VILNIAUS UNIVERSITETAS
CHEMIJOS IR GEOMOKSLŲ FAKULTETAS
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Simona Jonušienė
Chemija (6211CX003)
Magistro baigiamasis darbas

Jungiamąsias grupes turinčių 1,3,4,5-tetrahidro-2*H*-benzo[*b*][1,4]diazepin-2-ono darinių sintezė ir toksiškumo tyrimas

Darbo vadovas (-ė)
dr. Regina Jančienė

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(leidimas ginti, data, parašas)

Darbo įteikimo data _____
Registracijos Nr. _____

Vilnius 2023

VILNIUS UNIVERSITY
FACULTY OF CHEMISTRY AND GEOSCIENCES
INSTITUTE OF CHEMISTRY
DEPARTMENT OF ORGANIC CHEMISTRY

Simona Jonušienė
Chemistry (6211CX003)
Master thesis

Synthesis of linker groups containing 1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-one derivatives and toxicity study

Scientific adviser
dr. Regina Jančienė

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(permission to defend, date, signature)

Date of submission _____
Registration No. _____

Vilnius 2023

1. CONTENTS

2. ABBREVIATIONS

Ac	acetyl
API	active pharmaceutical ingredient
Bn	benzyl
CCK-B	cholecystokinin B
DCM	dichloromethane (methylene chloride)
dec.	decomposition
DIPEA	*N*,*N*-diisopropylethylamine
DMAD	dimethyl acetylenedicarboxylate
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
$DMSO_2$	dimethyl sulfone
ee	enantiomeric excess
GABA	gamma aminobutyric acid
HMDS	hexamethyldisiloxane
HPA	heteropolyacid (heteropolymetalates)
i.e.	in example
Mbz	meta-methoxybenzyl
MW	molecular weight
NHS	*N*-hydroxysuccinimide
Phth	phthalimide
Pmp	para-methoxyphenyl
PPA	polyphosphoric acid
PTC	phase transfer catalyst
Py	pyridine
r.t.	room temperature
TMS	tetramethylsilane

3. INTRODUCTION

Benzodiazepines (fig. 1) are bicyclic heterocyclic compounds in which the benzene ring is fused to a seven-membered ring with two nitrogen atoms - diazepine. Since Leo Sternbach accidentally invented Librium[1] in 1956, benzodiazepines became one of the most studied heterocycles in medicinal chemistry. Benzodiazepines were mostly studied and developed as new sedative drugs and were proven as superior replacement for previously popular barbiturates[2–5]. Many benzodiazepine analogs were developed with varying potencies and duration of action[6–8]. For example, medazepam[9] is mild daytime anxiolytic while midazolam[10,11] is used in surgery to achieve procedural sedation and induce temporary amnesia. Interestingly, not all benzodiazepines exhibit tranquilizing properties. For example, flumazenil has benzodiazepine scaffold but it is potent $GABA_A$ receptor antagonist and is used mainly as antidote for benzodiazepine overdose treatment[12]. Despite tranquilizing activity benzodiazepines are also studied for antimicrobial[13], antifungal, antiviral, blood-thinning, anticancer properties[14–20] (direct or as ligands[21]). The abundance of benzodiazepine analogues confirms its importance in medicinal chemistry.

There is no doubt that ongoing exploration of variously substituted benzodiazepines and development of new drug delivery methods will unveil new pharmacological activities and possible disease treatments in the near future[22].

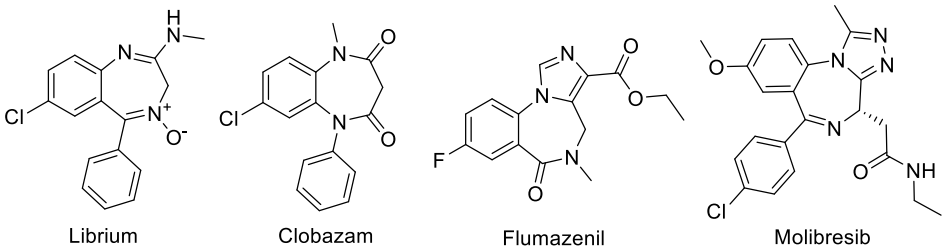


Figure 1. Selected examples of common benzodiazepines.

The aim of current MSc thesis is to study the synthesis of variously substituted benzodiazepines that could be further modified through the linker at the 4-th position (fig. 2) (i.e., attached to other molecules or surfaces by forming covalent bond) and investigation of toxicity on mice in collaboration with UAB "Innovita Research".

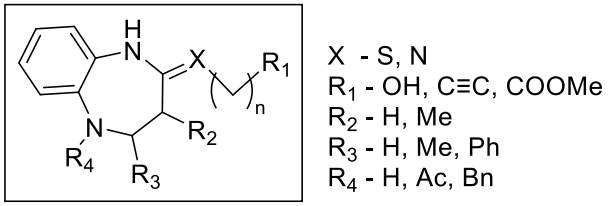
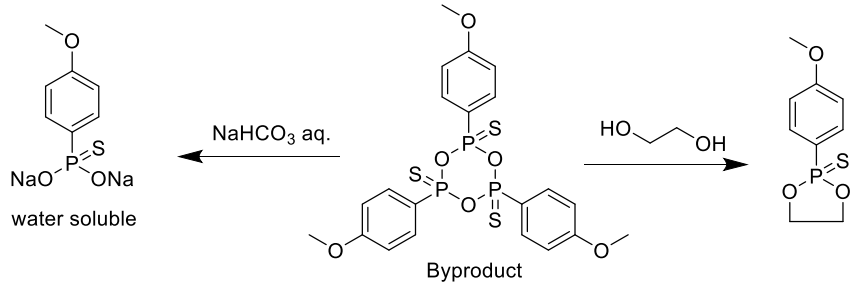


Figure 2. General structure of desired benzodiazepines.

4. LITERATURE REVIEW

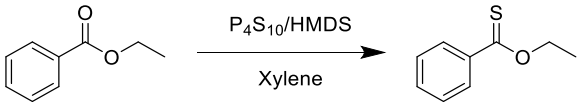
The scientific literature of the last decades on the subject of benzodiazepine ring formation, thionation reactions and various modifications of benzodiazepine scaffold is reviewed further in the following chapter.

K. Wu et al. [2021][23] developed new workup approach for thionations using Lawesson's reagent. Invention makes Lawesson's thionation attractive method for large scale synthesis because no solid or sticky byproducts are generated (sch. 1).



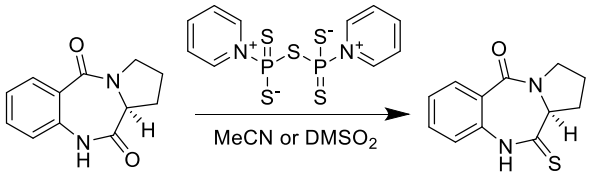
Scheme 1. Conversion of thionation byproduct to extractable compound.

T. J. Cuphey [2002][24] reported highly efficient thionation procedure of various substrates by using HMDS and P_4S_{10} combination in organic solvents (sch. 2). The procedure has been successfully applied by G. A. Erickson et al. [2022][25] for multikilogram scale synthesis of anticancer API Molibresib.



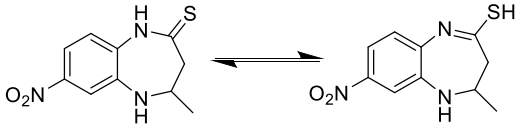
Scheme 2. Highly efficient thionation reaction example.

J. Bergman et al. [2011][26] reported in depth study on thionations of amides using pyridine and P_4S_{10} complex in various solvents (sch. 3). Reported yields were higher than traditional methods such as Lawesson's reagent or the same complex (P_4S_{10}+Py) in pyridine.



Scheme 3. Selective thionation with P_4S_{10} and Py complex.

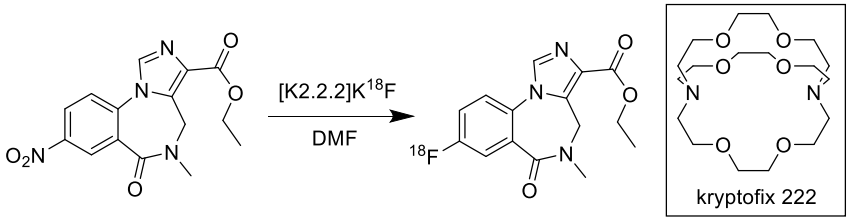
B. Puodziunaite et al. [2002][27] studied the tautomerism of the thioamide group in 4-methyl-7-nitro-2,3,4,5-tetrahydro-1,5-benzodiazepine-2-thione (sch. 4). Experimental data suggest that nitro group has significant effect on mobility of hydrogen atom of thioamide group.



Scheme 4. Tautomerism of the thioamide group.

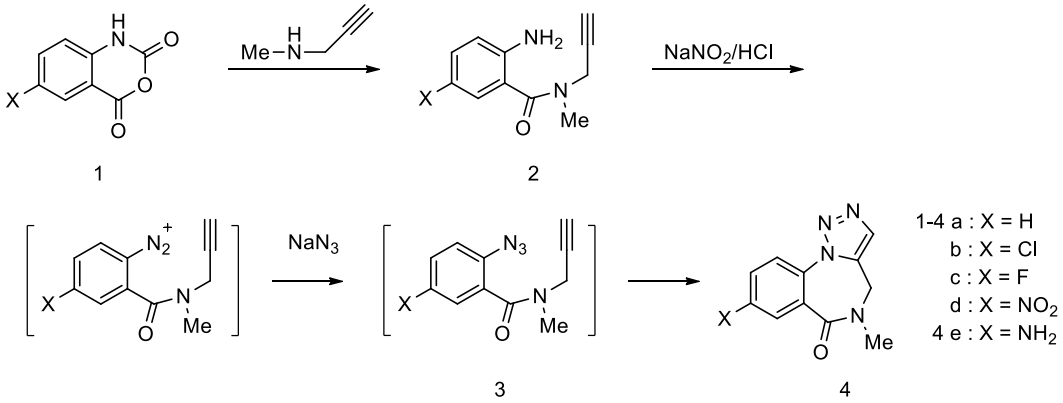
D. Vaulina et al. [2018][28] developed an automatic approach towards [18F] flumazenil. Implementation of automatic synthesis modules and replacing chromatographic purification method

with solid phase extraction gave superior product quality and improvement of synthesis time. Fluorination was achieved in DMF solvent by K[18F] complex with kryptofix 222 (sch. 5).



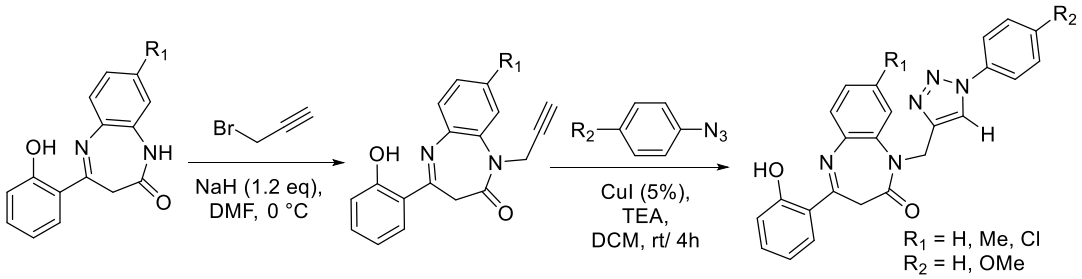
Scheme 5. Synthesis of [18F] flumazenil.

G. Broggini et al. [1999][29] synthesized series of compounds related to flumazenil by intramolecular cyclization of -C≡CH and azide. Precursors for cyclization reaction were made from 5-substituted isatoic anhydrides and *N*-proprargylmethylamine. Anthranilamides were converted to diazonium salts by sodium nitrite and hydrochloric acid and treated with sodium azide to obtain propargylic azides that spontaneously cyclized (sch. 6).



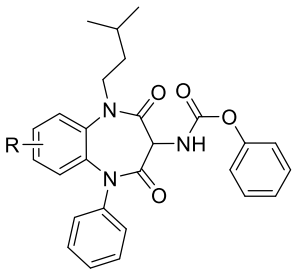
Scheme 6. Intramolecular cycloaddition reaction.

A. Nsira et al. [2022][30]. By establishing click reaction between alkyne and azide new *N*-triazolo-1,5-benzodiazepinones were synthesized (sch. 7). Some of obtained compounds performed as antimicrobial agents at µM concentrations.



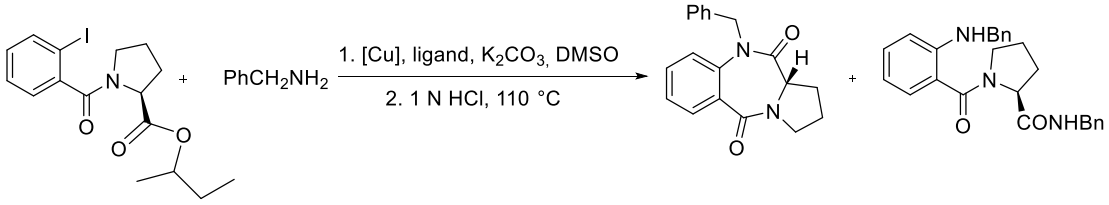
Scheme 7. Synthesis of benzodiazepine antimicrobial agents.

M. E. Tranquillini et al. [1997][31] developed 3-carbamic substituted 1,5-benzodiazepines witch are selective CCK-B ligands. The best affinity was observed in compounds with 8-Cl, 7,8-diCl substitution (sch. 8).



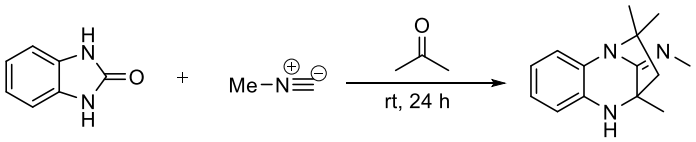
Scheme 8. Benzodiazepines with prominent anticancer activity.

H. Zhang et al. [2010][32] discovered new method for preparation of pyrrolo[2,1-*c*][1,4]-benzodiazepine-5,11-diones from substituted aryl iodides and primary amines in good yields. Reaction is catalyzed by copper complexes with K_2CO_3 in DMSO (sch. 9). L-proline is used as a ligand. Yields of crystallized products are above 50% with up to 93% ee.



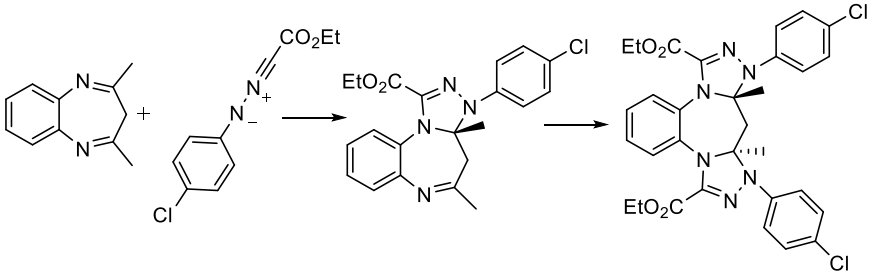
Scheme 9. Copper catalyzed cyclization.

R. L. Mohlala et al. [2021][33] developed new multicomponent reaction of benzimidazol-2-one, acetone and isocyanide that yields benzodiazepine backbone bearing compounds (sch. 10).



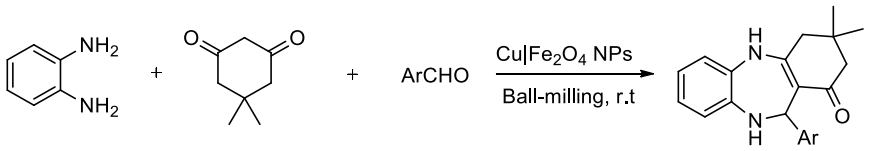
Scheme 10. Multicomponent benzodiazepine synthesis.

M. Maatallah et al. [2020][34] discovered that 2,4-dimethyl-3H-1,5-benzodiazepine react with excess of *N*-aryl-C-ethoxycarbonylnitrilimine (prepared in situ from ethyl *N*-arylhydrazono-bromoglyoxylate) to yield diethyl 8*a*,9*a*-dimethyl-8,10-diphenyl-8*a*,9,9*a*,10-tetrahydro-8*H*-benzo[*b*]bis([1,2,4]triazolo)[4,3-*d*:3',4'-*g*][1,4]diazepine-6,12-dicarboxylates (sch. 11).



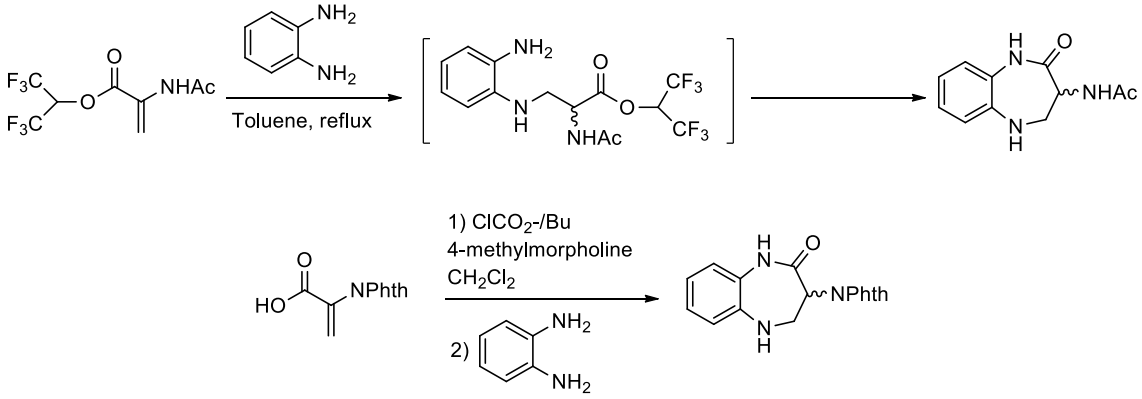
Scheme 11. Double cycloaddition towards bis([1,2,4]triazolo) derivative.

A. Maleki et al. [2018][35] developed nanocatalyst that catalyzes many solvent free reactions including the reaction between 1,2-phenylenediamine, aromatic aldehydes and 5,5-dimethylcyclohexane-1,3-dione that yields substituted benzodiazepines. Main advantage of catalyst - $CuFe_2O_4$ is magnetic recoverability, reusability and its low cost (sch. 12).



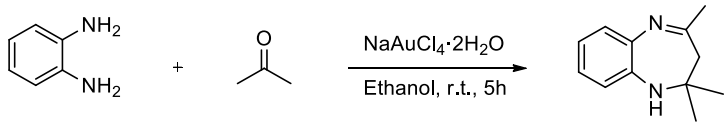
Scheme 12. Synthesis of benzodiazepines with magnetic catalyst.

N. Obara et al. [2019][36] developed two methods for synthesizing of 3-amino-1,5-benzodiazepine-2-one derivatives (sch. 13). The method is beneficial because of simplicity and ease of purification.



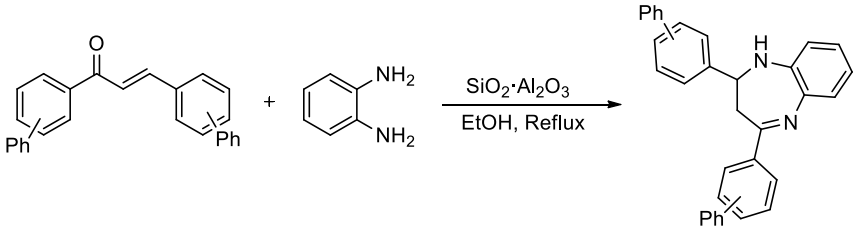
Scheme 13. Two methods for preparation of 3-amino-1,5-benzodiazepine-2-one derivatives.

R. Shi et al. [2010][37] developed gold catalyzed synthesis of 1,5-benzodiazepines from 1,2-diaminobenzene and ketones. Ethanol was used as a solvent with very low catalyst loading, good yields of product were obtained (sch. 14).



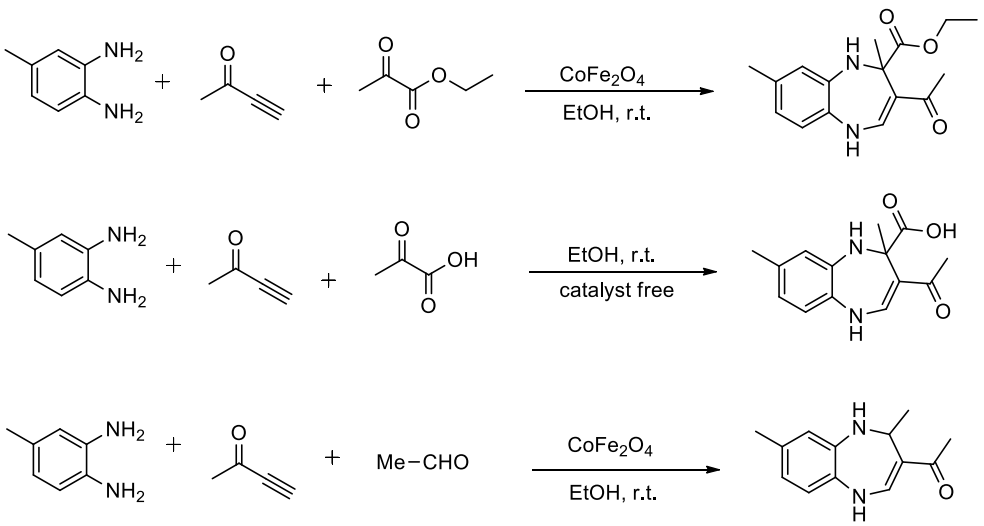
Scheme 14. Selected example of benzodiazepine synthesis.

D. Tayde et al. [2021][38] have reported that heating chalcone with ortho-phenylenediamine in ethanol in combination with mixed metal oxide catalyst (SiO_2-Al_2O_3) leads to the diphenyl substituted derivatives of 1,5-benzodiazepine in a highly efficient way (sch. 15).



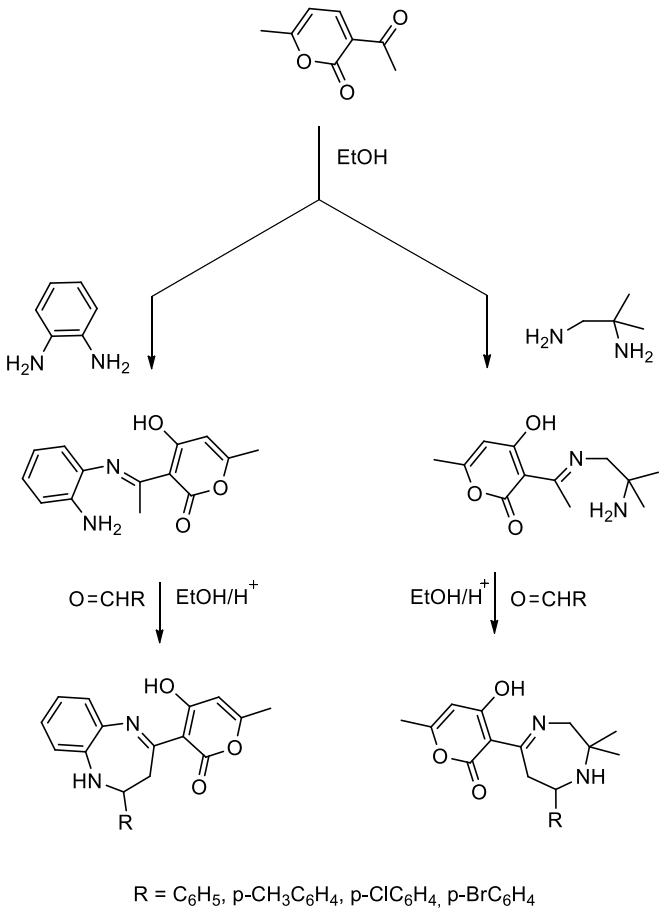
Scheme 15. Synthesis of diphenyl substituted benzodiazepines.

L. Wang et al. [2022][39] developed tandem reaction for the synthesis of substituted 1,5-benzodiazepines using 1,2-phenylenediamines, 3-butyn-2-one, dicarbonyl compounds or aldehyde carbonyl compounds as starting materials (sch. 16).



Scheme 16. Synthesis of substituted 1,5-benzodiazepines.

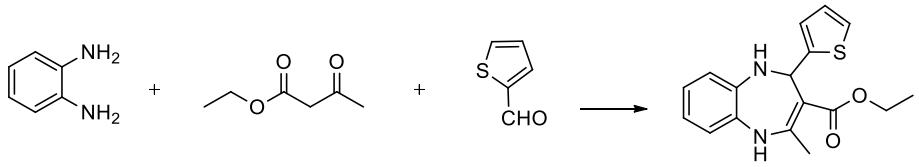
B. Nedjar-Kolli et al. [2011][40] developed acid catalyzed multicomponent reaction for the synthesis of diazepine derivatives starting from 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (sch. 17).



R = C_6H_5, p-$CH_3C_6H_4$, p-ClC_6H_4, p-BrC_6H_4

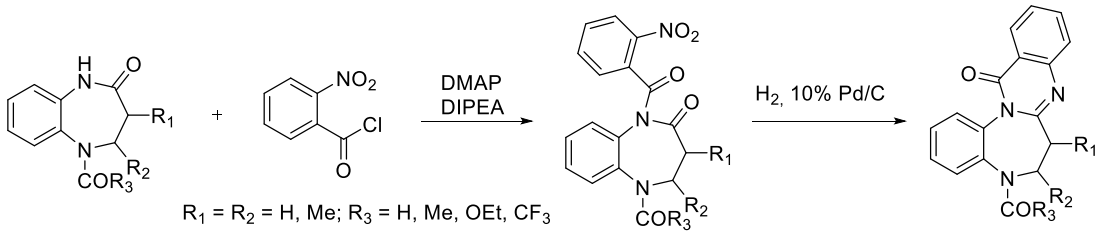
Scheme 17. Acid catalyzed multicomponent synthesis of diazepine derivatives.

L. Wang et al. [2014][41] described an efficient procedure for converting of thiophene aldehydes, substituted 1,2-phenylenediamines and ethyl acetoacetate towards 1,5-benzodiazepine derivatives (sch. 18).



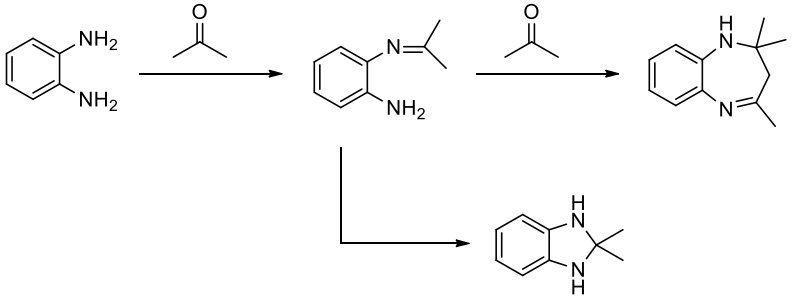
Scheme 18. Thiophene aldehydes towards benzodiazepines.

R. Janciene et al. [2015][42] described the preparation of dihydroquinazolino[3,2-*a*][1,5]benzodiazepines via the benzoylation of 1,5-benzodiazepinones with 2-nitrobenzoyl chloride in the presence of DMAP and DIPEA and subsequent reduction of nitro group with H_2 and Pd/C (sch. 19).



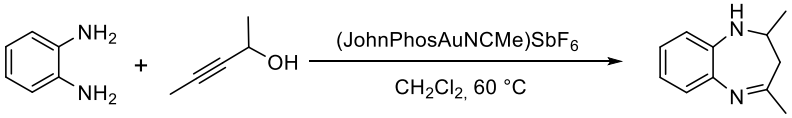
Scheme 19. Preparation of dihydroquinazolino[3,2-*a*][1,5]benzodiazepines.

M. N. Timofeeva et al. [2019][43] developed the montmorillonite[44] based catalyst that speeds up and enhances the yield of 1,2-ethylenediamine and acetone cyclocondensation reaction towards 1,5-benzodiazepine (sch. 20).



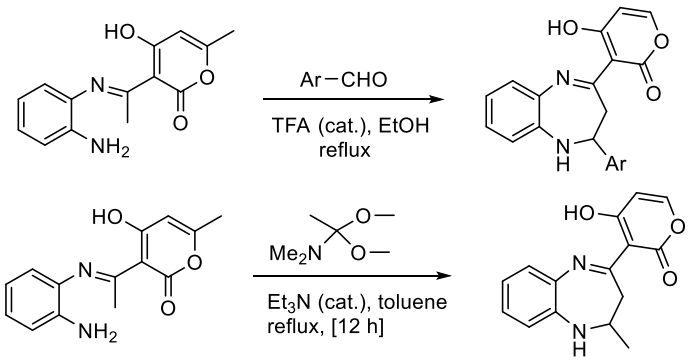
Scheme 20. Montmorillonite catalyzed cyclocondensation.

A. Goggiamani et al. [2016][45] reported an efficient synthesis of 1,5-benzodiazepines from propargylic alcohols catalyzed by gold complexes in DCM (sch. 21).



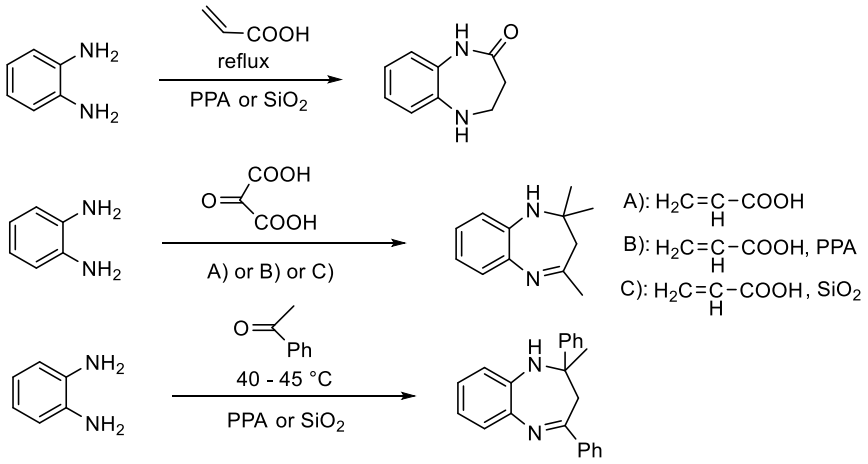
Scheme 21. Propargylic alcohols towards benzodiazepines.

M. Fodili et al. [1999][46] described an efficient synthesis of 2-pyronyl-1,5-benzodiazepine compounds starting from 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one 1,2-diaminobenzene and aldehydes or amide dimethyl acetals (sch. 22).



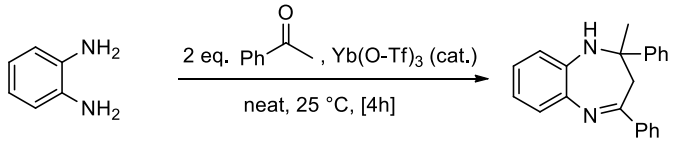
Scheme 22. Synthesis of 2-pyronyl-1,5-benzodiazepine compounds.

D. Jung et al. [1999][47] described the synthesis of 1,5-benzodiazepine derivatives by condensation of 1,2-diaminobenzene with acrylic acid, acetophenone or acetonedicarboxylic acid in the presence of various acid reagents (sch. 23).



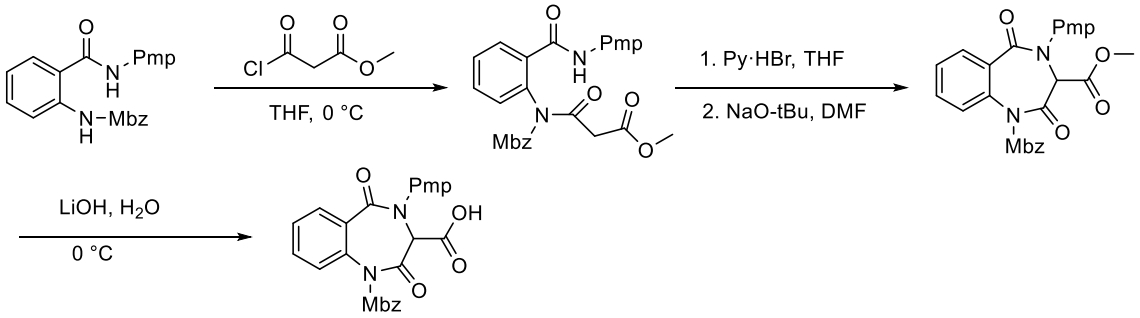
Scheme 23. Synthesis of 1,5-benzodiazepine derivatives.

M. Curini et al. [2001][48] developed highly efficient $Yb(OTf)_3$ catalyzed condensation of ketones and phenylenediamine towards 1,5-benzodiazepine derivatives (sch. 24).



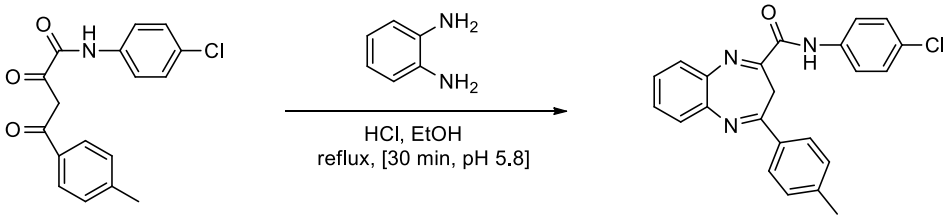
Scheme 24. $Yb(OTf)_3$ catalyzed synthesis towards benzodiazepines.

T. Ho et al. [2002][49] described the synthesis of benzodiazepine-2,5-diones starting from methyl malonyl chloride followed by monobromination and nucleophilic ring closure (sch. 25).



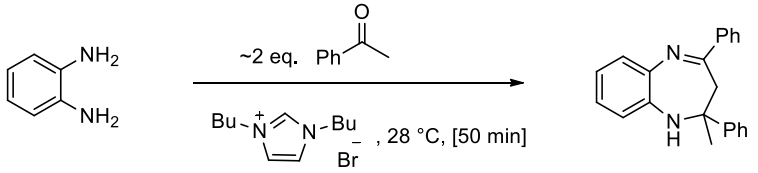
Scheme 25. Synthesis of benzodiazepine-2,5-diones.

L. Kurdina et al. [2002][50] described the synthesis of 4-aryl-1,5-benzodiazepine-2-carboxamides. Mechanism is also investigated in the manuscript (sch. 26).



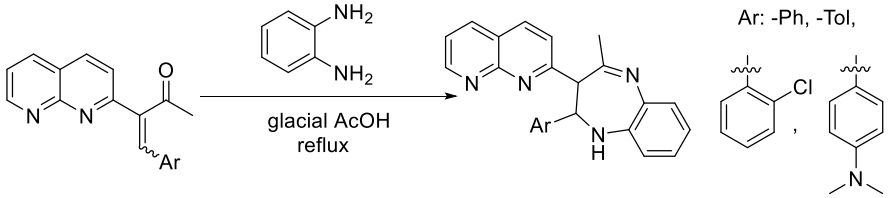
Scheme 26. Synthesis of 4-aryl-1,5-benzodiazepine-2-carboxamides.

K. V. Srinivasan et al. [2003][51] developed high yielding synthesis of 1,5-benzodiazepines promoted by ionic liquid under very mild conditions (sch. 27). 1,2-Diaminobenzene and substituted ketones were selected as starting materials.



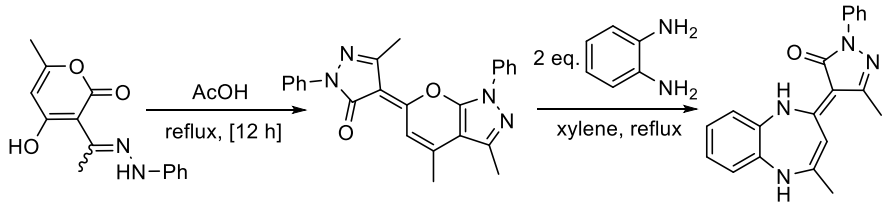
Scheme 27. Synthesis of benzodiazepines in ionic liquid.

K. Mogilaiah et al. [2003][52] reported synthesis of 1,8-naphthyridine substituted 1,5-benzodiazepine derivatives that show significant antibacterial activity against E. coli and Bacillus subtilis (sch. 28).



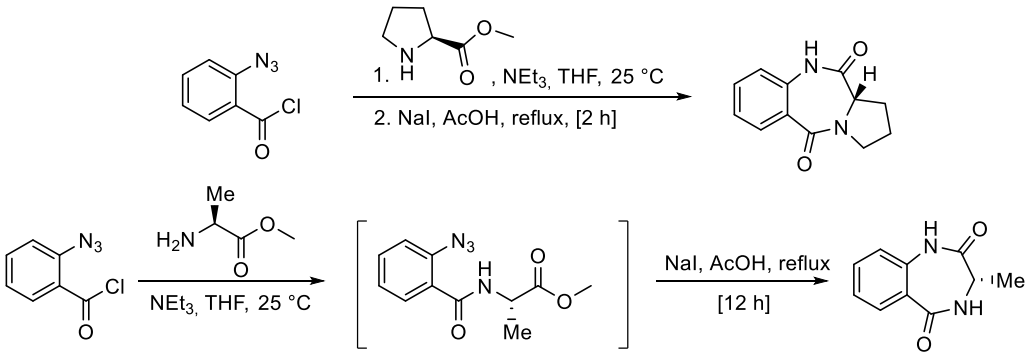
Scheme 28. Antibacterial benzodiazepines containing 1,8-naphthyridine.

E. Essassi et al. [2003][53] described the synthesis and crystal structure of 2-[1-Phenyl-3-methyl-5-oxo-pyrazol-4-ylidene]-4-methyl-1,5-benzodiazepine. 1,5-benzodiazepine ring was built in the last step by cyclo-addition of phenylenediamine (sch. 29).



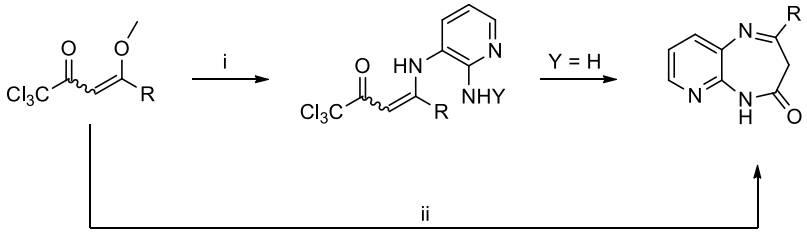
Scheme 29. Cyclo-addition reaction towards benzodiazepines.

A. Kamal et al. [2005][54] developed synthesis of 1,4-benzodiazepine-2,5-diones from azido arenes (sch. 30). Obtained benzodiazepines are enantiomerically pure because of natural amino acids used as building blocks.



Scheme 30. Synthesis of 1,4-benzodiazepine-2,5-diones from azido arenes

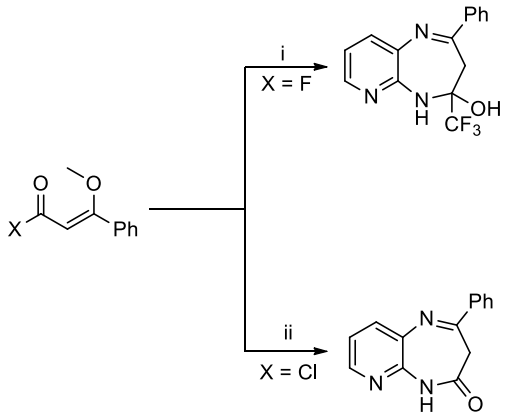
H. G. Bonacorso et al. [2009][55] described the synthesis of 3*H*-pyrido[2,3-*b*][1,4]diazepin-4(5*H*)-ones by direct cyclocondensation of 4-methoxy-1,1,1-trichloro-3-alken-2-ones with 2,3-diaminopyridine (sch. 31).



2,3-Diaminopyridine, MeOH, 0 °C (i) or 65 °C (ii); Y = H, SO_2Me; R = Alkyl, Heteroaryl

Scheme 31. Cyclocondensation of 4-methoxy-1,1,1-trichloro-3-alken-2-ones.

H. G. Bonacorso et al. [2007][56] described the regiospecific synthesis of dihydro-3*H*-pyrido[2,3-*b*][1,4]diazepinols. The synthesis is useful approach to obtaining 3*H*-pyrido[2,3-b][1,4]diazepin-4(5*H*)-ones (sch. 32).



(i): 2,3-$(NH_2)_2C_5H_3N$, MeOH, 60-65 °C, 24 h; (ii): 2,3-$(NH_2)_2C_5H_3N$, MeONa, MeOH, 60-65 °C, 24 h.

Scheme 32. Synthesis of 3*H*-pyrido[2,3-b][1,4]diazepin-4(5*H*)-ones.

5. CHEMICAL SYNTHESIS OF BENZODIAZEPINE DERIVATIVES

First of all, starting benzodiazepinones were prepared by slightly modified **1a-c** or directly by literature **1d**[57] methods (fig. 3). Obtained structures were confirmed by observing no melting point depression when mixed with authentic samples. Compounds **1a-c** were *N* benzylated[58] to **1aBn**, **1bBn** and **1cBn** accordingly in order to expand the scope of starting materials.

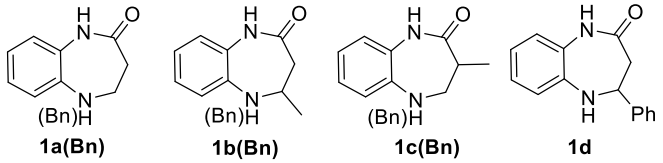
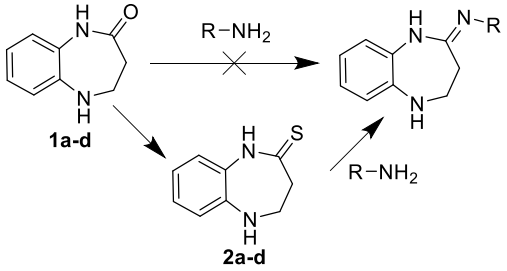


Figure 3. Starting 1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-ones.

It is known that amides do not react towards amidines directly. Oxygen atom has to be activated in order to displace it with linker molecules (sch. 33). Many methods of activation exist[59] but the formation of thioamide was selected in this work because of easy preparation/handling and most importantly good reactivity with nucleophiles such as amines[60].



Scheme 33. Synthesis of amidines from thioamide.

Refluxing in toluene with Lawesson's reagent seemed attractive method for thionation of benzodiazepinones **1a-d**. Unfortunately, yields were very low (table 1) and the work up was complicated because of tar formation. Heating with P_2S_5 in pyridine performed much better but the reaction outcome was still unsatisfactory (table 1). Despite good performance mentioned in literature, method with P_2S_5 and hexamethyldisiloxane (HMDS) gave worst results of thionation (table 1). Best performance was observed in thionations with $P_2S_5Py_2$ (synthesized separately) in MeCN (table 1).

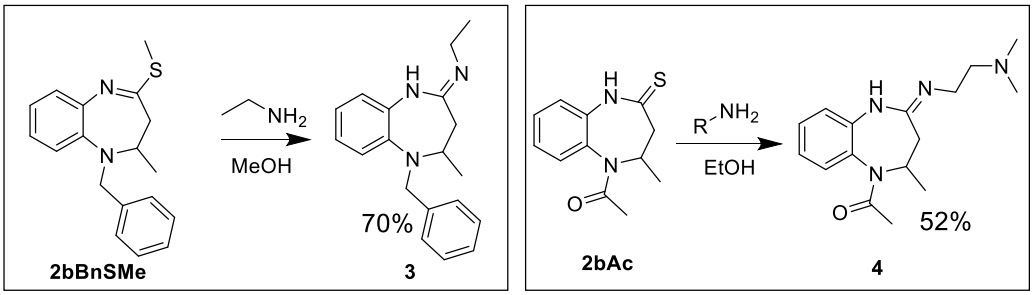
Table 1. Isolated yields (%) from experiments on thionation of benzodiazepinones.

=O	**Product**	**[a]Lawesson's**	**[b]P_2S_5+Py**	**[c]P_2S_5+HDMS**	**[d]$P_2S_5Py_2$+MeCN**
1a	**2a**	39	60	12	71
1b	**2b**	43	52	7	62
1c	**2c**	12	30	-	67
1d	**2d**	32	41	-	76
1aBn	**2aBn**	-	55	-	63
1bBn	**2bBn**	-	51	15	71
1cBn	**2bBn**	-	32	-	63

Conditions: a – benzodiazepinone (2.5 mmol) in toluene (0.5M), Lawesson's reagent (0.6 eq), reflux 12 h. b – benzodiazepinone (2.5 mmol) added to mixture of P_2S_5 (1 eq.) in pyridine (0.25M), 80°C 8 h. c –benzodiazepinone (2.5 mmol), MeCN (0.5M), P_2S_5 (0.6 eq), HMDS (1.8 eq.), 72°C 12 h. d - benzodiazepinone (2.5 mmol) in acetonitrile (0.25 M), $P_2S_5Py_2$ (1 eq.), reflux 10 h.

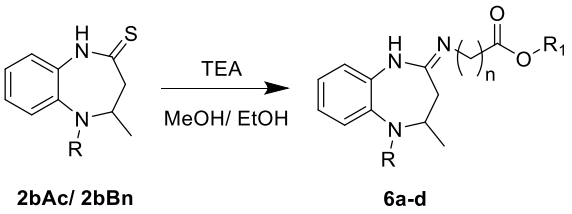
In order to further increase substrate scope and study reactivity of obtained thioamides **2b-c** were acetylated using acetic anhydride[61] and **2bBn** was S methylated using methyl iodide[62] to **2bBnSMe**.

It is well known that variously substituted benzodiazepin-2-thiones are quite readily involved in nucleophilic substitution reactions with various amines in the presence of mercury (II) salts to form amidines[63]. While keeping in mind that synthesized compounds will be tested in vitro and in vivo even negligible traces of mercury in the final products should be avoided because of its toxic nature and plausible interference with test results. It is known that hydrazides, hydrazine or hydroxylamine react with benzodiazepin-2-thiones without any catalyst. We have decided to test whether our thioamides **2** react with primary amines without catalyst (sch. 34). As expected after prolonged reflux with large excess of amine, amidine products **3** and **4** were obtained in moderate yields.



Scheme 34. Test reaction with primary amines.

After successful attempt to synthesize compounds **3**, **4** amino acid methyl ester hydrochlorides were prepared by modified literature procedure[64] **5b-c**. Ethyl glycine hydrochloride **5a** was obtained from commercial supplier Sigma-Aldrich (CAS: 623-33-6, catalog ID: G6503-100G-A). These amino acid ester hydrochlorides were obtained in order to form amidines **6a-d** with benzodiazepin-2-thiones **2** (sch.35). Later, ester group could be hydrolyzed and NHS ester formed in order to easily attach the benzodiazepine molecule to amino functionality in other compounds.



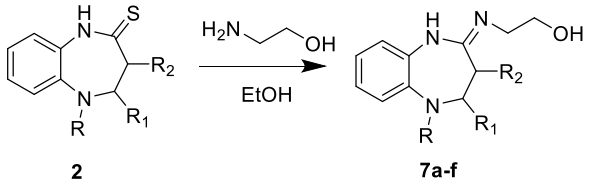
Scheme 35. Synthesis of amidines **6a-d**.

Amidines **6a-d** were synthesized in moderate to good yields (table 2). Reactions were performed in ethanol or methanol (selected by amino acid ester hydrochloride) using excess triethylamine as a base. If ethanol is used with methyl esters of amino acids significant transesterification takes place and the mixture of both ethyl and methyl esters is obtained.

Table 2. Amino acid ester modified benzodiazepines **6a-d**.

R	**R_1**	**n**	**=S**	**A.A. ester.**	**Product**	**Yield, %**
Ac	Et	1	**2bAc**	**5a**	**6a**	67
Ac	Me	2	**2bAc**	**5b**	**6b**	69
Bn	Me	2	**2bBn**	**5b**	**6c**	27
Bn	Me	3	**2bBn**	**5c**	**6d**	82

Another linker - ethanolamine was selected for the synthesis of amidines with easily functionable alcohol group (sch. 36).



2 **7a-f**

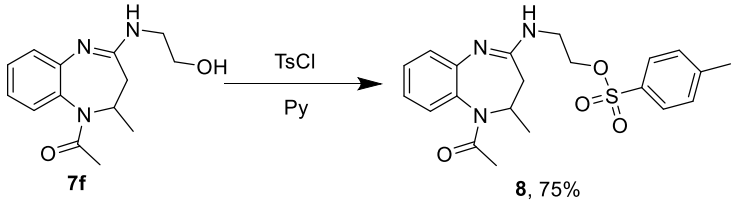
Scheme 36. Synthes of amidines **7a-f**.

Amidines were synthesized in good yields by refluxing thioamides **2a-d**, **2bBn**, **2bAc**, **2cAc** in absolute ethanol with seven-fold excess of ethanolamine for 3-4 h (table 3).

Table 3. Ethanolamine modified benzodiazepines **7a-f**.

=S	**R**	**R_1**	**R_2**	**Product**	**Yield, %**
2a	H	H	H	**7a**	54
2b	H	Me	H	**7b**	92
2c	H	H	Me	**7c**	63
2d	H	Ph	H	**7d**	83
2bBn	Bn	Me	H	**7e**	80
2bAc	Ac	Me	H	**7f**	87
2cAc	Ac	H	Me	**7g**	80

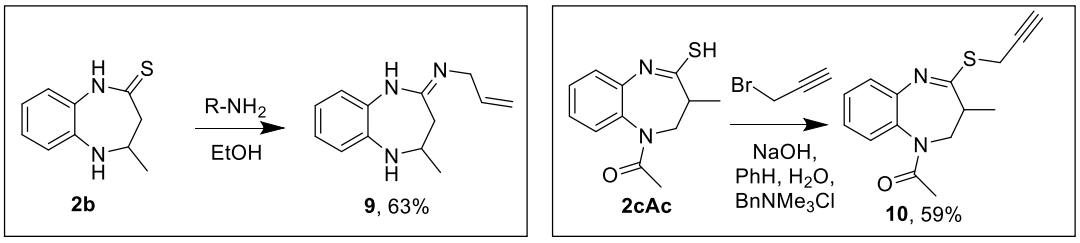
In order to demonstrate activation of alcohol functional group in compound **7f** it was tosylated with tosyl chloride to **8** (sch. 37).



7f **8**, 75%

Scheme 37. Tosylation of **7f** to **8**.

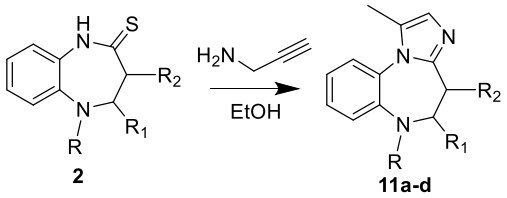
Moreover compound **9** was prepared from **2b** which is interesting because of remaining monosubstituted alkene that could be easily activated by epoxidation with peracetic acid[65] (sch. 38). Compound **10** was prepared from **2cAc** by treatment with commercially available solution of propargyl bromide in toluene using water and benzene solvent mixture together with sodium hydroxide as a base and PTC – benzyl trimethylammonium chloride (sch. 38).



2b **9**, 63% **2cAc** **10**, 59%

Scheme 38. Synthesis of compounds **9** and **10**.

Interestingly, alkyne functional group is widely used in biochemical research because it could easily participate in cycloaddition reaction with azides to form 1,2,3-triazoles[66]. It was decided to synthesize amidines by the reaction of thioamides **2** and propargyl amine (same conditions as **9** with allyl amine). Surprisingly the obtained compounds had no alkyne group. After careful examination of ^{1}H, ^{13}C, HSQC and HMBC 2D NMR spectra (fig. 4, table 5, suppl. 1-2) it was found that cyclic imidazo derivatives **11a-d** were obtained instead (sch. 39, table 4).



Scheme 39. Unexpected cyclization to **11a-d.**

Table 4. Imidazo compounds **11a-d**.

=S	R	R_1	R_2	Product	Yield, %
2a	H	H	H	**11a**	66
2b	H	Me	H	**11b**	80
2aBn	Bn	H	H	**11c**	65
2cAc	Ac	H	Me	**11d**	70

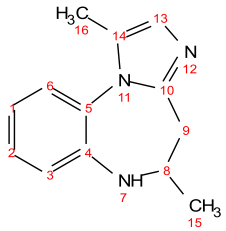


Figure 4. Numeration of **11b** atoms for assignment by NMR.

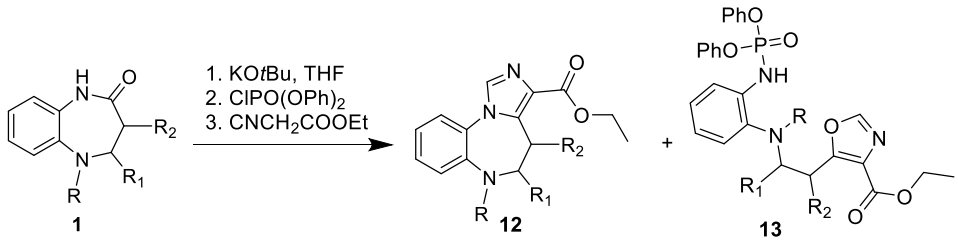
First of all, **11b** ^{1}H NMR spectrum was integrated and proton count was checked. It is evident that compound has 5 aromatic protons between 6.7 – 7.4 ppm. and 10 protons in aliphatic field between 1.2 – 4.2 ppm. No singlets with integral 2 and 1 were found which is the first sign that there is no propargylic fragment in the spectrum. Further in ^{13}C NMR carbon atoms were counted. It can be seen that molecule has 4 aliphatic carbons and 8 aromatic carbons + 1 (146.40 ppm) potentially carbonyl or similar carbon atom. It is evident that **11b** has one aliphatic and two aromatic carbons more than in starting material **2b** it means that propargyl amine has probably attached somehow. In both ^{1}H and ^{13}C NMR signal at 0 ppm represent TMS. In HSQC spectra signal at (1.26; 23,12) doublet in ^{1}H with integral 3 represents 15 (fig. 4). Doublet can be explained by existence of single proton on neighbor atom 8 (fig. 4). Signal in HSQC at (2.24, 10.83) singlet in ^{1}H (3H) represent 16 (fig. 4) shifts in ^{13}C spectrum suggest that it is aliphatic CH_3 attached to atom with 0 hydrogen atoms. By further analyzing HSQC spectrum (from right top corner to left) two hydrogen atoms on one carbon can be seen (2.56, 2.95; 32.42). In HMBC spectrum carbon at 32.42 ppm has interaction with protons at 1.26 ppm. It means that this carbon is 9 (fig. 4) and is separated by three bonds from 15 (fig. 4) protons. Also, to support above claim interaction in HMBC (4.01, 32.42) suggest that carbon 9 (fig. 4) is separated by two bonds from single proton on 8 (fig. 4) which gives rather interesting multiplicity - hexuplet. From HSQC it can now be solved that 8 (fig. 4) has single proton at 4.02 ppm and carbon at 58.77 ppm. Moreover ^{1}H signal (3.25 ppm, 1H, br. s) does not show interaction in HSQC nor HMBC it could be postulated that this signal belongs to NH 7 (fig. 4). In near aromatic field ^{1}H signal (6.80 ppm, 1H, s) has signal in HSQC at (6.80, 126.14) and HMBC (6.80, 127.26; 146.35) also (2.24, 127.26) it means that this proton is attached to aromatic carbon with other substituents without protons and it interacts with 3 protons 16 (fig. 4) also interaction of proton 13 (fig. 4) with carbon 10 (fig. 4) is evident. Above assignments leaves 6 aromatic carbons and 5 protons unassigned. By HSQC NMR it is not hard to find two carbons at 129.27 and 140.13 ppm which does not have protons. It means that to these carbons have other atoms attached. By HMBC spectrum interaction between protons 8 (fig. 4)

and carbon at 140.13 can easily be observed. This finding suggest that 140.13 ppm represents 4 (fig. 4) and 129.27 represents 5 (fig. 4). Atoms 1, 2, 3, 6 (fig. 4) were assigned by the same logic that HSQC signals represent which protons are on which carbon atoms and HMBC suggest interactions between hydrogen and carbon atoms separated by two to three bonds.

Table 5. Assignment of **H** and **C** atoms in **11b**.

Nr.	H	C
1	7.00	123.59
2	7.20	124.59
3	7.22	127.80
4	-	140.13
5	-	129.27
6	7.10	122.38
7	3.25	-
8	4.02	58.77
9	2.56; 2.95	32.42
10	-	146.4
13	6.80	126.14
14	-	127.3
15	1.26	23.12
16	2.24	10.83

Following it was decided to test activation of amide with diphenyl chlorophosphate and to perform synthesis with ethyl isocyanoacetate to obtain series of similar compounds to flumazenil[67] (GABA-A receptor antagonist). Unusually, desired imidazo compounds **12a-b** were obtained only in two cases and in extraordinary low yields. Major product in these experiments were oxazole **13** derivatives formed by diazepine ring opening (sch. 40, table 6).



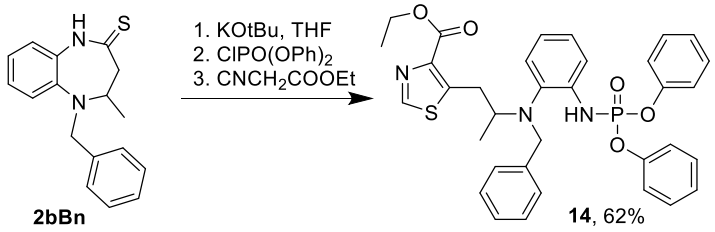
Scheme 40. Unexpected benzodiazepine ring opening.

In cases **13c-e** oxazole derivatives were the only products isolated.

Table 6. Imidazo **12a-b** and oxazole **13** compounds.

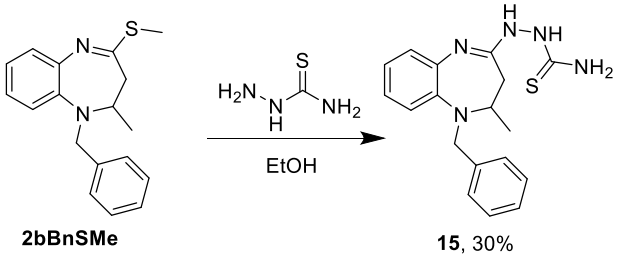
=O	R	R_1	R_2	Product	Yield, %
1bBn	Bn	Me	H	**12a; 13a**	11; 54
1cBn	Bn	H	Me	**12b**	35
1aBn	Bn	H	H	**13c**	47
1d	H	Ph	H	**13d**	44

Under the same reaction conditions as above (sch. 40) benzo[*b*][1,4]diazepine-2-thione **2bBn** was reacted and yielded **14** as a major product – thiazole derivative (sch. 41).



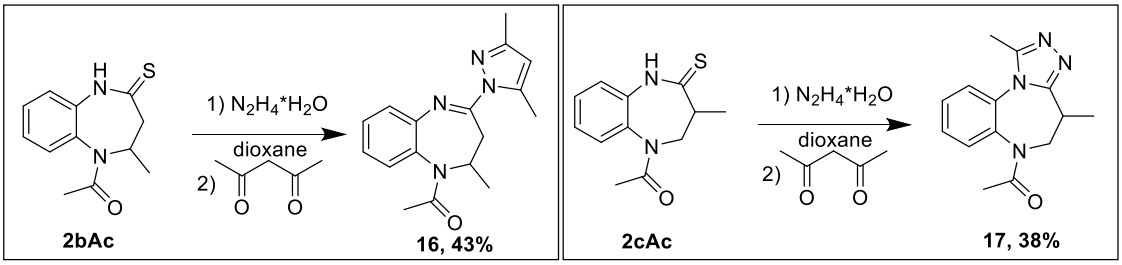
Scheme 41. Formation of thiazole derivative **14**.

In order to get better understanding of reactivity of studied diazepine-2-thiones and to increase scope of compounds for toxicity studies several additional reactions were performed. Upon **2bBnSMe** reaction with thiosemicarbazide in absolute ethanol compound **15** was obtained (sch 42).



Scheme 42. Synthesis of **15** with thiosemicarbazide.

Last but not least compounds **2cAc** and **2bAc** after treatment with hydrazine hydrate and refluxing in dioxane with acetylacetone behaved quite differently. **2cAc** gave expected[68] cyclocondensation of acetylacetone product **16** while **2bAc** gave unexpected 1,2,4-triazolo derivative **17** (sch. 43). Intermediate amidrazone derivatives were not isolated and studied because of low stability. In order to confirm formation of amidrazone known[69] product **18** was synthesized in 60% yield by reaction with thiophosgene.



Scheme 43. Different behavior of **2bAc** and **2cAc** under the same conditions.

Reaction course of **2bAc** towards **17** could be explained by acetylacetone hydrolysis[70] with residual humidity in dioxane or by the reaction of **2bAc** with another molecule of **2bAc** as acetyl group donor. Interestingly, formation of **2b** was not observed.

Atoms of **16** (fig. 5, table 7, suppl. 3-4) and **17** (fig. 5, table 8, suppl. 5-6) were assigned by analysis of ^{1}H, ^{13}C, HSQC and HMBC spectra. Compound **16** has very characteristic aromatic singlet at 6.01 ppm in ^{1}H spectrum (suppl. 4, table 7) and four signals with integrals 3H (15,21,22 – singlets, 17-doublet) in aliphatic fields representing methyl groups. Compound **17** has only 3 aliphatic methyl groups. Also, almost all **17** signals differ from **16** because of different placement of groups in the molecule and different solvent used for acquiring an NMR information.

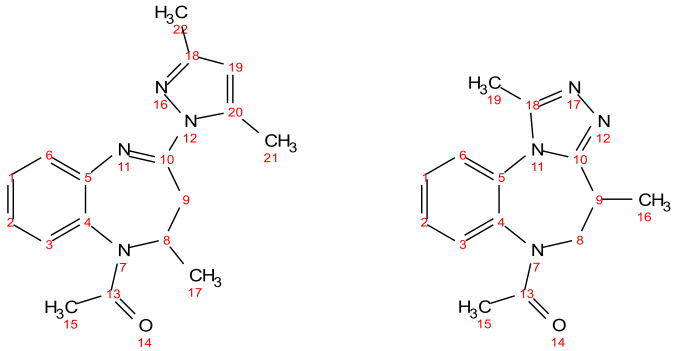


Figure 5. Numeration of **16** and **17** atoms for assignment by NMR.

Table 7. Assignment of **H** and **C** atoms in **16**.

Nr.	**H**	**C**
1.	7.17	125.37
2.	7.17	124.95
3.	7.40	129.05
4.	-	130.04
5.	-	146.01
6.	7.13	130.07
8.	5.44	59.47
9.	2.32; 3.81	34.82
10.	-	161.07
13.	-	170.01
15.	1.79	22.93
17.	1.25	18.73
18.	-	150.20
19.	6.01	111.04
20.	-	142.83
21.	2.60	15.59
22.	2.24	13.65

Table 8. Assignment of **H** and **C** atoms in **17**.

Nr.	**H**	**C**
1.	7.68	131.23
2.	7.68	125.71
3.	7.76	125.62
4.	-	135.54
5.	-	132.04
6.	7.62	130.48
8.	3.50, 4.35	54.57
9.	2.88	28.61
10.	-	155.22
13.	-	169.31
15.	1.59	22.50
16.	1.35	13.58
18.	-	150.17
19.	2.46	11.19

To conclude the current chapter several observations should be noted. Reactions of studied diazepine-2-thiones **2** and primary amines or amino acid esters performed satisfactorily to yield desired amidines without heavy metal catalysis. Propargylamine yielded cyclic derivatives **11a-d** by just prolonged heating. Pd, Au or I_2 as a catalyst was not necessary in this case to activate C≡C triple bond.

6. TOXICITY STUDY

Substances **6a**, **6b**, **7a-g**, **8**, **12a**, **12b** were selected for toxicity study on mice. In order to better evaluate synthesized benzodiazepines with linker groups several compounds without linkers **2bBnSMe, 3, 11b, 18** were selected together with acyclic – nonbenzodiazepine compounds **13a**, **14**. Obviously, selected compounds did not dissolve in sterile saline, so DMSO was used as a solvent. Saturated solutions of compounds in DMSO were mixed with saline solution 5%:95% (w/w). Final solutions were clear and no turbidity or deposits were observed. Unfortunately, compounds **6a**, **11b**, **7b**, **7c**, **8**, **12a**, **12b** were not soluble enough in DMSO to perform toxicity testing.

6.1 METHODS

6.1.1 Experimental animals and husbandry

A total of 36 Balb/c mice (13 weeks old) males were selected for study based on adequate body weight and absence of clinical signs of disease or injuries. The animals were kept in polypropylene cages in a temperature-controlled room at 21–23°C and relative humidity of 30%–70%, a 12-h light/dark cycle, and free access to food and water.

Mice were randomly assigned to the following twelve groups, each consisting of three animals (table 9)

Table 9. Groups of mice and compounds tested.

Group	Compound	Concentration of DMSO solution, %
1. vehicle control (VC)	-	5
2.	**7f**	5
3.	**7g**	5
4.	**7d**	5
5.	**2bBnSMe**	5
6.	**7e**	5
7.	**6b**	5
8.	**7a**	5
9.	**3**	5
10.	**13a**	5
11.	**14**	5
12.	**18**	5

6.1.2 Substances administration

Each mouse received a single bolus of the appropriate substance via subcutaneous injection. The shots were administered using a sterile hypodermic syringe and a stainless-steel needle (26 G). The volume was 10 mL/kg body weight and was adjusted based on the animal's body weight on the day of treatment.

6.1.3 Mortality and Clinical Signs

During the first four hours following injection, all animals were monitored for mortality at intervals of 30 min, 1, 2, 4, 8, and 24 h. In the same way, all animals were observed for indications of toxicity. The presence of any symptoms, their progression or disappearance, if any, would be documented.

6.1.4 Body Weight

The body weights were measured the day before treatment, and 24 h after it (just before sacrifice). The change in body weight of the individual animals with respect to the initial measurement and group mean values were calculated.

6.1.5 Hematology

Blood sampling for hematology was performed from the tail vein before and 24 h after the administration of tested substances. Sodium citrate was used as an anticoagulant. The following parameters were analyzed using a hematological autoanalyzer (ADVIA2120i Hematology analyzer, Bayer, USA): white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), platelets (PLT), neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS).

6.1.6 Statistical analysis

Experimental data were processed using the Statistica 10.0 software package. Data are shown in graphs as median ranges. For intergroup comparison, the nonparametric Kruskal–Wallis test was used, with adjustment for multiple comparisons. The differences were considered statistically significant with a P-value equal to or less than 0.05.

6.2 RESULTS

6.2.1 Clinical Signs and Mortality

On the day of subcutaneous treatment dosing and 24 h after the administration, clinical examinations at intervals of 30 min, 1, 2, and 4 h did not reveal any unusual clinical features or mortalities among the treated mice in the following groups: VC, 2, 3, 5, 7, 8, 10 – 12 (table 10). Mice of groups 4 (Substance **7d**), 6 (Substance **7e**), and 9 (Substance **3**) did not demonstrate toxicity symptoms till 8 hours post-injection of substances, whereas at 24 h, they all were found dead (table 10).

Table 10. Summary of Mortality.

Group	**Treatment**	**Incidence of mortality**	
		Absolute	**% Mortality**
Group 1/VC	0.2 ml of DMSO (5%) S.C.	0/3	0
Group 2	Substance **7f**, 0.2 ml in DMSO (5%) S.C.	0/3	0
Group 3	Substance **7g**, 0.2 ml in DMSO (5%) S.C.	0/3	0
Group 4	Substance **7d**, 0.2 ml in DMSO (5%) S.C.	3/3	100
Group 5	Substance **2bBnSMe**, 0.2 ml in DMSO (5%) S.C.	0/3	0
Group 6	Substance **7e**, 0.2 ml in DMSO (5%) S.C.	3/3	100
Group 7	Substance **6b**, 0.2 ml in DMSO (5%) S.C.	0/3	0
Group 8	Substance **7a**,0.2 ml in DMSO (5%) S.C.	0/3	0
Group 9	Substance **3**, 0.2 ml in DMSO (5%) S.C.	3/3	100
Group 10	Substance **13a**, 0.2 ml in DMSO (5%) S.C.	0/3	0
Group 11	Substance **14**, 0.2 ml in DMSO (5%) S.C.	0/3	0
Group 12	Substance **18**, 0.2 ml in DMSO (5%) S.C.	0/3	0

Absolute mortality is presented as the number of animals that died/numbers treated.

6.2.2 Body Weight

The body weights of groups VC, 2, 3, 5, 7, 8, 10 - 12 were not adversely affected by administered substances. Substances **7d** (group 4), **7e** (group 6), and **3** (group 9) decreased the weights of mice 24 h post-administration, but changes were not statistically significant (table 11).

Table 11. Body weights of mice before and after treatment.

Group	Treatment	Weight, g	
		Before treatment	24h after treatment
Group 1/VC	0.2 ml of DMSO (5%) S.C.	30,3±0,4	30,0±0,7
Group 2	Substance **7f**, 0.2 ml in DMSO (5%) S.C.	31,0±0,7	31,0±0,7
Group 3	Substance **7g**, 0.2 ml in DMSO (5%) S.C.	31,0±0,7	31,0±0,7
Group 4	Substance **7d**, 0.2 ml in DMSO (5%) S.C.	30,7±0,8	29,3±1,1
Group 5	Substance **2bBnSMe**, 0.2 ml in DMSO (5%) S.C.	30,3±0,4	30,7±0,4
Group 6	Substance **7e**, 0.2 ml in DMSO (5%) S.C.	30,7±0,4	29,7±0,4
Group 7	Substance **6b**, 0.2 ml in DMSO (5%) S.C.	31,3±0,4	30,7±0,4
Group 8	Substance **7a**,0.2 ml in DMSO (5%) S.C.	30,7±0,4	30,3±0,4
Group 9	Substance **3**, 0.2 ml in DMSO (5%) S.C.	30,3±0,8	29,7±1,2
Group 10	Substance **13a**, 0.2 ml in DMSO (5%) S.C.	31,0±0,7	30,7±0,4
Group 11	Substance **14**, 0.2 ml in DMSO (5%) S.C.	30,0±0,7	30,3±0,4
Group 12	Substance **18**, 0.2 ml in DMSO (5%) S.C.	31,3±0,8	31,0±0,7

6.2.3 Blood parameters

Hematological blood evaluations showed no statistically significant differences in counts of RBC, HCT, or HGB 24 h after injection. Tendencies to decrease were registered after injection for groups 1 and 11. (Table 12).

Table 12. Hematological blood evaluation.

		RBC, x10^{12}/L		HCT, %		HGB, g/dL	
Group	**Treatment**	**before**	**24 h after**	**before**	**24 h after**	**before**	**24 h after**
1	Vehicle (5% DMSO)	7,55±0,22	7,00±0,09	37,2±2,7	35,4±1,0	11,8±0,3	11,4±0,3
2	Substance **7f**	8,92±0,77	5,33±0,22↓	42,5±2,0	24,2±1,0↓	13,8±0,7	8,2±0,7↓
3	Substance **7g**	6,88±0,08	5,68±0,23	32,2±2,2	26,3±0,8	10,8±0,2	9,0±0,2
5	Substance **2bBnSMe**	6,47±0,38	5,56±0,17	30,3±1,3	25,8±1,0	10,1±0,1	8,7±0,2
7	Substance **6b**	6,92±0,31	5,50±0,36	32,2±1,1	25,3±1,3	10,7±0,2	8,3±0,2
8	Substance **7a**	6,02±0,67	6,38±0,28	30,4±0,4	32,2±2,2	10,2±0,5	10,6±0,5
10	Substance **13a**	7,11±0,43	6,26±0,11	33,4±0,6	28,9±1,7	10,9±0,1	9,6±0,3
11	Substance **14**	8,75±0,25	5,77±0,23↓	41,5±1,6	26,4±2,0↓	13,4±0,5	8,9±0,3↓
12	Substance **18**	8,84±0,17	9,13±0,69	42,3±2,0	43,7±3,2	13,7±0,3	13,8±0,3

No statistically significant changes of immune cell counts were registered after injections of vehicle or any tested substance. Tendencies to decrease in counts of WBC and LYMPH were observed 24 h after administration of substances **6b**, **7a**, **14**, and **18** (table 13). DMSO itself caused tendency to increase amount of EOS (probably due to irritative action), whereas tested substances did not evoke such a reaction (table 14). Substance **7g** insignificantly increased, whereas substance **7a** insignificantly lowered the number of MONO (table 14).

Table 13. Immune cell count evaluation.

		WBC, x10^{9}/L		NEU, x10^{9}/L		LYMPH, x10^{9}/L	
Group	**Treatment**	**before**	**24 h after**	**before**	**24 h after**	**before**	**24 h after**
1	Vehicle (5% DMSO)	9,97±0,53	9,25±0,25	3,12±0,09	3,12±0,1	4,37±0,21	5,54±0,34
2	Substance **7f**	6,33±0,24	7,08±0,10	2,86±0,04	1,65±0,18	3,16±0,16	4,98±0,06
3	Substance **7g**	6,64±0,04	8,26±0,32	1,79±0,12	1,36±0,02	4,47±0,15	6,43±0,09
5	Substance **2bBnSMe**	4,94±0,28	5,16±0,12	1,53±0,11	1,17±0,08	3,18±0,16	3,69±0,35
7	Substance **6b**	8,25±0,11	4,94±0,20↓	1,83±0,11	1,43±0,17	5,98±0,36	3,17±0,20↓
8	Substance **7a**	8,33±0,31	4,68±1,21↓	2,93±0,06	1,88±0,04	4,79±0,10	2,32±0,16↓
10	Substance **13a**	5,64±0,2	6,70±1,23	1,99±0,03	2,57±0,29	3,47±0,11	3,71±0,20
11	Substance **14**	7,74±0,29	5,43±0,09↓	2,27±0,06	1,73±0,21	5,15±0,29	3,36±0,39↓
12	Substance **18**	7,8±0,18	6,08±0,16↓	3,33±0,20	2,33±0,21	6,19±0,18	3,32±0,16↓

Even though the count of platelets increased at 24 h after administration of vehicle and substance **7f**, whereas it decreased after injection of substance **7a**, changes in this parameter were not statistically significant (table 14). The blood of mice injected with substances **7d**, **7e**, and **3** was not analyzed due to the death of animals.

Table 14. Blood parameters evaluation.

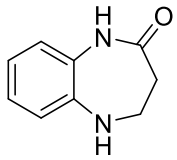
		MONO		**EOS**		**PLT**	
Group	**Treatment**	**before**	**24 h after**	**before**	**24 h after**	**before**	**24 h after**
1	Vehicle (5% DMSO)	0,14±0,01	0,17±0,01	0,16±0,01	0,41±0,01↑	504,0±28,1	804,0±64,7↑
2	Substance **7f**	0,11±0,01	0,17±0,01	0,19±0,01	0,27±0,01	555,0±23,4	826,0±63,4↑
3	Substance **7g**	0,12±0,01	0,19±0,01	0,25±0,01	0,26±0,01	505,0±59,1	629,0±46,9
5	Substance **2bBnSMe**	0,08±0,01	0,09±0,01	0,15±0,01	0,20±0,02	728,0±34,4	664,0±22,1
7	Substance **6b**	0,09±0,01	0,04±0,01	0,35±0,02	0,30±0,02	545,0±31,0	580,0±48,4
8	Substance **7a**	0,26±0,04	0,10±0,01↓	0,34±0,03	0,36±0,02	775,0±74,6	531,0±28,0
10	Substance **13a**	0,08±0,01	0,13±0,02	0,09±0,01	0,28±0,04	642,0±52,6	508,0±42,1
11	Substance **14**	0,09±0,01	0,09±0,01	0,23±0,03	0,24±0,03	657,0±34,8	637,0±27,0
12	Substance **18**	0,09±0,01	0,06±0,01	0,35±0,02	0,37±0,02	628,0±28,1	623,0±27,8

7. EXPERIMENTAL PART

The ^{1}H, ^{13}C, ^{31}P NMR spectra were recorded using a BRUKER spectrometer at a frequency of 400 MHz, 101 MHz, 162 MHz respectively. Residual values of deuterated solvents or TMS was used as internal standards. 85% H_3PO_4 was used as external standard (0.0 ppm) for ^{31}P NMR spectra. Chemical shift values are given on the δ-scale. The symbols used to describe NMR spectra are: s - singlet, d - doublet, t - triplet, br. s - broad singlet, dd - doublet of doublet, td - triplet of doublet, m - multiplet. IR spectra were recorded with PerkinElmer Spectrum GX FTIR spectrometer (KBr). Melting points of compounds were determined in open capillaries using a Stuart SMP 10 instrument and are uncorrected. Mass spectra were recorded on a Bruker maXis 4G Q-TOF spectrometer using ESI (positive ion mode) at capillary voltage of 4.0 kV. Elemental analyses were performed on a Thermo-Scientific Flash 2000 CHNS/O analyzer. The progress of the reactions was monitored by thin-layer chromatography using TLC silica gel 60 F^{254} (Merc) plates. Eluents: mixtures of hexane, ethyl acetate, methanol, and dichloromethane at various ratios. Developers such as vanillin, ninhydrin, UV light and potassium permanganate were used to develop the TLC plates. Silica gel 60 mesh (40-63 μm) was used for column chromatography.

7.1 Synthesis of 1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-one (1a)

To the 500 ml round-bottom flask with a reflux condenser, 0.36 mol (38.4 g) of ortho-phenylenediamine, 280 ml of water and 40 ml of sulfuric acid are added and heated slowly until full dissolution of solids. Then 0.54 mol (38.4 g) of acrylamide is added to the hot solution and refluxed for 2 hours. Afterward, the reaction mixture is cooled to r.t. and 300 ml of water is added to the flask, reaction mixture is neutralized with sodium carbonate to pH = 7. The precipitate is filtered, dried and crystallized from benzene and air dried.

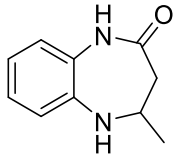


MW = 162.19 g/mol

White powder, m.p. 140-142 °C (lit. 140-142 °C[71]). Yield: 40.5 g (70 %).

7.2 Synthesis of 4-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-one (1b)

To the 100 ml round-bottom flask with an air condenser 0.25 mol (27 g) of ortho-phenylenediamine and 0.25 mol (21.5 g) of crotonic acid are added, then heated in a Wood's metal bath at 190 °C for 2 hours. After that, the reaction mixture is cooled and 30 ml of methanol is added. The flask is left in the refrigerator overnight. The precipitated crystals are filtered, recrystallized from dioxane, washed with isopropanol and air dried.



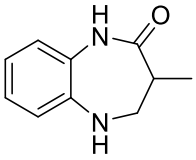
MW = 176.22 g/mol

White powder, m.p. 186-188 °C (lit. 185-186 °C[72]). Yield: 24 g (54 %).

7.3 Synthesis of 3-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-one (1c)

To the 250 ml round-bottom flask with an air condenser 0.37 mol (40.5 g) of ortho-phenylenediamine and 0.37 mol (31.5 g) of methacrylic acid are added, then heated in a Wood's metal bath at 190 °C. After 2 hours, the reaction mixture is cooled and 200 ml of methanol is slowly added to it. The flask

is left in the refrigerator overnight. The precipitated crystals are filtered and crystallized from methanol and air dried.

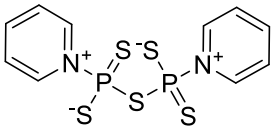


MW = 176.22 g/mol

White crystals, m.p. 196-198 °C (lit. 202-204 °C[73]). Yield: 41 g (62 %).

7.4 Synthesis of P_2S_5 and pyridine complex ($P_2S_5Py_2$)

Into the 500 ml round bottom flask 40.5 mmol (18 g) of P_2S_5 and 225 ml of dry pyridine are added. Mixture is refluxed for 4 hours and cooled to +4 °C overnight. After filtering, complex is stored in vacuum desiccator over P_2O_5.



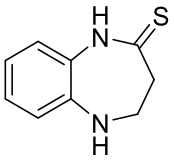
MW = 380.45 g/mol

Yellow solid (water sensitive), m.p. 115-124 °C (lit. 110-120 °C[26]). Yield: 20 g (65 %).

7.5 Synthesis of benzodiazepine-2-thiones (general procedure)

Into the 500 ml flask 50 mmol of starting benzodiazepine-2-one was added together with 16 mmol (6 g) of P_2S_5 pyridine complex and 250 ml acetonitrile. Reaction mixture refluxed for 4 hours. After cooling to room temperature half of solvent was evaporated under reduced pressure and the same amount of water slowly added. Remaining solids were filtered, air dried and crystallized from toluene.

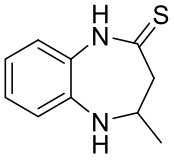
7.5.1 1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepine-2-thione (2a)



MW = 178.25 g/mol

Yellow solid, m.p. 161-162 °C (lit. 161-162 °C[63]). Yield: 6.2 g (71 %).

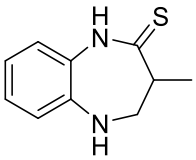
7.5.2 4-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepine-2-thione (2b)



MW = 192.28 g/mol

Yellow solid, m.p. 130-131 °C (lit. 129-130 °C[63]). Yield: 5.9 g (62 %).

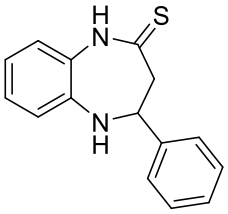
7.5.3 3-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepine-2-thione (2c)



MW = 192.28 g/mol

Yellow solid, m.p. 199-202 °C (lit. 198-200 °C[63]). Yield: 6.4 g (67 %).

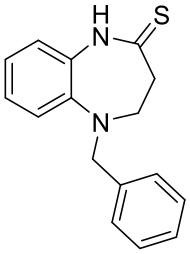
7.5.4 4-phenyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepine-2-thione (2d)



MW = 254.35 g/mol

Off white crystals, m.p. 186 ºC (lit. 186-188 ºC[14]). Yield: 9.6 g (76 %).

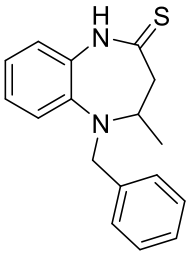
7.5.1 5-benzyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepine-2-thione (2aBn)



MW = 268.38 g/mol

Yellow crystals, m.p. 123-125 ºC (lit. 123-125 ºC[63]). Yield: 8.4 g (63 %).

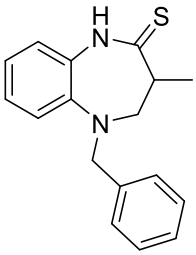
7.5.2 5-benzyl-4-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepine-2-thione (2bBn)



MW = 282.41 g/mol

Yellow solid, m.p. 141-144 ºC (lit. 144-146 ºC[63]). Yield: 10.0 g (71 %).

7.5.3 5-benzyl-3-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepine-2-thione (2cBn)



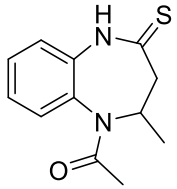
MW = 282.41 g/mol

Yellow solid, m.p. 129-130 ºC (lit. 128-130 ºC[63]). Yield: 8.9 g (63 %).

7.6 Acetylation of benzodiazepine-2-thiones (general procedure)

Into the 100 ml round bottom flask with magnetic stirrer and reflux condenser 20.8 mmol of starting benzodiazepin-2-thione was added together with 30 ml of chloroform and 25 mmol of acetic anhydride. The reaction mixture was refluxed for about 10 hours. At the end of the reaction, the mixture is transferred to a separating funnel and washed with 0.1 M HCl solution, then with saturated potassium carbonate solution and water. Solvent is evaporated under reduced pressure and the dry residue is crystallised from ethyl acetate.

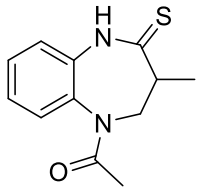
7.6.1 1-(2-methyl-4-thioxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one (2bAc)



MW = 234.32 g/mol

Off white crystals, m.p. 175 ºC (lit. 174-176 ºC[74]). Yield: 3.94 g (81 %).

7.6.2 1-(3-methyl-4-thioxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one (2cAc)

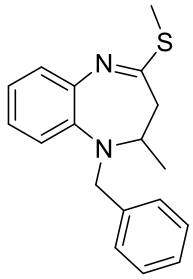


MW = 234.32 g/mol

Off white crystals, m.p. 174-175 ºC (lit. 172-175 ºC[75]). Yield: 4.0 g (82 %).

7.7 Synthesis of 1-benzyl-2-methyl-4-(methylthio)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (2bBnSMe)

Into the 100 ml round bottom flask 6.37 mmol (1.8 g) of **2bBn** was added together with 30 ml of benzene, 4.15 mmol (0.94 g) of benzyl triethylammonium chloride, 25.48 mmol (3.62 g) of MeI and 6.5 ml of 40% NaOH solution. The reaction mixture is stirred at room temperature for about 7 hours. At the end of the reaction the solution is filtered off, transferred to a separating funnel, the aqueous layer is separated and extracted with benzene. All the organic layers are then combined, washed with water, dried with magnesium sulphate. Solvent is evaporated under reduced pressure. The resulting solid is crystallised from absolute ethanol.



MW = 296.43 g/mol

White crystals, m.p. 161-165 ºC (lit. 38-40 ºC[62]). Yield: 1.6 g (85 %).

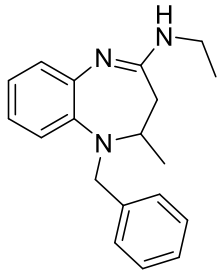
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.24 (m, 4H), 7.24 – 7.13 (m, 2H), 7.11 – 6.98 (m, 3H), 4.36 – 4.19 (m, 2H), 3.98 (dp, *J* = 12.1, 6.1 Hz, 1H), 2.61 (s, 3H), 2.45 – 2.25 (m, 2H), 1.03 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.83, 138.45, 128.34, 127.99, 126.97, 125.08, 124.96, 124.87, 123.95, 123.33, 123.00, 64.00, 54.02, 42.42, 14.61, 13.64.

7.8 1-benzyl-*N*-ethyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-amine (3)

Into the 50 ml round-bottomed flask fitted with a magnetic stirrer, a reflux condenser and a desiccant tube made of calcium chloride, 1.174 mmol (0.350 g) of the starting **2bBnSMe** was added together with 20 ml of absolute methanol and 10.7 mmol (1 ml) of 70 % aqueous ethylamine. The reaction mixture was refluxed for two days until no starting material remains. At the end of the reaction, the

methanol is evaporated under reduced pressure. The remaining oil is extracted with methylene chloride and washed with water. The solvent is evaporated under reduced pressure and the remaining oil is crystallised from a mixture of benzene and hexane.



MW = 293.41 g/mol

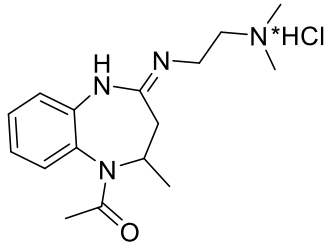
Off white crystals, m.p. 129-132 °C. Yield: 0.24 g (70 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.31 (m, 2H), 7.31 – 7.22 (m, 3H), 7.24 – 7.16 (m, 1H), 7.11 – 6.87 (m, 3H), 4.34 – 4.19 (m, 2H), 3.88 – 3.75 (m, 1H), 3.61 (s, 1H), 3.52 – 3.34 (m, 1H), 2.27 (t, *J* = 12.4 Hz, 1H), 1.94 (br. s, 2H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.85, 146.81, 139.16, 137.83, 127.82, 127.44, 126.28, 123.63, 122.41, 122.14, 120.75,61.43, 54.08, 39.97, 36.31, 14.84, 14.23.

7.9 (*E*)-1-(4-((2-(dimethylamino)ethyl)imino)-2-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one hydrochloride (4)

Into the 50 ml round-bottomed flask 5.3 mmol (1.24 g) of starting 1-(2-methyl-4-thioxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethanone is added together with 20 ml of absolute ethanol, and 37 mmol (4 ml) of *N*,*N*-dimethylethylenediamine. The reaction mixture is refluxed for about 5 hours. At the end of the reaction, the ethanol is evaporated under reduced pressure and the remaining oil is extracted with methylene chloride and washed with water. The methylene chloride is then evaporated under reduced pressure and the remaining oil is converted to hydrochloride salt.



MW = 324.85 g/mol

Yellow solid (humid sensitive), m.p. 116-120 °C (dec.). Yield: 0.9 g (52 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.9 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.43 – 7.33 (m, 1H), 7.18 (d, *J* = 6.4 Hz, 1H), 5.53 – 5.37 (m, 1H), 4.57 – 4.39 (m, 1H), 4.36 – 4.22 (m, 1H), 3.78 – 3.67 (m, 2H), 3.72 – 3.62 (m, 1H), 3.20 (dd, *J* = 13.6, 5.3 Hz, 1H), 3.09 – 2.98 (m, 6H), 2.26 (t, *J* = 13.1 Hz, 1H), 1.74 (s, 3H), 1.24 – 1.15 (m, 3H).

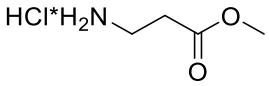
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.10, 164.91, 133.92, 131.89, 130.63, 130.26, 128.62, 125.66, 55.70, 55.14, 43.43, 43.31, 39.46, 36.82, 23.07, 18.82.

7.10 Synthesis of methyl esters of amino acids (general procedure)

Into the two neck 250 ml round bottom flask with magnetic stirrer, reflux condenser and gas inlet tube 0.22 mol of corresponding amino acid is added and 100 ml of dry methanol is poured subsequently. Dry HCl gas is passed through the suspension (without external cooling) until complete dissolution of solids observed. HCl flow is stopped and the reaction mixture is gently refluxed for 5 hours. After removal of excess methanol in vacuum 50 ml of acetone is added onto remaining solids.

Suspension is filtered and solids are washed with acetone, dried in vacuum exicator until constant mass reached.

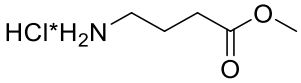
7.10.1 methyl 3-aminopropanoate hydrochloride (5b)



MW = 139.58 g/mol

White crystals, m.p. 90 °C (lit. 89-90 °C[76]). Yield: 27.8 g (89 %).

7.10.2 methyl 4-aminobutanoate hydrochloride (5c)



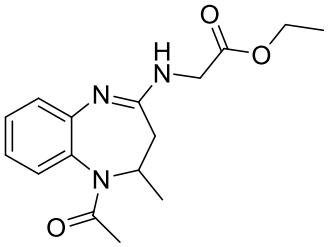
MW = 153.61 g/mol

White crystals, m.p. 119-121 °C (lit. 118-120 °C[77]). Yield: 27.5 g (92 %).

7.11 Reaction of benzodiazepine-2-thiones with amino acid esters

Into the 50 ml round-bottomed flask fitted with a magnetic stirred, reflux condenser and a desiccant tube made of calcium chloride, 4,26 mmol of starting benzodiazepine-2-thione was added together with 34 mmol of the methyl or ethyl ester of either glycine/beta-alanine or of the methyl or the ethyl ester of the gamma-aminobutyric acid, followed by the addition of 30 ml of absolute ethanol and 40 mmol of triethylamine. The reaction mixture was refluxed for about 7 hours. At the end of the reaction, the ethanol is evaporated under reduced pressure and the remaining oil is extracted with methylene chloride and washed with water. The solvent is evaporated under reduced pressure and the remaining oil is crystallised from ethyl acetate or diethyl ether.

7.11.1 ethyl (1-acetyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)glycinate (6a)



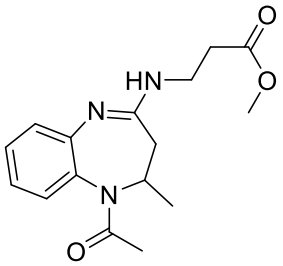
MW = 289.34 g/mol

White crystals, m.p. 198-201 °C. Yield: 0.82 g (67 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 3.8 Hz, 2H), 5.33 (tt, *J* = 12.2, 6.2 Hz, 1H), 4.30 – 4.18 (m, 3H), 4.03 (d, *J* = 18.5 Hz, 1H), 2.31 (t, *J* = 13.2 Hz, 1H), 2.14 (dd, *J* = 13.4, 5.1 Hz, 1H), 1.72 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.16 (t, *J* = 8.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.50, 170.24, 160.62, 148.10, 130.34, 129.88, 129.09, 125.45, 122.90, 61.49, 57.24, 43.30, 38.01, 22.87, 18.79, 14.18.

7.11.2 methyl 3-((1-acetyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)propanoate (6b)

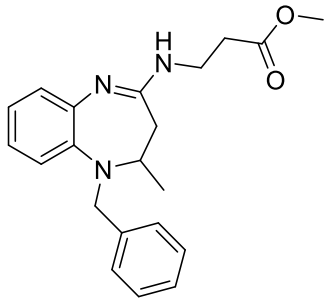


MW = 303.36 g/mol

Colorless oil. Yield: 0.89 g (69 %).

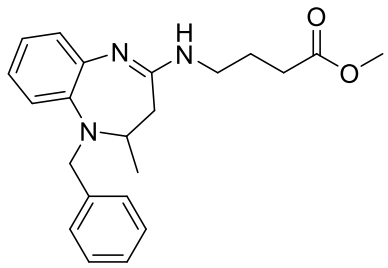
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32 – 7.26 (m, 1H), 7.06 (d, *J* = 7.9 Hz, 1H), 7.02 – 6.94 (m, 2H), 5.46 (br. s, 1H), 5.33 – 5.20 (m, 1H), 3.69 (s, 3H), 3.62 (t, *J* = 6.0 Hz, 2H), 2.76 – 2.56 (m, 2H), 2.28 (t, *J* = 25.7, 12.5 Hz, 1H), 2.05 – 2.00 (m, 1H), 1.72 (s, 3H), 1.13 (d, *J* = 6.3 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.23, 170.57, 161.02, 148.51, 130.26, 129.82, 129.10, 125.37, 122.60, 57.21, 51.81, 38.44, 36.64, 33.04, 22.79, 18.71.

7.11.3 methyl 3-((1-benzyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)propanoate (6c)



MW = 351.45 g/mol
White solid, m.p. 206-208 ºC. Yield: 0.4 g (27 %).
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 – 7.14 (m, 4H), 7.14 – 7.07 (m, 1H), 6.95 (d, *J* = 7.4 Hz, 1H), 6.91 – 6.67 (m, 3H), 4.26 – 4.11 (m, 2H), 3.77 – 3.65 (m, 1H), 3.58 – 3.48 (m, 2H), 3.45 – 3.34 (m, 1H), 3.26 – 3.13 (m, 1H), 2.57 – 2.48 (m, 3H), 2.24 (dd, *J* = 13.2, 6.0 Hz, 1H), 2.20 – 2.11 (m, 1H), 1.92 (t, *J* = 12.0 Hz, 1H), 0.91 (d, *J* = 6.0 Hz, 3H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 172.34, 162.99, 139.56, 138.67, 128.57, 128.22, 127.02, 127.16, 124.05, 123.41, 123.10, 122.64, 62.83, 53.26, 51.74, 37.55, 35.39, 33.97, 15.32.

7.11.4 methyl 4-((1-benzyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)butanoate (6d)



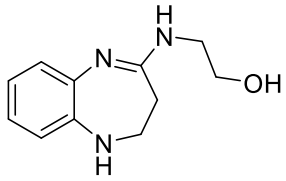
MW = 365.48 g/mol
White powder, m.p. 124-128 ºC. Yield: 1.3 g (82 %).
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 – 7.13 (m, 4H), 7.10 (t, *J* = 6.9 Hz, 1H), 6.92 – 6.85 (m, 2H), 6.81 – 6.75 (m, 1H), 6.72 – 6.64 (m, 2H), 4.15 (d, *J* = 3.3 Hz, 2H), 3.75 – 3.63 (m, 1H), 3.55 (s, 3H), 3.47 – 3.37 (m, 1H), 3.14 – 3.02 (m, 1H), 2.39 (t, *J* = 7.6 Hz, 2H), 2.09 (dd, *J* = 13.0, 5.9 Hz, 1H), 1.93 – 1.74 (m, 3H), 0.88 (d, *J* = 5.9 Hz, 3H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 173.80, 161.72, 147.81, 139.69, 138.58, 128.52, 128.26, 127.05, 124.37, 123.10, 122.69, 121.25, 62.87, 53.55, 51.74, 39.89, 38.56, 31.50, 24.63, 15.01.

7.12 Reaction of benzodiazepine-2-thiones with ethanolamine (general procedure)

Into the 100 ml round-bottomed flask fitted with a magnetic stirrer, a reflux condenser and a desiccant tube made of calcium chloride, 12.8 mmol of starting benzodiazepine-2-thione was added together with 50 ml of absolute ethanol and 89.6 mmol of ethanolamine. The reaction mixture was refluxed for about 3-4 hours. At the end of the reaction, the ethanol is evaporated under reduced pressure. The remaining oil is extracted with methylene chloride and then washed with water. The solvent is then

is evaporated under reduced pressure and the remaining oil is crystallised from ethyl acetate or a mixture of ethyl acetate and diethyl ether.

7.12.1 2-((2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)ethan-1-ol (7a)



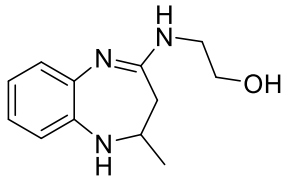
MW = 205.26 g/mol

Yellowish crystals, m.p. 143-146 °C. Yield: 1.4 g (54 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.75 – 6.59 (m, 4H), 5.26 (br. s, 1H), 4.94 (s, 1H), 3.54 (t, *J* = 5.6 Hz, 4H), 3.28 (t, *J* = 5.6 Hz, 2H), 2.35 (t, *J* = 5.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 161.82, 140.69, 139.47, 126.87, 122.20, 119.71, 119.67, 61.22, 51.20, 44.15, 31.93.

7.12.1 2-((2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)ethan-1-ol (7b)



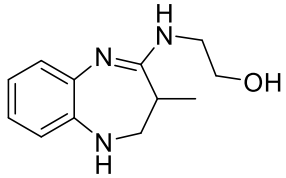
MW = 219.29 g/mol

White crystals, m.p. 163-168 °C. Yield: 2.6 g (92 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.98 (s, 1H), 6.77 – 6.61 (m, 4H), 5.15 (s, 1H), 4.60 (s, 1H), 3.90 (h, *J* = 6.2 Hz, 1H), 3.54 (t, *J* = 5.7 Hz, 2H), 3.41 – 3.21 (m, 3H), 2.35 (dd, *J* = 13.0, 4.8 Hz, 1H), 2.02 (dd, *J* = 13.0, 6.7 Hz, 1H), 1.14 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 161.07, 141.27, 139.67, 125.91, 121.92, 120.70, 120.57, 61.18, 58.99, 44.10, 37.47, 23.79.

7.12.2 2-((3-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)ethan-1-ol (7c)



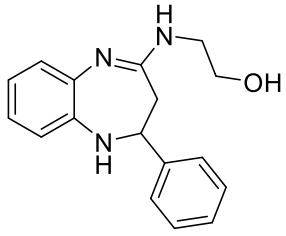
MW = 219.29 g/mol

Off white crystals, m.p. 192-195°C. Yield: 1.8 g (63 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.76 (d, *J* = 7.7 Hz, 1H), 6.66 – 6.51 (m, 4H), 5.39 (br. s, 1H), 5.27 (s, 1H), 3.52 (t, *J* = 5.6 Hz, 2H), 3.41 – 3.23 (m, 3H), 3.21 – 3.10 (m, 1H), 2.81 – 2.71 (m, 1H), 1.04 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.06, 141.57, 136.30, 128.09, 122.31, 118.53, 118.21, 61.62, 53.18, 44.39, 38.21, 15.34.

7.12.3 2-((2-phenyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)ethan-1-ol (7d)



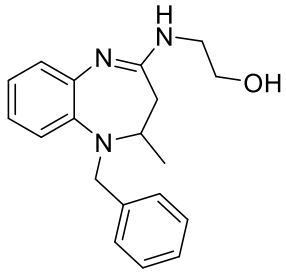
MW = 281.36 g/mol

Off white crystals, m.p. 109-112 ºC. Yield: 2.98 g (83 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.45 (d, *J* = 7.0 Hz, 2H), 7.39 – 7.32 (m, 5H), 7.08 – 7.02 (m, 1H), 6.99 – 6.87 (m, 2H), 6.80 – 6.73 (m, 1H), 5.13 – 5.08 (m, 1H), 3.75 (t, *J* = 4.4 Hz, 2H), 3.60 – 3.40 (m, 3H), 2.69 (dd, *J* = 13.4, 4.9 Hz, 1H), 2.46 (dd, *J* = 13.4, 7.0 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.45, 144.44, 138.14, 128.73, 128.33, 128.04, 126.44, 126.09, 123.62, 122.24, 120.86, 68.41, 64.46, 45.79, 39.02.

7.12.4 2-((1-benzyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)ethan-1-ol (7e)



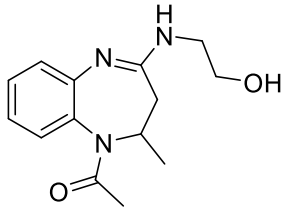
MW = 309.41 g/mol

Off white crystals, m.p. 129-132 ºC. Yield: 3.2 g (80 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 – 7.19 (m, 3H), 7.19 – 7.11 (m, 1H), 7.08 (s, 1H), 6.99 – 6.90 (m, 1H), 6.88 – 6.80 (m, 1H), 6.79 – 6.69 (m, 2H), 5.15 (br. s, 1H), 4.24 (q, *J* = 14.9 Hz, 2H), 3.78 (dt, *J* = 11.5, 5.8 Hz, 1H), 3.70 – 3.54 (m, 2H), 3.46 (d, *J* = 12.8 Hz, 1H), 3.39 – 3.27 (m, 1H), 2.23 (dd, *J* = 12.9, 5.8 Hz, 1H), 1.99 – 1.88 (m, 1H), 0.95 (d, *J* = 5.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 161.84, 146.79, 139.06, 137.82, 127.84, 127.43, 126.28, 123.64, 122.42, 122.13, 120.73, 62.38, 60.38, 52.83, 43.31, 37.76, 14.47.

7.12.5 1-(4-((2-hydroxyethyl)amino)-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one (7f)



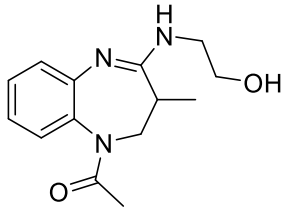
MW = 261.33 g/mol

Off white crystals, m.p. 163-165 ºC. Yield: 2.9 g (87 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (t, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 7.5 Hz, 1H), 6.96 – 6.87 (m, 2H), 5.06 (dp, *J* = 12.0, 6.1 Hz, 1H), 4.35 (br. s, 1H), 3.50 (hept, *J* = 5.8, 5.3 Hz, 2H), 3.41 – 3.21 (m, 3H), 2.30 (dd, *J* = 13.1, 5.3 Hz, 1H), 1.99 (t, *J* = 12.9 Hz, 1H), 1.56 (s, 3H), 1.03 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.19, 162.14, 149.54, 130.63, 130.38, 129.13, 125.15, 121.94, 60.11, 57.04, 43.76, 37.26, 22.97, 19.05.

7.12.6 1-(4-((2-hydroxyethyl)amino)-3-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one (7g)



MW = 261.33 g/mol

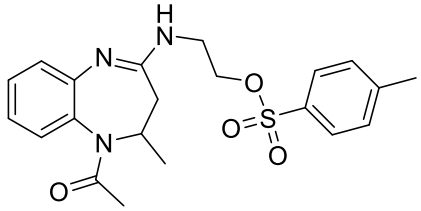
White crystals, m.p. 112-114 ºC. Yield: 2.5 g (80 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 – 7.18 (m, 1H), 7.18 – 7.08 (m, 1H), 6.97 – 6.85 (m, 2H), 6.60 (t, *J* = 5.1 Hz, 1H), 4.84 (s, 1H), 4.41 (t, *J* = 12.8 Hz, 1H), 3.59 – 3.45 (m, 2H), 3.39 (dd, *J* = 12.3, 6.3 Hz, 1H), 3.35 – 3.28 (m, 2H), 2.77 – 2.62 (m, 1H), 1.61 (s, 3H), 1.06 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.74, 163.94, 148.78, 132.79, 129.10, 128.88, 124.91, 122.00, 60.07, 57.92, 43.83, 34.26, 22.66, 13.05.

7.13 Synthesis of 2-((1-acetyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)amino)ethyl 4-methylbenzenesulfonate (8)

Into the 100 ml round-bottomed flask fitted with a magnetic stirrer, thermocouple and reflux condenser 5.14 mmol (1.343 g) of the starting 2-[(5-acetyl-4-methyl-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-2-yl)amino]ethanol was added together with 20 ml of dry pyridine, and stirred until starting material dissolves. Then 7.71 mmol (1.47 g) of tosyl chloride was added. After the addition, stir at room temperature for 15 minutes, then heat at 80 °C for about 30 to 45 minutes (check by thin-layer chromatography that no starting compound remains). After the reaction is complete, dry pyridine is evaporated under reduced pressure, poured into water and extracted with methylene chloride, the organic layer is washed several times with water. The solvent is evaporated under reduced pressure and the remaining solid is crystallised from ethyl acetate.



MW = 415.51 g/mol

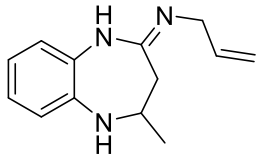
White crystals, m.p. 110 ºC (dec.). Yield: 1.6 g (75 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (t, *J* = 6.1 Hz, 1H), 7.63 – 7.51 (m, 4H), 7.42 – 7.33 (m, 4H), 4.98 (dp, *J* = 12.1, 6.1 Hz, 1H), 4.05 – 3.90 (m, 1H), 3.65 – 3.54 (m, 1H), 2.87 – 2.74 (m, 1H), 2.69 – 2.56 (m, 1H), 2.37 (s, 3H), 2.31 (dd, *J* = 12.9, 5.2 Hz, 1H), 2.14 – 1.98 (m, 1H), 1.58 (s, 3H), 0.99 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 170.60, 168.60, 143.33, 140.44, 137.36, 133.09, 131.44, 130.14, 130.06, 127.43, 127.00, 124.45, 53.96, 47.05, 40.76, 40.70, 22.79, 21.40, 18.74.

7.14 (*E*)-*N*-allyl-4-methyl-1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-imine (9)

Into the 50 ml round-bottomed flask 5.3 mmol (1 g) of starting 4-methyl-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2(3*H*)-thione is added together with 20ml of absolute ethanol, and 37 mmol (2.7 ml) of allylamine. The reaction mixture is refluxed for about 8 hours. At the end of the reaction, the ethanol is evaporated under reduced pressure and the remaining oil is extracted with methylene chloride and washed with water. The methylene chloride is than evaporated under reduced pressure and the remaining oil is crystallised from ethyl acetate.



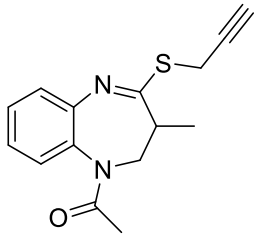
MW = 215.30 g/mol

White solid, m.p. 135-145 ºC. Yield: 0.72 g (63 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.19 – 7.08 (m, 2H), 7.04 – 6.97 (m, 1H), 6.96 – 6.89 (m, 1H), 6.02 – 5.88 (m, 1H), 5.49 (s, 1H), 5.38 (d, *J* = 17.2 Hz, 1H), 5.29 (d, *J* = 10.3 Hz, 1H), 4.19 (s, 2H), 4.06 – 3.94 (m, 1H), 2.79 (dd, *J* = 13.6, 5.0 Hz, 1H), 2.47 (s, 1H), 1.22 (d, *J* = 6.3 Hz, 3H).
^{13}C NMR (101 MHz, DMSO-d_6) δ 162.68, 139.50, 131.02, 126.58, 126.48, 123.57, 120.82, 120.05, 116.99, 56.49, 43.78, 35.00, 22.12.

7.15 Synthesis of 1-(3-methyl-4-(prop-2-yn-1-ylthio)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one (10)

Into a 50 ml round-bottomed flask with a magnetic stirrer, 5.12 mmol (1.2 g) of the starting benzodiazepine-2-thione was added together with 20 ml of benzene, 3.33 mmol (0.75 g) of benzyl triethylammonium chloride, 10.24 mmol (0.78 ml) of propargyl bromide, and 20.5 mmol (0.82 g) of sodium hydroxide dissolved in 12 ml water. The reaction mixture is stirred at room temperature for 6-8 hours. At the end of the reaction, the aqueous layer is separated in a separating funnel and washed several times with benzene, then organic layers are combined and washed several times with water and dried with magnesium sulphate. The solvent is evaporated under reduced pressure. The substance is purified chromatographically 1:1 with ethyl acetate and hexane. After chromatography, crystallised from diethyl ether.

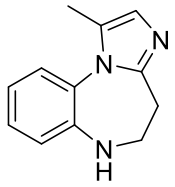


MW = 272.37 g/mol
White solid, m.p. 81-84 ºC. Yield: 0.82 g (59 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.34 (m, 1H), 7.18 – 7.07 (m, 3H), 4.72 (t, *J* = 12.9 Hz, 1H), 3.91 – 3.71 (m, 2H), 3.55 (dd, *J* = 12.8, 6.1 Hz, 1H), 3.01 (dp, *J* = 13.3, 6.7 Hz, 1H), 2.19 (t, *J* = 2.7 Hz, 1H), 1.80 (s, 3H), 1.20 (d, *J* = 6.9 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 175.28, 170.75, 146.42, 131.50, 129.08, 128.46, 125.02, 124.83, 78.89, 70.74, 59.35, 37.86, 22.65, 18.20, 12.92.

7.16 Reaction of benzodiazepin-2-thiones with propargylamine (general procedure)

Into the 50 ml round-bottomed flask fitted with a magnetic stirrer, a reflux condenser and a desiccant tube made of calcium chloride, 3.92 mmol of starting benzodiazepin-2-thione was added together with 20 ml of absolute ethanol and 27.45 mmol of propargylamine. The reaction mixture was refluxed for 5-12 hours (depending on the starting material). At the end of the reaction, the ethanol is evaporated in reduced pressure. The remaining oil is extracted with methylene chloride and washed with water. The solvent is evaporated under reduced pressure and the remaining oil is crystallised from ethyl acetate or diethyl ether.

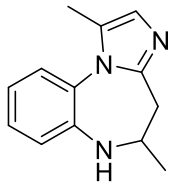
7.16.1 1-methyl-5,6-dihydro-4*H*-benzo[*b*]imidazo[1,2-*d*][1,4]diazepine (11a)



MW = 199.26 g/mol
White crystals, m.p. 107-110 ºC. Yield: 0.51 g (66 %).

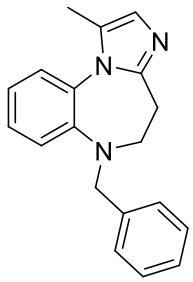
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.15 (m, 2H), 7.11 – 7.02 (m, 1H), 7.03 – 6.96 (m, 1H), 6.80 (s, 1H), 3.73 (t, *J* = 6.4 Hz, 2H), 3.59 – 3.43 (m, 1H), 2.92 (t, *J* = 6.4 Hz, 2H), 2.23 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 147.28, 140.96, 128.58, 127.98, 127.44, 126.18, 124.88, 122.83, 121.93, 52.28, 25.74, 10.84.

7.16.2 1,5-dimethyl-5,6-dihydro-4*H*-benzo[*b*]imidazo[1,2-*d*][1,4]diazepine (11b)



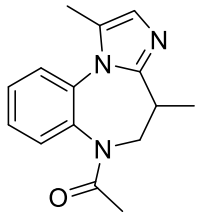
MW = 213.28 g/mol
White crystals, m.p. 170-172 ºC. Yield: 0.7 g (80 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.17 (m, 2H), 7.14 – 7.06 (m, 1H), 7.03 – 6.97 (m, 1H), 6.80 (s, 1H), 4.01 (h, *J* = 6.2 Hz, 1H), 3.25 (s, 1H), 2.95 (dd, *J* = 14.5, 5.5 Hz, 1H), 2.56 (dd, *J* = 14.5, 6.7 Hz, 1H), 2.24 (s, 3H), 1.26 (d, *J* = 6.4 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 146.40, 140.18, 129.27, 127.81, 127.27, 126.14, 124.60, 123.57, 122.39, 58.79, 32.42, 23.13, 10.85.

7.16.3 6-benzyl-1-methyl-5,6-dihydro-4*H*-benzo[*b*]imidazo[1,2-*d*][1,4]diazepine (11c)



MW = 289.38 g/mol
White crystals, m.p. 90-94 ºC. Yield: 0.73 g (65 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.01 (m, 9H), 6.83 (s, 1H), 4.27 (br. s, 2H), 3.41 (br. s, 2H), 2.83 (br. s, 2H), 2.25 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 147.72, 143.47, 138.05, 132.13, 128.33, 127.97, 127.71, 126.96, 126.83, 125.75, 124.51, 122.91, 122.46, 58.54, 57.53, 25.89, 10.49.

7.16.4 1-(1,4-dimethyl-4,5-dihydro-6*H*-benzo[*b*]imidazo[1,2-*d*][1,4]diazepin-6-yl)ethan-1-one (11d)

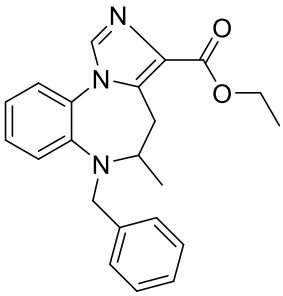


MW = 255.32 g/mol
Off white crystals, m.p. 168-172 ºC. Yield: 0.7 g (70 %).
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 – 7.51 (m, 1H), 7.51 – 7.42 (m, 1H), 7.41 – 7.32 (m, 2H), 6.85 (s, 1H), 4.54 (t, *J* = 12.6 Hz, 1H), 3.53 (dd, *J* = 12.5, 6.1 Hz, 1H), 2.85 (dp, *J* = 13.1, 6.6 Hz, 1H), 2.27 (s, 3H), 1.71 (s, 3H), 1.45 (d, *J* = 6.7 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.08, 149.26, 136.13, 134.35, 130.30, 129.41, 128.47, 126.59, 126.40, 125.27, 56.99, 30.11, 22.73, 13.53, 10.29.

7.17 Synthesis of imidazo-benzodiazepine and oxazole derivatives by activation with diphenyl chlorophosphate (general procedure)

Solution of 10 mmol benzodiazepinone **1** in 80 ml of absolute THF was cooled to -38 °C, and 12 ml of 1 M *t*-BuOK (12 mmol) solution in THF was added dropwise under N_2 atmosphere. The mixture was allowed to warm to room temperature and stirred for 1 h. Reaction mixture was cooled to -38°C, and solution of diphenyl chlorophosphate (2.4 ml, 12 mmol) in 10 ml THF was added dropwise. After stirring at room temperature for 2 h, the solution was cooled to -38°C and 1.3 ml of ethyl isocyanoacetate (12 mmol) in 5 ml THF and 1 M *t*-BuOK solution in dry THF (12 ml, 12 mmol) were added subsequently. The mixture was stirred for 20 h at r.t. After addition of AcOH (2 ml), the reaction mixture was stirred for additional 20 min. The formed precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in 20 ml dichloroethane and washed with water (2x20 ml). After removal of the solvent under reduced pressure, the oily residue was purified by column chromatography using benzene–dichloroethane as eluent (gradient elution from 0 to 100% dichloroethane).

7.17.1 ethyl 6-benzyl-5-methyl-5,6-dihydro-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]diazepine-3-carboxylate (12a)



MW = 361.45 g/mol

Yellowish crystals, m.p. 149–151 °C (Et_2O, EtOAc). Yield: 0.4 g (11 %).

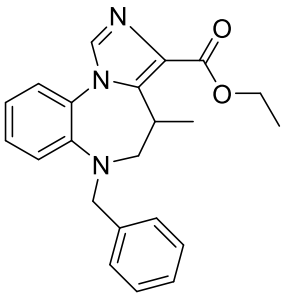
IR, ν, cm^{-1}: 1688 (C=O), 1573, 1501 (C=C, C=N), 1099 (C–O).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.27–7.34 (m, 2H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.09–7.17 (m, 4H), 6.97 (d, *J* = 6.9 Hz, 2H), 4.39 (q, *J* = 7.1Hz, 2H), 4.37 (d, *J* = 14.1 Hz, 1H), 4.24 (d, *J* = 14.4 Hz, 1H), 3.81–3.92 (m, 2H), 2.31–2.38 (m, 1H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.17 (d, *J* = 5.7 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.8, 140.4, 138.0, 137.7, 134.7, 132.0, 128.8, 128.3, 128.0, 127.6, 127.0, 125.0, 123.8, 122.8, 61.1, 60.3, 54.6, 30.3, 15.6, 14.4.

Elemental analysis ($C_{22}H_{23}N_3O_2$): Found (C 73.32%; H 6.65%; N 11.69%), Calculated (C 73.11%; H 6.41%; N 11.63%).

7.17.2 ethyl 6-benzyl-4-methyl-5,6-dihydro-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]diazepine-3-carboxylate (12b)



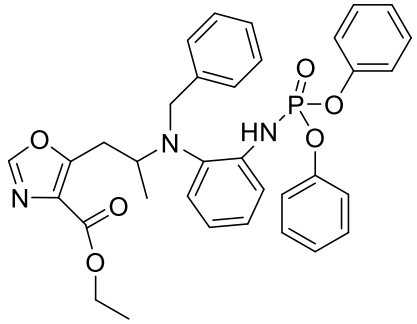
MW = 361.45 g/mol

White solid, m.p. 130-131 °C. Yield: 1.2 g (35 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.34 – 7.06 (m, 9H), 4.43 – 4.35 (m, 3H), 4.35 – 4.26 (m, 1H), 4.19 (d, *J* = 14.4 Hz, 1H), 3.73 (dd, *J* = 11.4, 7.4 Hz, 1H), 3.10 (dd, *J* = 11.4, 3.1 Hz, 1H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.04 (d, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.80, 144.17, 141.92, 137.71, 135.91, 130.73, 128.93, 128.55, 127.95, 127.67, 127.23, 122.85, 122.75, 121.03, 62.77, 60.38, 58.05, 27.96, 18.72, 14.45.

7.17.3 ethyl 5-(2-(benzyl(2-((diphenoxyphosphoryl)amino)phenyl)amino)propyl)oxazole-4-carboxylate (13a)



MW = 611.63 g/mol

White crystals, m.p. 104-106 ºC (Et_2O). Yield: 3.30 g (54 %).

IR, ν, cm^{-1}: 3343, 3137, 3105 (NH), 1703 (C=O), 1606, 1589 (C=C, C=N), 1489 (P=O), 1186 (P–O), 750 (P–N).

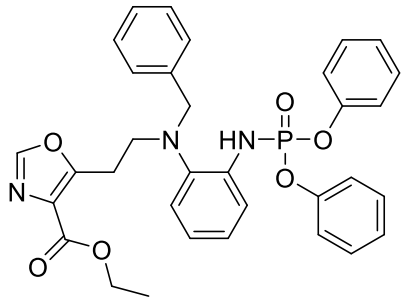
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.50 (d, *J* = 8.1 Hz, 1H), 6.94–7.32 (m, 18H), 6.89 (d, *J* = 13.4 Hz, 1H), 4.31 (m, 2H), 4.11 (s, 2H), 3.55 (br. s, 1H), 3.30–3.37 (m, 1H), 3.05 (dd, *J* = 6.8; 14.8 Hz, 1H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.03 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 157.0, 150.3 (d, $^{2}J_{PC}$ = 6.1 Hz), 150.2, 150.1, 137.6, 136.7, 136.5, 136.1 (d, $^{3}J_{PC}$ = 11.6 Hz), 129.7 (d, $^{4}J_{PC}$ = 5.1 Hz), 128.4, 128.3, 128.2, 127.0, 126.2, 126.1, 125.9, 125.3, 121.5, 120.5 (d, $^{3}J_{PC}$ = 4.8 Hz), 120.3, 116.5, 61.0, 56.2, 52.3, 30.4, 16.2, 14.2.

^{31}P NMR (125 MHz, $CDCl_3$) δ –6.05.

Elemental analysis ($C_{34}H_{34}N_3O_6P$): Found (C 66.61%; H 5.68%; N 6.65%), Calculated (C 66.77%; H 5.60%; N 6.87%).

7.17.4 ethyl 5-(2-(benzyl(2-((diphenoxyphosphoryl)amino)phenyl)amino)ethyl)oxazole-4-carboxylate (13c)



MW = 597.61 g/mol

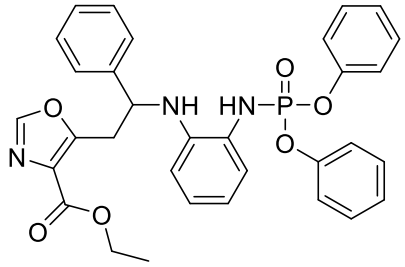
White crystals, m.p. 98–101°C ºC (Et_2O). Yield: 2.82 g (47 %).

IR, ν, cm^{-1}: 3315, 3186, 3061 (NH), 1732 (C=O), 1601, 1587 (C=C, C=N), 1489 (P=O), 1186 (P–O), 753 (P–N).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.28 - 7.18 (m, 12H), 7.12 – 7.09 (m, 3H), 7.02 (d, *J* = 10.2 Hz, 1H), 6.99 – 6.96 (m, 3H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.78 (s, 2H), 3.14 (t, *J* = 6.7 Hz, 2H), 2.91 (t, *J* = 6.7 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.7, 157.6, 150.3 (d, $^2J_{PC}$ = 6.3 Hz), 149.3, 137.6 (d, $^3J_{PC}$ = 11.5 Hz), 136.5, 136.3 (d, $^2J_{PC}$ = 2.5 Hz), 129.7, 129.0, 128.3, 127.4, 127.3, 126.5, 125.3 (d, $^5J_{PC}$ = 0.8 Hz), 124.0, 122.0, 120.3 (d, $^3J_{PC}$ = 4.6 Hz), 116.7, 60.9, 59.6, 50.6, 24.2, 14.2.
^{31}P NMR (125 MHz, $CDCl_3$) δ –5.85.
Found, *m*/*z*: 620.2030 $[M+Na]^+$ $C_{33}H_{32}N_3NaO_6P$. Calculated, *m*/*z*: 620.1926.
Elemental analysis ($C_{33}H_{32}N_3O_6P$): Found (C 66.21%; H 5.38%; N 6.90%), Calculated (C 66.32%; H 5.40%; N 7.03%).

7.17.5 ethyl 5-(2-((2-((diphenoxyphosphoryl)amino)phenyl)amino)-2-phenylethyl)oxazole-4-carboxylate (13d)

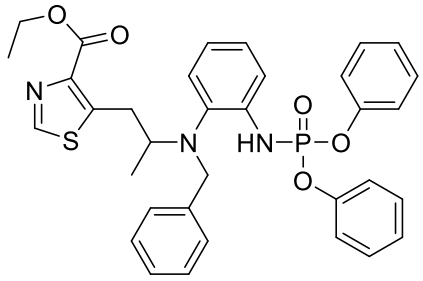


MW = 583.58 g/mol
White solid, m.p. 148-150 °C (Et_2O). Yield: 2.56 g (44 %).
IR spectrum, ν, cm^{-1}: 3124, 3077, 3060 (NH), 1734, 1709 (C=O), 1603, 1588 (C=C, C=N), 1484 (P=O), 1193 (P–O), 743 (P–N).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.29 – 7.13 (m, 15H), 6.84 (t, *J* = 7.6 Hz, 1H), 6.68 (t, *J* = 7.6 Hz, 1H), 6.37 (d, *J* = 8.1 Hz, 1H), 5.84 – 5.80 (m, 1H), 5.09 (s, 1H), 4.69 – 4.65 (m, 1H), 4.44 – 4.31 (m, 2H), 3.44 – 3.31 (m, 2H), 1.33 (t, *J* = 7.1, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5, 156.5, 150.5 (d, $^2J_{PC}$ = 6.8 Hz), 149.3, 141.5, 139.3 (d, $^3J_{PC}$ = 8.5 Hz), 129.7, 129.0, 128.7, 127.5, 126.1, 125.2 (d, $^5J_{PC}$ = 1.9 Hz), 125.1, 124.7, 122.6, 120.3 (d, $^3J_{PC}$ = 4.5 Hz), 118.2, 113.5, 61.6, 57.4, 34.7, 14.2.
^{31}P NMR (125 MHz, $CDCl_3$) δ –5.82.
Elemental analysis ($C_{32}H_{30}N_3O_6P$): Found (C 66.01%; H 5.38%; N 6.99%), Calculated (C 65.86%; H 5.18%; N 7.20%).

7.18 ethyl 5-(2-(benzyl(2-((diphenoxyphosphoryl)amino)phenyl)amino)propyl)thiazole-4-carboxylate (14)

The reaction is carried out in an inert atmosphere. Into a 250 ml flask, 7.08 mmol (2 g) of the starting benzodiazepin-2-thione **2bBn** is added together with 30 ml of tetrahydrofuran. This solution is then cooled to -40 °C and 8.5 ml (8.5 mmol) of 1M potassium tert-butoxide solution is added over 15-20 minutes. Exothermicity is minimal. After addition of potassium tert-butoxide, the reaction mixture is warmed to room temperature and left to stir for one hour. After one hour of stirring, the mixture is cooled again to -40 °C and 1,7 ml (7.97 mmol) of diphenyl chlorophosphate dissolved in 7 ml of tetrahydrofuran is added. The reaction mixture is again cooled to room temperature and left to stir for two hours. The whole mixture is then cooled again to - 40 °C and 7.3 mmol (0.92 ml) of distilled ethyl isocyanate dissolved in 5 ml of tetrahydrofuran is added over a period of 10 to 15 minutes. After addition of ethyl isocyanate, 8,5 ml (8.5 mmol) of 1M potassium tert-butoxide solution is added immediately to the reaction mixture. The reaction mixture is then allowed to warm to room temperature and stirred overnight in an inert atmosphere. Then 1,5 ml of glacial acetic acid is added to the whole mixture and stirred for 20 minutes. The mixture is then filtered through celite and tetrahydrofuran is evaporated under reduced pressure. The remaining oil is dissolved in dichloroethane and washed several times with water. The dichloroethane is evaporated under reduced

pressure and the remaining oil is purified by chromatography, firstly with a 4:9 eluent of ethyl acetate and hexane, followed by a 1:1 eluent of ethyl acetate and hexane. A yellowish oil is obtained.



MW = 627.70 g/mol

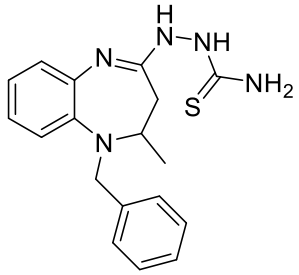
Yellow oil. Yield: 2.75 g (62 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.31 – 6.91 (m, 18H), 4.35 (qd, *J* = 7.2, 3.2 Hz, 2H), 4.26 (d, *J* = 29.5 Hz, 1H), 3.65 (br. s, 2H), 3.40 (br. s, 2H), 1.37 (t, *J* = 7.1 Hz, 3H), 0.99 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.95, 157.20, 150.29, 141.72, 136.83, 130.19, 129.76, 129.12, 128.35, 128.19, 127.83, 126.99, 126.00, 125.15, 120.99, 120.45, 116.72, 111.81, 61.22, 60.25, 29.83, 21.21, 16.02, 14.10.

7.19 Synthesis of 2-(1-benzyl-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)hydrazine-1-carbothioamide (15)

Into the 50 ml round-bottomed flask fitted with a magnetic stirrer, reflux condenser and a desiccant tube made of calcium chloride, 4.26 (1.27 g) of the **2bBnSMe** was added together with 4.26 mmol (0.388 g) of the thiosemicarbazide and 30 ml of absolute ethanol. The reaction mixture was refluxed for about 20 hours. At the end of the reaction, the reaction mixture is cooled and the precipitated crystals are filtered off and washed with ethanol and diethyl ether.



MW = 339.46 g/mol

White crystals, m.p. 135-138 ºC (dec.). Yield: 0.43 g (30 %).

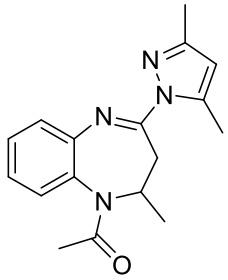
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (s, 1H), 13.10 (br. s, 1H), 7.16 (d, *J* = 7.5 Hz, 2H), 7.08 (t, *J* = 7.4 Hz, 2H), 6.99 (dd, *J* = 21.8, 7.5 Hz, 2H), 6.62 (t, *J* = 7.5 Hz, 1H), 6.49 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.32 (td, *J* = 7.5, 1.6 Hz, 1H), 4.71 (br. s, 2H), 4.13 (s, 2H), 3.43 – 3.35 (m, 1H), 2.94 (dd, *J* = 15.0, 7.1 Hz, 1H), 2.67 (dd, *J* = 14.9, 7.7 Hz, 1H), 1.06 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 166.24, 151.58, 144.79, 140.21, 133.93, 128.53, 128.27, 126.82, 124.82, 124.11, 116.02, 115.07, 54.73, 48.43, 30.07, 16.23.

7.20 1-(4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-1-yl)ethan-1-one (16)

Into the 100 ml round bottomed flask fitted with a magnetic stirrer and reflux condenser, 5 mmol (1.16 g) of the starting 4-hydrazinyl-3-methyl-1-(prop-1-en-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine was added together with 20 ml of dioxane and 19.5 mmol (2 ml) of acetyl acetone. The reaction mixture is refluxed for 5 hours. The precipitate was filtered off and washed with dioxane. Then dioxane is evaporated under reduced pressure and remaining oil is extracted with

dichloroethane. The solvent is evaporated under reduced pressure. The resulting solid is crystallised from ethyl acetate.



MW = 296.37 g/mol

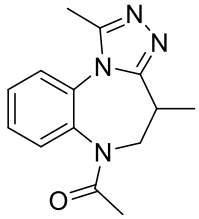
White solid, m.p. 215-217 °C. Yield: 0.6 g (43 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (td, *J* = 7.5, 1.7 Hz, 1H), 7.21 – 7.15 (m, 2H), 7.16 – 7.09 (m, 1H), 6.01 (s, 1H), 5.46 (dp, *J* = 17.7, 6.1 Hz, 1H), 3.83 (dd, *J* = 13.0, 5.1 Hz, 1H), 2.60 (s, 3H), 2.31 (t, *J* = 13.0 Hz, 1H), 2.24 (s, 3H), 1.77 (s, 3H), 1.26 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.09, 161.09, 150.27, 146.03, 142.90, 130.21, 130.07, 129.08, 125.40, 124.96, 111.16, 59.58, 35.01, 22.97, 18.81, 15.68, 13.66.

7.21 1-(1,4-dimethyl-4,5-dihydro-6*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]diazepin-6-yl)ethan-1-one (17)

Into the 100 ml round bottomed flask fitted with a magnetic stirrer and reflux condenser, 5 mmol (1.16 g) of the starting 4-hydrazinyl-3-methyl-1-(prop-1-en-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine was added together with 20 ml of dioxane. The reaction mixture is refluxed for 5 hours. The precipitate was filtered off and washed with dioxane and then dried.



MW = 256.31 g/mol

Pink solid, m.p. 254-257 °C. Yield: 0.5 g (38 %).

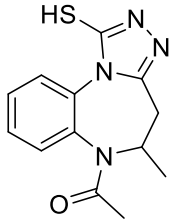
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (d, *J* = 7.7 Hz, 1H), 7.72 – 7.63 (m, 2H), 7.65 – 7.57 (m, 1H), 4.34 (t, *J* = 12.4 Hz, 1H), 3.49 (dd, *J* = 12.5, 6.7 Hz, 1H), 2.86 (dq, *J* = 13.3, 6.7 Hz, 1H), 2.46 (s, 3H), 1.58 (s, 3H), 1.35 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 169.57, 155.21, 150.17, 135.56, 132.04, 131.26, 130.48, 130.20, 125.66, 54.84, 28.79, 22.86, 13.62, 11.22.

7.22 1-(1-mercapto-5-methyl-4,5-dihydro-6*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]diazepin-6-yl)ethan-1-one (18)

Into the 100 ml round bottomed flask fitted with a magnetic stirrer, reflux condenser, dropping funnel and a thermocouple 4.3 mmol (1 g) of starting 4-hydrazinyl-3-methyl-1-(prop-1-en-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine was added together with 25 ml tetrahydrofuran and 10 mmol (1.425 ml) of triethylamine. The reaction mixture than is cooled to -10 °C in a salt-ice bath and a solution of 5,21 mmol (0.4 ml) of thiophosgene in 10 ml of tetrahydrofuran was added drop by drop at -5-(-10) °C. Very violent reaction. After that, the reaction mixture is allowed to warm to room temperature and then refluxed for a further three hours. Then the reaction mixture is cooled, the precipitate is filtered off and washed with tetrahydrofuran. The solvent is evaporated under reduced pressure. The remaining solid is extracted with methylene chloride, washed with sodium bicarbonate

solution and then with water. Organic layer is dried with magnesium sulphate. Methylene chloride is evaporated under reduced pressure. The substance is purified chromatographically 1:9 with methanol and methylene chloride. The resulting solid was crystallised from ethyl acetate.



MW = 274.34 g/mol

White solid, m.p. 300-302°C (lit. 301-303 °C[69]). Yield: 0.7 g (60 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.91 (s, 1H), 8.07 – 8.01 (m, 1H), 7.70 (td, *J* = 7.6, 1.9 Hz, 1H), 7.66 – 7.55 (m, 2H), 5.08 (dp, *J* = 12.7, 6.3 Hz, 1H), 3.19 (dd, *J* = 14.9, 6.2 Hz, 1H), 2.21 (dd, *J* = 14.9, 12.0 Hz, 1H), 1.54 (s, 3H), 1.13 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.27, 166.21, 150.56, 133.09, 132.07, 131.99, 130.18, 129.74, 127.70, 52.95, 30.18, 23.06, 19.03.

8. PUBLICATIONS AND CONFERENCES

- M. Jonušis, A. Vektarienė, G. Mikulskienė, **S. Jonušienė**, D. Vektarytė, R. Jančienė; *Chem. Heterocycl. Compd.* **2023**, (accepted manuscript).
- **S. Jonušienė**, D. Vektarytė, M. Jonušis, A. Vektarienė, R. Jančienė; Study of unexpected rearrangement of 1,5-benzodiazepin-2-one derivatives under modified Wittig-Horner reaction conditions // Balticum organicum syntheticum [BOS] **2022**: in memory of prof. Victor Sniečkus: July 3-6, 2022, Vilnius, Lithuania: program and abstracts. Vilnius: UAB Kalanis, 2022. ISBN 9786099603940. eISBN 9786099603933. p. 92.

9. CONCLUSIONS

1) The best thionation of **1a-d**, **1a-cBn** towards **2a-d**, **2a-bBn** performance was achieved by using $P_2S_5Py_2$ complex in MeCN. In all cases isolated yields were above 63%.
2) Thioamides **2** react with amines without heavy metal catalysts to form amidines in moderate yields. In this work amidine derivatives **3, 4, 6a-d, 7a-f, 8, 9** were successfully synthesized and characterized.
3) Thioamides **2a-b**, **2aBn**, **2cAc** in the reaction with propargylamine gave unexpected imidazo derivatives **11a-d.** Interestingly the reaction proceeded smoothly without any catalyst added (isolated yields between 65-80 %).
4) Unexpected benzodiazepine ring opening was observed during the reaction of **1a-bBn**, **1d** under known imidazo annulation conditions with potassium t-butoxide, diphenyl phosphoryl chloride and ethyl isocyanoacetate. Imidazo compounds **12a-b** were obtained together with oxazole derivatives **13a**, **13c-d**. Interestingly, under the same reaction conditions benzodiazepine-2-thione **2bBn** gave thiazole product **14** in moderate yield - 62 %.
5) Starting compounds **2bAc** and **2cAc** after the reaction with acetylacetone gave different products **16** (pyrazole derivative) and **17** (1,2,4-triazole derivative).
6) Full assignment of H and C atoms in compounds **11b**, **16**, **17** was accomplished by the analysis of ^{1}H, ^{13}C, HSQC and HMBC spectra (supplement material).
7) Substances **7d**, **7e**, and **3** are considered toxic due to the death of mice at 24 h after their single subcutaneous injection. One-time administration of other tested substances did not evoke death, any visible signs of toxicity, or statistically significant changes in counts of blood cells. However, some tested substances could affect counts of RBC and connection parameters as well as immune cells and platelets. Further experiments on their toxicity of them are needed.

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SANTRAUKA

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Jungiamąsias grupes turinčių 1,3,4,5-tetrahidro-2*H*-benzo[*b*][1,4]diazepin-2-ono darinių sintezė ir toksiškumo tyrimas

Nuo 1956 m., kai Leo Sternbachas atsitiktinai išrado raminamąjį vaistą "Librium", benzodiazepinai tapo vienais iš labiausiai tyrinėjamų heterociklų medicininėje chemijoje. Benzodiazepinų analogų gausa patvirtina šių heterociklų svarbą vaistų chemijoje. Neabejotina, kad įvairiai pakeistų benzodiazepinų tyrimai netolimoje ateityje atskleis naują benzodiazepinų farmakologinį aktyvumą ir galimus ligų gydymo būdus. Šio magistro darbo tikslas - bendradarbiaujant su UAB "Innovita Research" ištirti įvairiai pakeistų benzodiazepinų, kurie galėtų būti toliau modifikuojami per jungiamąją grupę, sintezę ir toksiškumo pelėms tyrimą. Nustatyta, kad geriausia amidus **1a-d**, **1a-cBn** versti tioamidais **2a-d**, **2a-bBn** naudojant $P_2S_5Py_2$ kompleksą acetonitrile. Visais atvejais išskirta išeiga viršijo 63 %. Be to, pastebėta, kad tioamidai **2** reaguoja su aminais be sunkiųjų metalų (Hg) katalizatorių, susidarant amidinams. Šiame darbe sėkmingai susintetinti ir ištirti amidinų dariniai **3, 4, 6a-d, 7a-f, 8, 9**. Pastebėta, kad tioamidai **2a-b, 2aBn, 2cAc**, reaguodami su propargilaminu, sudaro netikėtus imidazo darinius **11a-d**. Reakcija vyko sklandžiai, nepridedant jokio katalizatoriaus (išeiga: 65-80 %). **1a-bBn, 1d** reaguojant anksčiau aprašytomis imidazo žiedo sudarymo sąlygomis su kalio t-butilatu, difenil-fosforilchloridu ir etilo izocianoacetatu buvo pastebėtas netikėtas benzodiazepino žiedo atsivėrimas. Imidazo junginiai **12a-b** buvo gauti kartu su oksazolo dariniais **13a, 13c-d**. Įdomu tai, kad tomis pačiomis reakcijos sąlygomis benzodiazepino-2-tionas **2bBn** sudarė tiazolą **14**. Pradiniai junginiai **2bAc** ir **2cAc** po reakcijos su acetilacetonu sudarė skirtingus produktus **16** (pirazolo darinį) ir **17** (1,2,4-triazolo darinį). Galiausiai, atlikus ^{1}H, ^{13}C, HSQC ir HMBC spektrų analizę, pavyko pilnai priskirti H ir C atomus junginiuose **11b**, **16**, **17**. Medžiagos **6a**, **6b**, **7a-g**, **8**, **12a**, **12b** atrinktos toksiškumo pelėms tyrimams. Siekiant geriau įvertinti susintetintus benzodiazepinus su jungiamosiomis grupėmis, buvo atrinkti keli junginiai be šių grupių **2bBnSMe**, **3**, **11b, 18** ir acikliniai - ne benzodiazepino dariniai **13a**, **14**. Sotūs junginių tirpalai DMSO buvo sumaišyti su fiziologiniu tirpalu 5 %:95 % (v/v). Galutiniai tirpalai buvo skaidrūs, drumstumo ir nuosėdų nepastebėta. Deja, junginiai **6a, 11b, 7b, 7c, 8, 12a, 12b** nebuvo pakankamai tirpūs DMSO, kad būtų galima atlikti toksiškumo tyrimus. Medžiagos **7d, 7e** ir **3** laikomos toksiškomis dėl to, kad praėjus 24 val. po jų vienkartinės poodinės injekcijos pelės nugaišo. Vienkartinis kitų tirtų medžiagų suleidimas nesukėlė mirties, nepastebėta jokių matomų toksiškumo požymių ar statistiškai reikšmingų kraujo ląstelių skaičiaus pokyčių. Tačiau kai kurios tirtos medžiagos galėjo turėti įtakos kraujo kūnelių skaičiui, taip pat imuninėms ląstelėms ir trombocitams. Siekiant geriau suprasti šių junginių toksiškumą reikia atlikti tolimesnius eksperimentus.

SUMMARY

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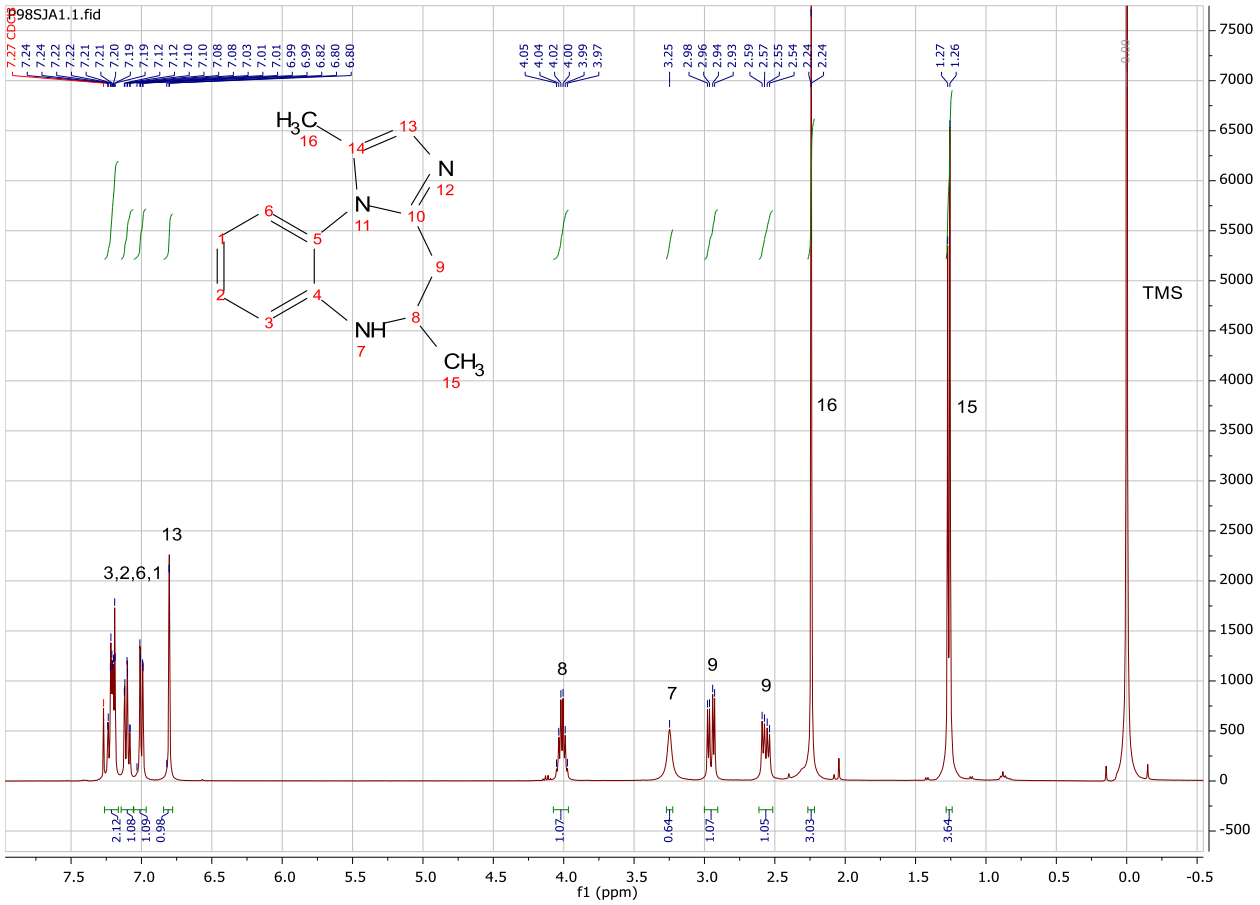
Synthesis of linker groups containing 1,3,4,5-tetrahydro-2*H*-benzo[*b*][1,4]diazepin-2-one derivatives and toxicity study

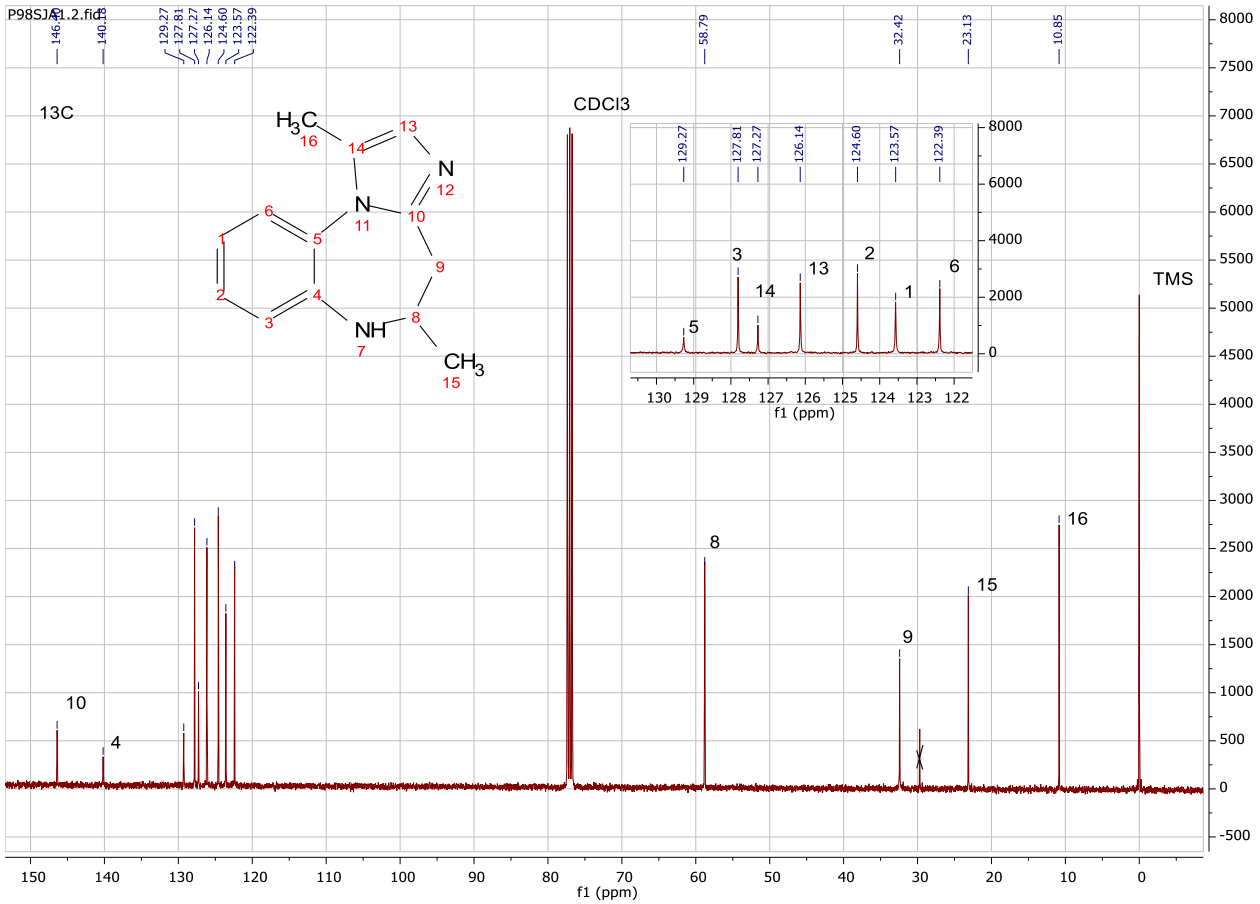
Since Leo Sternbach accidentally invented sedative Librium in 1956, benzodiazepines became one of the most studied heterocycles in medicinal chemistry. The abundance of benzodiazepine analogues confirms the importance of benzodiazepine backbone in medicinal chemistry. There is no doubt that ongoing exploration of variously substituted benzodiazepines and development of new drug delivery methods will unveil new pharmacological activities and possible disease treatments in the near future. The aim of current MSc thesis is to study the synthesis of variously substituted benzodiazepines that could be further modified through the linker and investigation of toxicity on mice in collaboration with UAB "Innovita Research". It was found that the best thionation of **1a-d**, **1a-cBn** towards **2a-d**, **2a-bBn** performance was achieved by using $P_2S_5Py_2$ complex in MeCN. In all cases isolated yields were above 63%. Moreover, it was observed that thioamides **2** react with amines without heavy metal catalysts to form amidines in moderate yields. In this work amidine derivatives **3, 4, 6a-d, 7a-f, 8, 9** were successfully synthesized and characterized. Surprisingly, thioamides **2a-b**, **2aBn**, **2cAc** in the reaction with propargylamine gave unexpected imidazo derivatives **11a-d.** The reaction proceeded smoothly without any catalyst added (isolated yields between 65-80 %). Unexpected benzodiazepine ring opening was observed during the reaction of **1a-bBn**, **1d** under known imidazo annulation conditions with potassium t-butoxide, diphenyl phosphoryl chloride and ethyl isocyanoacetate. Imidazo compounds **12a-b** were obtained together with oxazole derivatives **13a**, **13c-d**. Interestingly, under the same reaction conditions benzodiazepine-2-thione **2bBn** gave thiazole product **14** in moderate yield - 62 %. Starting compounds **2bAc** and **2cAc** after the reaction with acetylacetone gave different products **16** (pyrazole derivative) and **17** (1,2,4-triazole derivative). Last but not least, full assignment of H and C atoms in compounds **11b**, **16**, **17** was accomplished by the analysis of ^{1}H, ^{13}C, HSQC and HMBC spectra. Substances **6a**, **6b**, **7a-g**, **8**, **12a**, **12b** were selected for toxicity study on mice. In order to better evaluate synthesized benzodiazepines with linker groups several compounds without linkers **2bBnSMe, 3, 11b, 18** were selected together with acyclic – nonbenzodiazepine compounds **13a**, **14**. Saturated solutions of compounds in DMSO were mixed with saline solution 5%:95% (w/w). Final solutions were clear and no turbidity or deposits were observed. Unfortunately, compounds **6a**, **11b**, **7b**, **7c**, **8**, **12a**, **12b** were not soluble enough in DMSO to perform toxicity testing. Substances **7d**, **7e**, and **3** are considered toxic due to the death of mice at 24h after their single subcutaneous injection. One-time administration of other tested substances did not evoke death, any visible signs of toxicity, or statistically significant changes in counts of blood cells. However, some tested substances could affect counts of RBC and connection parameters as well as immune cells and platelets. Further experiments on their toxicity of them are needed.

SUPPLEMENTS

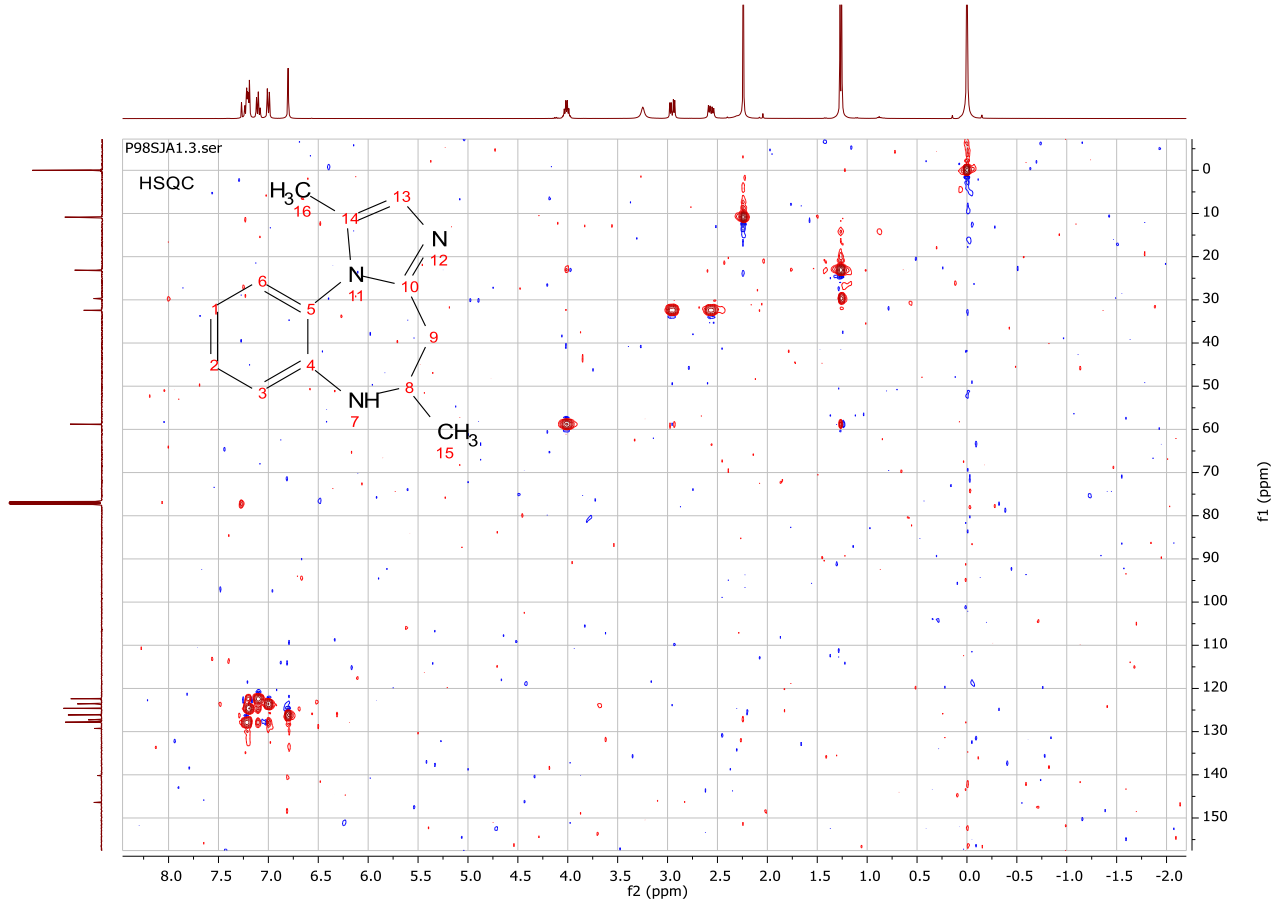
1. Supplement 1. ^{1}H and ^{13}C NMR spectra of compound **11b**
2. Supplement 2. HSQC and HMBC NMR spectra of compound **11b**
3. Supplement 3. ^{1}H and ^{13}C NMR spectra of compound **16**
4. Supplement 4. HSQC and HMBC NMR spectra of compound **16**
5. Supplement 5. ^{1}H and ^{13}C NMR spectra of compound **17**
6. Supplement 6. HSQC and HMBC NMR spectra of compound **17**

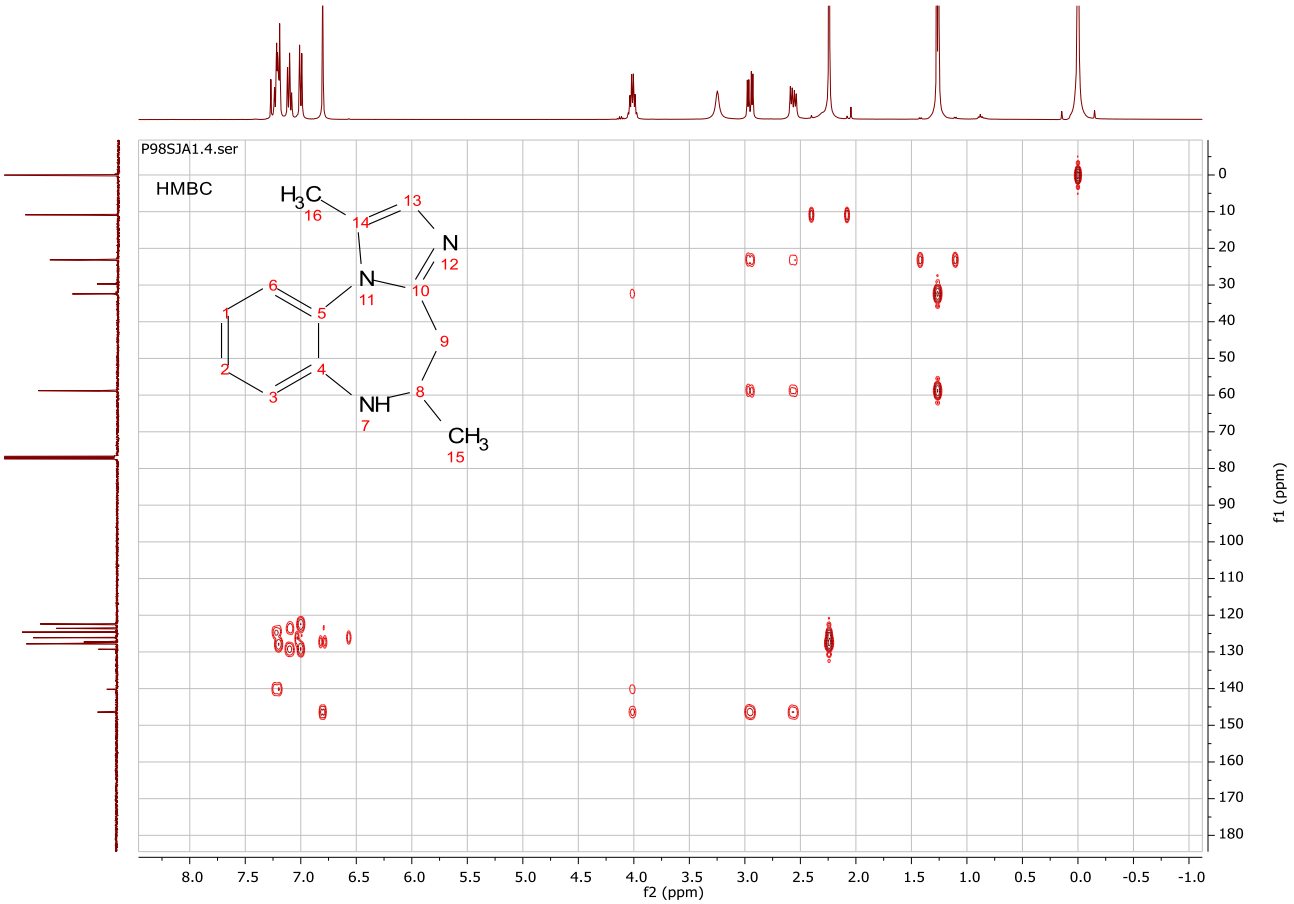
^{1}H and ^{13}C NMR spectra of compound 11b



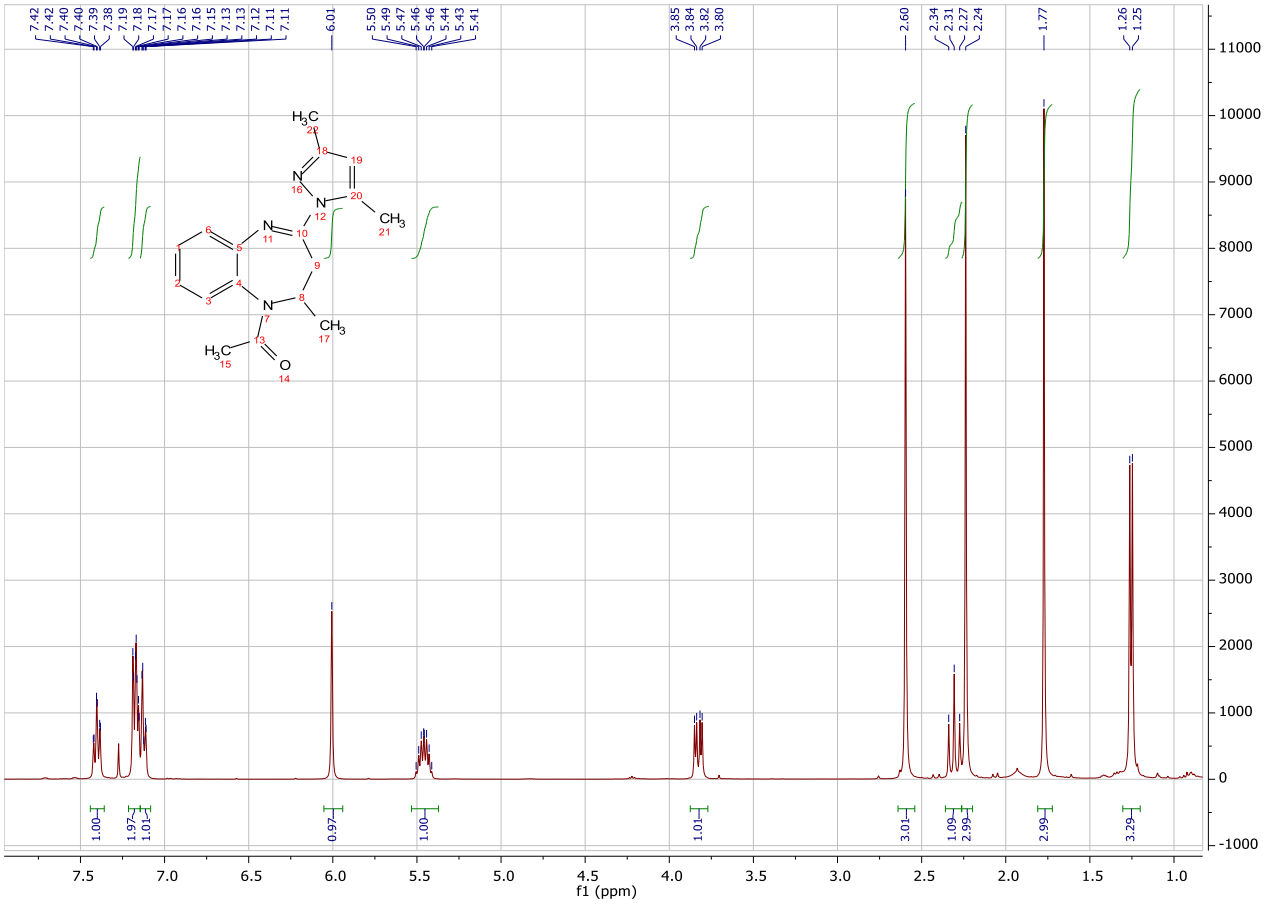


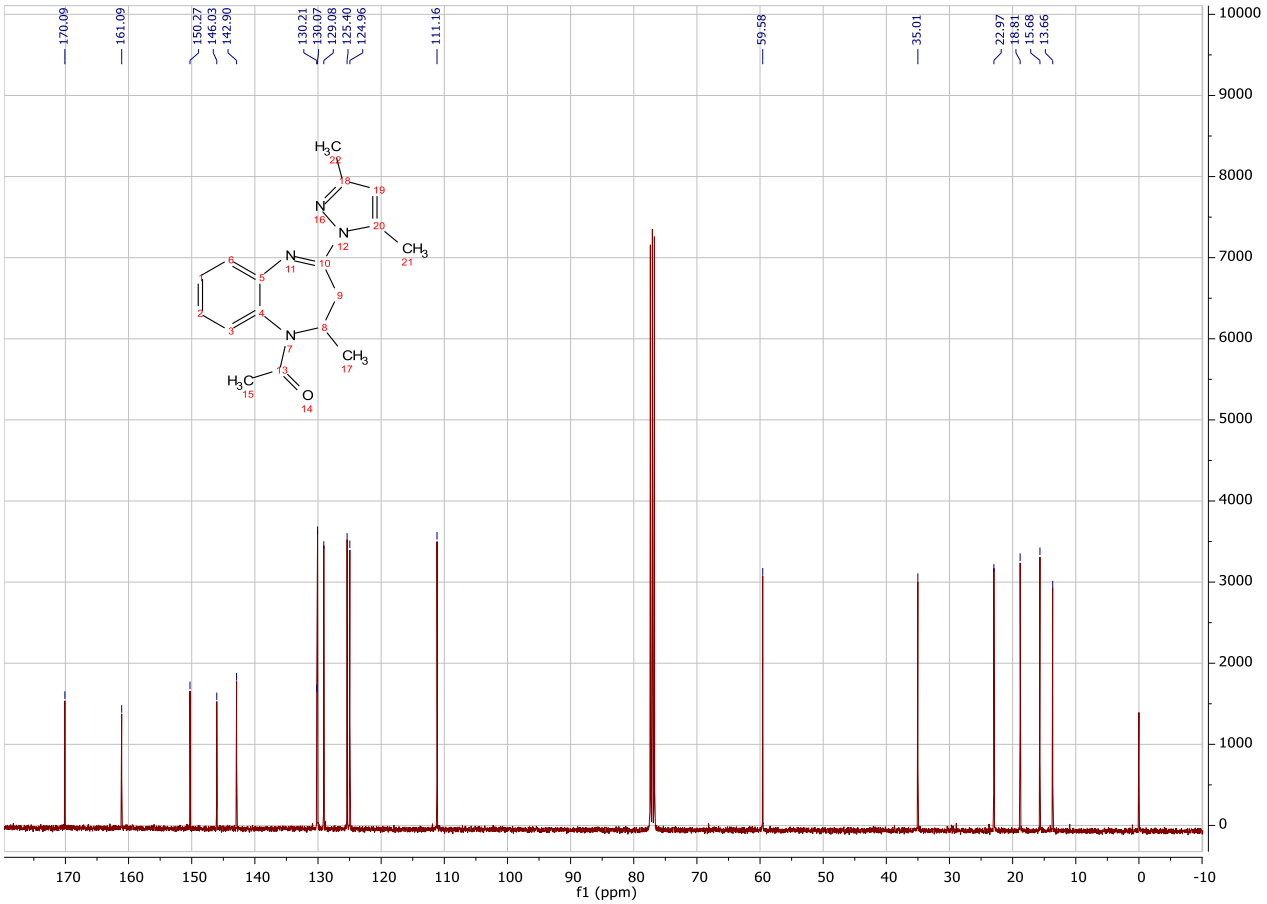
HSQC and HMBC NMR spectra of compound 11b



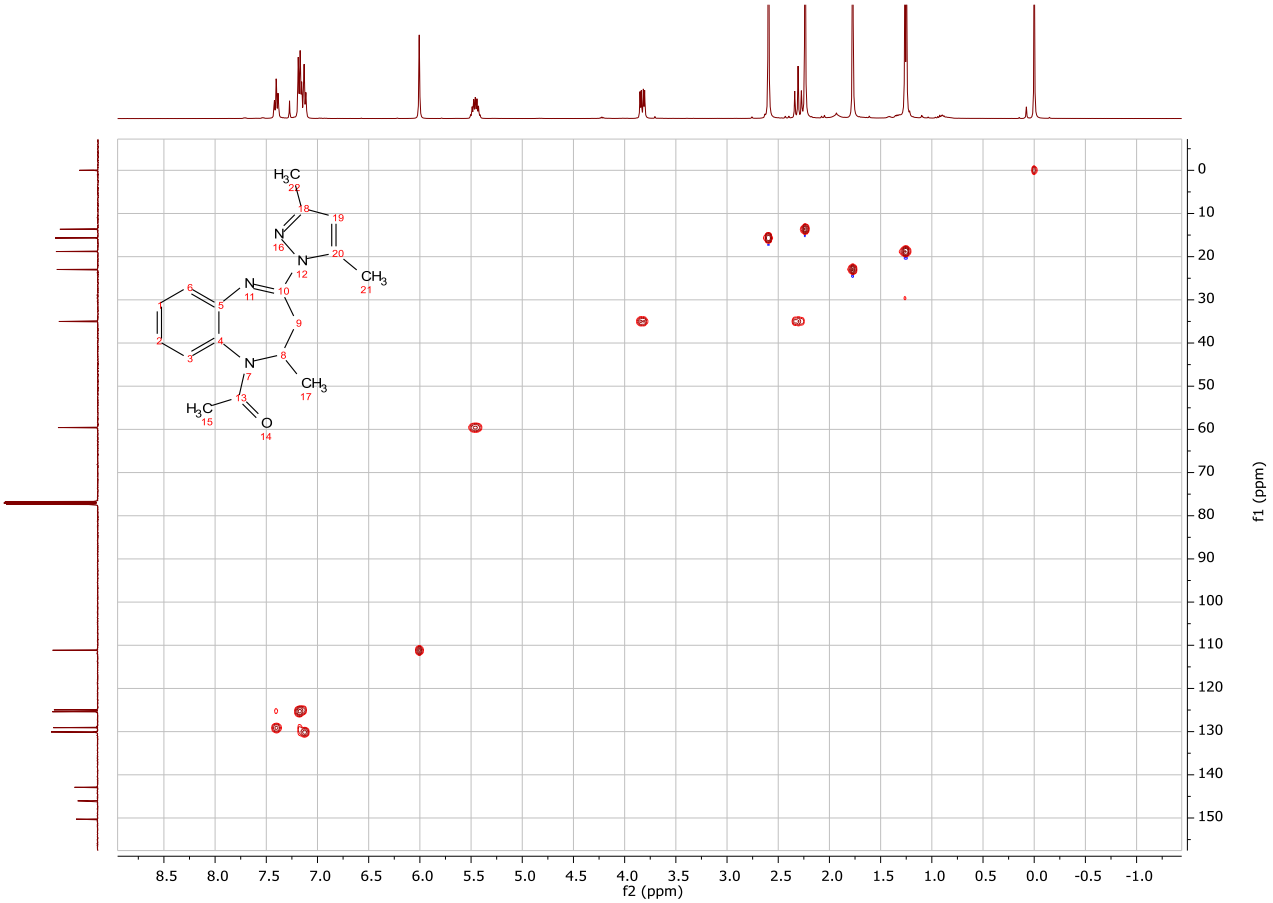


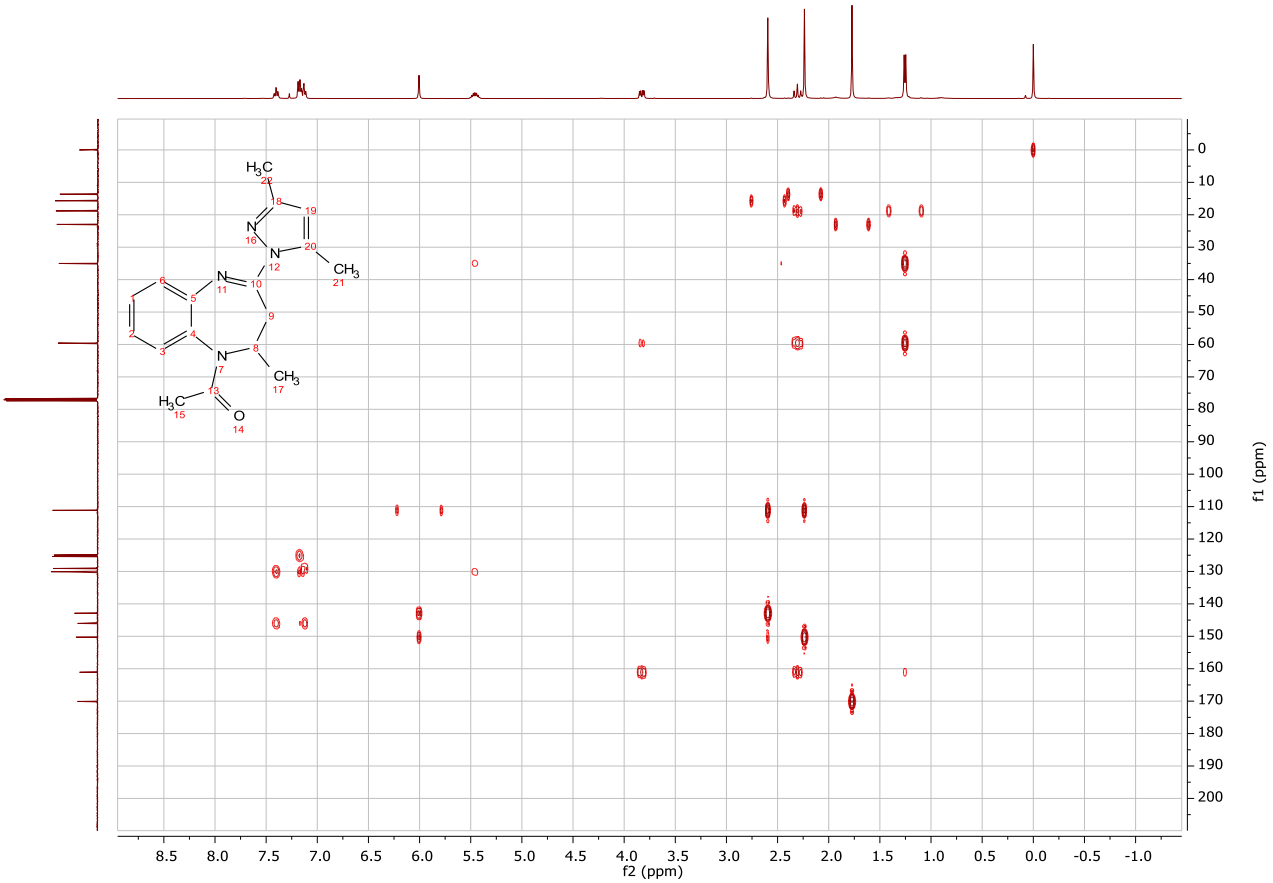
^{1}H and ^{13}C NMR spectra of compound 16



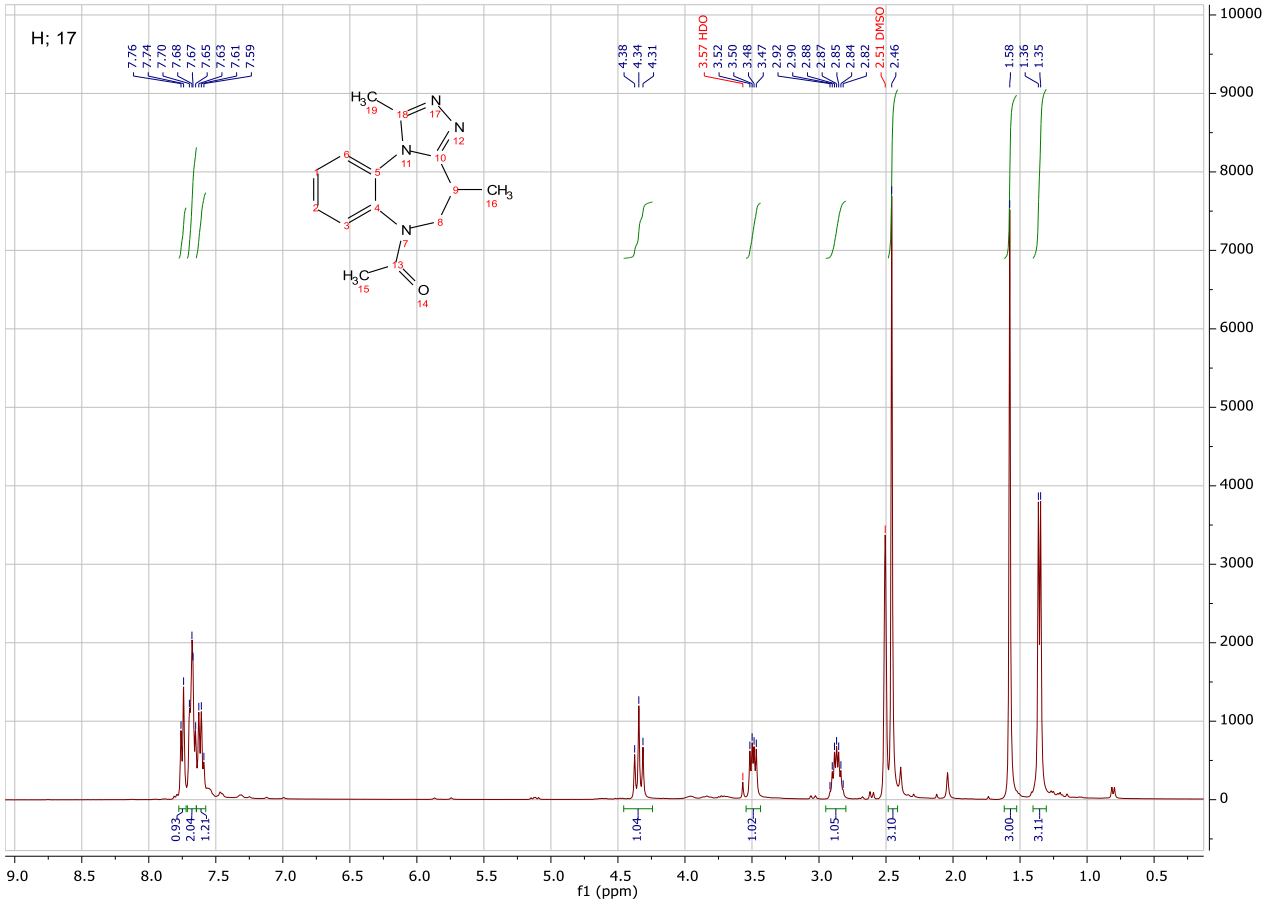


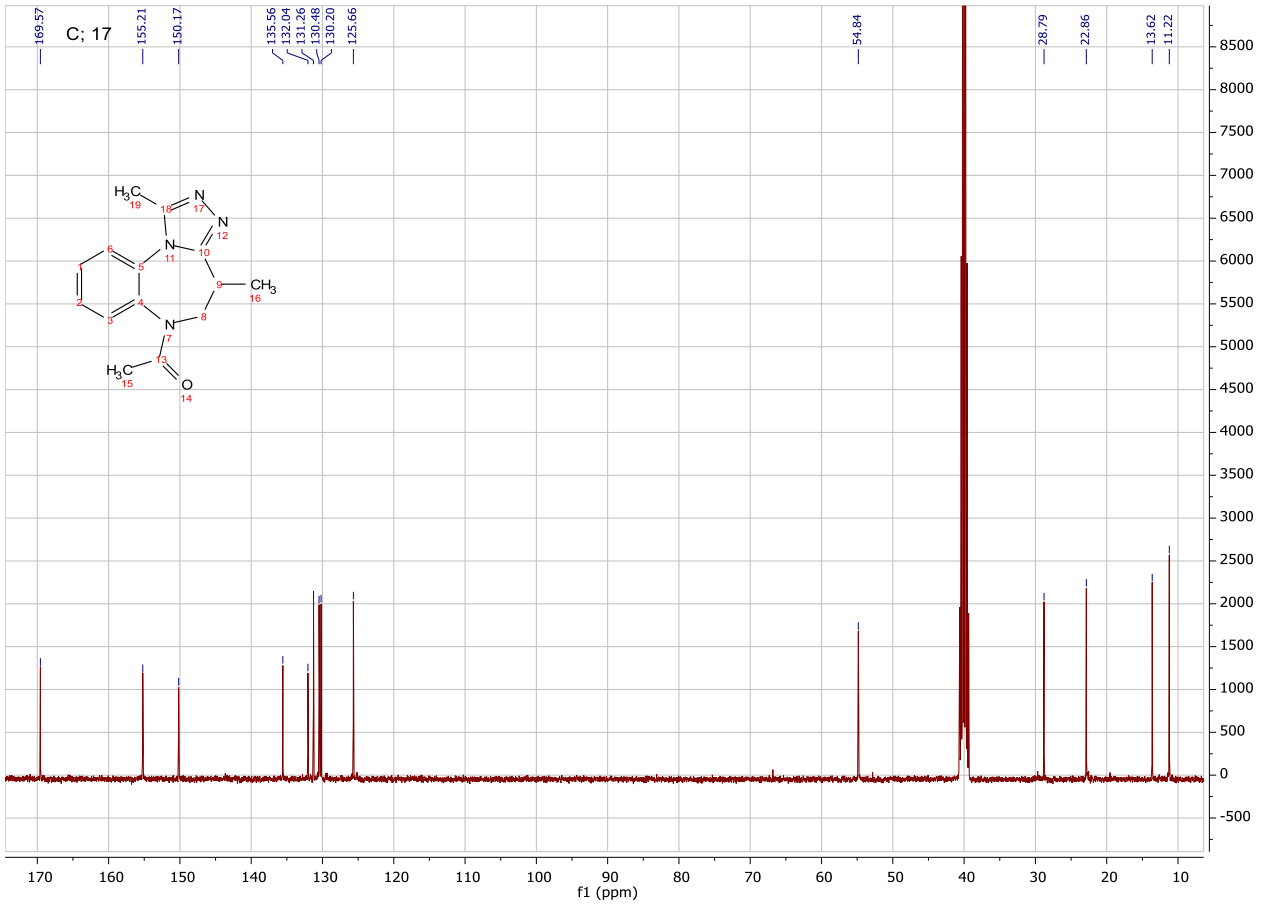
HSQC and HMBC NMR spectra of compound 16





^{1}H and ^{13}C NMR spectra of compound 17





HSQC and HMBC NMR spectra of compound 17