 (C-1''), 45.4 (C-4), 53.6 (C-1'), 126.2 (C-10), 128.6 (C-7), 130.5 (C-10a), 131.2 (C-6a), 132.0 (C-9), 134.4 (C-8), 153.7 (C-1), 154.9 (C-3a), 172.0 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 2935 (w), 1633 (s), 1538 (m), 1487 (s), 1443 (s), 1290 (w), 1113 (m), 1055 (m), 1024 (m), 827 (m)

MS (CI): *m/z* (%) = 279 (25), 277 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 278 (5), 276 (20) $[M]^{+\cdot}$, 241 (100)

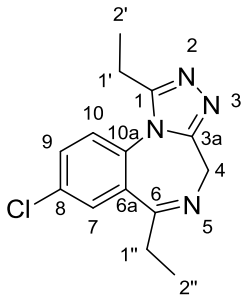
HR-MS (EI): calcd. for $C_{13}H_{13}ClN_4O$ $[M]^{+\cdot}$ 276.0778; found 276.0778

Elemental analysis calcd. (%) for $C_{13}H_{13}ClN_4O$ (276.7): C 56.42, H 4.74, N 20.25; found C 56.03, H 4.84, N 19.89

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{13}H_{13}ClN_4O$

MW: 276.72 g/mol



8-Chloro-1,6-diethyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepine (29c)

According to **GP1**, compound **29c** was synthesized, using 7-chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (900 mg, 3.77 mmol, 1.00 equiv) and propanoic acid hydrazide **27c** (664 mg, 7.54 mmol, 2.00 equiv) dissolved in 15 mL of n-butanol. After purification (R_f = 0.47), product **29c** (464 mg, 1.69 mmol, 45 %) was obtained as yellow solid.

mp: 203.6 – 205.9 °C

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.07 (t, $^3J_{HH}$ = 7.4 Hz, 3 H, 2''-H), 1.34 (t, $^3J_{HH}$ = 7.5 Hz, 3 H, 2'-H), 2.60 – 2.70 (m, 1 H, 1''-*H*H), 2.77 – 2.89 (m, 2 H, 1'-*H*H, 1''-H*H*), 3.00 – 3.11 (m, 1 H, 1'-H*H*), 3.92 (d, $^2J_{HH}$ = 13.0 Hz, 1 H, 4-*H*H), 5.28 (d, $^2J_{HH}$ = 13.0 Hz, 1 H, 4-H*H*), 7.37 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 10-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H), 7.67 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 11.1 (C-2''), 11.5 (C-2'), 19.8 (C-1'), 32.4 (C-1''), 45.7 (C-4), 124.6 (C-10), 128.8 (C-7), 130.9 (C-10a), 131.1 (C-9), 131.8 (C-6a), 133.8 (C-8), 154.8 (C-1), 155.1 (C-3a), 171.0 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2986 (m), 2934 (m), 2873 (m), 2362 (w), 2343 (w), 1630 (m), 1540 (m), 1485 (s), 1430 (s), 1261 (s), 1108 (s), 1027 (s), 818 (s), 799 (s)

MS (CI): *m/z* (%) = 277 (30), 275 (100) $[M + H]^+$

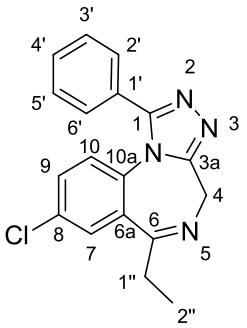
MS (EI): *m/z* (%) = 276 (10), 274 (30) $[M]^{+\cdot}$, 245 (40), 239 (100)

HR-MS (EI): calcd. for $C_{14}H_{15}ClN_4$ $[M]^{+\cdot}$ 274.0985; found 274.0987

HPLC purity: 91 % [λ = 210 nm], 93 % [λ = 254 nm]

MF: $C_{14}H_{15}ClN_4$

MW: 274.75 g/mol



8-Chloro-6-ethyl-1-phenyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepine (29d)

According to **GP1**, compound **29d** was synthesized, using 7-chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (515 mg, 2.16 mmol, 1.00 equiv) and benzhydrazide **27d** (587 mg, 4.31 mmol, 2.00 equiv) dissolved in 10 mL of n-butanol. After purification (R_f = 0.49), product **29d** (422 mg, 1.31 mmol, 61 %) was obtained as yellow solid.

mp: 176.8 °C

^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) = 1.13 (t, $^3J_{HH}$ = 7.3 Hz, 3 H, 2''-H), 2.67 – 2.80 (m, 1 H, 1''-*H*H), 2.82 – 2.96 (m, 1 H, 1''-H*H*), 4.00 (d, $^2J_{HH}$ = 13.0 Hz, 1 H, 4-*H*H), 5.21 (d, $^2J_{HH}$ = 13.1 Hz, 1 H, 4-H*H*), 6.87 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.30 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.37 – 7.52 (m, 5 H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 7.69 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 11.2 (C-2''), 32.8 (C-1''), 46.2 (C-4), 126.8 (C-10), 127.2 (C-1'), 128.8 (C-2', C-6'), 128.9 (C-7), 129.3 (C-3', C-5'), 130.7 (C-4'), 131.1 (C-9), 132.0 (C-10a), 132.2 (C-6a), 133.9 (C-8), 153.6 (C-1), 157.3 (C-3a), 171.9 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3057 (w), 2970 (m), 2930 (m), 2854 (m), 2365 (w), 2345 (w), 1631 (m), 1534 (m), 1485 (s), 1472 (s), 1422 (s), 1287 (s), 1107 (s), 977 (m), 821 (m), 768 (m), 696 (m)

MS (CI): *m/z* (%) = 325 (35), 323 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 324 (15), 322 (50) $[M]^{+\cdot}$, 293 (50), 287 (100)

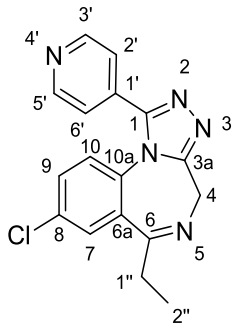
HR-MS (EI): calcd. for $C_{18}H_{15}ClN_4$ $[M]^{+\cdot}$ 322.0985; found 322.0963

Elemental analysis calcd. (%) for $C_{18}H_{15}ClN_4$ (322.8): C 66.98, H 4.68, N 17.36; found C 65.57, H 4.78, N 17.01

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{18}H_{15}ClN_4$

MW: 322.79 g/mol



8-Chloro-6-ethyl-1-(pyridin-4-yl)-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]-diazepine (29e)

According to **GP1**, compound **29e** was synthesized, using 7-chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (55 mg, 0.23 mmol, 1.0 equiv) and isonicotinic acid hydrazide **27e** (63 mg, 0.46 mmol, 2.0 equiv) dissolved in 4 mL of n-butanol. After purification (R_f = 0.49), product **29e** (13 mg, 0.04 mmol, 17 %) was obtained as yellow solid.

mp: 157.3 – 159.9 °C

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.16 (t, $^3J_{HH}$ = 7.4 Hz, 3 H, 2''-H), 2.72 – 2.82 (m, 1 H, 1''-*H*H), 2.88 – 2.98 (m, 1 H, 1''-H*H*), 4.01 (d, $^2J_{HH}$ = 13.3 Hz, 1 H, 4-*H*H), 5.36 (d, $^2J_{HH}$ = 13.3 Hz, 1 H, 4-H*H*), 6.90 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 (dd, $^4J_{HH}$ = 2.3 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.41 (dd, $^3J_{HH}$ = 6.2 Hz, 2 H, 2'-H, 6'-H), 7.72 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H), 8.72 (d, $^3J_{HH}$ = 5.7 Hz, 2 H, 3'-H, 5'-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 11.3 (C-2''), 32.7 (C-1''), 45.9 (C-4), 122.3 (C-2', C-6'), 126.4 (C-10), 129.0 (C-7), 131.1 (C-10a), 131.3 (C-9), 131.9 (C-6a), 134.3 (C-1'), 134.7 (C-8), 150.9 (C-3', C-5'), 151.2 (C-1), 158.0 (C-3a), 171.7 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3036 (m), 2971 (m), 2927 (m), 2853 (m), 2366 (w), 2344 (w), 2225 (w), 1633 (m), 1604 (m), 1531 (m), 1484 (s), 1467 (s), 1434 (s), 1290 (m), 1110 (m), 985 (m), 827 (s), 728 (m), 576 (m)

MS (CI): *m/z* (%) = 326 (35), 324 (100) $[M + H]^+$

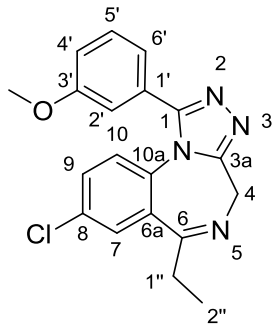
MS (EI): *m/z* (%) = 325 (10), 323 (25) $[M]^{+\cdot}$, 294 (50), 288 (100), 78 (40)

HR-MS (EI): calcd. for $C_{17}H_{14}ClN_5$ $[M]^{+\cdot}$ 323.0938; found 323.0934

HPLC purity: 80 % [λ = 210 nm], 80 % [λ = 254 nm]

MF: $C_{17}H_{14}ClN_5$

MW: 323.78 g/mol



8-Chloro-6-ethyl-1-(3-methoxyphenyl)-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]-diazepine (29f)

According to **GP1**, compound **29f** was synthesized, using 7-chloro-5-ethyl-1*H*-benzo[*e*][1,4]diazepine-2(3*H*)-thione **2** (66 mg, 0.28 mmol, 1.0 equiv) and 3-methoxybenzoic acid hydrazide **27f** (92 mg, 0.55 mmol, 2.0 equiv) dissolved in 3 mL of n-butanol. After purification (R_f = 0.50), product **29f** (65 mg, 0.18 mmol, 67 %) was obtained as yellow solid.

mp: 132.6 – 133.7 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.14 (t, $^3J_{HH}$ = 7.4 Hz, 3 H, 2''-H), 2.70 – 2.82 (m, 1 H, 1''-*H*H), 2.83 – 2.96 (m, 1 H, 1''-H*H*), 3.81 (s, 3 H, OCH_3), 4.00 (d, $^2J_{HH}$ = 13.4 Hz, 1 H, 4-*H*H), 5.33 (d, $^2J_{HH}$ = 13.2 Hz, 1 H, 4-H*H*), 6.88 (ddd, $^4J_{HH}$ = 1.0 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.7 Hz, 1 H, 6'-H), 6.91 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.00 (ddd, $^4J_{HH}$ = 1.0 Hz, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 8.4 Hz, 1 H, 4'-H), 7.14 (dd, $^4J_{HH}$ = 1.5 Hz, $^4J_{HH}$ = 2.4 Hz, 1 H, 2'-H), 7.30 (dd, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 8.4 Hz, 1 H, 5'-H), 7.32 (dd, 1 H, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 9-H), 7.66 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 11.2 (C-2''), 32.6 (C-1''), 45.9 (C-4), 55.4 (OCH_3), 113.7 (C-2'), 116.4 (C-4'), 120.6 (C-6'), 126.4 (C-10), 127.6 (C-1'), 128.4 (C-7), 130.0 (C-5'), 130.8 (C-9), 131.4 (C-10a), 131.5 (C-6a), 133.7 (C-8), 153.2 (C-1), 156.9 (C-3a), 159.9 (C-3'), 171.7 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3072 (w), 2970 (w), 2935 (w), 1632 (m), 1583 (m), 1535 (m), 1485 (s), 1466 (m), 1435 (m), 1319 (w), 1287 (m), 1238 (m), 1108 (m), 1045 (m), 994 (w), 753 (m)

MS (CI): *m/z* (%) = 355 (35), 353 (100) $[M + H]^+$

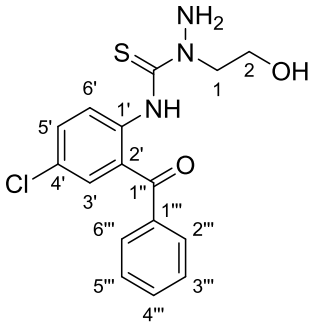
MS (EI): *m/z* (%) = 354 (30), 352 (80) $[M]^{+\cdot}$, 323 (65), 317 (100)

HR-MS (EI): calcd. for $C_{19}H_{17}ClN_4O$ $[M]^{+\cdot}$ 352.1091; found 352.1098

HPLC purity: 89 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{19}H_{17}ClN_4O$

MW: 352.82 g/mol



N-(2-Benzoyl-4-chlorophenyl)-1-(2-hydroxyethyl)hydrazinecarbothioamide (42)

(Literature known compound but different procedure[109])

(5-Chloro-2-isothiocyanatophenyl)(phenyl)methanone **6** (600 mg, 2.19 mmol, 1.00 equiv) and 2-hydroxyethylhydrazine **41** (178 µL, 2.63 mmol, 1.2 equiv) were dissolved in tetrahydrofuran (8 mL) under ice-cooling and stirred for 15 min, followed by additional 45 min at room temperature. The reaction mixture was concentrated under reduced pressure and crystallization was done in diethyl ether (80 mL) yielding compound **42** (641 mg, 1.22 mmol, 84 %) as pale yellow solid.

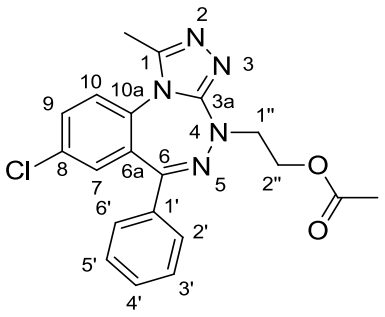
^{1}H NMR (500 MHz, MeOD): δ (ppm) = 3.89 (t, $^{3}J_{HH}$ = 5.2 Hz, 2 H, 2-H), 4.22 (t, $^{3}J_{HH}$ = 5.2 Hz, 2 H, 1-H), 7.42 (d, $^{4}J_{HH}$ = 2.5 Hz, 1 H, 3'-H), 7.49 – 7.54 (m, 3 H, 5'-H, 3'''-H, 5'''-H), 7.62 – 7.67 (m, 1 H, 4'''-H), 7.78 – 7.82 (m, 2 H, 2'''-H, 6'''-H), 8.35 (d, $^{3}J_{HH}$ = 8.8 Hz, 1 H, 6-H)

^{13}C NMR (100 MHz, MeOD): δ (ppm) = 56.9 (C-2), 61.1 (C-1), 123.1 (C-4'), 129.3 (C-6'), 129.5 (C-3''', C-5'''), 130.2 (C-2'), 131.2 (C-3'), 131.2 (C-2''', C-6'''), 132.3 (C-5'), 134.4 (C-4'''), 138.4 (C-1'''), 139.3 (C-1'), 181.1 (CS), 197.2 (C-1'')

MS (CI): *m/z* (%) = 352 (2), 350 (7) $[M + H]^{+}$, 332 (70), 274 (100)

MF: $C_{16}H_{16}ClN_3O_2S$

MW: 349.84 g/mol



4-(2-Acetoxyethyl)-8-chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (44a)

8-Chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-[1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) was suspended in 5 mL of acetic acid anhydride and treated with 0.2 mL of conc. sulfuric acid. After stirring for 1 h at 80 °C the mixture was cooled to room temperature. Water (5 mL) was added slowly to the vigorously stirred solution and the mixture was extracted with dichloromethane (3 x 7 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 9:1, R_f = 0.64), giving compound **44a** (87 mg, 0.22 mmol, 78 %) as pale yellow solid.

mp: 83.2 – 85.4 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 1.92 (s, 3 H, O(CO)CH_3), 2.55 (s, 3 H, 1-CH_3), 3.90 – 4.03 (m, 1 H, 1''-*H*H), 4.07 – 4.18 (m, 1 H, 1''-H*H*), 4.32 – 4.42 (m, 1 H, 2''-*H*H), 4.43 – 4.54 (m, 1 H, 2''-H*H*), 7.26 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.33 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.45 – 7.50 (m, 1 H, 4'-H), 7.53 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 21.0 (O(CO)CH_3), 53.1 (C-1''), 61.9 (C-2''), 124.3 (C-10), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.8 (C-6a),

130.9 (C-4'), 131.5 (C-7), 132.1 (C-9), 132.8 (C-8), 133.8 (C-10a), 136.5 (C-1'), 148.6 (C-1), 160.2 (C-3a), 161.6 (C-6), 171.0 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3061 (w), 2958 (w), 2925 (w), 2363 (w), 2342 (w), 1739 (s), 1519 (s), 1490 (m), 1434 (m), 1231 (s), 1050 (w), 829 (w), 696 (w)

MS (CI): *m/z* (%) = 398 (35), 396 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 397 (8), 395 (20) $[M]^{+\cdot}$, 240 (100), 77 (65)

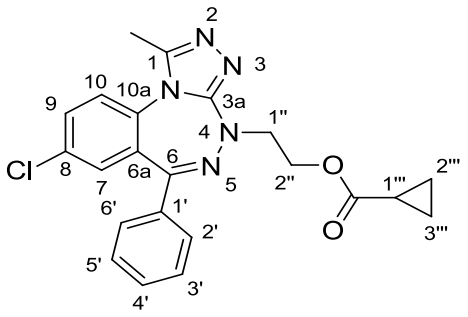
HR-MS (EI): calcd. for $C_{20}H_{18}ClN_5O_2$ $[M]^{+\cdot}$ 395.1149; found 395.1150

Elemental analysis calcd. (%) for $C_{20}H_{18}ClN_5O_2$ (395.8): C 60.68, H 4.58, N 17.69; found C 60.22, H 4.63, N 17.32

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{20}H_{18}ClN_5O_2$

MW: 395.84 g/mol



8-Chloro-4-{2-[(cyclopropanecarbonyl)oxy]ethyl}-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44b)

8-Chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-[1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) was dissolved in 2 mL of anhydrous tetrahydrofuran and treated with *N*,*N*-diisopropylethylamine (48 µL, 0.28 mmol, 1.0 equiv). After stirring for 10 min at room temperature cyclopropanecarbonyl chloride (26 µL, 0.28 mmol, 1.0 equiv) was added and stirring was continued for 5 min. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.60), giving compound **44b** (32 mg, 0.08 mmol, 38 %) as pale yellow solid.

mp: 100.9 – 101.8 °C

^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) = 0.67 – 0.82 (m, 4 H, 2'''-H, 3'''-H), 1.43 – 1.52 (m, 1 H, 1'''-H), 2.55 (s, 3 H, 1-CH_3), 3.90 – 4.03 (m, 1 H, 1''-*H*H), 4.04 – 4.17 (m, 1 H, 1''-H*H*), 4.28 – 4.39 (m, 1 H, 2''-*H*H), 4.48 – 4.60 (m, 1 H, 2''-H*H*), 7.26 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.46 – 7.51 (m, 1 H, 4'-H), 7.53 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 8.4 (C-2''', C-3'''), 12.8 (1-CH_3), 13.0 (C-1'''), 53.3 (C-1''), 61.7 (C-2''), 124.3 (C-10), 128.9 (C-3', C-5'), 129.6 (C-2', C-6'), 130.9 (C-4'), 130.9 (C-6a), 131.6 (C-7), 132.0 (C-9), 132.9 (C-8), 133.9 (C-10a), 136.5 (C-1'), 148.5 (C-1), 160.2 (C-3a), 161.7 (C-6), 174.7 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3063 (w), 2924 (w), 2368 (w), 1726 (s), 1535 (m), 1520 (s), 1490 (m), 1446 (m), 1381 (m), 1350 (m), 1320 (m), 1272 (m), 1174 (s), 1100 (m), 1031 (m), 826 (w), 777 (w), 696 (w)

MS (CI): *m/z* (%) = 424 (30), 422 (100) $[M + H]^+$, 159 (20), 113 (25), 101 (10)

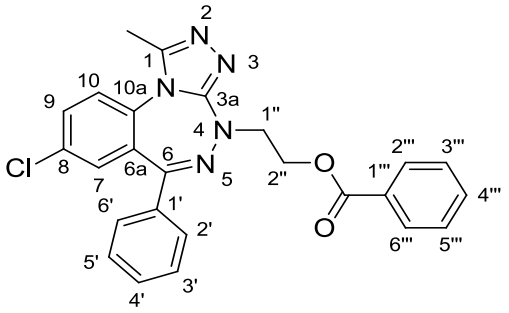
MS (EI): *m/z* (%) = 423 (4), 421 (15) $[M]^{+\cdot}$, 335 (10), 267 (15), 240 (100), 219 (25)

HR-MS (EI): calcd. for $C_{22}H_{20}ClN_5O_2$ $[M]^{+\cdot}$ 421.1306; found 421.1305

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{22}H_{20}ClN_5O_2$

MW: 421.88 g/mol



4-[2-(Benzoyloxy)ethyl]-8-chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (44c)

Benzoic acid anhydride (96 mg, 0.42 mmol, 3.0 equiv) and 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **5** (50 mg, 0.14 mmol, 1.0 equiv) were dissolved in 2 mL of anhydrous tetrahydrofuran. Then 0.2 mL of conc. sulfuric acid was added before the mixture was heated to reflux for 1 h. Water (5 mL) was added slowly to the vigorously stirred solution and the mixture was extracted with dichloromethane (3 x 7 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.55), giving compound **44c** (24 mg, 0.04 mmol, 37 %) as pale yellow solid.

mp: 101.6 – 102.7 °C

^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) = 2.53 (s, 3 H, 1-CH_3), 4.04 – 4.15 (m, 1 H, 1''-*H*H), 4.20 – 4.33 (m, 1 H, 1''-H*H*), 4.54 – 4.66 (m, 1 H, 2''-*H*H), 4.73 – 4.86 (m, 1 H, 2''-H*H*), 7.20 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.23 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.33 – 7.43 (m, 4 H, 3'-H, 5'-H, 3'''-H, 5'''-H), 7.44 – 7.57 (m, 5 H, 9-H, 2'-H, 4'-H, 6'-H, 4'''-H), 7.81 – 7.87 (m, 2 H, 2'''-H, 6'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 53.0 (C-1''), 62.3 (C-2''), 124.1 (C-10), 128.6 (C-3''', C-5'''), 128.8 (C-3', C-5'), 129.6 (C-2', C-6'), 129.8 (C-2''', C-6'''), 130.6 (C-1'''), 130.9 (C-6a), 130.9 (C-4'), 130.9 (C-6a), 131.5 (C-7),

131.9 (C-9), 132.8 (C-8), 133.2 (C-4'''), 133.9 (C-10a), 136.4 (C-1'), 148.5 (C-1), 160.2 (C-3a), 162.0 (C-6), 166.5 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2926 (w), 2363 (w), 1719 (s), 1535 (m), 1520 (m), 1490 (m), 1449 (m), 1273 (s), 1175 (m), 1102 (m), 1027 (w), 713 (m)

MS (CI): *m/z* (%) = 460 (30), 458 (100) $[M + H]^+$, 165 (25), 149 (20), 105 (25)

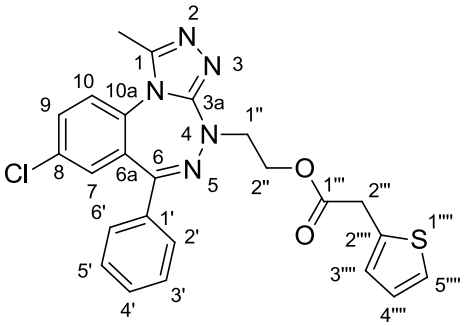
MS (EI): *m/z* (%) = 457 (8) $[M]^{+\cdot}$, 240 (80), 149 (30), 105 (55), 77 (100)

HR-MS (EI): calcd. for $C_{25}H_{20}ClN_5O_2$ $[M]^{+\cdot}$ 457.1306; found 457.1306

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{25}H_{20}ClN_5O_2$

MW: 457.91 g/mol



8-Chloro-1-methyl-6-phenyl-4-{2-[2-(thiophen-2-yl)acetoxy]ethyl}-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44d)

A 10 mL flask was charged with 5 mL of dichloromethane, 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **5** (150 mg, 0.424 mmol, 1.00 equiv), 2-thiopheneacetic acid (121 mg, 0.848 mmol, 2.00 equiv), *N*,*N*'-dicyclohexylcarbodiimide (192 mg, 0.933 mmol, 2.20 equiv) and 4-(dimethylamino)pyridine (16 mg, 0.13 mmol, 0.3 equiv). After stirring for 20 h at room temperature the solvent was evaporated. Purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 9:1, R_f = 0.51), giving compound **44d** (124 mg, 0.259 mmol, 61 %) as yellow solid.

mp: 78.2 – 80.5 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 3.71 (d, $^4J_{HH}$ = 2.9 Hz, 2 H, 2'''-H), 3.93 – 4.02 (m, 1 H, 1''-*H*H), 4.12 – 4.22 (m, 1 H, 1''-H*H*), 4.37 – 4.47 (m, 1 H, 2''-*H*H), 4.56 – 4.66 (m, 1 H, 2''-H*H*), 6.75 – 6.79 (m, 1 H, 3''''-H), 6.86 (dd, $^3J_{HH}$ = 3.5 Hz, $^3J_{HH}$ = 5.2 Hz, 1 H, 4''''-H), 7.13 (dd, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 5.2 Hz, 1 H, 5''''-H), 7.23 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.37 – 7.43 (m, 2 H, 3'-H, 5'-H), 7.43 – 7.48 (m, 1 H, 4'-H), 7.53 – 7.56 (m, 2 H, 2'-H, 6'-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 35.4 (C-2'''), 53.1 (C-1''), 62.3 (C-2''), 124.3 (C-10), 125.3 (C-5''''), 127.0 (C-4''''), 127.0 (C-3''''), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.7 (C-6a), 130.8 (C-4'), 131.5 (C-7), 132.1 (C-9),

132.8 (C-8), 133.7 (C-10a), 135.5 (C-2''''), 136.5 (C-1'), 148.6 (C-1), 160.0 (C-3a), 161.6 (C-6), 170.4 (C-1''')

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2957 (w), 2914 (w), 2360 (w), 1736 (s), 1536 (m), 1519 (s), 1489 (m), 1433 (m), 1320 (m), 1172 (s), 825 (m), 695 (s)

MS (CI): *m/z* (%) = 480 (40), 478 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 479 (10), 477 (35) $[M]^{+\cdot}$, 354 (60), 295 (100), 97 (55), 77 (45)

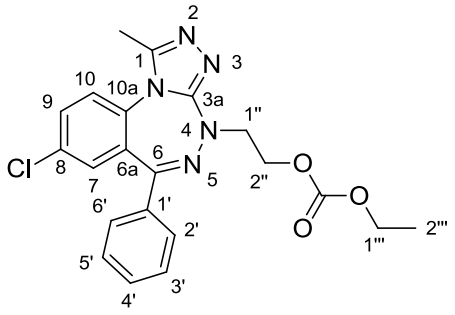
HR-MS (EI): calcd. for $C_{24}H_{20}ClN_5O_2S$ $[M]^{+\cdot}$ 477.1026; found 477.1022

Elemental analysis calcd. (%) for $C_{24}H_{20}ClN_5O_2S$ (478.0): C 60.31, H 4.22, N 14.65; found C 59.03, H 4.22, N 14.17

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{20}ClN_5O_2S$

MW: 477.97 g/mol



8-Chloro-4-{2-[(ethoxycarbonyl)oxy]ethyl}-1-methyl-6-phenyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44e)

8-Chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-[1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) was dissolved in 5 mL of diethyl carbonate. Sodium hydride (23 mg, 0.57 mmol, 2.0 equiv) was added portionwise as a 60 % dispersion in mineral oil to the well stirred solution and the mixture was heated for 3 h at 130 °C. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.55), giving compound **44e** (109 mg, 0.256 mmol, 91 %) as pale yellow solid.

mp: 81.0 – 82.1 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 1.16 (t, $^3J_{HH}$ = 7.1 Hz, 3 H, 2'''-H), 2.55 (s, 3 H, 1-CH_3), 3.93 – 4.01 (m, 1 H, 1''-*H*H), 4.02 (q, $^3J_{HH}$ = 7.1 Hz, 2 H, 1'''-H), 4.11 – 4.21 (m, 1 H, 1''-H*H*), 4.38 – 4.47 (m, 1 H, 2''-*H*H), 4.53 – 4.63 (m, 1 H, 2''-H*H*), 7.27 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.34 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.46 – 7.50 (m, 1 H, 4'-H), 7.54 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.62 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 14.3 (C-2'''), 53.4 (C-1''), 64.3 (C-1'''), 65.0 (C-2''), 124.4 (C-10), 128.8 (C-3', C-5'), 129.6 (C-2', C-6'), 130.8 (C-6a), 130.9 (C-4'), 131.6 (C-7), 132.1 (C-9), 132.9 (C-8), 133.8 (C-10a), 136.5 (C-1'), 148.6 (C-1), 155.4 (CO), 160.0 (C-3a), 161.8 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3063 (w), 2982 (w), 2933 (w), 2346 (w), 1745 (s), 1520 (m), 1490 (m), 1445 (m), 1373 (w), 1320 (w), 1262 (s), 1173 (w), 1103 (w), 1022 (w)

MS (CI): *m/z* (%) = 428 (35), 426 (100) $[M + H]^+$

MS (EI): *m/z* (%) = 427 (4), 425 (15) $[M]^{+\cdot}$, 253 (25), 240 (100), 219 (30)

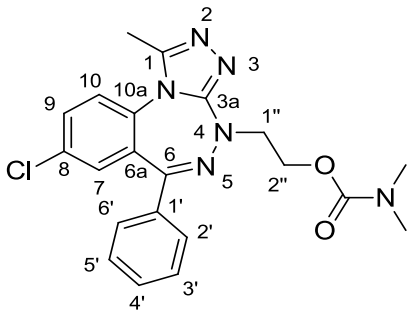
HR-MS (EI): calcd. for $C_{21}H_{20}ClN_5O_3$ $[M]^{+\cdot}$ 425.1255; found 425.1254

Elemental analysis calcd. (%) for $C_{21}H_{20}ClN_5O_3$ (425.9): C 59.23, H 4.73, N 16.44; found C 59.12, H 4.78, N 16.30

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{21}H_{20}ClN_5O_3$

MW: 425.87 g/mol



8-Chloro-4-{2-[(dimethylcarbamoyl)oxy]ethyl}-1-methyl-6-phenyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44f)

Dimethylcarbamoyl chloride (117 µL, 1.27 mmol, 3.00 equiv) was added to a solution of 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **5** (150 mg, 0.424 mmol, 1.00 equiv) in 2 mL of anhydrous tetrahydrofuran. Sodium hydride (34 mg, 0.85 mmol, 2.0 equiv) was added portionwise as a 60 % dispersion in mineral oil to the well stirred solution. After stirring for 18 h at room temperature, the solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane / methanol 9:1, R_f = 0.56), giving compound **44f** (124 mg, 0.292 mmol, 69 %) as pale yellow solid.

mp: 61.0 – 62.3 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 2.75 (s, 6 H, N(CH_3)$_2$), 3.88 – 4.03 (m, 1 H, 1''-*H*H), 4.06 – 4.20 (m, 1 H, 1''-H*H*), 4.29 – 4.39 (m, 1 H, 2''-*H*H), 4.39 – 4.51 (m, 1 H, 2''-H*H*), 7.26 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.33 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.38 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.45 – 7.50 (m, 1 H, 4'-H), 7.54 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 38.8 (N(CH_3)$_2$), 53.4 (C-1''), 62.7 (C-2''), 124.3 (C-10), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.8 (C-4'), 130.9 (C-6a), 131.4 (C-7), 132.0 (C-9), 132.8 (C-8), 133.9 (C-10a), 136.5 (C-1'), 148.5 (C-1), 156.5 (CO), 160.3 (C-3a), 161.6 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2928 (w), 2369 (w), 1703 (s), 1641 (m), 1520 (m), 1490 (m), 1445 (m), 1379 (m), 1188 (m), 696 (w)

MS (CI): *m/z* (%) = 427 (10), 425 (30) $[M + H]^+$, 117 (100)

MS (EI): *m/z* (%) = 424 (5) $[M]^{+\cdot}$, 240 (100), 72 (65)

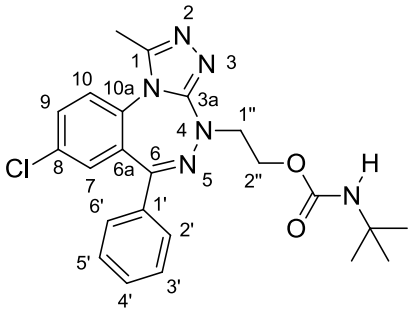
HR-MS (EI): calcd. for $C_{21}H_{21}ClN_6O_2$ $[M]^{+\cdot}$ 424.1415; found 424.1413

Elemental analysis calcd. (%) for $C_{21}H_{21}ClN_6O_2$ (424.9): C 59.36, H 4.98, N 19.78; found C 57.85, H 5.38, N 19.54

HPLC purity: 94 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{21}H_{21}ClN_6O_2$

MW: 424.88 g/mol



4-{2-[(tert-Butylcarbamoyl)oxy]ethyl}-8-chloro-1-methyl-6-phenyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44g)

tert-Butyl isocyanate (65 µL, 0.57 mmol, 2.0 equiv) was added to a solution of 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-[1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) in 2 mL of anhydrous tetrahydrofuran. Sodium hydride (17 mg, 0.42 mmol, 1.5 equiv) was added portionwise as a 60 % dispersion in mineral oil to the well stirred solution. After stirring for 3 h at room temperature, the solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.60), giving compound **44g** (85 mg, 0.19 mmol, 66 %) as pale yellow solid.

mp: 119.6 – 120.0 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 1.18 (bs, 9 H, C(CH_3)$_3$), 2.54 (s, 3 H, 1-CH_3), 3.85 – 3.96 (m, 1 H, 1''-*H*H), 4.00 – 4.14 (m, 1 H, 1''-H*H*), 4.21 – 4.36 (m, 1 H, 2''-*H*H), 4.40 – 4.52 (m, 1 H, 2''-H*H*), 4.71 (bs, 1 H, NH), 7.25 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.39 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.45 – 7.50 (m, 1 H, 4'-H), 7.54 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 28.9 (C(*C*H_3)$_3$), 50.4 (*C*(CH_3)$_3$), 53.9 (C-1''), 61.3 (C-2''), 124.3 (C-10), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.8 (C-4'), 130.9 (C-6a), 131.6 (C-7), 132.0 (C-9), 132.9 (C-8), 133.8 (C-10a), 136.6 (C-1'), 148.5 (C-1), 154.8 (CO), 160.2 (C-3a), 161.4 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3061 (w), 2967 (m), 2933 (m), 2371 (w), 2345 (w), 1720 (s), 1520 (s), 1446 (m), 1267 (s), 1096 (s), 824 (m), 777 (m), 696 (m)

MS (CI): *m/z* (%) = 455 (40), 453 (100) $[M + H]^+$, 354 (20)

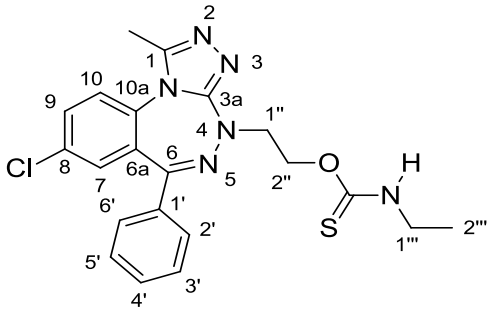
MS (EI): *m/z* (%) = 452 (3) $[M]^{+\cdot}$, 335 (20), 295 (35), 240 (100), 219 (20), 77 (30)

HR-MS (EI): calcd. for $C_{23}H_{25}ClN_6O_2$ $[M]^{+\cdot}$ 452.1728; found 452.1730

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{25}ClN_6O_2$

MW: 452.94 g/mol



8-Chloro-4-{2-[(ethylcarbamothioyl)oxy]ethyl}-1-methyl-6-phenyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44h)

Ethyl isothiocyanate (50 µL, 0.57 mmol, 2.0 equiv) was added to a solution of 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*]-[1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) in 2 mL of anhydrous tetrahydrofuran. Sodium hydride (17 mg, 0.42 mmol, 1.5 equiv) was added portionwise as a 60 % dispersion in mineral oil to the well stirred solution. After stirring for 3 h at room temperature, the solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.50), giving compound **44h** (106 mg, 0.240 mmol, 85 %) as pale yellow solid.

mp: 115.6 – 117.3 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 0.89 (t, $^3J_{HH}$ = 7.3 Hz, 0.37 x 3 H, 2'''-H), 1.11 (t, $^3J_{HH}$ = 7.3 Hz, 0.63 x 3 H, 2'''-H), 2.54 (s, 0.63 x 3 H, 1-CH_3), 2.55 (s, 0.37 x 3 H, 1-CH_3), 3.04 – 3.12 (m, 0.37 x 2 H, 1'''-H), 3.39 – 3.51 (m, 0.63 x 2 H, 1'''-H), 3.93 – 4.33 (m, 2 H, 1''-H), 4.67 – 4.93 (m, 2 H, 2''-H), 6.58 (bs, 0.63 x 1 H, NH), 6.81 (bs, 0.37 x 1 H, NH), 7.26 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.38 – 7.45 (m, 2 H, 3'-H, 5'-H), 7.45 – 7.51 (m, 1 H, 4'-H), 7.54 – 7.59 (m, 2 H, 2'-H, 6'-H), 7.60 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 12.8 (1-CH_3), 13.8 (C-2'''), 14.2 (C-2'''), 38.4 (C-1'''), 40.5 (C-1'''), 52.8 (C-1''), 53.4 (C-1''), 67.2 (C-2''), 68.6 (C-2''), 124.3 (C-10), 128.8 (C-3', C-5'), 128.9 (C-3', C-5'), 129.6 (C-2', C-6'),

129.6 (C-2', C-6'), 130.8 (C-6a), 130.9 (C-4'), 130.9 (C-4'), 131.5 (C-7), 131.7 (C-7), 132.0 (C-9), 132.8 (C-8), 133.8 (C-10a), 133.9 (C-10a), 136.3 (C-1'), 136.5 (C-1'), 148.6 (C-1), 160.2 (C-3a), 161.6 (C-6), 161.8 (C-6), 190.0 (CS), 190.3 (CS)

IR [cm^{-1}]: $\tilde{\nu}$ = 3209 (w), 3056 (w), 2933 (m), 2972 (w), 2932 (w), 1733 (w), 1536 (s), 1520 (s), 1489 (m), 1432 (m), 1321 (m), 1194 (m), 824 (w), 695 (w)

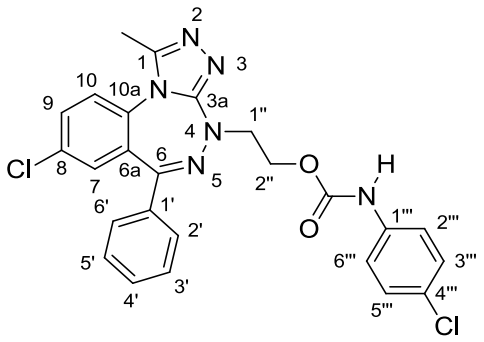
MS (CI): *m/z* (%) = 443 (20), 441 (50) $[M + H]^+$, 354 (100)

HR-MS (EI): calcd. for $C_{21}H_{21}ClN_6OS$ $[M]^{+\cdot}$ 440.1186; found 440.1186

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{21}H_{21}ClN_6OS$

MW: 440.95 g/mol



8-Chloro-4-{2-[((4-chlorophenyl)carbamoyl)oxy]ethyl}-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44i)

4-Chlorophenyl isocyanate (72 µL, 0.57 mmol, 2.0 equiv) and 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) were dissolved in 2 mL of anhydrous tetrahydrofuran. Sodium hydride (17 mg, 0.42 mmol, 1.5 equiv) was added portionwise as a 60 % dispersion in mineral oil to the well stirred solution. The mixture was stirred at room temperature for 18 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.58), giving compound **44i** (92 mg, 0.18 mmol, 64 %) as pale yellow solid.

mp: 112.8 – 113.9 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 2.53 (s, 0.07 x 3 H, 1-CH_3), 2.54 (s, 0.93 x 3 H, 1-CH_3), 3.92 – 4.04 (m, 1 H, 1''-*H*H), 4.12 – 4.26 (m, 1 H, 1''-H*H*), 4.35 – 4.49 (m, 1 H, 2''-*H*H), 4.54 – 4.68 (m, 1 H, 2''-H*H*), 7.18 – 7.21 (m, 2 H, 3'''-H, 5'''-H), 7.23 (d, $^4J_{HH}$ = 2.3 Hz, 0.93 x 1 H, 7-H), 7.25 (d, $^3J_{HH}$ = 8.7 Hz, 0.93 x 1 H, 10-H), 7.26 – 7.30 (m, 0.07 x 1 H, 7-H, 2 H, 2'''-H, 6'''-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 0.07 x 1 H, 10-H), 7.37 – 7.43 (m, 2 H, 3'-H, 5'-H), 7.44 – 7.52 (m, 2 H, 4'-H, NH), 7.50 (dd, $^4J_{HH}$ = 2.3 Hz, $^3J_{HH}$ = 8.7 Hz, 0.93 x 1 H, 9-H), 7.53 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 0.07 x 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 53.6 (C-1''), 62.4 (C-2''), 120.1 (C-2''', C-6'''), 124.2 (C-10), 128.1 (C-4'''), 128.9 (C-3', C-5'), 129.2 (C-3''',

C-5'''), 129.6 (C-2', C-6'), 130.8 (C-6a), 130.9 (C-4'), 131.6 (C-7), 132.0 (C-9), 132.9 (C-8), 133.7 (C-10a), 136.5 (C-1'), 137.5 (C-1'''), 148.6 (C-1), 153.6 (CO), 160.1 (C-3a), 161.9 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3240 (m), 3183 (m), 3054 (m), 2959 (m), 1731 (s), 1599 (m), 1537 (s), 1520 (s), 1493 (s), 1308 (m), 1221 (s), 1091 (m), 1074 (m), 826 (m), 695 (m)

MS (CI): *m/z* (%) = 509 (10), 507 (3) $[M + H]^+$, 396 (20), 354 (60), 154 (90), 128 (100)

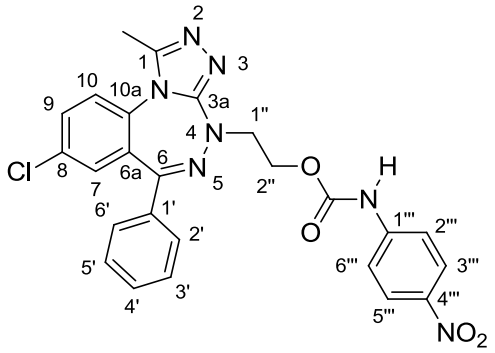
MS (EI): *m/z* (%) = 506 (2) $[M]^{+\cdot}$, 295 (100), 219 (25), 153 (75)

HR-MS (ESI): calcd. for $C_{25}H_{21}Cl_2N_6O_2^+$ $[M + H]^+$ 507.1098; found 507.1100

HPLC purity: 93 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{25}H_{20}Cl_2N_6O_2$

MW: 507.37 g/mol



8-Chloro-1-methyl-4-{2-[((4-nitrophenyl)carbamoyl)oxy]ethyl}-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (44j)

4-Nitrophenyl isocyanate (46 µL, 0.34 mmol, 1.2 equiv) and 8-chloro-4-(2-hydroxyethyl)-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **5** (100 mg, 0.283 mmol, 1.00 equiv) were dissolved in 2 mL of anhydrous tetrahydrofuran. Sodium hydride (17 mg, 0.42 mmol, 1.5 equiv) was added portionwise as a 60 % dispersion in mineral oil to the well stirred solution. The mixture was stirred at room temperature for 3 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / methanol 9:1, R_f = 0.53), giving compound **44j** (53 mg, 0.10 mmol, 36 %) as yellow solid.

mp: 153.2 – 155.6 °C

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 2.54 (s, 0.92 x 3 H, 1-CH_3), 2.55 (s, 0.08 x 3 H, 1-CH_3), 3.95 – 4.08 (m, 1 H, 1''-*H*H), 4.16 – 4.31 (m, 1 H, 1''-H*H*), 4.36 – 4.51 (m, 1 H, 2''-*H*H), 4.64 – 4.77 (m, 1 H, 2''-H*H*), 7.25 (d, $^4J_{HH}$ = 2.4 Hz, 0.92 x 1 H, 7-H), 7.26 (d, $^3J_{HH}$ = 8.7 Hz, 0.92 x 1 H, 10-H), 7.27 (d, $^4J_{HH}$ = 2.4 Hz, 0.08 x 1 H, 7-H), 7.33 (d, $^3J_{HH}$ = 8.7 Hz, 0.08 x 1 H, 10-H), 7.38 – 7.44 (m, 2 H, 3'-H, 5'-H), 7.45 – 7.50 (m, 2 H, 9-H, 4'-H), 7.54 (d, $^3J_{HH}$ = 9.2 Hz, 0.92 x 2 H, 2'''-H, 6'''-H), 7.55 – 7.58 (m, 2 H, 2'-H, 6'-H), 7.62 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 0.08 x 1 H, 9-H), 7.74 (d, $^3J_{HH}$ = 9.2 Hz, 0.08 x 2 H, 2'''-H, 6'''-H), 8.10 (d, $^3J_{HH}$ = 9.2 Hz, 0.08 x 2 H, 3'''-H, 5'''-H), 8.15 (d, $^3J_{HH}$ = 9.2 Hz, 0.92 x 2 H, 3'''-H, 5'''-H), 8.42 (bs, 0.92 x 1 H, NH), 9.67 (bs, 0.08 x 1 H, NH)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 53.5 (C-1''), 62.8 (C-2''), 118.1 (C-2''', C-6'''), 124.3 (C-10), 125.3 (C-3''', C-5'''), 128.9 (C-3', C-5'), 129.6 (C-2', C-6'), 130.8 (C-6a), 131.0 (C-4'), 131.7 (C-7), 132.1 (C-9), 133.0 (C-8), 133.6 (C-10a), 136.3 (C-1'), 143.0 (C-4'''), 145.1 (C-1'''), 148.8 (C-1), 153.3 (CO), 160.1 (C-3a), 162.3 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3248 (w), 3150 (w), 3046 (w), 2924 (w), 1736 (m), 1598 (m), 1540 (m), 1508 (s), 1435 (m), 1331 (s), 1305 (m), 1216 (s), 1176 (m), 1110 (m), 1071 (m), 854 (m), 694 (m)

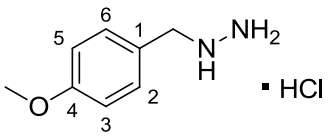
MS (ESI): *m/z* = 520, 518 $[M + H]^+$, 433, 396, 299

HR-MS (ESI): calcd. for $C_{25}H_{21}ClN_7O_4^+$ $[M + H]^+$ 518.1338; found 518.1339

HPLC purity: 95 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{25}H_{20}ClN_7O_4$

MW: 517.92 g/mol



(4-Methoxybenzyl)hydrazine hydrochloride (48)

(Literature known compound but different procedure[178])

tert-Butoxycarbonyl hydrazide **46** (2.5 g, 19 mmol, 1.0 equiv) and 4-methoxybenzaldehyde **45** (2.3 mL, 19 mmol, 1 equiv) were dissolved in ethanol (50 mL) and heated to reflux for 1 h. Reaction progress was monitored by TLC (ethyl acetate / isohexane 1:1, R_f = 0.53). The solvent was evaporated and the colorless solid was re-dissolved in tetrahydrofuran (10 mL), then treated with a solution of BH_3 x SMe_2 in tetrahydrofuran (2.0 M, 9.9 mL, 1.1 equiv) under cooling with an ice bath. After complete addition, the ice bath was removed and the solution was stirred for 15 min at room temperature. Then concentrated hydrochloric acid (4.8 mL, 3.0 equiv) was added dropwise at room temperature and the mixture was stirred subsequently at 60 °C for 10 min to complete the deprotection of the boc-group. The reaction mixture was evaporated, tetrahydrofuran was added and the colorless precipitate was filtered off. Product **48** (3.51 g, 18.6 mmol, 98 %) was yielded as colorless hydrochloride.

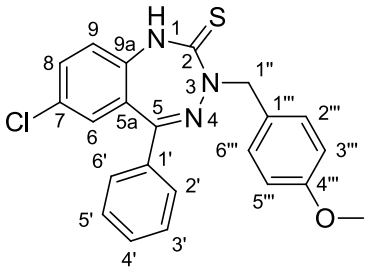
^{1}H NMR (400 MHz, MeOD): δ (ppm) = 3.81 (s, 3 H, OCH_3), 4.15 (s, 2 H, CH_2), 6.97 (d, $^3J_{HH}$ = 8.6 Hz, 2 H, 3-H, 5-H), 7.40 (d, $^3J_{HH}$ = 8.5 Hz, 2 H, 2-H, 6-H)

^{13}C NMR (100 MHz, MeOD): δ (ppm) = 56.0 (OCH_3), 56.2 (CH_2), 115.6 (C-3, C-5), 124.6 (C-1), 132.7 (C-2, C-6), 162.1 (C-4)

MS (CI): *m/z* (%) = 153 (35) $[M + H]^+$, 136 (75), 121 (100)

MF: $C_8H_{13}ClN_2O$ ($C_8H_{12}N_2O$)

MW: 188.65 g/mol (152.19 g/mol)



7-Chloro-3-(4-methoxybenzyl)-5-phenyl-1H-benzo[e][1,2,4]triazepine-2(3H)-thione (50)

(Literature known compound but different procedure[110])

(4-Methoxybenzyl)hydrazine hydrochloride **48** (569 mg, 3.01 mmol, 1.10 equiv) was dissolved in 15 mL of methanol, cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (563 µL, 3.29 mmol, 1.20 equiv). After stirring for 30 min at room temperature, a solution of (5-chloro-2-isothiocyanatophenyl)(phenyl)-methanone **6** (750 mg, 2.74 mmol, 1.00 equiv) in 8 mL of tetrahydrofuran was added and stirring was continued for 1 h. The mixture was concentrated under reduced pressure, the residue dissolved in ethanol (12 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (13 mg, 68 µmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane, R_f = 0.56), giving compound **50** (751 mg, 1.84 mmol, 67 %) as pale yellow solid.

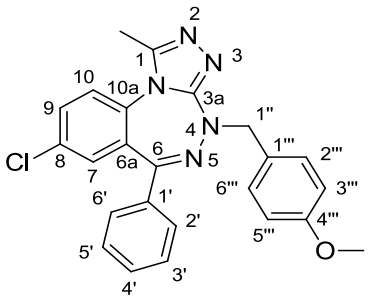
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 3.79 (s, 3 H, OCH_3), 5.28 (s, 2 H, 1''-H), 6.83 – 6.88 (m, 2 H, 3'''-H, 5'''-H), 6.93 (d, $^3J_{HH}$ = 8.4 Hz, 1 H, 9-H), 6.93 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 6-H), 7.22 – 7.29 (m, 4 H, 3'-H, 5'-H, 2'''-H, 6'''-H), 7.32 – 7.37 (m, 2 H, 2'-H, 6'-H), 7.38 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 8-H), 7.42 – 7.47 (m, 1 H, 4'-H), 7.73 (bs, 1 H, 1-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 55.2 (OCH_3), 59.4 (C-1''), 113.8 (C-3''', C-5'''), 121.7 (C-9), 127.4 (C-5a), 128.5 (C-3', C-5'), 128.6 (C-1'''), 129.3 (C-2', C-6'), 129.5 (C-7), 130.1 (C-2''', C-6'''), 130.1 (C-6), 131.0 (C-4'), 132.7 (C-8), 135.6 (C-1'), 142.8 (C-9a), 158.9 (C-4'''), 166.7 (C-5), 192.1 (C-2)

MS (CI): *m/z* (%) = 410 (20), 408 (30) $[M + H]^+$, 376 (10), 300 (10), 158 (10), 136 (20), 121 (100)

MF: $C_{22}H_{18}ClN_3OS$

MW: 407.92 g/mol



8-Chloro-4-(4-methoxybenzyl)-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo [3,4-c][1,2,4]triazepine (51)

(Literature known compound but different procedure[110])

Synthesis of the triazole-ring followed **GP3**. 7-Chloro-3-(4-methoxybenzyl)-5-phenyl-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **50** (375 mg, 0.919 mmol, 1.00 equiv) was treated first with hydrazine hydrate (223 µL, 4.60 mmol, 5.00 equiv). Triethyl orthoacetate **43** (218 µL, 1.20 mmol, 1.30 equiv) and para-toluenesulfonic acid monohydrate (35 mg, 0.18 mmol, 0.20 equiv) were used in the second step. Product **51** (285 mg, 0.663 mmol, 72 %) was obtained after purification (R_f = 0.37) as pale yellow solid.

mp: 118.1 °C (Lit. 116 – 118 °C)[110]

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 3.77 (s, 3 H, OCH_3), 4.81 (d, $^2J_{HH}$ = 13.4 Hz, 1 H, 1''-*H*H), 5.02 (d, $^2J_{HH}$ = 13.5 Hz, 1 H, 1''-H*H*), 6.83 – 6.88 (m, 2 H, 3'''-H, 5'''-H), 7.21 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.32 – 7.47 (m, 7 H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H, 2'''-H, 6'''-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 55.6 (OCH_3), 57.6 (C-1''), 113.9 (C-3''', C-5'''), 124.3 (C-10), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 129.7 (C-1'''), 130.7 (C-4'), 130.8 (C-6a), 131.0 (C-2''', C-6'''), 131.5 (C-7), 132.0 (C-9), 132.8 (C-8), 133.9 (C-10a), 136.6 (C-1'), 148.5 (C-1), 159.4 (C-4'''), 160.3 (C-3a), 161.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2924 (w), 2833 (w), 1612 (m), 1514 (s), 1489 (m), 1438 (m), 1320 (m), 1250 (m), 1173 (m), 1102 (m), 1032 (m), 822 (m), 697 (m)

MS (CI): *m/z* (%) = 432 (35), 430 (100) $[M + H]^+$, 121 (30)

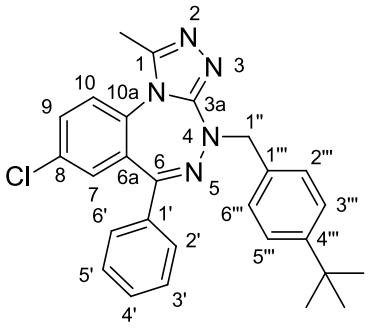
MS (EI): *m/z* (%) = 431 (8), 429 (20) $[M]^{+\cdot}$, 121 (100)

HR-MS (EI): calcd. for $C_{24}H_{20}ClN_5O$ $[M]^{+\cdot}$ 429.1356; found 429.1364

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{20}ClN_5O$

MW: 429.90 g/mol



4-[4-(tert-Butyl)benzyl]-8-chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (53)

Following **GP4**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 4-(*tert*-butyl)benzyl bromide **54** (143 µL, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.55), product **53** (50 mg, 0.11 mmol, 42 %) was obtained as pale yellow solid.

mp: 111.3 – 111.8 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 1.30 (s, 9 H, $C(CH_3)_3$), 2.54 (s, 3 H, 1-CH_3), 4.84 (d, $^2J_{HH}$ = 13.6 Hz, 1 H, 1''-*H*H), 5.09 (d, $^2J_{HH}$ = 13.6 Hz, 1 H, 1''-H*H*), 7.23 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.33 – 7.35 (m, 4 H, 2'''-H, 3'''-H, 5'''-H, 6'''-H), 7.35 – 7.40 (m, 2 H, 3'-H, 5'-H), 7.42 – 7.47 (m, 3 H, 2'-H, 4'-H, 6'-H), 7.60 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 31.5 ($C(CH_3)_3$), 34.8 ($C(CH_3)_3$), 57.9 (C-1''), 124.3 (C-10), 125.6 (C-3''', C-5'''), 128.8 (C-3', C-5'), 129.3 (C-2''', C-6'''), 129.5 (C-2', C-6'), 130.7 (C-4'), 130.8 (C-6a), 131.5 (C-7), 132.1 (C-9), 132.9 (C-8), 133.9 (C-10a), 134.7 (C-1'''), 136.4 (C-1'), 148.5 (C-1), 150.7 (C-4'''), 160.4 (C-3a), 161.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3059 (w), 2961 (m), 2866 (w), 1535 (m), 1518 (s), 1489 (m), 1445 (m), 1431 (m), 1319 (m), 1270 (m), 1171 (w), 1107 (w), 1031 (w), 826 (m), 695 (m)

MS (CI): *m/z* (%) = 458 (30), 456 (100) $[M + H]^+$, 190 (20), 147 (70), 132 (20)

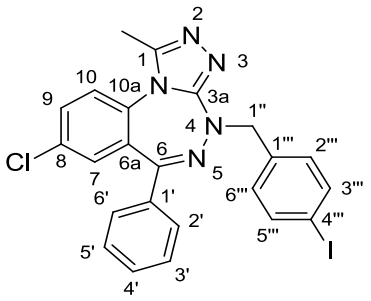
MS (EI): *m/z* (%) = 457 (3), 455 (10) $[M]^{+\cdot}$, 427 (10), 295 (15), 147 (100), 132 (20), 117 (25), 105 (20), 77 (20)

HR-MS (EI): calcd. for $C_{27}H_{26}ClN_5$ $[M]^{+\cdot}$ 455.1877; found 455.1877

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{27}H_{26}ClN_5$

MW: 455.98 g/mol



8-Chloro-4-(4-iodobenzyl)-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (56a)

Following **GP4**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 4-iodobenzyl bromide **55a** (248 µL, 0.775 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.60), product **56a** (104 mg, 0.198 mmol, 77 %) was obtained as pale yellow solid.

mp: 175.2 – 176.1 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 4.82 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-*H*H), 5.03 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-H*H*), 7.18 (d, $^3J_{HH}$ = 8.4 Hz, 2 H, 2'''-H, 6'''-H), 7.22 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.34 – 7.42 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.42 – 7.48 (m, 1 H, 4'-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.66 (d, $^3J_{HH}$ = 8.4 Hz, 2 H, 3'''-H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.6 (C-1''), 93.1 (C-4'''), 124.3 (C-10), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.7 (C-6a), 130.9 (C-4'), 131.5 (C-7), 131.6 (C-2''', C-6'''), 132.2 (C-9), 132.9 (C-8), 133.8 (C-10a), 136.4 (C-1'), 137.6 (C-1'''), 137.8 (C-3''', C-5'''), 148.6 (C-1), 160.1 (C-3a), 161.5 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3059 (w), 2924 (w), 2362 (w), 1734 (w), 1637 (w), 1535 (m), 1519 (s), 1487 (m), 1444 (m), 1431 (m), 1345 (m), 1319 (m), 1272 (w), 1171 (w), 1008 (m), 825 (m), 695 (m)

MS (CI): *m/z* (%) = 528 (35), 526 (100) $[M + H]^+$, 217 (15), 124 (20)

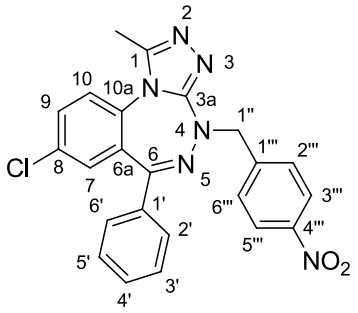
MS (EI): *m/z* (%) = 527 (8), 525 (25) $[M]^{+\cdot}$, 497 (35), 295 (65), 280 (70), 239 (20), 217 (75), 90 (70), 77 (100)

HR-MS (EI): calcd. for $C_{23}H_{17}ClIN_5$ $[M]^{+\cdot}$ 525.0217; found 525.0215

HPLC purity: 95 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}ClIN_5$

MW: 525.77 g/mol



8-Chloro-1-methyl-4-(4-nitrobenzyl)-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (56b)

Following **GP4**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 4-nitrobenzyl bromide **55b** (168 mg, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.58), product **56b** (50 mg, 0.11 mmol, 43 %) was obtained as dark yellow solid.

mp: 93.0 °C (decomposition)

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.57 (s, 3 H, 1-CH_3), 5.01 (d, $^2J_{HH}$ = 14.5 Hz, 1 H, 1''-*H*H), 5.19 (d, $^2J_{HH}$ = 14.4 Hz, 1 H, 1''-H*H*), 7.25 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H), 7.35 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 10-H), 7.36 – 7.38 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.42 – 7.48 (m, 1 H, 4'-H), 7.56 – 7.61 (m, 2 H, 2'''-H, 6'''-H), 7.65 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 8.15 – 8.20 (m, 2 H, 3'''-H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.4 (C-1''), 123.9 (C-3''', C-5'''), 124.4 (C-10), 128.9 (C-3', C-5'), 129.5 (C-2', C-6'), 130.2 (C-2''', C-6'''), 130.6 (C-6a), 131.0 (C-4'), 131.6 (C-7), 132.4 (C-9), 133.1 (C-8), 133.7 (C-10a), 136.3 (C-1'), 145.4 (C-1'''), 147.7 (C-4'''), 148.9 (C-1), 160.0 (C-3a), 162.0 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2923 (w), 2852 (w), 1602 (w), 1519 (s), 1489 (m), 1432 (w), 1344 (s), 1321 (m), 1170 (w), 1108 (w), 1066 (w), 831 (w), 738 (w), 696 (w)

MS (CI): *m/z* (%) = 447 (35), 445 (100) $[M + H]^+$, 415 (20), 154 (100)

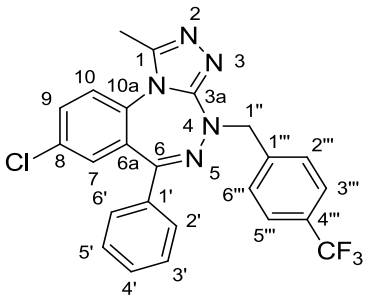
MS (EI): *m/z* (%) = 444 (5) $[M]^{+\cdot}$, 416 (10), 295 (15), 219 (10), 106 (100), 77 (50), 51 (20)

HR-MS (EI): calcd. for $C_{23}H_{17}ClN_6O_2$ $[M]^{+\cdot}$ 444.1102; found 444.1082

HPLC purity: 96 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}ClN_6O_2$

MW: 444.87 g/mol



8-Chloro-1-methyl-6-phenyl-4-[4-(trifluoromethyl)benzyl]-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (56c)

Following **GP4**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 4-(trifluoromethyl)benzyl bromide **55c** (120 µL, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.51), product **56c** (104 mg, 0.222 mmol, 86 %) was obtained as pale yellow solid.

mp: 107.4 – 108.6 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.56 (s, 3 H, 1-CH_3), 4.96 (d, $^2J_{HH}$ = 14.2 Hz, 1 H, 1''-*H*H), 5.17 (d, $^2J_{HH}$ = 14.1 Hz, 1 H, 1''-H*H*), 7.24 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.34 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.34 – 7.48 (m, 5 H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 7.55 (d, $^3J_{HH}$ = 8.2 Hz, 2 H, 2'''-H, 6'''-H), 7.59 (d, $^3J_{HH}$ = 8.3 Hz, 2 H, 3'''-H, 5'''-H), 7.63 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.6 (C-1''), 124.3 (C-10), 124.8 (q, $^1J_{CF}$ = 271.8 Hz, CF_3), 125.5 (q, $^3J_{CF}$ = 3.9 Hz, C-3''', C-5'''), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 129.6 (q, $^2J_{CF}$ = 32.0 Hz, C-4'''), 129.8 (C-2''', C-6'''), 130.7 (C-6a), 130.9 (C-4'), 131.6 (C-7), 132.2 (C-9), 133.0 (C-8), 133.8 (C-10a), 136.4 (C-1'), 142.1 (q, $^5J_{CF}$ = 1.3 Hz, C-1'''), 148.7 (C-1), 160.1 (C-3a), 161.7 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3063 (w), 2926 (w), 1618 (m), 1535 (m), 1519 (m), 1490 (m), 1437 (m), 1325 (s), 1165 (m), 1123 (m), 1066 (m), 1019 (m), 824 (m), 695 (m)

MS (CI): *m/z* (%) = 470 (35), 468 (100) $[M + H]^+$, 159 (20), 107 (35)

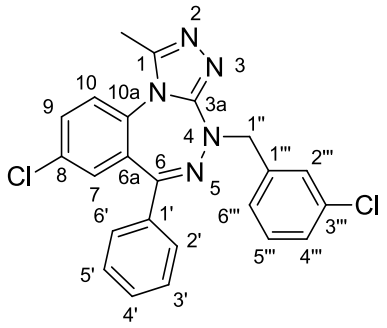
MS (EI): *m/z* (%) = 469 (8), 467 (20) $[M]^{+\cdot}$, 439 (35), 295 (30), 280 (20), 253 (15), 239 (15), 219 (25), 159 (30), 109 (20), 77 (100), 51 (20)

HR-MS (EI): calcd. for $C_{24}H_{17}ClF_3N_5$ $[M]^{+\cdot}$ 467.1125; found 467.1131

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{17}ClF_3N_5$

MW: 467.87 g/mol



8-Chloro-4-(3-chlorobenzyl)-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (56d)

Following **GP4**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 3-chlorobenzyl bromide **55d** (102 µL, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.51), product **56d** (78 mg, 0.18 mmol, 70 %) was obtained as pale yellow solid.

mp: 104.5 – 105.8 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 4.85 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-*H*H), 5.08 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1''-H*H*), 7.23 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.25 – 7.27 (m, 1 H, 4'''-H), 7.28 (td, $^5J_{HH}$ = 0.6 Hz, $^3J_{HH}$ = 7.8 Hz, 1 H, 5'''-H), 7.31 (d, $^3J_{HH}$ = 8.8 Hz, 1 H, 10-H), 7.31 – 7.34 (m, 1 H, 2'''-H), 7.34 – 7.43 (m, 5 H, 2'-H, 3'-H, 5'-H, 6'-H, 6'''-H), 7.43 – 7.48 (m, 1 H, 4'-H), 7.62 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.6 (C-1''), 124.3 (C-10), 127.8 (C-5'''), 127.9 (C-2'''), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 129.7 (C-6'''), 130.0 (C-4'''), 130.7 (C-6a), 130.9 (C-4'), 131.6 (C-7), 132.2 (C-9), 133.0 (C-8), 133.8 (C-10a), 134.3 (C-3'''), 136.5 (C-1'), 139.9 (C-1'''), 148.7 (C-1), 160.1 (C-3a), 161.5 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3058 (w), 2923 (w), 2361 (m), 2342 (m), 1598 (m), 1576 (m), 1535 (m), 1518 (s), 1488 (m), 1429 (m), 1344 (m), 1319 (m), 1171 (m), 1031 (m), 832 (m), 775 (m), 694 (m)

MS (CI): *m/z* (%) = 438 (20), 436 (75), 434 (100) $[M + H]^+$, 255 (10), 230 (25), 125 (35)

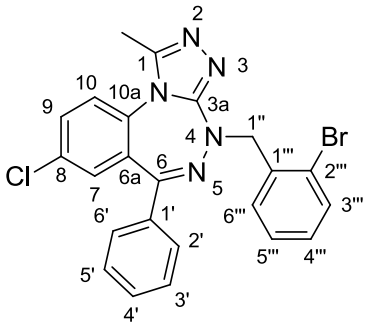
MS (EI): *m/z* (%) = 437 (2), 435 (10), 433 (20) $[M]^{+\cdot}$, 405 (30), 295 (40), 208 (35), 219 (30), 125 (40), 77 (100), 51 (25)

HR-MS (EI): calcd. for $C_{23}H_{17}Cl_2N_5$ $[M]^{+\cdot}$ 433.0861; found 433.0860

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}Cl_2N_5$

MW: 434.32 g/mol



4-(2-Bromobenzyl)-8-chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (56e)

Following **GP4**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 2-bromobenzyl bromide **55e** (194 mg, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.66), product **56e** (76 mg, 0.16 mmol, 61 %) was obtained as pale yellow solid.

mp: 215.2 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.56 (s, 3 H, 1-CH_3), 5.00 (d, $^2J_{HH}$ = 14.2 Hz, 1 H, 1''-*H*H), 5.23 (d, $^2J_{HH}$ = 14.1 Hz, 1 H, 1''-H*H*), 7.15 (td, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.7 Hz, 1 H, 4'''-H), 7.23 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.28 (td, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 5'''-H), 7.33 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.35 – 7.39 (m, 2 H, 3'-H, 5'-H), 7.41 – 7.47 (m, 4 H, 2'-H, 4'-H, 6'-H, 6'''-H), 7.56 (dd, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3'''-H), 7.61 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.9 (1-CH_3), 58.1 (CH_2), 124.3 (C-10), 124.9 (C-2'''), 127.6 (C-5'''), 128.8 (C-3', C-5'), 129.3 (C-4'''), 129.5 (C-2', C-6'), 130.7 (C-6a), 130.8 (C-4'), 131.5 (C-7), 131.6 (C-6'''), 132.2 (C-9), 132.9 (C-8), 133.1 (C-3'''), 133.9 (C-10a), 136.5 (C-1'), 137.0 (C-1'''), 148.7 (C-1), 160.2 (C-3a), 161.6 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2927 (w), 2360 (m), 2341 (m), 1635 (m), 1537 (m), 1520 (s), 1489 (m), 1439 (m), 1381 (m), 1346 (m), 1319 (m), 1272 (m), 1170 (m), 1101 (m), 1027 (m), 829 (m), 744 (m), 696 (m)

MS (CI): *m/z* (%) = 482 (25), 480 (95), 478 (65) $[M + H]^+$, 400 (10), 283 (10), 255 (15), 230 (10), 182 (10), 171 (15), 124 (30), 107 (100)

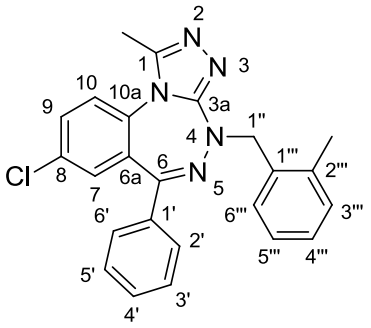
MS (EI): *m/z* (%) = 481 (1), 479 (5), 477 (4) $[M]^{+\cdot}$, 451 (15), 398 (70), 382 (20), 280 (10), 219 (10), 169 (15), 77 (100), 51 (30)

HR-MS (EI): calcd. for $C_{23}H_{17}BrClN_5$ $[M]^{+\cdot}$ 477.0356; found 477.0352

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}BrClN_5$

MW: 478.77 g/mol



8-Chloro-1-methyl-4-(2-methylbenzyl)-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (58a)

Following **GP5**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 2-methylbenzyl chloride **57a** (102 µL, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.52), product **58a** (79 mg, 0.19 mmol, 74 %) was obtained as pale yellow solid.

mp: 236.5 – 238.2 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.30 (s, 3 H, 2'''-CH_3), 2.55 (s, 3 H, 1-CH_3), 4.88 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-*H*H), 5.13 (d, $^2J_{HH}$ = 13.9 Hz, 1 H, 1''-H*H*), 7.12 – 7.19 (m, 3 H, 3'''-H, 4'''-H, 5'''-H), 7.21 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.34 – 7.42 (m, 5 H, 2'-H, 3'-H, 5'-H, 6'-H, 6'''-H), 7.42 – 7.46 (m, 1 H, 4'-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 19.6 (2'''-CH_3), 56.2 (C-1''), 124.3 (C-10), 126.0 (C-5'''), 127.8 (C-4'''), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.5 (C-3'''), 130.6 (C-6'''), 130.7 (C-6a), 130.7 (C-4'), 131.5 (C-7), 132.1 (C-9), 132.8 (C-8), 133.9 (C-10a), 135.7 (C-2'''), 136.6 (C-1'), 137.9 (C-1'''), 148.6 (C-1), 160.4 (C-3a), 161.2 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 3023 (w), 2924 (w), 2361 (w), 2343 (w), 1595 (w), 1560 (w), 1535 (m), 1518 (s), 1489 (m), 1445 (m), 1430 (m), 1379 (w), 1344 (m),

1319 (m), 1272 (w), 1171 (w), 1099 (w), 1030 (w), 830 (w), 775 (w), 742 (m), 695 (m)

MS (CI): *m/z* (%) = 416 (55), 414 (100) $[M + H]^+$, 255 (10), 190 (5), 176 (5), 105 (30)

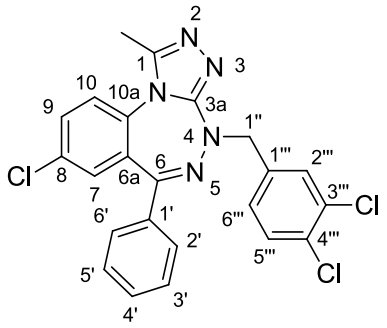
MS (EI): *m/z* (%) = 413 (5) $[M]^{+\cdot}$, 397 (10), 385 (15), 295 (15), 281 (20), 105 (100), 77 (60), 51 (15)

HR-MS (EI): calcd. for $C_{24}H_{20}ClN_5$ $[M]^{+\cdot}$ 413.1407; found 413.1406

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{20}ClN_5$

MW: 413.90 g/mol



8-Chloro-4-(3,4-dichlorobenzyl)-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (58b)

Following **GP5**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 3,4-dichlorobenzyl chloride **57b** (107 µL, 0.778 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.62), product **58b** (87 mg, 0.19 mmol, 72 %) was obtained as pale yellow solid.

mp: 166.5 – 167.8 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 4.83 (d, $^2J_{HH}$ = 13.6 Hz, 1 H, 1''-*H*H), 5.04 (d, $^2J_{HH}$ = 13.6 Hz, 1 H, 1''-H*H*), 7.23 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.28 (dd, $^4J_{HH}$ = 2.0 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 6'''-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.35 – 7.41 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.41 (d, $^3J_{HH}$ = 8.3 Hz, 1 H, 5'''-H), 7.43 – 7.48 (m, 1 H, 4'-H), 7.53 (d, $^4J_{HH}$ = 2.0 Hz, 1 H, 2'''-H), 7.62 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.1 (C-1''), 124.3 (C-10), 128.9 (C-3', C-5'), 129.3 (C-6'''), 129.5 (C-2', C-6'), 130.6 (C-5'''), 130.6 (C-6a), 130.9 (C-4'), 131.5 (C-4'''), 131.6 (C-7), 131.6 (C-2'''), 132.3 (C-9), 132.4 (C-3'''), 133.0 (C-8), 133.7 (C-10a), 136.4 (C-1'), 138.2 (C-1'''), 148.8 (C-1), 160.0 (C-3a), 161.7 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2925 (w), 2366 (w), 1637 (w), 1561 (m), 1535 (m), 1518 (s), 1489 (m), 1472 (m), 1432 (m), 1398 (m), 1380 (m), 1345 (m), 1319 (m), 1271 (w), 1171 (w), 1133 (w), 1101 (w), 1131 (m), 821 (m), 694 (m)

MS (CI): *m/z* (%) = 474 (8), 472 (35), 470 (100), 468 (100) $[M + H]^+$, 280 (10), 255 (6), 176 (5), 159 (20), 124 (75), 107 (55), 101 (7)

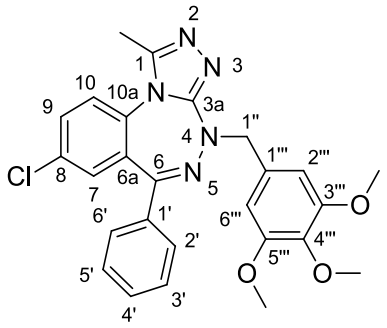
MS (EI): *m/z* (%) = 471 (3), 469 (10), 467 (10) $[M]^{+\cdot}$, 439 (150), 295 (35), 280 (40), 253 (5), 239 (15), 219 (15), 204 (5), 177 (10), 159 (20), 151 (5), 123 (10), 110 (7), 77 (100), 51 (20),

HR-MS (EI): calcd. for $C_{23}H_{16}Cl_3N_5$ $[M]^{+\cdot}$ 467.0472; found 467.0475

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{16}Cl_3N_5$

MW: 468.77 g/mol



8-Chloro-1-methyl-6-phenyl-4-(3,4,5-trimethoxybenzyl)-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (58c)

Following **GP5**, N-alkylation of 8-chloro-1-methyl-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **11** (80 mg, 0.26 mmol, 1.0 equiv) was carried out using 3,4,5-trimethoxybenzyl chloride **57c** (168 mg, 0.775 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (R_f = 0.49), product **58c** (112 mg, 0.229 mmol, 89 %) was obtained as pale yellow solid.

mp: 220.5 – 221.8 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.56 (s, 3 H, 1-CH_3), 3.74 (s, 3 H, 4'''-OCH_3), 3.77 (s, 6 H, 3'''-OCH_3, 5'''-OCH_3), 4.81 (d, $^2J_{HH}$ = 14.0 Hz, 1 H, 1''-*H*H), 5.04 (d, $^2J_{HH}$ = 14.0 Hz, 1 H, 1''-H*H*), 6.64 (s, 2 H, 2'''-H, 6'''-H), 7.24 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.34 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.36 – 7.42 (m, 2 H, 3'-H, 5'-H), 7.42 – 7.49 (m, 3 H, 2'-H, 4'-H, 6'-H), 7.62 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 56.3 (3'''-OCH_3, 5'''-OCH_3), 58.1 (C-1''), 60.8 (4'''-OCH_3), 106.3 (C-2''', C-6'''), 124.4 (C-10), 128.8 (C-3', C-5'), 129.5 (C-2', C-6'), 130.8 (C-4'), 130.8 (C-6a), 131.3 (C-7), 132.1 (C-9), 132.9 (C-8), 133.4 (C-1'''), 133.9 (C-10a), 136.4 (C-1'), 137.5 (C-4'''), 148.6 (C-1), 153.5 (C-3''', C-5'''), 160.3 (C-3a), 161.5 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3431 (m), 3062 (w), 2934 (m), 2836 (w), 2360 (w), 2344 (w), 1592 (m), 1535 (m), 1518 (s), 1508 (s), 1458 (m), 1422 (m), 1345 (m), 1327 (m),

1272 (w), 1234 (m), 1173 (w), 1126 (s), 1033 (w), 1008 (m), 825 (m), 778 (m), 695 (m)

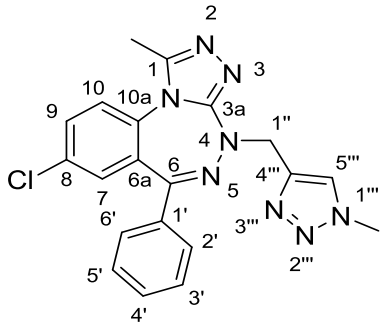
MS (APCI): *m/z* = 492, 490 $[M + H]^+$, 295, 255, 196, 181

HR-MS (ESI): calcd. for $C_{26}H_{25}ClN_5O_3^+$ $[M + H]^+$ 490.1640; found 490.1646

HPLC purity: 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{26}H_{24}ClN_5O_3$

MW: 489.95 g/mol



8-Chloro-1-methyl-4-[(1-methyl-1H-1,2,3-triazol-4-yl)methyl]-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (59)

Formation of the 1,2,3-triazole was accomplished according to **GP6**, using 8-chloro-1-methyl-6-phenyl-4-(prop-2-yn-1-yl)-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4] triazepine **13** (80 mg, 0.23 mmol, 1.1 equiv), sodium azide (15 mg, 0.23 mmol, 1.1 equiv), iodomethane (13 µL, 0.21 mmol, 1.0 equiv), sodium L-ascorbate (8.2 mg, 42 µmol, 0.2 equiv) and copper(II) sulfate pentahydrate (5.8 mg, 10 µmol, 5.0 mol%). After purification (R_f = 0.18), product **59** (41 mg, 0.10 mmol, 49 %) was yielded as pale yellow solid.

mp: 191.6 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 4.03 (s, 3 H, 1'''-CH_3), 4.95 (d, $^2J_{HH}$ = 14.0 Hz, 1 H, 1''-*H*H), 5.15 (d, $^2J_{HH}$ = 13.9 Hz, 1 H, 1''-H*H*), 7.24 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.30 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.38 – 7.43 (m, 2 H, 3'-H, 5'-H), 7.45 – 7.50 (m, 3 H, 2'-H, 4'-H, 6'-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.66 (s, 1 H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 36.9 (1'''-CH_3), 49.7 (C-1''), 124.4 (C-10), 125.5 (C-5'''), 128.9 (C-3', C-5'), 129.5 (C-2', C-6'), 130.7 (C-6a), 130.9 (C-4'), 131.6 (C-7), 132.2 (C-9), 133.0 (C-8), 133.7 (C-10a), 136.5 (C-1'), 144.0 (C-4'''), 148.7 (C-1), 159.9 (C-3a), 161.2 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3424 (s), 3049 (w), 2928 (w), 2361 (w), 2344 (w), 1793 (m), 1637 (w), 1596 (w), 1560 (m), 1535 (s), 1518 (s), 1489 (s), 1437 (m), 1381 (w),

1347 (m), 1320 (m), 1272 (w), 1220 (w), 1172 (m), 1140 (w), 1101 (w), 1050 (m), 831 (m), 775 (m), 744 (w), 696 (m), 661 (w), 593 (w), 534 (m)

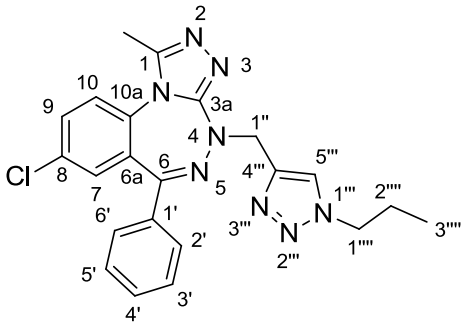
MS (ESI): *m/z* = 407, 405 $[M + H]^+$, 366, 207, 184

HR-MS (EI): calcd. for $C_{20}H_{17}ClN_8$ $[M]^{+\cdot}$ 404.1265; found 404.1264

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{20}H_{17}ClN_8$

MW: 404.86 g/mol



8-Chloro-1-methyl-6-phenyl-4-[(1-propyl-1H-1,2,3-triazol-4-yl)methyl]-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (61a)

Formation of the 1,2,3-triazole was accomplished according to **GP6**, using 8-chloro-1-methyl-6-phenyl-4-(prop-2-yn-1-yl)-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4] triazepine **13** (80 mg, 0.23 mmol, 1.1 equiv), sodium azide (15 mg, 0.23 mmol, 1.1 equiv), 1-bromopropane **60a** (19 µL, 0.21 mmol, 1.0 equiv), sodium L-ascorbate (8.3 mg, 42 µmol, 0.2 equiv) and copper(II) sulfate pentahydrate (5.8 mg, 10 µmol, 5.0 mol%). After purification (R_f = 0.25), product **61a** (37 mg, 85 µmol, 41 %) was yielded as pale yellow solid.

mp: 143.9 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 0.93 (t, $^3J_{HH}$ = 7.4 Hz, 3 H, 3''''-H), 1.87 – 1.95 (m, 2 H, 2''''-H), 2.55 (s, 3 H, 1-CH_3), 4.24 – 4.32 (m, 2 H, 1''''-H), 4.96 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-*H*H), 5.14 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-H*H*), 7.23 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.37 – 7.42 (m, 2 H, 3'-H, 5'-H), 7.44 – 7.49 (m, 3 H, 2'-H, 4'-H, 6'-H), 7.59 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.68 (s, 1 H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 11.2 (C-3''''), 12.8 (1-CH_3), 24.1 (C-2''''), 49.8 (C-1''), 52.2 (C-1''''), 124.4 (C-10), 125.5 (C-5'''), 128.9 (C-3', C-5'), 129.5 (C-2', C-6'), 130.7 (C-6a), 130.9 (C-4'), 131.6 (C-7), 132.2 (C-9), 133.0 (C-8), 133.7 (C-10a), 136.5 (C-1'), 143.6 (C-4'''), 148.7 (C-1), 160.0 (C-3a), 161.2 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3136 (w), 3064 (w), 2965 (m), 2932 (m), 2875 (w), 2362 (w), 2344 (w), 1595 (w), 1536 (s), 1519 (s), 1489 (s), 1436 (s), 1381 (m), 1347 (m), 1320 (m), 1273 (w), 1221 (w), 1172 (w), 1140 (w), 1101 (w), 1049 (m), 1032 (m), 830 (m), 775 (w), 744 (w), 696 (w), 662 (w), 593 (m), 534 (m)

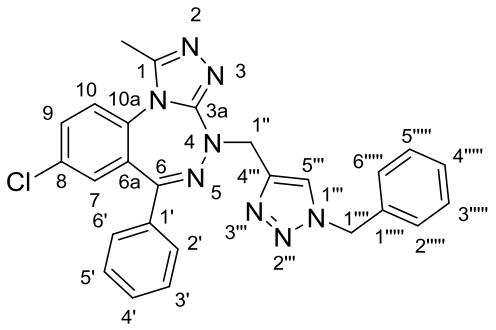
MS (ESI): *m/z* = 435, 433 $[M + H]^+$

HR-MS (EI): calcd. for $C_{22}H_{21}ClN_8$ $[M]^{+\cdot}$ 432.1578; found 432.1581

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{22}H_{21}ClN_8$

MW: 432.91 g/mol



4-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]-8-chloro-1-methyl-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (61b)

Formation of the 1,2,3-triazole was accomplished according to **GP6**, using 8-chloro-1-methyl-6-phenyl-4-(prop-2-yn-1-yl)-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4] triazepine **13** (80 mg, 0.23 mmol, 1.1 equiv), sodium azide (15 mg, 0.23 mmol, 1.1 equiv), benzyl bromide **60b** (25 µL, 0.21 mmol, 1.0 equiv), sodium L-ascorbate (8.3 mg, 42 µmol, 0.2 equiv) and copper(II) sulfate pentahydrate (5.8 mg, 10 µmol, 5.0 mol%). After purification (dichloromethane / methanol 9:1, R_f = 0.43), product **61b** (81 mg, 0.17 µmol, 80 %) was yielded as pale yellow solid.

mp: 152.1 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 4.95 (d, $^2J_{HH}$ = 13.8 Hz, 1 H, 1''-*H*H), 5.09 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1''-H*H*), 5.46 (d, $^2J_{HH}$ = 14.4 Hz, 1 H, 1''''-*H*H), 5.54 (d, $^2J_{HH}$ = 14.4 Hz, 1 H, 1''''-H*H*), 7.18 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.26 – 7.29 (m, 2 H, 2''''-H, 6''''-H), 7.30 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.33 – 7.38 (m, 7 H, 2'-H, 3'-H, 5'-H, 6'-H, 3''''-H, 4''''-H, 5''''-H), 7.42 – 7.48 (m, 1 H, 4'-H), 7.58 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 7.64 (s, 1 H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 49.7 (C-1''), 54.4 (C-1''''), 124.4 (C-10), 124.6 (C-5'''), 128.4 (C-2'''', C-6''''), 128.8 (C-3', C-5'), 128.9 (C-4''''), 129.4 (C-3'''', C-5''''), 129.5 (C-2', C-6'), 130.6 (C-6a), 130.8 (C-4'), 131.6 (C-7), 132.2 (C-9), 133.0 (C-8), 133.7 (C-10a), 135.6 (C-1''''), 136.5 (C-1'), 144.2 (C-4'''), 148.7 (C-1), 159.9 (C-3a), 161.3 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2924 (w), 2362 (w), 2344 (w), 1637 (w), 1535 (m), 1518 (s), 1490 (m), 1432 (m), 1380 (w), 1347 (w), 1320 (m), 1272 (w), 1221 (w), 1172 (w), 1129 (w), 1102 (w), 1048 (m), 1031 (w), 827 (w), 696 (m)

MS (CI): *m/z* (%) = 483 (35), 481 (90) $[M + H]^+$, 295 (5), 255 (15), 210 (100), 199 (25), 144 (40), 103 (40)

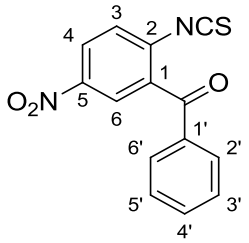
MS (EI): *m/z* (%) = 482 (10), 480 (30) $[M]^{+\cdot}$, 295 (20), 281 (55), 253 (10), 219 (15), 205 (20), 177 (10), 144 (10), 111 (15), 91 (100), 77 (45), 65 (15), 51 (10)

HR-MS (EI): calcd. for $C_{26}H_{21}ClN_8$ $[M]^{+\cdot}$ 480.1578; found 480.1579

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{26}H_{21}ClN_8$

MW: 480.95 g/mol



(2-Isothiocyanato-5-nitrophenyl)(phenyl)methanone (64)

(Literature known compound but different procedure[110])

Compound **64** was prepared according to **GP2** using 2-amino-5-nitro-benzophenone **62** (5.0 g, 21 mmol, 1.0 equiv) as starting material, calcium carbonate (3.1 g, 31 mmol, 1.5 equiv) and thiophosgene **16** (1.7 mL, 23 mmol, 1.1 equiv). After purification (dichloromethane, R_f = 0.74), product **64** (4.0 g, 14 mmol, 69 %) was yielded as yellow solid.

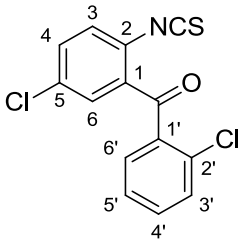
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.50 (d, $^3J_{HH}$ = 8.6 Hz, 1 H, 3-H), 7.52 – 7.57 (m, 2 H, 3'-H, 5'-H), 7.67 – 7.71 (m, 1 H, 4'-H), 7.80 – 7.83 (m, 2 H, 2'-H, 6'-H), 8.36 (d, $^4J_{HH}$ = 2.5 Hz, 1 H, 6-H), 8.38 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 4-H),

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 125.4 (C-6), 126.6 (C-4), 128.3 (C-3), 129.1 (C-3', C-5'), 130.1 (C-2', C-6'), 134.6 (C-4'), 135.5 (C-1'), 135.8 (C-1), 135.9 (C-2), 140.9 (NCS), 145.0 (C-5), 192.2 (CO)

MS (CI): *m/z* (%) = 285 (100) $[M + H]^+$

MF: $C_{14}H_8N_2O_3S$

MW: 284.29 g/mol



(5-Chloro-2-isothiocyanatophenyl)(2-chlorophenyl)methanone (65)

(Literature known compound but different procedure[110])

Compound **65** was prepared according to **GP2** using 2-amino-2,5-dichloro-benzophenone **63** (5.0 g, 19 mmol, 1.0 equiv) as starting material, calcium carbonate (2.8 g, 28 mmol, 1.5 equiv) and thiophosgene **16** (1.6 mL, 21 mmol, 1.1 equiv). No purification was needed after workup procedure, yielding product **65** (5.5 g, 18 mmol, 96 %) as red-brown solid.

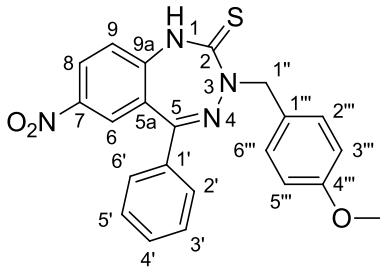
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.27 (d, $^3J_{HH}$ = 8.5 Hz, 1 H, 3-H), 7.42 (ddd, $^4J_{HH}$ = 2.7 Hz, $^3J_{HH}$ = 6.0 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 5'-H), 7.49 (dd, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 4-H), 7.47 – 7.49 (m, 2 H, 3'-H, 4'-H), 7.52 (ddd, $^5J_{HH}$ = 0.7 Hz, $^4J_{HH}$ = 1.4 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'-H), 7.57 (d, $^4J_{HH}$ = 2.5 Hz, 1 H, 6-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 127.3 (C-5'), 128.7 (C-2), 129.4 (C-3), 130.3 (C-6'), 130.6 (C-3'), 130.8 (C-6), 131.9 (C-2'), 132.8 (C-4'), 132.8 (C-5), 133.3 (C-4), 134.7 (C-1), 137.3 (NCS), 137.4 (C-1'), 192.0 (CO)

MS (CI): *m/z* (%) = 312 (15), 310 (70), 308 (100) $[M + H]^+$, 196 (20), 139 (40)

MF: $C_{14}H_7Cl_2NOS$

MW: 308.18 g/mol



3-(4-Methoxybenzyl)-7-nitro-5-phenyl-1H-benzo[e][1,2,4]triazepine-2(3H)-thione (66)

(Literature known compound but different procedure[110])

(4-Methoxybenzyl)hydrazine hydrochloride **48** (2.1 g, 11 mmol, 1.1 equiv) was dissolved in 35 mL of methanol, cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (2.1 mL, 12 mmol, 1.2 equiv). After stirring for 30 min at room temperature, a solution of (2-isothiocyanato-5-nitrophenyl)(phenyl)-methanone **64** (2.9 g, 10 mmol, 1.0 equiv) in 20 mL of tetrahydrofuran was added and stirring was continued for 1 h. The mixture was concentrated under reduced pressure, the residue dissolved in ethanol (30 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (49 mg, 0.26 mmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane, R_f = 0.39), giving compound **66** (2.67 g, 6.38 mmol, 63 %) as yellow solid.

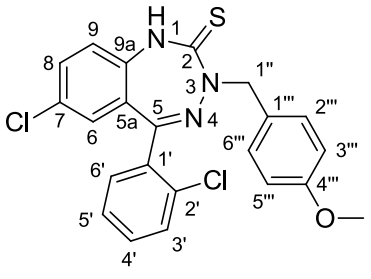
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.80 (s, 3 H, OCH_3), 5.30 (s, 2 H, 1''-H), 6.84 – 6.89 (m, 2 H, 3'''-H, 5'''-H), 7.09 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H), 7.20 – 7.24 (m, 2 H, 2'-H', 6'-H), 7.27 – 7.32 (m, 2 H, 2'''-H, 6'''-H), 7.35 – 7.40 (m, 2 H, 3'-H, 5'-H), 7.46 – 7.52 (m, 1 H, 4'-H), 7.86 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 6-H), 7.87 (bs, 1 H, 1-H), 8.30 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.8 Hz, 1 H, 8-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 55.3 (OCH_3), 59.7 (C-1''), 113.9 (C-3''', C-5'''), 121.0 (C-9), 126.0 (C-5a), 126.6 (C-6), 127.8 (C-8), 128.0 (C-1'''), 128.8 (C-3', C-5'), 129.2 (C-2', C-6'), 130.2 (C-2''', C-6'''), 131.5 (C-4'), 135.2 (C-1'), 143.4 (C-7), 148.8 (C-9a), 159.1 (C-4'''), 166.2 (C-5), 190.3 (C-2)

MS (CI): *m/z* (%) = 419 (20) $[M + H]^+$, 391 (15), 121 (100)

MF: $C_{22}H_{18}N_4O_3S$

MW: 418.47 g/mol



7-Chloro-5-(2-chlorophenyl)-3-(4-methoxybenzyl)-1H-benzo[e][1,2,4]-triazepine-2(3H)-thione (67)

(Literature known compound but different procedure[110])

(4-Methoxybenzyl)hydrazine hydrochloride **48** (673 mg, 3.57 mmol, 1.10 equiv) was dissolved in 20 mL of methanol, cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (667 µL, 3.89 mmol, 1.20 equiv). After stirring for 30 min at room temperature, a solution of (5-chloro-2-isothiocyanatophenyl)(2-chlorophenyl)-methanone **65** (1.0 g, 3.2 mmol, 1.0 equiv) in 10 mL of tetrahydrofuran was added and stirring was continued for 1 h. The mixture was concentrated under reduced pressure, the residue dissolved in ethanol (20 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (15 mg, 81 µmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane, R_f = 0.46), giving compound **67** (915 mg, 2.07 mmol, 64 %) as yellow solid.

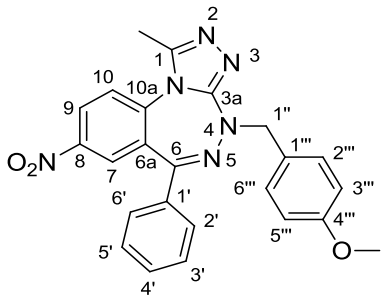
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.82 (s, 3 H, OCH_3), 5.26 (s, 2 H, 1''-H), 6.63 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 6-H), 6.83 – 6.90 (m, 4 H, 9-H, 6'-H, 3'''-H, 5'''-H), 7.23 – 7.28 (m, 3 H, 5'-H, 2'''-H, 6'''-H), 7.34 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 8-H), 7.36 – 7.39 (m, 2 H, 3'-H, 4'-H), 7.62 (bs, 1 H, 1-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 55.3 (OCH_3), 59.5 (C-1''), 113.8 (C-3''', C-5'''), 121.4 (C-9), 127.0 (C-5'), 128.1 (C-5a), 128.6 (C-1'''), 128.7 (C-6), 129.9 (C-7), 130.2 (C-2''', C-6'''), 130.2 (C-3'), 131.3 (C-6'), 131.4 (C-4'), 132.7 (C-8), 133.3 (C-2'), 135.0 (C-1'), 141.7 (C-9a), 159.0 (C-4'''), 165.5 (C-5), 192.3 (C-2)

MS (CI): *m/z* (%) = 446 (5), 444 (25), 442 (30) $[M + H]^+$, 121 (100)

MF: $C_{22}H_{17}Cl_2N_3OS$

MW: 442.36 g/mol



4-(4-Methoxybenzyl)-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (68)

(Literature known compound but different procedure[110])

Synthesis of the triazole-ring followed **GP3**. 3-(4-Methoxybenzyl)-7-nitro-5-phenyl-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **66** (2.6 g, 6.2 mmol, 1.0 equiv) was treated first with hydrazine hydrate (1.5 mL, 31 mmol, 5.0 equiv). Triethyl orthoacetate (1.5 mL, 8.1 mmol, 1.3 equiv) and para-toluenesulfonic acid monohydrate (236 mg, 1.24 mmol, 0.20 equiv) were used in the second step. Product **68** (2.1 g, 4.9 mmol, 78 %) was obtained after purification (R_f = 0.34) as bright yellow solid.

mp: 188.7 °C (lit. 189 °C)[110]

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.58 (s, 3 H, 1-CH_3), 3.76 (s, 3 H, OCH_3), 3.72 – 3.79 (m, 1 H, 1''-*H*H), 4.77 – 4.92 (m, 1 H, 1''-*H*H), 6.81 – 6.87 (m, 2 H, 3'''-H, 5'''-H), 7.32 – 7.36 (m, 2 H, 2'''-H, 6'''-H), 7.36 – 7.44 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.44 – 7.49 (m, 1 H, 4'-H), 7.53 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.05 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.44 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 55.6 (OCH_3), 57.7 (C-1''), 114.0 (C-3''', C-5'''), 124.0 (C-10), 126.8 (C-9), 126.9 (C-7), 129.0 (C-3', C-5'), 129.4 (C-1'''), 129.4 (C-2', C-6'), 130.4 (C-6a), 131.1 (C-2''', C-6'''), 131.1 (C-4'), 136.1 (C-1'), 140.4 (C-10a), 145.8 (C-8), 148.6 (C-1), 159.5 (C-4'''), 160.5 (C-3a), 160.7 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2934 (w), 1685 (m), 1613 (m), 1596 (m), 1535 (m), 1515 (s), 1445 (m), 1400 (m), 1346 (s), 1326 (m), 1250 (m), 1174 (m), 1100 (m), 1032 (m), 695 (w)

MS (CI): *m/z* (%) = 441 (100) $[M + H]^+$, 411 (30), 181 (30), 121 (75)

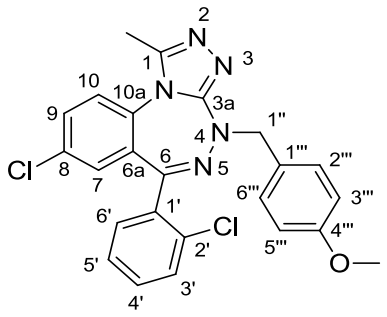
MS (EI): *m/z* (%) = 440 (3) $[M]^{+\cdot}$, 410 (5), 121 (100), 77 (15)

HR-MS (EI): calcd. for $C_{24}H_{20}N_6O_3$ $[M]^{+\cdot}$ 440.1597; found 440.1597

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{20}N_6O_3$

MW: 440.45 g/mol



8-Chloro-6-(2-chlorophenyl)-4-(4-methoxybenzyl)-1-methyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (69)

(Literature known compound but different procedure[110])

Synthesis of the triazole-ring followed **GP3**. 7-Chloro-5-(2-chlorophenyl)-3-(4-methoxybenzyl)-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **67** (850 mg, 1.92 mmol, 1.00 equiv) was treated first with hydrazine hydrate (467 µL, 9.61 mmol, 5.00 equiv). Triethyl orthoacetate (456 µL, 2.50 mmol, 1.30 equiv) and para-toluenesulfonic acid monohydrate (73 mg, 0.38 mmol, 0.2 equiv) were used in the second step. Product **69** (675 g, 1.45 mmol, 76 %) was obtained after purification (R_f = 0.29) as yellow solid.

mp: 192.8 °C (lit. 188 – 191 °C)[110]

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.52 (s, 3 H, 1-CH_3), 3.78 (s, 3 H, OCH_3), 4.91 (bs, 2 H, 1''-H), 6.82 – 6.88 (m, 2 H, 3'''-H, 5'''-H), 6.96 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.29 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.30 – 7.44 (m, 6 H, 3'-H, 4'-H, 5'-H, 6'-H, 2'''-H, 6'''-H), 7.55 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 55.6 (OCH_3), 57.5 (C-1''), 113.9 (C-3''', C-5'''), 124.2 (C-10), 127.6 (C-5'), 129.5 (C-7), 129.6 (C-1'''), 130.6 (C-3'), 130.9 (C-2''', C-6'''), 131.6 (C-6'), 131.7 (C-6a), 132.0 (C-4'), 132.0 (C-9), 133.1 (C-8), 133.2 (C-2'), 133.3 (C-10a), 135.7 (C-1'), 148.6 (C-1), 159.4 (C-4'''), 159.7 (C-3a), 160.3 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2929 (w), 2833 (w), 2361 (m), 2343 (m), 1612 (m), 1514 (s), 1493 (m), 1436 (m), 1320 (m), 1249 (m), 1174 (m), 1100 (w), 1032 (m), 820 (m)

MS (CI): *m/z* (%) = 468 (8), 466 (30), 464 (55) $[M + H]^+$, 211 (100), 121 (80)

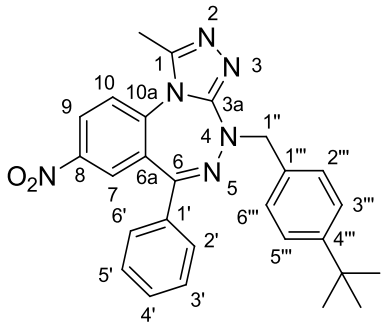
MS (EI): *m/z* (%) = 463 (2) $[M]^{+\cdot}$, 329 (5), 121 (100), 77 (10)

HR-MS (EI): calcd. for $C_{24}H_{19}Cl_2N_5O$ $[M]^{+\cdot}$ 463.0967; found 463.0966

HPLC purity: 96 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{19}Cl_2N_5O$

MW: 464.35 g/mol



4-[4-(tert-Butyl)benzyl]-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (71a)

Following **GP4**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 4-(*tert*-butyl)benzyl bromide **54** (138 µL, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate / methanol 9:1, R_f = 0.55), product **71a** (84 mg, 0.18 mmol, 72 %) was obtained as yellow solid.

mp: 245.6 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 1.29 (s, 9 H, $C(CH_3)_3$), 2.59 (s, 3 H, 1-CH_3), 4.77 – 5.24 (m, 2 H, 1''-H), 7.31 – 7.36 (m, 4 H, 2'''-H, 3'''-H, 5'''-H, 6'''-H), 7.37 – 7.41 (m, 2 H, 3'-H, 5'-H), 7.42 – 7.45 (m, 2 H, 2'-H, 6'-H), 7.45 – 7.50 (m, 1 H, 4'-H), 7.54 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.08 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.45 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 31.5 ($C(CH_3)_3$), 34.8 ($C(CH_3)_3$), 57.9 (C-1''), 124.0 (C-10), 125.6 (C-3''', C-5'''), 126.8 (C-9), 127.0 (C-7), 129.0 (C-3', C-5'), 129.3 (C-2''', C-6'''), 129.4 (C-2', C-6'), 130.5 (C-6a), 131.2 (C-4'), 134.4 (C-1'''), 136.0 (C-1'), 140.4 (C-10a), 145.8 (C-8), 148.6 (C-1), 150.8 (C-4'''), 160.5 (C-3a), 160.7 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3087 (w), 2961 (m), 2867 (w), 2360 (m), 2342 (m), 1618 (m), 1535 (s), 1521 (s), 1489 (m), 1445 (m), 1432 (m), 1346 (s), 1325 (m), 1268 (w),

1253 (w), 1100 (w), 1032 (w), 916 (w), 885 (w), 840 (w), 794 (m), 750 (m), 737 (m), 695 (m)

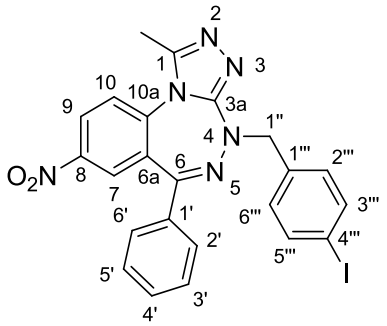
MS (ESI): *m/z* = 467 $[M + H]^+$, 229

HR-MS (EI): calcd. for $C_{27}H_{26}N_6O_2$ $[M]^{+\cdot}$ 466.2117; found 466.2115

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{27}H_{26}N_6O_2$

MW: 466.53 g/mol



4-(4-Iodobenzyl)-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c]-[1,2,4]triazepine (71b)

Following **GP4**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 4-iodobenzyl bromide **55a** (240 µL, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.42), product **71b** (91 mg, 0.17 mmol, 68 %) was obtained as yellow solid.

mp: 201.2 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.60 (s, 3 H, 1-CH_3), 4.79 – 4.91 (m, 1 H, 1''-*H*H), 4.98 – 5.11 (m, 1 H, 1''-H*H*), 7.14 – 7.20 (m, 2 H, 2'''-H, 6'''-H), 7.36 – 7.43 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.45 – 7.51 (m, 1 H, 4'-H), 7.55 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 7.62 – 7.67 (m, 2 H, 3'''-H, 5'''-H), 8.08 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.46 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 57.6 (C-1''), 93.3 (C-4'''), 124.1 (C-10), 126.9 (C-9), 127.0 (C-7), 129.0 (C-3', C-5'), 129.4 (C-2', C-6'), 130.3 (C-6a), 131.3 (C-4'), 131.7 (C-2''', C-6'''), 135.9 (C-1'), 137.2 (C-1'''), 140.2 (C-10a), 137.8 (C-3''', C-5'''), 145.7 (C-8), 148.7 (C-1), 160.3 (C-3a), 161.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3079 (w), 3057 (w), 2924 (w), 2859 (w), 2361 (w), 2342 (w), 1731 (m), 1618 (m), 1596 (m), 1536 (s), 1519 (s), 1486 (s), 1445 (m), 1432 (m), 1400 (m), 1344 (s), 1326 (s), 1250 (m), 1099 (m), 1059 (m), 1032 (m), 1007 (m), 915 (m), 885 (m), 842 (m), 790 (m), 748 (m), 737 (m), 694 (w)

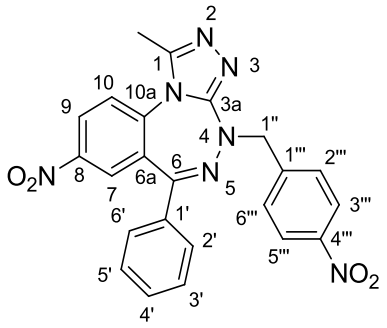
MS (ESI): *m/z* = 537 $[M + H]^+$, 491, 368, 340, 337, 335, 170

HR-MS (EI): calcd. for $C_{23}H_{17}IN_6O_2$ $[M]^{+\cdot}$ 536.0458; found 536.0463

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}IN_6O_2$

MW: 536.32 g/mol



1-Methyl-8-nitro-4-(4-nitrobenzyl)-6-phenyl-4H-benzo[e][1,2,4]triazolo[3,4-c]-[1,2,4]triazepine (71c)

Following **GP4**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 4-nitrobenzyl bromide **55b** (162 mg, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.38), product **71c** (68 mg, 0.15 mmol, 60 %) was obtained as yellow solid.

mp: 207.1 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.62 (s, 3 H, 1-CH_3), 4.98 – 5.10 (m, 1 H, 1''-*H*H), 5.15 – 5.28 (m, 1 H, 1''-H*H*), 7.35 – 7.42 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.44 – 7.52 (m, 1 H, 4'-H), 7.59 (d, $^3J_{HH}$ = 8.8 Hz, 2 H, 2'''-H, 6'''-H), 7.60 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.11 (d, $^4J_{HH}$ = 2.5 Hz, 1 H, 7-H), 8.16 (d, $^3J_{HH}$ = 8.8 Hz, 2 H, 3'''-H, 5'''-H), 8.50 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 57.5 (C-1''), 123.9 (C-3''', C-5'''), 124.2 (C-10), 127.1 (C-9), 127.1 (C-7), 129.1 (C-3', C-5'), 129.4 (C-2', C-6'), 130.2 (C-6a), 130.2 (C-2''', C-6'''), 131.4 (C-4'), 135.7 (C-1'), 140.2 (C-10a), 145.0 (C-1'''), 145.9 (C-8), 147.7 (C-4'''), 149.0 (C-1), 160.1 (C-3a), 161.6 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3080 (w), 2925 (w), 2855 (w), 2362 (w), 2344 (w), 1735 (w), 1618 (m), 1603 (m), 1519 (s), 1490 (m), 1445 (m), 1433 (m), 1401 (w), 1381 (w), 1345 (s), 1252 (m), 1177 (w), 1100 (m), 1032 (m), 913 (m), 843 (m), 737 (m), 695 (m), 592 (m)

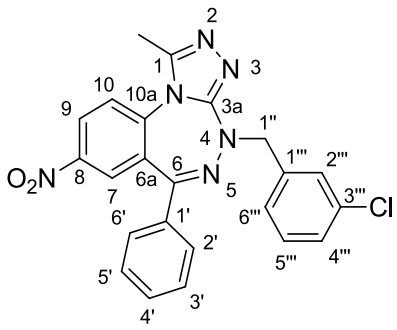
MS (ESI): *m/z* = 456 $[M + H]^+$, 397, 235, 229, 157

HR-MS (EI): calcd. for $C_{23}H_{17}N_7O_4$ $[M]^{+\cdot}$ 455.1342; found 455.1350

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}N_7O_4$

MW: 455.43 g/mol



4-(3-Chlorobenzyl)-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (71d)

Following **GP4**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 3-chlorobenzyl bromide **55d** (98 µL, 0.75 mmol, 3.0 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.25), product **71d** (78 mg, 0.18 mmol, 70 %) was obtained as yellow solid.

mp: 178.2 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.60 (s, 3 H, 1-CH_3), 4.79 – 4.99 (m, 1 H, 1''-*H*H), 4.99 – 5.18 (m, 1 H, 1''-H*H*), 7.23 – 7.34 (m, 3 H, 2'''-H, 4'''-H, 5'''-H), 7.36 – 7.44 (m, 5 H, 2'-H, 3'-H, 5'-H, 6'-H, 6'''-H), 7.45 – 7.51 (m, 1 H, 4'-H), 7.56 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.08 (d, $^4J_{HH}$ = 2.5 Hz, 1 H, 7-H), 8.47 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 57.7 (C-1''), 124.1 (C-10), 127.0 (C-9), 127.0 (C-7), 128.0 (C-5'''), 128.0 (C-2'''), 129.1 (C-3', C-5'), 129.4 (C-2', C-6'), 129.7 (C-6'''), 130.1 (C-4'''), 130.3 (C-6a), 131.3 (C-4'), 134.3 (C-3'''), 135.9 (C-1'), 139.5 (C-1'''), 140.2 (C-10a), 145.9 (C-8), 148.8 (C-1), 160.2 (C-3a), 161.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2924 (w), 2857 (w), 2367 (w), 2345 (w), 1618 (m), 1598 (m), 1578 (m), 1535 (s), 1519 (s), 1488 (m), 1445 (m), 1431 (m), 1401 (m), 1381 (m), 1345 (s), 1325 (s), 1282 (m), 1252 (m), 1210 (m), 1099 (m), 1077 (m),

1032 (m), 1001 (w), 974 (w), 915 (w), 886 (m), 840 (w), 784 (m), 748 (m), 737 (m), 694 (m), 654 (m), 596 (m)

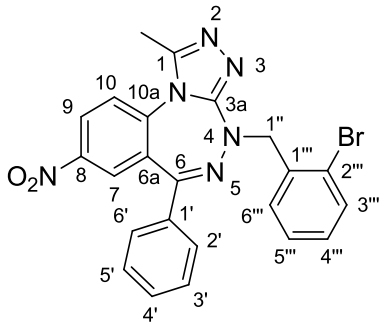
MS (ESI): *m/z* = 447, 445 $[M + H]^+$, 366, 239

HR-MS (EI): calcd. for $C_{23}H_{17}ClN_6O_2$ $[M]^{+\cdot}$ 444.1102; found 444.1101

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}ClN_6O_2$

MW: 444.87 g/mol



4-(2-Bromobenzyl)-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (71e)

Following **GP4**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 2-bromobenzyl bromide **55e** (187 mg, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.49), product **71e** (77 mg, 0.16 mmol, 63 %) was obtained as yellow solid.

mp: 231.7 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.61 (s, 3 H, 1-CH_3), 5.02 (d, $^2J_{HH}$ = 13.9 Hz, 1 H, 1''-*H*H), 5.25 (d, $^2J_{HH}$ = 14.0 Hz, 1 H, 1''-H*H*), 7.16 (td, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.7 Hz, 1 H, 4'''-H), 7.29 (td, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 5'''-H), 7.36 – 7.51 (m, 6 H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H, 6'''-H), 7.53 – 7.57 (m, 2 H, 10-H, 3'''-H), 8.08 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.46 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 58.1 (C-1''), 124.0 (C-10), 125.0 (C-2'''), 127.0 (C-9), 127.0 (C-7), 127.6 (C-5'''), 129.0 (C-3', C-5'), 129.4 (C-2', C-6'), 129.5 (C-4'''), 130.4 (C-6a), 131.3 (C-4'), 131.9 (C-6'''), 133.2 (C-3'''), 135.9 (C-1'), 136.6 (C-1'''), 140.4 (C-10a), 145.8 (C-8), 148.7 (C-1), 160.3 (C-3a), 161.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3058 (w), 2927 (w), 2362 (w), 2343 (w), 1734 (w), 1618 (m), 1596 (m), 1536 (s), 1519 (s), 1489 (m), 1434 (m), 1345 (s), 1325 (s), 1252 (w),

1099 (w), 1027 (m), 915 (w), 885 (w), 840 (w), 774 (w), 748 (m), 695 (m), 657 (w), 597 (w)

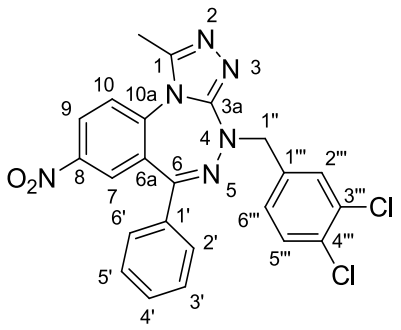
MS (ESI): *m/z* = 491, 489 $[M + H]^+$, 264

HR-MS (EI): calcd. for $C_{23}H_{17}BrN_6O_2$ $[M]^{+\cdot}$ 488.0596; found 488.0601

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{17}BrN_6O_2$

MW: 489.32 g/mol



4-(3,4-Dichlorobenzyl)-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (71f)

Following **GP5**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 3,4-dichlorobenzyl chloride **57b** (104 µL, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.34), product **71f** (57 mg, 0.12 mmol, 48 %) was obtained as yellow solid.

mp: 193.6 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.61 (s, 3 H, 1-CH_3), 4.79 – 4.94 (m, 1 H, 1''-*H*H), 5.00 – 5.13 (m, 1 H, 1''-H*H*), 7.29 (dd, $^4J_{HH}$ = 1.9 Hz, $^3J_{HH}$ = 8.3 Hz, 1 H, 6'''-H), 7.37 – 7.44 (m, 5 H, 2'-H, 3'-H, 5'-H, 6'-H, 5'''-H), 7.46 – 7.51 (m, 1 H, 4'-H), 7.53 (d, $^4J_{HH}$ = 1.9 Hz, 1 H, 2'''-H), 7.56 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.09 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.47 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 57.2 (C-1''), 124.1 (C-10), 127.0 (C-9), 127.1 (C-7), 129.1 (C-3', C-5'), 129.4 (C-6'''), 129.4 (C-2', C-6'), 130.3 (C-6a), 130.7 (C-5'''), 131.4 (C-4'), 131.7 (C-4'''), 131.7 (C-2'''), 132.5 (C-3'''), 135.9 (C-1'), 137.8 (C-1'''), 140.2 (C-10a), 145.9 (C-8), 148.8 (C-1), 160.1 (C-3a), 161.3 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3081 (w), 2926 (w), 2859 (w), 2361 (w), 2342 (w), 1733 (w), 1618 (m), 1596 (m), 1536 (s), 1519 (s), 1489 (m), 1472 (m), 1445 (m), 1433 (m),

1398 (m), 1381 (m), 1345 (s), 1325 (s), 1282 (w), 1251 (w), 1132 (w), 1099 (w), 1031 (m), 915 (w), 886 (w), 790 (m), 749 (m), 736 (m), 694 (m)

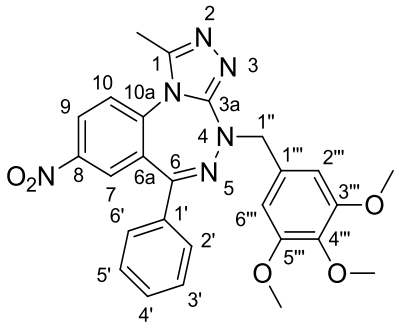
MS (ESI): *m/z* = 483, 481, 479 $[M + H]^+$, 130

HR-MS (EI): calcd. for $C_{23}H_{16}Cl_2N_6O_2$ $[M]^{+\cdot}$ 478.0712; found 478.0718

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{16}Cl_2N_6O_2$

MW: 479.32 g/mol



1-Methyl-8-nitro-6-phenyl-4-(3,4,5-trimethoxybenzyl)-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (71g)

Following **GP5**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 3,4,5-trimethoxybenzyl chloride **57c** (162 mg, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate / *N*,*N*-dimethylethylamine 39:1, R_f = 0.38), product **71g** (48 mg, 96 µmol, 38 %) was obtained as yellow solid.

mp: 212.8 – 214.3 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.61 (s, 3 H, 1-CH_3), 3.74 (s, 3 H, 4'''-OCH_3), 3.76 (s, 6 H, 3'''-OCH_3, 5'''-OCH_3), 4.78 – 4.87 (m, 1 H, 1''-*H*H), 5.00 – 5.09 (m, 1 H, 1''-H*H*), 6.64 (s, 2 H, 2'''-H, 6'''-H), 7.38 – 7.43 (m, 2 H, 3'-H, 5'-H), 7.44 – 7.47 (m, 2 H, 2'-H, 6'-H), 7.47 – 7.51 (m, 1 H, 4'-H), 7.56 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.09 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.46 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 56.3 (3'''-OCH_3, 5'''-OCH_3), 58.3 (C-1''), 60.8 (4'''-OCH_3), 106.6 (C-2''', C-6'''), 124.1 (C-10), 126.8 (C-9), 126.9 (C-7), 129.1 (C-3', C-5'), 129.4 (C-2', C-6'), 130.5 (C-6a), 131.3 (C-4'), 133.0 (C-1'''), 136.0 (C-1'), 137.7 (C-4'''), 140.4 (C-10a), 145.8 (C-8), 148.7 (C-1), 153.6 (C-3''', C-5'''), 160.4 (C-3a), 161.0 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3425 (w), 3081 (w), 2936 (m), 2837 (w), 2362 (w), 2343 (w), 1618 (m), 1592 (m), 1536 (m), 1521 (s), 1508 (m), 1459 (m), 1422 (m), 1345 (s), 1328 (w), 1235 (m), 1182 (w), 1126 (s), 1033 (w), 1006 (m), 916 (w), 884 (w), 841 (w), 793 (w), 780 (w), 749 (w), 737 (w), 695 (w)

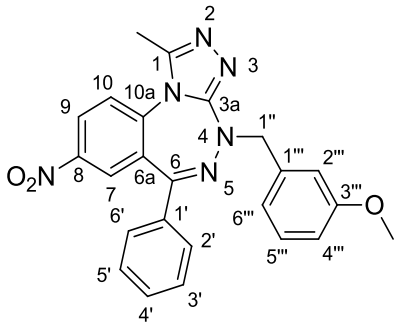
MS (APCI): *m/z* = 501 $[M + H]^+$, 471, 276, 266, 236, 196, 181

HR-MS (ESI): calcd. for $C_{26}H_{25}N_6O_5^+$ $[M + H]^+$ 501.1881; found 501.1888

HPLC purity: 91 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{26}H_{24}N_6O_5$

MW: 500.51 g/mol



4-(3-Methoxybenzyl)-1-methyl-8-nitro-6-phenyl-4H-benzo[e][1,2,4]triazolo-[3,4-c][1,2,4]triazepine (71h)

Following **GP4**, N-alkylation of 1-methyl-8-nitro-6-phenyl-4*H*-benzo[*e*][1,2,4]-triazolo[3,4-*c*][1,2,4]triazepine **14** (80 mg, 0.25 mmol, 1.0 equiv) was carried out using 3-methoxybenzyl bromide **70** (105 µL, 0.749 mmol, 3.00 equiv) and sodium hydride (11 mg, 0.28 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.33), product **71h** (68 mg, 0.15 mmol, 62 %) was obtained as pale yellow solid.

mp: 169.8 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.60 (s, 3 H, 1-CH_3), 3.74 (s, 3 H, OCH_3), 4.81 – 4.96 (m, 1 H, 1''-*H*H), 5.02 – 5.19 (m, 1 H, 1''-H*H*), 6.80 (dd, $^4J_{HH}$ = 2.3 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 4'''-H), 6.92 – 6.95 (m, 1 H, 2'''-H), 6.96 – 7.01 (m, 1 H, 6'''-H), 7.23 (t, $^3J_{HH}$ = 7.9 Hz, 1 H, 5'''-H), 7.35 – 7.45 (m, 4 H, 2'-H, 3'-H, 5'-H, 6'-H), 7.45 – 7.51 (m, 1 H, 4'-H), 7.55 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 8.08 (d, $^4J_{HH}$ = 2.6 Hz, 1 H, 7-H), 8.46 (dd, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 55.5 (OCH_3), 58.1 (C-1''), 113.1 (C-4'''), 115.1 (C-2'''), 121.7 (C-6'''), 124.1 (C-10), 126.9 (C-9), 126.9 (C-7), 129.0 (C-3', C-5'), 129.4 (C-2', C-6'), 129.7 (C-5'''), 130.4 (C-6a), 131.2 (C-4'), 136.0 (C-1'), 139.0 (C-1'''), 140.3 (C-10a), 145.8 (C-8), 148.7 (C-1), 160.0 (C-3'''), 160.5 (C-3a), 160.9 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3056 (w), 2962 (w), 2927 (w), 2834 (w), 2368 (w), 1617 (m), 1597 (m), 1535 (s), 1519 (s), 1489 (m), 1445 (m), 1432 (m), 1400 (w), 1345 (s),

1324 (m), 1263 (s), 1157 (m), 1098 (m), 1031 (m), 918 (w), 883 (w), 800 (m), 785 (m), 747 (w), 736 (w), 694 (m)

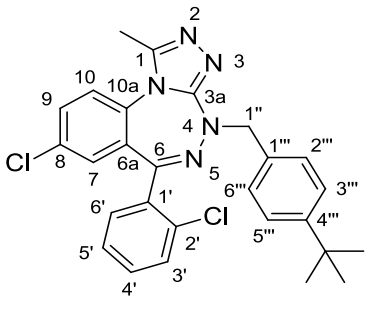
MS (ESI): *m/z* = 441 $[M + H]^+$, 248, 235

HR-MS (EI): calcd. for $C_{24}H_{20}N_6O_3$ $[M]^{+\cdot}$ 440.1597; found 440.1598

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{20}N_6O_3$

MW: 440.45 g/mol



4-[4-(tert-Butyl)benzyl]-8-chloro-6-(2-chlorophenyl)-1-methyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (72a)

Following **GP4**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 4-(*tert*-butyl)benzyl bromide **54** (128 µL, 0.697 mmol, 3.00 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate / methanol 9:1, R_f = 0.78), product **72a** (111 mg, 0.226 mmol, 97 %) was obtained as pale yellow solid.

mp: 190.2 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 1.31 (s, 9 H, $C(CH_3)_3$), 2.52 (s, 3 H, 1-CH_3), 4.96 (bs, 2 H, 1''-H), 6.97 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.30 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.31 – 7.38 (m, 7 H, 3'-H, 5'-H, 6'-H, 2'''-H, 3'''-H, 5'''-H, 6'''-H), 7.38 – 7.42 (m, 1 H, 4'-H), 7.55 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 31.5 ($C(CH_3)_3$), 34.8 ($C(CH_3)_3$), 57.8 (C-1''), 124.2 (C-10), 125.5 (C-3''', C-5'''), 127.6 (C-5'), 129.2 (C-2''', C-6'''), 129.6 (C-7), 130.6 (C-3'), 131.6 (C-4'), 131.7 (C-6a), 132.0 (C-6'), 132.0 (C-9), 133.1 (C-10a), 133.3 (C-8), 133.3 (C-2'), 134.5 (C-1'''), 135.7 (C-1'), 148.6 (C-1), 150.7 (C-4'''), 159.7 (C-6), 160.3 (C-3a)

IR [cm^{-1}]: $\tilde{\nu}$ = 3059 (w), 2961 (m), 2866 (w), 1594 (m), 1535 (s), 1518 (s), 1494 (s), 1436 (s), 1380 (m), 1361 (m), 1345 (m), 1320 (m), 1268 (w), 1174 (m), 1101 (m), 1053 (m), 1034 (m), 828 (m), 762 (m), 749 (m), 733 (m), 649 (m), 595 (m), 561 (m)

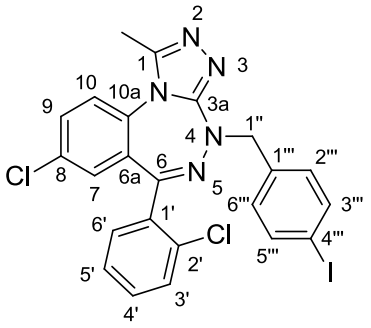
MS (ESI): *m/z* = 494, 492, 490 $[M + H]^+$, 317, 239

HR-MS (EI): calcd. for $C_{27}H_{25}Cl_2N_5$ $[M]^{+\cdot}$ 489.1487; found 489.1495

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{27}H_{25}Cl_2N_5$

MW: 490.43 g/mol



8-Chloro-6-(2-chlorophenyl)-4-(4-iodobenzyl)-1-methyl-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (72b)

Following **GP4**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 4-iodobenzyl bromide **55a** (223 µL, 0.697 mmol, 3.00 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate / methanol 9:1, R_f = 0.63), product **72b** (87 mg, 0.16 mmol, 67 %) was obtained as colorless solid.

mp: 217.3 – 218.3 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.53 (s, 3 H, 1-CH_3), 4.93 (bs, 2 H, 1''-H), 6.97 (dd, $^5J_{HH}$ = 0.3 Hz, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.14 – 7.19 (m, 2 H, 2'''-H, 6'''-H), 7.29 (ddd, $^5J_{HH}$ = 0.7 Hz, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'-H), 7.31 (dd, $^5J_{HH}$ = 0.3 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.35 (ddd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 5'-H), 7.37 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3'-H), 7.41 (ddd, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 8.1 Hz, 1 H, 4'-H), 7.57 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.6 Hz, 1 H, 9-H), 7.63 – 7.68 (m, 2 H, 3'''-H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 57.5 (C-1''), 93.1 (C-4'''), 124.2 (C-10), 127.6 (C-5'), 129.6 (C-7), 130.6 (C-3'), 131.5 (C-2''', C-6'''), 131.6 (C-6a), 131.7 (C-4'), 131.9 (C-6'), 132.1 (C-9), 133.0 (C-10a), 133.2 (C-8), 133.4 (C-2'), 135.6 (C-1'), 137.4 (C-1'''), 137.8 (C-3''', C-5'''), 148.7 (C-1), 160.1 (C-3a), 160.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2926 (w), 2360 (w), 2341 (w), 1793 (w), 1636 (m), 1593 (m), 1576 (w), 1535 (m), 1519 (s), 1494 (m), 1435 (m), 1400 (w), 1381 (w), 1346 (m), 1320 (m), 1281 (w), 1250 (w), 1175 (m), 1101 (w), 1056 (w), 1034 (w), 1008 (m), 827 (m)

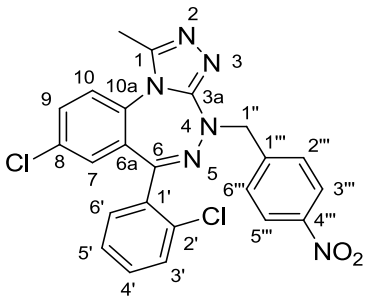
MS (ESI): *m/z* = 564, 562, 560 $[M + H]^+$, 405, 315, 301, 279, 229, 207, 184, 59

HR-MS (EI): calcd. for $C_{23}H_{16}Cl_2IN_5$ $[M]^{+\cdot}$ 558.9828; found 558.9808

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{16}Cl_2IN_5$

MW: 560.22 g/mol



8-Chloro-6-(2-chlorophenyl)-1-methyl-4-(4-nitrobenzyl)-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (72c)

Following **GP4**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 4-nitrobenzyl bromide **55b** (151 mg, 0.697 mmol, 3.00 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate / methanol 9:1, R_f = 0.69), product **72c** (73 mg, 0.15 mmol, 66 %) was obtained as yellow solid.

mp: 199.1 °C (decomposition)

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 5.09 (bs, 2 H, 1''-H), 6.99 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.22 (dd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.6 Hz, 1 H, 6'-H), 7.32 (ddd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.2 Hz, $^3J_{HH}$ = 7.6 Hz, 1 H, 5'-H), 7.34 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.36 (dd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 8.1 Hz, 1 H, 3'-H), 7.40 (ddd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.2 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 4'-H), 7.56 – 7.60 (m, 2 H, 2'''-H, 6'''-H), 7.60 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H), 8.14 – 8.20 (m, 2 H, 3'''-H, 5'''-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.4 (C-1''), 123.9 (C-3''', C-5'''), 124.3 (C-10), 127.7 (C-5'), 129.7 (C-7), 130.1 (C-2''', C-6'''), 130.7 (C-3'), 131.4 (C-6a), 131.8 (C-4'), 131.8 (C-6'), 132.3 (C-9), 133.0 (C-10a), 133.2 (C-2'), 133.5 (C-8), 135.4 (C-1'), 145.2 (C-1'''), 147.7 (C-4'''), 148.9 (C-1), 159.9 (C-3a), 162.6 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3440 (s), 2924 (w), 2364 (w), 1735 (w), 1637 (m), 1606 (m), 1519 (s), 1493 (m), 1436 (m), 1381 (w), 1345 (s), 1320 (m), 1281 (w), 1249 (w), 1174 (w), 1108 (w), 1053 (w), 1053 (w), 832 (m), 763 (w), 739 (m)

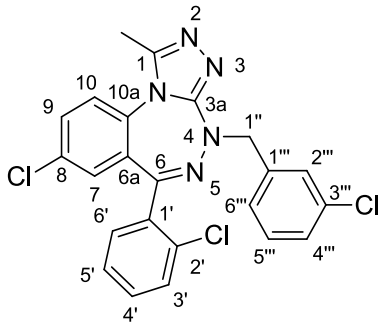
MS (ESI): *m/z* = 481, 479 $[M + H]^+$, 380, 229

HR-MS (EI): calcd. for $C_{23}H_{16}Cl_2N_6O_2$ $[M]^{+\cdot}$ 478.0712; found 478.0719

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{16}Cl_2N_6O_2$

MW: 479.32 g/mol



8-Chloro-4-(3-chlorobenzyl)-6-(2-chlorophenyl)-1-methyl-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (72d)

Following **GP4**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 3-chlorobenzyl bromide **55d** (92 µL, 0.70 mmol, 3.0 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.35), product **72d** (84 mg, 0.18 mmol, 77 %) was obtained as pale yellow solid.

mp: 116.1 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 4.81 – 5.13 (m, 2 H, 1''-H), 6.98 (d, $^4J_{HH}$ = 2.3 Hz, 1 H, 7-H), 7.24 – 7.43 (m, 9 H, 10-H, 3'-H, 4'-H, 5'-H, 6'-H, 2'''-H, 4'''-H, 5'''-H, 6'''-H), 7.58 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 57.6 (C-1''), 124.2 (C-10), 127.6 (C-5'), 127.9 (C-5'''), 127.9 (C-2'''), 129.6 (C-7), 129.7 (C-6'''), 130.0 (C-4'''), 130.6 (C-3'), 131.7 (C-4'), 131.5 (C-6a), 131.9 (C-6'), 132.1 (C-9), 133.0 (C-10a), 133.2 (C-8), 133.4 (C-2'), 134.3 (C-3'''), 135.6 (C-1'), 139.7 (C-1'''), 148.8 (C-1), 160.0 (C-3a), 160.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3061 (w), 2924 (w), 2360 (w), 2345 (w), 1746 (m), 1598 (m), 1576 (m), 1534 (s), 1518 (s), 1493 (s), 1474 (m), 1431 (s), 1379 (m), 1343 (m), 1320 (m), 1280 (m), 1252 (m), 1208 (m), 1174 (m), 1099 (m), 1077 (w), 1052 (m), 1033 (m), 884 (m), 833 (m), 768 (m), 747 (m), 734 (m), 712 (m), 682 (m), 598 (m)

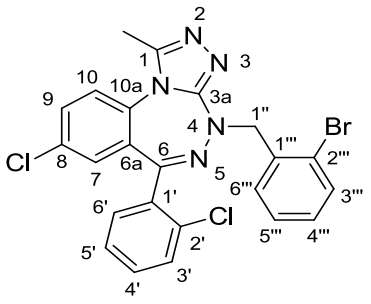
MS (ESI): *m/z* = 472, 472, 468 $[M + H]^+$, 280, 263, 248

HR-MS (EI): calcd. for $C_{23}H_{16}Cl_3N_5$ $[M]^{+\cdot}$ 467.0472; found 467.0480

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{16}Cl_3N_5$

MW: 468.77 g/mol



4-(2-Bromobenzyl)-8-chloro-6-(2-chlorophenyl)-1-methyl-4H-benzo[e][1,2,4]-triazolo[3,4-c][1,2,4]triazepine (72e)

Following **GP4**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 2-bromobenzyl bromide **55e** (174 mg, 0.697 mmol, 3.00 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate / methanol 9:1, R_f = 0.73), product **72e** (91 mg, 0.18 mmol, 76 %) was obtained as colorless solid.

mp: 245.0 – 246.4 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 4.84 – 5.31 (m, 2 H, 1''-H), 6.98 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.16 (ddd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.5 Hz, $^3J_{HH}$ = 7.9 Hz, 1 H, 4'''-H), 7.29 (td, $^4J_{HH}$ = 1.3 Hz, $^3J_{HH}$ = 7.6 Hz, 1 H, 5'''-H), 7.31 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.9 Hz, $^3J_{HH}$ = 7.6 Hz, 1 H, 6'-H), 7.31 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.34 (ddd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.0 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 5'-H), 7.37 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3'-H), 7.41 (ddd, $^4J_{HH}$ = 1.9 Hz, $^3J_{HH}$ = 6.9 Hz, $^3J_{HH}$ = 8.1 Hz, 1 H, 4'-H), 7.46 (dd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.7 Hz, 1 H, 6'''-H), 7.56 (dd, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3'''-H), 7.57 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 58.0 (C-1''), 124.2 (C-10), 124.9 (C-2'''), 127.6 (C-5'), 127.6 (C-5'''), 129.4 (C-4'''), 129.6 (C-7), 130.6 (C-3'), 131.6 (C-4'), 131.6 (C-6a), 131.7 (C-6'''), 132.0 (C-6'), 132.1 (C-9), 133.1 (C-10a),

133.2 (C-3'''), 133.3 (C-8), 133.4 (C-2'), 135.6 (C-1'), 136.8 (C-1'''), 148.8 (C-1), 160.1 (C-3a), 160.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3062 (w), 2917 (w), 2864 (w), 2365 (w), 1772 (w), 1594 (m), 1568 (m), 1536 (s), 1512 (s), 1494 (s), 1473 (s), 1439 (s), 1380 (m), 1364 (m), 1344 (m), 1323 (s), 1282 (m), 1238 (m), 1208 (m), 1177 (m), 1098 (m), 1046 (m), 1024 (s), 910 (m), 887 (m), 832 (s), 769 (s), 746 (s), 732 (s)

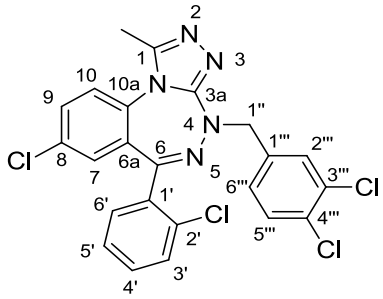
MS (ESI): *m/z* = 518, 516, 514, 512 $[M + H]^+$, 404, 391

HR-MS (EI): calcd. for $C_{23}H_{16}BrCl_2N_5$ $[M]^{+\cdot}$ 510.9966; found 510.9959

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{16}BrCl_2N_5$

MW: 513.22 g/mol



8-Chloro-6-(2-chlorophenyl)-4-(3,4-dichlorobenzyl)-1-methyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (72f)

Following **GP5**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 3,4-dichlorobenzyl chloride **57b** (97 µL, 0.70 mmol, 3.0 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate / methanol 9:1, R_f = 0.63), product **72f** (69 mg, 0.14 mmol, 59 %) was obtained as colorless solid.

mp: 219.6 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 4.95 (bs, 2 H, 1''-H), 6.98 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.28 (dd, $^4J_{HH}$ = 2.0 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 6'''-H), 7.30 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'-H), 7.32 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.36 (ddd, $^4J_{HH}$ = 1.6 Hz, $^3J_{HH}$ = 7.0 Hz, $^3J_{HH}$ = 7.4 Hz, 1 H, 5'-H), 7.37 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3'-H), 7.41 (ddd, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.0 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 4'-H), 7.41 (d, $^3J_{HH}$ = 8.2 Hz, 1 H, 5'''-H), 7.53 (d, $^4J_{HH}$ = 2.0 Hz, 1 H, 2'''-H), 7.58 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.8 (1-CH_3), 57.0 (C-1''), 124.2 (C-10), 127.7 (C-5'), 129.2 (C-6'''), 129.7 (C-7), 130.6 (C-3'), 130.6 (C-5'''), 131.4 (C-4'''), 131.6 (C-2'''), 131.6 (C-6a), 131.7 (C-4'), 131.9 (C-6'), 132.2 (C-9), 132.4 (C-3'''), 132.9 (C-10a), 133.2 (C-8), 133.4 (C-2'), 135.5 (C-1'), 138.0 (C-1'''), 148.8 (C-1), 159.9 (C-3a), 160.3 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3067 (w), 2914 (w), 2360 (w), 2343 (w), 1735 (w), 1637 (m), 1594 (m), 1538 (m), 1511 (s), 1496 (s), 1473 (m), 1432 (m), 1378 (m), 1361 (m), 1347 (m), 1323 (m), 1280 (w), 1215 (m), 1178 (m), 1132 (m), 1032 (m), 926 (w), 884 (m), 832 (m), 818 (s), 777 (w), 595 (m)

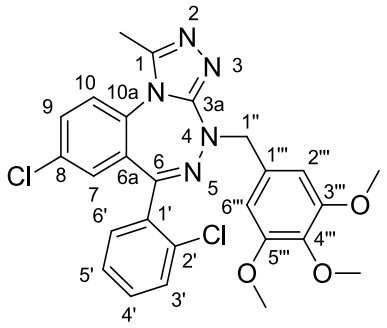
MS (ESI): *m/z* = 506, 504, 502 $[M + H]^+$, 239

HR-MS (EI): calcd. for $C_{23}H_{15}Cl_4N_5$ $[M]^{+\cdot}$ 501.0081; found 501.0082

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{15}Cl_4N_5$

MW: 503.21 g/mol



8-Chloro-6-(2-chlorophenyl)-1-methyl-4-(3,4,5-trimethoxybenzyl)-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (72g)

Following **GP5**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 3,4,5-trimethoxybenzyl chloride **57c** (151 mg, 0.697 mmol, 3.00 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (R_f = 0.48), product **72g** (83 mg, 0.16 mmol, 68 %) was obtained as pale yellow solid.

mp: 258.1 – 260.0 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.54 (s, 3 H, 1-CH_3), 3.75 (s, 3 H, 4'''-OCH_3), 3.76 (s, 6 H, 3'''-OCH_3, 5'''-OCH_3), 4.76 – 5.08 (m, 2 H, 1''-H), 6.64 (s, 2 H, 2'''-H, 6'''-H), 6.98 (d, $^4J_{HH}$ = 2.4 Hz, 1 H, 7-H), 7.33 (d, $^3J_{HH}$ = 8.7 Hz, 1 H, 10-H), 7.34 – 7.43 (m, 4 H, 3'-H, 4'-H, 5'-H, 6'-H), 7.57 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 56.3 (3'''-OCH_3, 5'''-OCH_3), 58.1 (C-1''), 60.8 (4'''-OCH_3), 106.4 (C-2''', C-6'''), 124.3 (C-10), 127.6 (C-5'), 129.4 (C-7), 130.6 (C-3'), 131.7 (C-4'), 131.7 (C-6a), 131.9 (C-6'), 132.1 (C-9), 133.1 (C-10a), 133.2 (C-8), 133.2 (C-2'), 133.3 (C-1'''), 135.6 (C-1'), 137.6 (C-4'''), 148.6 (C-1), 153.5 (C-3''', C-5'''), 160.0 (C-6), 160.2 (C-3a)

IR [cm^{-1}]: $\tilde{\nu}$ = 3422 (w), 3061 (w), 2992 (w), 2930 (w), 2835 (w), 2361 (w), 2343 (m), 1591 (m), 1537 (m), 1521 (m), 1508 (m), 1494 (m), 1456 (m), 1421 (m),

1351 (m), 1327 (m), 1236 (w), 1181 (m), 1125 (s), 1055 (m), 1010 (m), 832 (m), 760 (m)

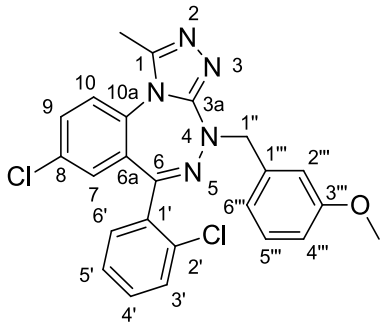
MS (APCI): *m/z* = 528, 526, 524 $[M + H]^+$, 490, 329, 291, 289, 259, 196, 181

HR-MS (ESI): calcd. for $C_{26}H_{24}Cl_2N_5O_3^+$ $[M + H]^+$ 524.1251; found 524.1259

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{26}H_{23}Cl_2N_5O_3$

MW: 524.40 g/mol



8-Chloro-6-(2-chlorophenyl)-4-(3-methoxybenzyl)-1-methyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (72h)

Following **GP4**, N-alkylation of 8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*-benzo[*e*][1,2,4]triazolo[3,4-*c*][1,2,4]triazepine **15** (80 mg, 0.23 mmol, 1.0 equiv) was carried out using 3-methoxybenzyl bromide **70** (98 µL, 0.70 mmol, 3.0 equiv) and sodium hydride (10 mg, 0.26 mmol, 1.1 equiv). After purification (ethyl acetate, R_f = 0.26), product **72h** (87 mg, 0.19 mmol, 81 %) was obtained as pale yellow solid.

mp: 184.4 °C

^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm) = 2.53 (s, 3 H, 1-CH_3), 3.74 (s, 3 H, OCH_3), 4.73 – 5.20 (m, 2 H, 1''-H), 6.78 – 6.83 (m, 1 H, 4'''-H), 6.92 – 6.95 (m, 1 H, 2'''-H), 6.96 – 7.00 (m, 2 H, 7-H, 6'''-H), 7.23 (t, $^3J_{HH}$ = 7.9 Hz, 1 H, 5'''-H), 7.29 – 7.42 (m, 5 H, 10-H, 3'-H, 4'-H, 5'-H, 6'-H), 7.56 (dd, $^4J_{HH}$ = 2.4 Hz, $^3J_{HH}$ = 8.7 Hz, 1 H, 9-H)

^{13}C NMR (100 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 55.5 (OCH_3), 58.0 (C-1''), 113.1 (C-4'''), 114.8 (C-2'''), 121.6 (C-6'''), 124.2 (C-10), 127.6 (C-5'), 129.5 (C-7), 129.6 (C-5'''), 130.6 (C-3'), 131.6 (C-4'), 131.6 (C-6a), 132.0 (C-6'), 132.0 (C-9), 133.1 (C-10a), 133.2 (C-8), 133.3 (C-2'), 135.6 (C-1'), 139.2 (C-1'''), 148.7 (C-1), 159.9 (C-3'''), 160.0 (C-6), 160.2 (C-3a)

IR [cm^{-1}]: $\tilde{\nu}$ = 3058 (w), 2997 (w), 2930 (w), 2833 (w), 2363 (w), 2343 (w), 1596 (m), 1535 (s), 1518 (s), 1492 (s), 1434 (s), 1380 (m), 1344 (m), 1320 (s),

1265 (s), 1172 (m), 1156 (m), 1099 (m), 1050 (m), 1034 (m), 884 (w), 831 (m), 769 (m), 746 (m), 693 (m), 598 (m)

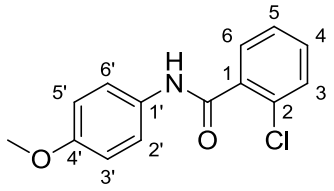
MS (ESI): *m/z* = 468, 466, 464 $[M + H]^+$, 280, 239

HR-MS (EI): calcd. for $C_{24}H_{19}Cl_2N_5O$ $[M]^{+\cdot}$ 463.0967; found 463.0983

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{19}Cl_2N_5O$

MW: 464.35 g/mol



2-Chloro-N-(4-methoxyphenyl)benzamide (75)

(Literature known compound but different procedure[159])

4-Aminoanisole **73** (3.5 g, 28 mmol, 1.0 equiv) was dissolved in anhydrous tetrahydrofuran (25 mL) and cooled with an ice bath to 0 °C before 2-chlorobenzoyl chloride **74** (4.5 mL, 31 mmol, 1.1 equiv) and *N*,*N*-diisopropylethylamine (5.0 mL, 28 mmol, 1.0 equiv) were added consecutively to the well stirred solution. After complete addition the mixture was allowed to warm to room temperature and stirring was continued for 15 min. The reaction mixture was concentrated under reduced pressure and 60 mL of a water / methanol (2:1) mixture were added. The precipitate was filtered off and washed three times with methanol (10 mL) to obtain product **75** (7.3 g, 28 mmol, 98 %) as colorless solid.

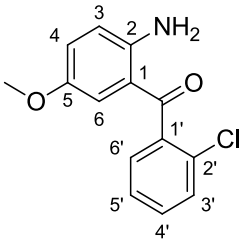
^{1}H NMR (500 MHz, acetone-d_6) δ (ppm) = 3.80 (s, 3 H, OCH_3), 6.91 – 6.96 (m, 2 H, 3'-H, 5'-H), 7.41 (ddd, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.1 Hz, $^3J_{HH}$ = 7.3 Hz, 1 H, 5-H), 7.46 (ddd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 7.0 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 4-H), 7.49 (dd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3-H), 7.58 (dd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.4 Hz, 1 H, 6-H), 7.71 – 7.76 (m, 2 H, 2'-H, 6'-H), 9.39 (s, 1 H, NH)

^{13}C NMR (125 MHz, acetone-d_6): δ (ppm) = 55.8 (OCH_3), 114.8 (C-3', C-5'), 122.1 (C-2', C-6'), 128.0 (C-5), 130.0 (C-6), 130.7 (C-3), 131.5 (C-2), 131.8 (C-4), 133.3 (C-1'), 138.3 (C-1), 157.3 (C-4'), 165.4 (CO)

MS (APCI): *m/z* = 264, 262 $[M + H]^+$

MF: $C_{14}H_{12}ClNO_2$

MW: 261.70 g/mol



(2-Amino-5-methoxyphenyl)(2-chlorophenyl)methanone (76)

(Literature known compound but different procedure[161])

2-Chloro-*N*-(4-methoxyphenyl)benzamide[157] **75** (2.0 g, 7.6 mmol, 1.0 equiv) was dissolved in 75 mL of deoxygenated acetonitrile and filled into a quartz glass tube. Photo-Fries rearrangement was carried out by irradiation for 1 d with 254 nm while using a cooling fan to maintain the reaction mixture at room temperature. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / isohexane / TEA 13:26:1, R_f = 0.57), yielding product **76** (248 mg, 0.948 mmol, 12 %) as yellow solid.

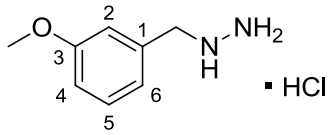
^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 3.55 (s, 3 H, OCH_3), 6.19 (bs, 2 H, NH_2), 6.61 (d, $^4J_{HH}$ = 3.0 Hz, 1 H, 6-H), 6.70 (d, $^3J_{HH}$ = 9.0 Hz, 1 H, 3-H), 7.00 (dd, $^4J_{HH}$ = 3.0 Hz, $^3J_{HH}$ = 9.0 Hz, 1 H, 4-H), 7.32 (dd, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'-H), 7.37 (ddd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.3 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 5'-H), 7.41 (ddd, $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 7.3 Hz, $^3J_{HH}$ = 7.9 Hz, 1 H, 4'-H), 7.46 (dd, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.9 Hz, 1 H, 3'-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 56.1 (OCH_3), 116.8 (C-6), 117.4 (C-1), 118.7 (C-3), 124.7 (C-4), 127.2 (C-5'), 128.8 (C-6'), 130.2 (C-3'), 130.8 (C-2'), 130.8 (C-4'), 140.2 (C-1'), 146.8 (C-2), 150.2 (C-5), 196.9 (CO)

MS (APCI): *m/z* = 264, 262 $[M + H]^+$, 226, 150, 141, 139

MF: $C_{14}H_{12}ClNO_2$

MW: 261.70 g/mol



(3-Methoxybenzyl)hydrazine hydrochloride (86)

(Literature known compound but different procedure[178])

tert-Butoxycarbonyl hydrazide **46** (2.5 g, 19 mmol, 1.0 equiv) and 3-methoxybenzaldehyde **84** (2.3 mL, 19 mmol, 1.0 equiv) were dissolved in ethanol (50 mL) and heated to reflux for 1 h. Reaction progress was monitored by TLC (ethyl acetate / isohexane 1:1, R_f = 0.53). The solvent was evaporated and the colorless solid was re-dissolved in tetrahydrofuran (10 mL), then treated with a solution of BH_3 x SMe_2 in tetrahydrofuran (2.0 M, 9.9 mL, 1.1 equiv) under cooling with an ice bath. After complete addition, the ice bath was removed and the solution was stirred for 15 min at room temperature. Then concentrated hydrochloric acid (4.8 mL, 3.0 equiv) was added dropwise at room temperature and the mixture was stirred subsequently at 60 °C for 10 min to complete the deprotection of the boc-group. After the reaction mixture was taken to dryness, tetrahydrofuran was added and the colorless precipitate was filtered off. Product **86** (3.2 g, 17 mmol, 90 %) was yielded as colorless hydrochloride.

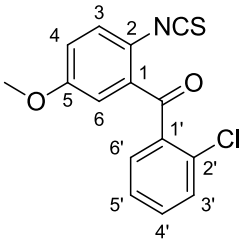
^{1}H NMR (500 MHz, MeOD): δ (ppm) = 3.82 (s, 3 H, OCH_3), 4.16 (s, 2 H, CH_2), 6.95 – 6.98 (m, 1 H, 4-H), 7.01 – 7.04 (m, 1 H, 6-H), 7.04 – 7.05 (m, 1 H, 2-H), 7.32 – 7.36 (m, 1 H, 5-H)

^{13}C NMR (100 MHz, MeOD): δ (ppm) = 55.9 (OCH_3), 56.3 (CH_2), 115.9 (C-4), 116.3 (C-2), 123.0 (C-6), 131.3 (C-5), 134.9 (C-1), 161.7 (C-3)

MS (APCI): *m/z* = 153 $[M + H]^+$, 136, 121

MF: $C_8H_{13}ClN_2O$ ($C_8H_{12}N_2O$)

MW: 188.65 g/mol (152.19 g/mol)



(2-Chlorophenyl)(2-isothiocyanato-5-methoxyphenyl)methanone (87)

Compound **87** was prepared according to **GP2** using (2-amino-5-methoxyphenyl)(2-chlorophenyl)methanone **76** (277 mg, 1.06 mmol, 1.00 equiv) as starting material, calcium carbonate (159 mg, 1.59 mmol, 1.50 equiv) and thiophosgene **16** (89 µL, 1.2 mmol, 1.1 equiv). After purification (R_f = 0.73), product **87** (227 mg, 0.747 mmol, 71 %) was yielded as yellow solid.

mp: 79.1 – 80.2 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 3.83 (s, 3 H, OCH_3), 7.06 (dd, $^4J_{HH}$ = 3.0 Hz, $^3J_{HH}$ = 8.8 Hz, 1 H, 4-H), 7.14 (d, $^4J_{HH}$ = 3.0 Hz, 1 H, 6-H), 7.25 (d, $^3J_{HH}$ = 8.8 Hz, 1 H, 3-H), 7.41 (ddd, $^4J_{HH}$ = 2.2 Hz, $^3J_{HH}$ = 6.5 Hz, $^3J_{HH}$ = 7.4 Hz, 1 H, 5'-H), 7.45 (ddd, $^4J_{HH}$ = 1.7 Hz, $^3J_{HH}$ = 6.5 Hz, $^3J_{HH}$ = 7.9 Hz, 1 H, 4'-H), 7.47 (ddd, $^5J_{HH}$ = 0.6 Hz, $^4J_{HH}$ = 2.2 Hz, $^3J_{HH}$ = 7.9 Hz, 1 H, 3'-H), 7.50 (ddd, $^5J_{HH}$ = 0.6 Hz, $^4J_{HH}$ = 1.6 Hz, $^3J_{HH}$ = 7.4 Hz, 1 H, 6'-H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 55.8 (OCH_3), 115.2 (C-6), 119.6 (C-4), 122.7 (C-2), 127.2 (C-5'), 129.5 (C-3), 130.0 (C-6'), 130.5 (C-3'), 131.8 (C-2'), 132.4 (C-4'), 134.5 (C-1), 135.1 (NCS), 138.0 (C-1'), 158.2 (C-5), 193.1 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3433 (w), 3086 (w), 3001 (w), 2965 (w), 2937 (w), 2903 (w), 2835 (w), 2361 (w), 2343 (w), 2183 (w), 2085 (s), 1648 (s), 1591 (m), 1567 (m), 1488 (s), 1461 (m), 1432 (m), 1410 (m), 1333 (s), 1286 (m), 1231 (s), 1146 (m), 1095 (m), 1054 (m), 1035 (m), 981 (w), 955 (w), 934 (w), 879 (w), 833 (m), 826 (m), 760 (m), 743 (m), 711 (w), 633 (m)

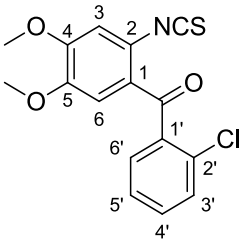
MS (APCI): *m/z* = 306, 304 $[M + H]^+$, 290, 288, 274, 272, 264, 262, 237, 226, 192, 139

HR-MS (ESI): calcd. for $C_{15}H_{11}ClNO_2S^+$ $[M + H]^+$ 304.0194; found 304.0198

HPLC purity: 96 % [λ = 210 nm], 97 % [λ = 254 nm]

MF: $C_{15}H_{10}ClNO_2S$

MW: 303.76 g/mol



(2-Chlorophenyl)(2-isothiocyanato-4,5-dimethoxyphenyl)methanone (88)

Compound **88** as prepared according to **GP2** using (2-amino-4,5-dimethoxyphenyl)(2-chlorophenyl)methanone[162] **81** (990 mg, 3.39 mmol, 1.00 equiv) as starting material, calcium carbonate (509 g, 5.09 mmol, 1.50 equiv) and thiophosgene **16** (286 µL, 3.73 mmol, 1.10 equiv). After purification (ethyl acetate / isohexane 1:2, R_f = 0.49), product **88** (1.0 g, 3.1 mmol, 91 %) was yielded as red oily substance.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 3.91 (s, 3 H, 5-OCH_3), 3.95 (s, 3 H, 4-OCH_3), 6.73 (s, 1 H, 3-H), 7.26 (s, 1 H, 6-H), 7.39 – 7.47 (m, 3 H, 4'-H, 5'-H, 6'-H), 7.47 – 7.51 (m, 1 H, 3'-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 56.3 (5-OCH_3), 56.5 (4-OCH_3), 110.6 (C-3), 112.1 (C-6), 124.9 (C-2), 125.4 (C-1), 127.3 (C-5'), 129.3 (C-6'), 130.3 (C-3'), 131.2 (C-2'), 131.9 (C-4'), 135.4 (NCS), 139.1 (C-1'), 148.2 (C-5), 153.5 (C-4), 191.9 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3065 (w), 3009 (w), 2964 (w), 2936 (w), 2850 (w), 2642 (w), 2432 (w), 2360 (w), 2342 (w), 2115 (s), 1657 (m), 1591 (s), 1566 (m), 1514 (s), 1464 (m), 1436 (m), 1399 (m), 1360 (m), 1282 (s), 1225 (s), 1209 (s), 1179 (m), 1161 (w), 1122 (s), 1057 (m), 1034 (m), 1007 (m), 963 (w), 942 (w), 868 (m), 840 (m), 787 (m), 754 (m), 709 (w), 629 (m)

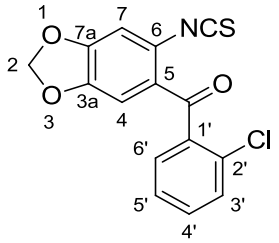
MS (ESI): *m/z* = 336, 334 $[M + H]^+$, 314, 292, 237, 222, 139, 102

HR-MS (ESI): calcd. for $C_{16}H_{13}ClNO_3S^+$ $[M + H]^+$ 334.0299; found 334.0305

HPLC purity: 90 % [λ = 210 nm], 91 % [λ = 254 nm]

MF: $C_{16}H_{12}ClNO_3S$

MW: 333.79 g/mol



(2-Chlorophenyl)(6-isothiocyanatobenzo[d][1,3]dioxol-5-yl)methanone (89)

Compound **89** was prepared according to **GP2** using (6-aminobenzo[*d*][1,3]dioxol-5-yl)(2-chlorophenyl)methanone[162] **83** (170 mg, 0.617 mmol, 1.00 equiv) as starting material, calcium carbonate (93 mg, 0.93 mmol, 1.5 equiv) and thiophosgene **16** (52 µL, 0.68 mmol, 1.1 equiv). After purification (ethyl acetate / isohexane 1:3, R_f = 0.33), product **89** (124 mg, 0.390 mmol, 63 %) was yielded as orange oily substance.

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 6.10 (s, 2 H, 2-H), 6.79 (s, 1 H, 7-H), 7.04 (s, 1 H, 4-H), 7.40 – 7.50 (m, 4 H, 3'-H, 4'-H, 5'-H, 6'-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 103.7 (C-2), 108.7 (C-7), 110.2 (C-4), 126.3 (C-6), 127.6 (C-5'), 127.8 (C-5), 129.9 (C-6'), 130.7 (C-3'), 131.6 (C-2'), 132.3 (C-4'), 135.8 (NCS), 139.2 (C-1'), 147.5 (C-3a), 152.4 (C-7a), 191.9 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3060 (w), 2983 (w), 2908 (w), 2772 (w), 2642 (w), 2461 (w), 2286 (w), 2111 (s), 1729 (w), 1664 (m), 1612 (m), 1589 (w), 1503 (s), 1481 (s), 1434 (m), 1421 (m), 1380 (m), 1282 (m), 1262 (s), 1243 (m), 1201 (m), 1156 (w), 1124 (w), 1089 (m), 1035 (s), 934 (m), 872 (w), 849 (w), 824 (w), 756 (m)

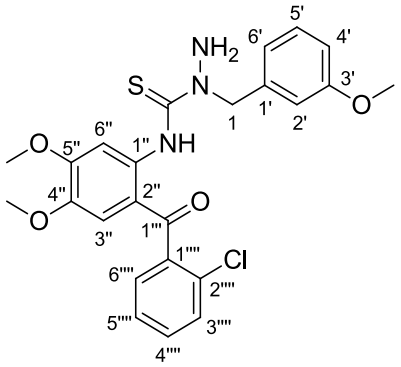
MS (APCI): *m/z* = 320, 318 $[M + H]^+$, 304, 302, 278, 276, 206

HR-MS (ESI): calcd. for $C_{15}H_9ClNO_3S^+$ $[M + H]^+$ 317.9986; found 317.9991

HPLC purity: 90 % [λ = 210 nm], 94 % [λ = 254 nm]

MF: $C_{15}H_8ClNO_3S$

MW: 317.75 g/mol



N-[2-(2-Chlorobenzoyl)-4,5-dimethoxyphenyl]-1-(3-methoxybenzyl)hydrazine-carbothioamide (91)

(3-Methoxybenzyl)hydrazine hydrochloride **86** (615 mg, 3.26 mmol, 1.10 equiv) was dissolved in 5 mL of methanol, cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (609 µL, 3.56 mmol, 1.20 equiv). After stirring for 1 h at room temperature, a solution of (2-chlorophenyl)(2-isothiocyanato-4,5-dimethoxy-phenyl)methanone **88** (990 mg, 2.97 mmol, 1.00 equiv) in 7 mL of tetrahydrofuran was added and stirring was continued for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / isohexane 1:1, R_f = 0.48), giving compound **91** (1.3 g, 2.7 mmol, 91 %) as pale yellow solid.

mp: 167.5 – 168.1 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 3.66 (s, 3 H, 4''-OCH_3), 3.80 (s, 3 H, 3'-OCH_3), 3.90 (bs, 2 H, NH_2), 4.04 (s, 3 H, 5''-OCH_3), 5.48 (s, 2 H, 1-H), 6.78 (s, 1 H, 3''-H), 6.86 (ddd, $^4J_{HH}$ = 0.8 Hz, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 8.3 Hz, 1 H, 4'-H), 6.93 – 6.97 (m, 2 H, 2'-H, 6'-H), 7.26 – 7.32 (m, 1 H, 5'-H), 7.32 – 7.38 (m, 2 H, 5''''-H, 6''''-H), 7.41 (ddd, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 6.5 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 4''''-H), 7.46 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.1 Hz, $^3J_{HH}$ = 8.0 Hz, 1 H, 3''''-H), 9.33 (s, 1 H, 6''-H), 13.09 (s, 1 H, NH)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 55.3 (3'-OCH_3), 56.2 (4''-OCH_3), 56.6 (5''-OCH_3), 57.2 (C-1), 106.0 (C-6''), 113.6 (C-4'), 113.8 (C-2'), 115.7 (C-3''), 117.0 (C-

2''), 120.4 (C-6'), 126.7 (C-5''''), 129.1 (C-6''''), 130.1 (C-5'), 130.1 (C-3''''), 131.0 (C-4''''), 131.1 (C-2''''), 136.9 (C-1'), 138.7 (C-1''), 139.3 (C-1''''), 143.9 (C-4''), 154.0 (C-5''), 160.2 (C-3'), 180.2 (CS), 195.9 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3334 (w), 3094 (w), 3002 (w), 2935 (w), 2832 (w), 2366 (w), 2345 (w), 1611 (m), 1573 (m), 1521 (s), 1489 (m), 1463 (m), 1433 (m), 1408 (w), 1377 (w), 1348 (m), 1321 (m), 1284 (m), 1263 (s), 1213 (m), 1184 (m), 1160 (m), 1117 (m), 1039 (m), 866 (w), 836 (w), 787 (w), 758 (w), 739 (w), 699 (w), 649 (w), 595 (w)

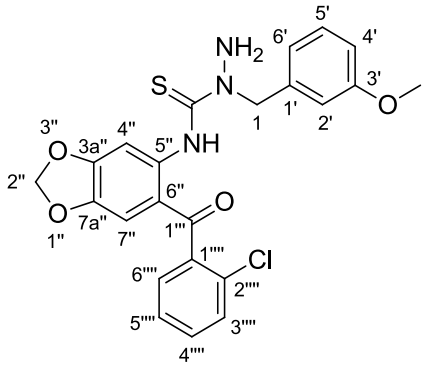
MS (ESI): *m/z* = 488, 486 $[M + H]^+$, 334, 292, 238, 193, 153

HR-MS (ESI): calcd. for $C_{24}H_{24}ClN_3NaO_4S^+$ $[M + Na]^+$ 508.1068; found 508.1075

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{24}ClN_3O_4S$

MW: 485.98 g/mol



N-[6-(2-Chlorobenzoyl)benzo[d][1,3]dioxol-5-yl]-1-(3-methoxybenzyl)-hydrazinecarbothioamide (92)

(3-Methoxybenzyl)hydrazine hydrochloride **86** (72 mg, 0.38 mmol, 1.1 equiv) was dissolved in 5 mL of methanol, cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (71 µL, 0.42 mmol, 1.2 equiv). After stirring for 1 h at room temperature, a solution of (2-chlorophenyl)(6-isothiocyanatobenzo[*d*][1,3]dioxol-5-yl)methanone **89** (110 mg, 0.346 mmol, 1.00 equiv) in 5 mL of tetrahydrofuran was added and stirring was continued for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / isohexane 2:3, R_f = 0.42), giving compound **92** (82 mg, 0.17 mmol, 50 %) as yellow solid.

mp: 170.5 – 172.1 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 3.79 (s, 3 H, 3'-OCH_3), 3.87 (bs, 2 H, NH_2), 5.46 (s, 2 H, 1-H), 6.02 (s, 2 H, 2''-H), 6.73 (s, 1 H, 7''-H), 6.86 (ddd, $^4J_{HH}$ = 1.0 Hz, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 8.3 Hz, 1 H, 4'-H), 6.93 – 6.97 (m, 2 H, 2'-H, 6'-H), 7.26 – 7.32 (m, 1 H, 5'-H), 7.32 – 7.35 (m, 2 H, 5''''-H, 6''''-H), 7.37 – 7.43 (m, 1 H, 4''''-H), 7.43 – 7.46 (m, 1 H, 3''''-H), 8.92 (s, 1 H, 4''-H), 12.94 (s, 1 H, NH)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 55.3 (3'-OCH_3), 57.3 (C-1), 102.2 (C-2''), 104.5 (C-4''), 111.5 (C-7''), 113.7 (C-2'), 113.7 (C-4'), 119.0 (C-6''), 120.5 (C-6'), 126.7 (C-5''''), 129.0 (C-6''''), 130.1 (C-5'), 130.1 (C-3''''), 131.1 (C-4''''), 131.1 (C-

2’’’’), 136.9 (C-1’), 139.2 (C-1’’’’), 140.0 (C-5’’), 143.0 (C-7a’’), 152.1 (C-3a’’), 160.2 (C-3’), 180.5 (CS), 195.7 (CO)

IR [cm^{-1}]: $\tilde{\nu}$ = 3094 (w), 3002 (w), 2905 (w), 2364 (m), 2345 (m), 1654 (w), 1637 (m), 1618 (m), 1601 (m), 1507 (s), 1490 (s), 1432 (m), 1387 (m), 1350 (m), 1315 (m), 1271 (m), 1244 (m), 1141 (w), 1083 (m), 1036 (s), 935 (w), 866 (w), 847 (w), 782 (w), 762 (w), 741 (w), 693 (w)

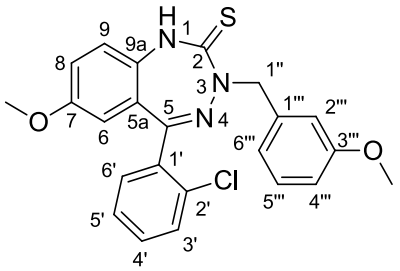
MS (APCI): *m/z* = 472, 470 $[M + H]^+$, 320, 318, 302, 286, 278, 276, 240, 206, 193, 153, 136

HR-MS (ESI): calcd. for $C_{23}H_{20}ClN_3NaO_4S^+$ $[M + Na]^+$ 492.0755; found 492.0762

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{20}ClN_3O_4S$

MW: 469.94 g/mol



5-(2-Chlorophenyl)-7-methoxy-3-(3-methoxybenzyl)-1H-benzo[e][1,2,4]-triazepine-2(3H)-thione (93)

(3-Methoxybenzyl)hydrazine hydrochloride **86** (102 mg, 0.543 mmol, 1.10 equiv) was dissolved in 5 mL of methanol, cooled to 0 °C and treated with *N*,*N*-diisopropylethylamine (101 µL, 0.593 mmol, 1.20 equiv). After stirring for 30 min at room temperature, a solution of (2-chlorophenyl)(2-isothiocyanato-5-methoxyphenyl)methanone **87** (150 mg, 0.494 mmol, 1.00 equiv) in 5 mL of tetrahydrofuran was added and stirring was continued for 3 h. The mixture was concentrated under reduced pressure, dissolved in ethanol (10 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (2.0 mg, 12 µmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (dichloromethane, R_f = 0.33), giving compound **93** (126 mg, 0.288 mmol, 58 %) as yellow solid.

mp: 134.5 – 136.7 °C

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 3.63 (s, 3 H, 7-OCH_3), 3.70 (s, 3 H, 3'''-OCH_3), 5.31 (s, 2 H, 1''-H), 6.19 (d, $^4J_{HH}$ = 2.0 Hz, 1 H, 6-H), 6.82 (dd, $^4J_{HH}$ = 2.5 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 4'''-H), 6.84 – 6.88 (m, 2 H, 6'-H, 2'''-H), 6.88 – 6.95 (m, 3 H, 8-H, 9-H, 6'''-H), 7.17 – 7.35 (m, 2 H, 5'-H, 5'''-H), 7.30 – 7.35 (m, 2 H, 3'-H, 4'-H), 8.02 (bs, 1 H, 1-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 55.1 (3'''-OCH_3), 55.6 (7-OCH_3), 59.6 (C-1''), 113.3 (C-4'''), 113.4 (C-2'''), 113.9 (C-6), 118.0 (C-8), 120.8 (C-6'''), 121.4 (C-9), 126.9 (C-5'), 128.0 (C-5a), 129.3 (C-5'''), 130.1 (C-3'), 131.1 (C-4'), 131.3 (C-

6'), 133.4 (C-2'), 135.4 (C-1'), 136.8 (C-9a), 138.6 (C-1'''), 156.2 (C-7), 159.6 (C-3'''), 166.6 (C-5), 193.4 (C-2)

IR [cm^{-1}]: $\tilde{\nu}$ = 3432 (w), 3087 (w), 3005 (w), 2930 (w), 2832 (w), 2360 (s), 2342 (m), 1587 (m), 1500 (s), 1434 (m), 1373 (w), 1328 (m), 1300 (m), 1261 (m), 1235 (m), 1219 (s), 1174 (m), 1152 (s), 1112 (m), 1035 (m), 1002 (m), 975 (w), 872 (w), 814 (w), 758 (m), 727 (m), 690 (m), 668 (m)

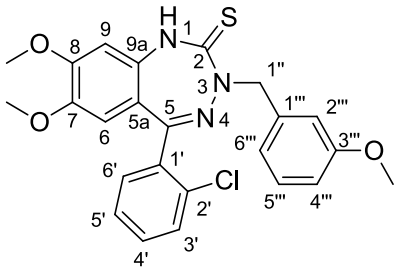
MS (APCI): *m/z* = 440, 438 $[M + H]^+$, 422, 406, 303, 262, 241, 185, 136

HR-MS (ESI): calcd. for $C_{23}H_{21}ClN_3O_2S^+$ $[M + H]^+$ 438.1038; found 438.1044

HPLC purity: 96 % [λ = 210 nm], 97 % [λ = 254 nm]

MF: $C_{23}H_{20}ClN_3O_2S$

MW: 437.94 g/mol



5-(2-Chlorophenyl)-7,8-dimethoxy-3-(3-methoxybenzyl)-1H-benzo[e][1,2,4]-triazepine-2(3H)-thione (94)

N-[2-(2-Chlorobenzoyl)-4,5-dimethoxyphenyl]-1-(3-methoxybenzyl)hydrazine-carbothioamide **91** (580 mg, 1.19 mmol, 1.00 equiv) was dissolved in ethyl acetate (15 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (6.0 mg, 30 µmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / isohexane 2:3, R_f = 0.46), giving compound **94** (469 mg, 1.00 mmol, 84 %) as yellow solid.

mp: 152.5 – 154.8 °C

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 3.59 (s, 3 H, 7-OCH_3), 3.71 (s, 3 H, 3'''-OCH_3), 3.88 (s, 3 H, 8-OCH_3), 5.31 (s, 2 H, 1''-H), 6.09 (s, 1 H, 6-H), 6.55 (s, 1 H, 9-H), 6.82 (ddd, $^4J_{HH}$ = 0.8 Hz, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 4'''-H), 6.85 (ddd, $^5J_{HH}$ = 0.4 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.7 Hz, 1 H, 6'-H), 6.87 (dd, $^4J_{HH}$ = 1.5 Hz, $^4J_{HH}$ = 2.5 Hz, 1 H, 2'''-H), 6.93 (ddd, $^4J_{HH}$ = 0.8 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.6 Hz, 1 H, 6'''-H), 7.19 – 7.23 (m, 1 H, 5'-H), 7.23 (dd, $^3J_{HH}$ = 7.6 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 5'''-H), 7.31 – 7.36 (m, 2 H, 3'-H, 4'-H), 8.08 (s, 1 H, 1-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 55.1 (3'''-OCH_3), 56.3 (8-OCH_3), 56.3 (7-OCH_3), 59.5 (C-1''), 103.6 (C-9), 111.0 (C-6), 113.3 (C-4'''), 113.3 (C-2'''), 118.8 (C-5a), 120.8 (C-6'''), 126.9 (C-5'), 129.3 (C-5'''), 130.1 (C-3'), 131.1 (C-4'), 131.5 (C-6'), 133.4 (C-2'), 135.4 (C-1'), 138.3 (C-9a), 138.6 (C-1'''), 145.9 (C-7), 152.8 (C-8), 159.6 (C-3'''), 167.2 (C-5), 193.3 (C-2)

IR [cm^{-1}]: $\tilde{\nu}$ = 3072 (w), 3000 (w), 2933 (w), 2834 (w), 2645 (w), 2363 (w), 2344 (w), 1609 (m), 1520 (s), 1492 (s), 1463 (m), 1435 (m), 1374 (m), 1339 (m), 1315 (m), 1270 (s), 1222 (s), 1202 (m), 1175 (m), 1123 (s), 1036 (m), 998 (m), 945 (w), 852 (w), 756 (m), 737 (w), 691 (w), 609 (w)

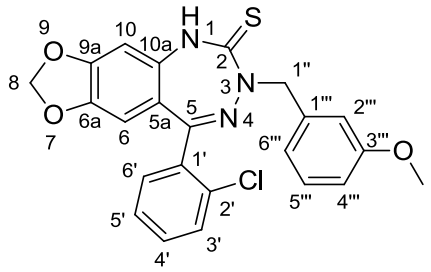
MS (ESI): *m/z* = 470, 468 $[M + H]^+$, 238, 406, 303, 262, 241, 185, 136

HR-MS (ESI): calcd. for $C_{24}H_{23}ClN_3O_3S^+$ $[M + H]^+$ 468.1143; found 468.1150

HPLC purity: 98 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{24}H_{22}ClN_3O_3S$

MW: 467.97 g/mol



5-(2-Chlorophenyl)-3-(3-methoxybenzyl)-1H-[1,3]dioxolo[4',5':4,5]benzo-[1,2-e][1,2,4]triazepine-2(3H)-thione (95)

N-[6-(2-Chlorobenzoyl)benzo[*d*][1,3]dioxol-5-yl]-1-(3-methoxybenzyl)hydrazine-carbothioamide **92** (70 mg, 0.15 mmol, 1.0 equiv) was dissolved in ethyl acetate (6 mL), treated with a catalytic amount of para-toluenesulfonic acid monohydrate (0.7 mg, 4 µmol, 2.5 mol%) and heated to reflux for 1 h. The solvent was evaporated and purification was done by flash column chromatography on flash silica gel (ethyl acetate / isohexane 2:3, R_f = 0.53), giving compound **95** (64 mg, 0.14 mmol, 95 %) as yellow solid.

mp: 191.2 – 193.5 °C

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 3.74 (s, 3 H, 3'''-OCH_3), 5.29 (s, 2 H, 1''-H), 5.99 (s, 2 H, 8-H), 6.10 (s, 1 H, 6-H), 6.44 (s, 1 H, 10-H), 6.80 (ddd, $^5J_{HH}$ = 0.5 Hz, $^4J_{HH}$ = 1.4 Hz, $^3J_{HH}$ = 7.7 Hz, 1 H, 6'-H), 6.83 (ddd, $^4J_{HH}$ = 0.8 Hz, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.3 Hz, 1 H, 4'''-H), 6.87 (dd, $^4J_{HH}$ = 1.6 Hz, $^4J_{HH}$ = 2.5 Hz, 1 H, 2'''-H), 6.91 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.6 Hz, 1 H, 6'''-H), 7.18 – 7.21 (m, 1 H, 5'-H), 7.21 – 7.25 (m, 1 H, 5'''-H), 7.31 – 7.36 (m, 2 H, 3'-H, 4'-H), 7.43 (s, 1 H, 1-H)

^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 55.2 (3'''-OCH_3), 59.5 (C-1''), 101.3 (C-10), 102.3 (C-8), 107.6 (C-6), 113.2 (C-4'''), 113.5 (C-2'''), 120.2 (C-5a), 120.8 (C-6'''), 126.9 (C-5'), 129.4 (C-5'''), 130.1 (C-3'), 131.1 (C-4'), 131.3 (C-6'), 133.4 (C-2'), 135.5 (C-1'), 138.4 (C-1'''), 139.3 (C-10a), 144.7 (C-6a), 151.2 (C-9a), 159.6 (C-3'''), 166.8 (C-5), 193.4 (C-2)

IR [cm^{-1}]: $\tilde{\nu}$ = 3074 (w), 2909 (w), 2364 (w), 2345 (w), 1602 (m), 1503 (s), 1485 (s), 1434 (m), 1409 (m), 1364 (w), 1340 (w), 1312 (m), 1255 (s), 1200 (m), 1162 (m), 1081 (w), 1037 (s), 1003 (w), 930 (w), 853 (w), 760 (w), 740 (w), 692 (w)

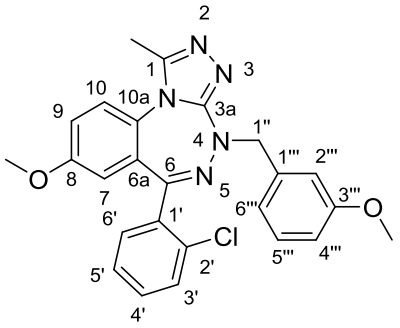
MS (APCI): *m/z* = 454, 452 $[M + H]^+$, 420, 316, 283

HR-MS (ESI): calcd. for $C_{23}H_{19}ClN_3O_3S^+$ $[M + H]^+$ 452.0830; found 452.0838

HPLC purity: 94 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{23}H_{18}ClN_3O_3S$

MW: 451.93 g/mol



6-(2-Chlorophenyl)-8-methoxy-4-(3-methoxybenzyl)-1-methyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (96)

Synthesis of the triazole-ring followed **GP3**. 5-(2-Chlorophenyl)-7-methoxy-3-(3-methoxybenzyl)-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **93** (110 mg, 0.251 mmol, 1.00 equiv) was treated first with hydrazine hydrate (61 µL, 1.3 mmol, 5.0 equiv). Triethyl orthoacetate **43** (60 µL, 0.33 mmol, 1.3 equiv) and para-toluenesulfonic acid monohydrate (10 mg, 0.05 mmol, 0.2 equiv) were used in the second step. Product **96** (59 mg, 0.13 mmol, 51 %) was obtained after purification (R_f = 0.24) as yellow solid.

mp: 170.9 – 172.4 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.52 (s, 3 H, 1-CH_3), 3.70 (s, 3 H, 8-OCH_3), 3.74 (s, 3 H, 3'''-OCH_3), 4.75 – 5.19 (m, 2 H, 1''-H), 6.47 (d, $^4J_{HH}$ = 2.9 Hz, 1 H, 7-H), 6.80 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 4'''-H), 6.95 (dd, $^4J_{HH}$ = 1.6 Hz, $^4J_{HH}$ = 2.6 Hz, 1 H, 2'''-H), 6.98 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'''-H), 7.10 (dd, $^4J_{HH}$ = 2.9 Hz, $^3J_{HH}$ = 8.9 Hz, 1 H, 9-H), 7.22 (dd, $^3J_{HH}$ = 7.5 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 5'''-H), 7.29 (d, $^3J_{HH}$ = 8.9 Hz, 1 H, 10-H), 7.31 – 7.33 (m, 2 H, 5'-H, 6'-H), 7.33 – 7.35 (m, 1 H, 3'-H), 7.35 – 7.40 (m, 1 H, 4'-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 12.7 (1-CH_3), 55.5 (3'''-OCH_3), 56.1 (8-OCH_3), 57.9 (C-1''), 113.1 (C-4'''), 114.8 (C-2'''), 115.3 (C-7), 116.8 (C-9), 121.6 (C-6'''), 124.1 (C-10), 127.4 (C-5'), 127.4 (C-10a), 129.6 (C-5'''), 130.4 (C-3'),

131.3 (C-6a), 131.3 (C-4'), 131.9 (C-6'), 133.3 (C-2'), 136.3 (C-1'), 139.6 (C-1'''), 148.7 (C-1), 158.4 (C-8), 160.0 (C-3'''), 160.1 (C-3a), 160.9 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3431 (w), 3058 (w), 3000 (w), 2932 (m), 2835 (m), 1794 (w), 1683 (w), 1602 (m), 1585 (m), 1520 (s), 1505 (s), 1491 (m), 1455 (m), 1436 (s), 1380 (w), 1338 (m), 1316 (m), 1297 (m), 1268 (s), 1229 (m), 1189 (w), 1157 (m), 1136 (w), 1051 (m), 1006 (m), 973 (w), 882 (w), 823 (w), 773 (m), 745 (m), 734 (m), 690 (w), 621 (w)

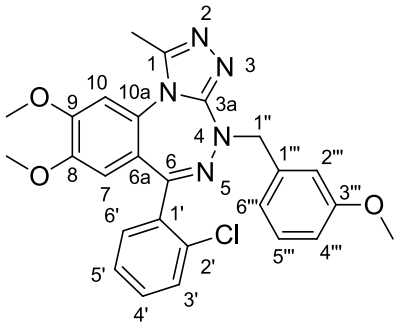
MS (APCI): *m/z* = 462, 460 $[M + H]^+$, 325, 315, 287, 285

HR-MS (ESI): calcd. for $C_{25}H_{23}ClN_5O_2^+$ $[M + H]^+$ 460.1535; found 460.1542

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{25}H_{22}ClN_5O_2$

MW: 459.93 g/mol



6-(2-Chlorophenyl)-8,9-dimethoxy-4-(3-methoxybenzyl)-1-methyl-4H-benzo-[e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (97)

Synthesis of the triazole-ring followed **GP3**. 5-(2-Chlorophenyl)-7,8-dimethoxy-3-(3-methoxybenzyl)-1*H*-benzo[*e*][1,2,4]triazepine-2(3*H*)-thione **94** (470 mg, 1.00 mmol, 1.00 equiv) was treated first with hydrazine hydrate (244 µL, 5.02 mmol, 5.00 equiv). Triethyl orthoacetate **43** (238 µL, 1.31 mmol, 1.30 equiv) and para-toluenesulfonic acid monohydrate (38 mg, 0.20 mmol, 0.20 equiv) were used in the second step. Product **97** (171 mg, 0.349 mmol, 35 %) was obtained after purification (R_f = 0.21) as colorless solid.

mp: 232.0 – 233.9 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.55 (s, 3 H, 1-CH_3), 3.61 (s, 3 H, 8-OCH_3), 3.75 (s, 3 H, 3'''-OCH_3), 3.92 (s, 3 H, 9-OCH_3), 4.75 – 4.95 (m, 1 H, 1''-*H*H), 4.96 – 5.17 (m, 1 H, 1''-H*H*), 6.37 (s, 1 H, 7-H), 6.80 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.3 Hz, 1 H, 4'''-H), 6.81 (s, 1 H, 10-H), 6.96 (dd, $^5J_{HH}$ = 0.4 Hz, $^4J_{HH}$ = 1.6 Hz, $^4J_{HH}$ = 2.6 Hz, 1 H, 2'''-H), 6.99 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'''-H), 7.22 (dd, $^5J_{HH}$ = 0.4 Hz, $^3J_{HH}$ = 7.6 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 5'''-H), 7.29 – 7.39 (m, 4 H, 3'-H, 4'-H, 5'-H, 6'-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 13.0 (1-CH_3), 55.5 (3'''-OCH_3), 56.5 (8-OCH_3), 56.7 (9-OCH_3), 57.9 (C-1''), 106.2 (C-10), 111.9 (C-7), 113.1 (C-4'''), 114.8 (C-2'''), 121.6 (C-6'''), 122.5 (C-6a), 127.4 (C-5'), 128.3 (C-10a), 129.5 (C-5'''), 130.5 (C-3'), 131.2 (C-4'), 132.0 (C-6'), 133.4 (C-2'), 136.3 (C-1'), 139.6 (C-1'''), 148.1 (C-8), 148.4 (C-1), 151.9 (C-9), 160.0 (C-3'''), 160.3 (C-3a), 161.1 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3089 (w), 3061 (w), 2998 (w), 2954 (w), 2931 (w), 2835 (w), 2364 (w), 2345 (w), 1610 (m), 1588 (m), 1526 (s), 1513 (s), 1493 (w), 1459 (m), 1439 (m), 1373 (m), 1341 (m), 1290 (m), 1263 (s), 1220 (m), 1147 (m), 1048 (m), 1019 (m), 865 (m), 811 (m), 761 (w), 741 (w), 694 (w)

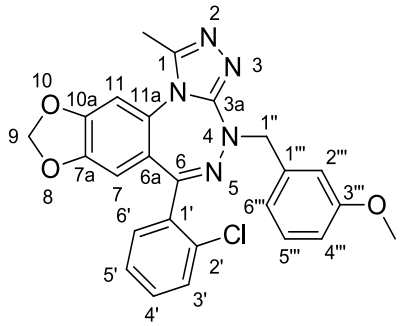
MS (APCI): *m/z* = 492, 490 $[M + H]^+$, 355, 315

HR-MS (ESI): calcd. for $C_{26}H_{25}ClN_5O_3^+$ $[M + H]^+$ 490.1640; found 490.1647

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{26}H_{24}ClN_5O_3$

MW: 489.95 g/mol



6-(2-Chlorophenyl)-4-(3-methoxybenzyl)-1-methyl-4H-[1,3]dioxolo[4',5':4,5]-benzo[1,2-e][1,2,4]triazolo[3,4-c][1,2,4]triazepine (98)

Synthesis of the triazole-ring followed **GP3**. 5-(2-Chlorophenyl)-3-(3-methoxybenzyl)-1*H*-[1,3]dioxolo[4',5':4,5]benzo[1,2-*e*][1,2,4]triazepine-2(3*H*)-thione **95** (50 mg, 0.11 mmol, 1.0 equiv) was treated first with hydrazinie hydrate (27 µL, 0.55 mmol, 5.0 equiv). Triethyl orthoacetate **43** (26 µL, 0.14 mmol, 1.3 equiv) and para-toluenesulfonic acid monohydrate (4.2 mg, 22 µmol, 0.2 equiv) were used in the second step. Product **98** (21 mg, 44 µmol, 40 %) was obtained after purification (R_f = 0.46) as colorless solid.

mp: 213.1 – 214.4 °C

^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm) = 2.52 (s, 3 H, 1-CH_3), 3.76 (s, 3 H, 3'''-OCH_3), 4.80 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1''-*H*H), 5.09 (d, $^2J_{HH}$ = 13.7 Hz, 1 H, 1''-H*H*), 6.08 (s, 2 H, 9-H), 6.39 (s, 1 H, 7-H), 6.81 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 8.2 Hz, 1 H, 4'''-H), 6.83 (s, 1 H, 11-H), 6.96 (dd, $^4J_{HH}$ = 1.6 Hz, $^4J_{HH}$ = 2.5 Hz, 1 H, 2'''-H), 6.99 (ddd, $^4J_{HH}$ = 0.9 Hz, $^4J_{HH}$ = 1.5 Hz, $^3J_{HH}$ = 7.5 Hz, 1 H, 6'''-H), 7.22 (dd, $^3J_{HH}$ = 7.5 Hz, $^3J_{HH}$ = 8.1 Hz, 1 H, 5'''-H), 7.27 – 7.39 (m, 4 H, 3'-H, 4'-H, 5'-H, 6'-H)

^{13}C NMR (125 MHz, CD_2Cl_2): δ (ppm) = 12.9 (1-CH_3), 55.5 (3'''-OCH_3), 57.8 (C-1''), 103.4 (C-9), 103.8 (C-11), 108.7 (C-7), 113.0 (C-4'''), 114.8 (C-2'''), 121.5 (C-6'''), 124.2 (C-6a), 127.5 (C-5'), 129.4 (C-11a), 129.6 (C-5'''), 130.5 (C-3'), 131.3 (C-4'), 131.9 (C-6'), 133.4 (C-2'), 136.3 (C-1'), 139.6 (C-1'''), 147.0 (C-7a), 148.6 (C-1), 150.5 (C-10a), 160.0 (C-3'''), 160.3 (C-3a), 161.0 (C-6)

IR [cm^{-1}]: $\tilde{\nu}$ = 3057 (w), 2912 (w), 2834 (w), 2362 (w), 2344 (w), 1708 (w), 1626 (w), 1594 (m), 1520 (m), 1506 (s), 1489 (s), 1455 (m), 1436 (m), 1400 (m), 1358 (w), 1342 (w), 1303 (w), 1283 (m), 1265 (m), 1235 (s), 1155 (m), 1105 (w), 1035 (s), 989 (w), 926 (w), 867 (w), 836 (w), 775 (m), 758 (m), 742 (m), 688 (w)

MS (APCI): *m/z* = 476, 474 $[M + H]^+$, 460, 341, 339, 304, 301, 299, 136

HR-MS (ESI): calcd. for $C_{25}H_{21}ClN_5O_3^+$ $[M + H]^+$ 474.1327; found 474.1335

HPLC purity: > 99 % [λ = 210 nm], > 99 % [λ = 254 nm]

MF: $C_{25}H_{20}ClN_5O_3$

MW: 473.91 g/mol

ABBREVIATIONS

°C	degree Celsius
5mC	5-methylcytosine
Å	Ångström
Ac	acetyl
ADT	agar diffusion test
APCI	atmospheric-pressure chemical ionization
BET	bromodomain and extra-terminal
bp	base pair
BRD	bromodomain
BRD [*number*]	bromodomain-containing protein [*number*]
BzD	benzodiazepine
BzT	benzotriazepine
c	concentration
C	cytosine
cc	compound concentration
CI	chemical ionization
COSY	correlation spectroscopy
CpG	C-phosphate-G
CuAAC	copper-catalyzed azide-alkyne cycloaddition
DAD	diode array detector
DCC	*N*,*N*‘-dicyclohexylcarbodiimide
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
DFT	density functional theory
DIAD	diisopropyl azodicarboxylate
DIC	*N*,*N*‘-diisopropylcarbodiimide
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide

DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
DSF	differential scanning fluorimetry
EDG	electron donating group
EI	electronic ionization
ESI	electron spray ionization
EWG	electron withdrawing group
FT	Fourier transformed
G	guanine
GABA	γ-aminobutyric acid
GP	general procedure
H [*number*]	histone [*number*]
HAT	histone acetyltransferase
HDAC	histone deacetylase
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond coherence
HMQC	heteronuclear multiple quantum coherence
HPLC	high performance liquid chromatography
HR	high resolution
HTS	high throughput screening
Hz	Hertz
IR	infrared
ITC	isothermal titration calorimetry
K	lysine
K_{ac}	ε-N-acetylated lysine
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MF	molecular formula

MLL	mixed lineage leukaemia
mp	melting point
MS	mass spectrometry
MW	molecular weight
NAD(P)	nicotine adenine dinucleotide (phosphate)
NMR	nuclear magnetic resonance
P	proline
PCR	polymerase chain reaction
PDB	protein data bank
PMB	para-methoxybenzyl
ppm	parts per million
R	arginine
r.t.	room temperature
S	serine
SAR	structure-activity relationship(s)
SGC	Structural Genomics Consortium
T	threonine
TBzD	triazolobenzodiazepine
TBzT	triazolobenzotriazepine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultraviolet
Y	tyrosine

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