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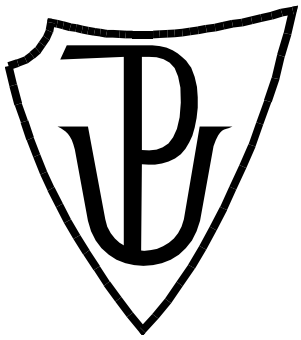

Solid-Phase Synthesis of New Purine Derivatives and Study of Their Properties

Ph.D. Thesis

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In Olomouc, ..., 2013 .....

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Abstract

Research in a field of discovery and development of novel drugs is very demanding process in which usually large number of new chemical entities has to be studied in a short time. One of the basic goals of medicinal chemistry is to develop and improve synthetic pathways in a fashion that allows simple preparation of novel drug-like molecules in sufficient number for biological screening. Such collections of compounds are called chemical libraries which contain tens to thousands of chemical substances. This justifies the need of development of synthetic methods that can simply be applied for production of variously substituted derivatives of a target scaffold or even different scaffolds to increase molecular diversity inside the chemical libraries. Solid-phase synthesis and combinatorial chemistry have been proved to be efficient tools for completion this mission. This Ph.D. thesis describes synthesis of selected nitrogenous heterocycles with use of polysterene resin. In each case the applicability of developed procedures was tested by the preparation of smaller collections of compounds. These sets of compounds were prepared to evaluate limitation and scopes of developed methods for possible future preparation of larger chemical libraries.

The main part of the work was focused on a synthesis of novel purine/deazapurine derivatives and subsequent study of their biological and also spectral properties. We have combined both traditional approaches for the preparation of target compounds: (i) use of cyclization methods from suitable linear precursors, (ii) use of suitably substituted purine scaffold for further chemical decoration. The latter approach was applied in our research of purine derivatives with 2,6-dichloropurine as a key building block. Bisheterocyclic molecules of purine and 3-hydroxyquinolin-4(1*H*)-one were prepared with use of this strategy and similar approach was used for study of palladium catalyzed C^8-H arylation of solid-supported purine moiety. In a following research leading to some purine deaza-analogues we used heterocyclization technique giving the target scaffolds from suitably immobilized precursors. The last solved topic was dedicated to solid-phase preparation of selected tetrahydrobenzodiazepinones. In this topic 4-chloro-2-fluoro-5-nitrobenzoic acid was used as the starting material.

All synthesized derivatives were subjected to biological screening of cytotoxicity using MTT test and the results obtained are discussed. Additionally, fluorescence properties of bisheterocyclic derivatives were studied.

Souhrn

Výzkum v oblasti vývoje nových léčiv je velmi náročný proces, který zahrnuje studium velkého množství strukturně odlišných látek v relativně krátkém čase. Jedním z tradičních cílů medicinální chemie je vyvinout a zdokonalit syntetické přístupy tak, aby umožňovaly jednoduchou přípravu nových molekul (potenciálních léčiv) v dostatečném počtu pro primární biologický screening. Tyto kolekce sloučenin, nazývané také chemické knihovny, obsahují desítky až tisíce látek. Z tohoto důvodu je významné vyvíjet metody syntézy, které mohou být jednoduše použity pro přípravu různě substituovaných derivátů cílového skeletu, nebo dokonce zcela jiných skeletů za účelem zvýšení různorodosti chemické knihovny. Syntéza na pevné fázi a kombinatoriální chemie jsou velmi efektivní nástroje k dosažení tohoto cíle. Tato dizertační práce popisuje syntézu vybraných dusíkatých heterocyklů na pevné fázi. Aplikovatelnost vyvinutých metod pro syntézu knihoven byla ve všech případech testována přípravou menších souborů látek. Tyto skupiny látek vždy sloužily k vyhodnocení aplikovatelnosti a omezení každé metody pro budoucí přípravu větší chemické knihovny.

Hlavní část práce byla zaměřena na syntézu různých derivátů purinu nebo deazapurinu a studium jejich biologických a spektrálních vlastností. V rámci práce byly kombinovány oba tradiční přístupy pro přípravu cílových sloučenin: (i) použití cyklizačních metod, které poskytují požadované deriváty; (ii) využití vhodně substituovaného purinového skeletu k modifikaci jeho struktury. Výzkum purinových derivátů nebyl založen na syntéze purinu z vhodných prekurzorů, ale naopak byla využita metoda imobilizace 2,6-dichlorpurinu na pevnou fázi a jeho následná modifikace. Bisheterocyklické deriváty purinu a 3-hydroxy-4(1*H*)-chinolonu byly připraveny právě touto strategií a podobná metoda byla využita i pro studium palladiem katalyzované C^8-H arylace purinového jádra. Heterocyklizační technika byla využita pro syntézu deaza-analog, kde cílové sloučeniny vznikaly přímo na pevné fázi. Poslední řešené téma bylo zaměřeno na přípravu tetrasubstituovaných tetrahydrobenzodiazepinonů na pevné fázi. Zde byla jako výchozí komponenta využita 2-fluoro-4-chloro-5-nitrobenzoová kyselina, která již v minulosti byla na naší katedře studována jako vhodný materiál pro přípravu různých typů heterocyklů.

Všechny získané strukturně nové sloučeniny byly podrobeny biologickému testování v tzv. MTT cytotoxickém testu. Bisheterocyklické sloučeniny purinu a hydroxychinolonu byly navíc studovány z pohledu fluorescenčních vlastností.

Contents

1 Introduction

It was the year 1828, when the German chemist Friedrich Wöhler for the first time ever carried out an artificial synthesis of an organic compound. The nowadays-called Wöhler synthesis afforded an organic molecule - urea from inorganic reagents.[1] This discovery is considered to be one of the starting points of a large field of chemical science – synthetic organic chemistry. Within hundred years after Wöhler´s discovery, more than one million of organic substances were prepared with use of organic synthesis. Its domain became synthesis in solution which can be also called as "traditional synthesis". During the 20th century, traditional organic synthesis became an important discipline of modern chemistry. It's results helped mankind to reach significant progress in a broad spectrum of activities, especially in medicine,[2] agriculture[3] and industry.[4] Almost one and half century after the first organic synthesis, american chemist Robert Bruce Merrifield introduced synthesis of organic substances on polystyrene support and initiated a new chapter of organic chemistry called "solid-phase synthesis". At the end of the 20th century, this approach to the synthesis allowed incredible boom of combinatorial chemistry,[5] particularly in the field of pharmaceutical industry. Combinatorial solid-phase organic synthesis (CSPOS) dramatically changed the capacity of synthetic chemistry: with its use, chemists were able to synthesize million of organic compounds not within a century but within a couple of days. Despite some disillusions that appeared over the time CSPOS still represents an important part of drug research and development process.

Solid-phase synthesis can be used as a powerful tool for high-throughput synthesis of chemical libraries particularly due to simple isolation of intermediates during the multistep synthesis which allows rapid preparation of large numbers of chemical entities.[6] In our long-term research, we have been focused on synthesis of compounds with potential anticancer activity. From 2007 we have been interested (except the traditional organic synthesis) in development of solid-phase syntheses of drug-like molecules to increase our capacity of compounds production for primary biological screening. This thesis contains some currently developed solid-phase methodologies and also biological data of selected prepared compounds.

2 Aims of the work

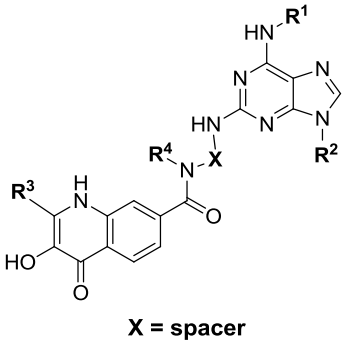
The main part of this thesis describes development of solid-phase syntheses of selected heterocyclic derivatives (see chapters 2.1-2.4). The target compounds were particularly novel purine derivatives: (i) bisheterocyclic compounds containing purine scaffold, (i) novel tetrasubstituted purine derivatives prepared with the use of Pd-catalyzed reactions, (iii) some deazapurines. Except this, we paid attention to high throughput synthesis of some tetrahydrobenzodiazepinone derivatives. The major part of this research was carried out at the Department of Organic Chemistry, Palacky University Olomouc. This included study of fluorescent properties of some prepared compounds. Pd-catalyzed reactions on solid-supported purines were developed during fellowship at the University of Notre Dame, USA in which the author became a member of a research group focused on preparation of diversity-oriented heterocyclic scaffolds from polymer-supported acyclic intermediates via *N*-alkyl- and *N*-acyliminiums.[A5,A6] Laboratory of experimental medicine at Palacky University Olomouc provided biological screening of all synthetized derivatives with the use of MTT cytotoxicity test on representative cancer cell lines derived from normal tissues and malignant tumors. The MTT test was performed on human myeloid leukemia (K562), human myeloid leukemia resistant to paclitaxel (K562-tax), T-lymphoblastic leukemia (CEM), T-lymphoblastic leukemia resistant to dorubicine (CEM-DNR-bulk), lung adenocarcinoma cell line (A549), colorectal cancer cells_12 Gy gamma radiation (HCT116 p53 WT) and Colorectal cancer cells_12 Gy gamma radiation (HCT116 p53 MUT HETEROZYGOUS).

2.1 Synthetic Method 1: Solid-phase Synthesis of Highly Diverse Purine-Hydroxyquinolinone Bisheterocycles

Based on: Barbora Vaňková, Jan Hlaváč and Miroslav Soural, J. Comb. Chem. 2010, 6, 890-894[A1] *and Kamil Motyka, Barbora Vaňková, Jan Hlaváč, Miroslav Soural and Petr Funk, J.Fluoresc, 2011, 21, 2207-2212.*[A2]

The first experimental chapter is dedicated to solid-phase synthesis of bisheterocyclic compounds that contain purine and 3-hydroxyquinolin-4(1*H*)-one scaffolds connected with an aliphatic spacer of various length/structure. The proposed target molecules contain five diversity positions. Two positions at purine scaffold, two positions at hydroxyquinolinone scaffold and the last diversity position coming from type of the spacer between both heterocyclic scaffolds.

Figure 1: Suggested general structure of the target bisheterocycles.



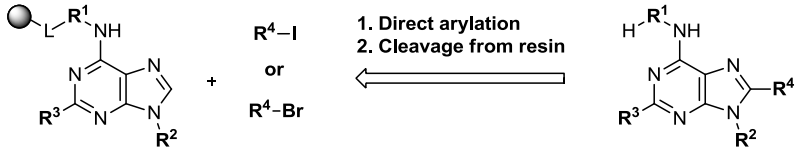
The reaction sequence includes nine reaction steps which were carried out on aminomethyl polystyrene resin equipped with an acid-labile benzaldehyde linker (4-(4-formyl-3-methoxyphenoxy)butyric acid). The attention was paid particularly to optimization of each reaction step which finally allowed to get the final compounds in an excellent crude purity. The versatility of the method was demonstrated by the preparation, isolation and full characterization of 20 compounds. This set of compounds was subsequently used for study of the effect of purine scaffold to hydroxyquinolinone fluorescence properties. Prepared compounds were also subjected to biological screening of anticancer activity and the obtained results are reported and discussed in the thesis.

2.2 Synthetic Method 2: Direct Arylation of Purine on Solid Phase and Its Use for Chemical Libraries Synthesis

Based on: Barbora Vaňková, Viktor Krchňák, Miroslav Soural and Jan Hlaváč, ACS Comb. Sci. 2011, 12, 496-500.[A3]

The second experimental part reports C^8-H direct arylation of purine derivatives immobilized on Wang resin. The purine skeleton was attached via C^6-regioselective substitution of 2,6-dichloropurine with polymer-supported amines. Subsequently N^9 alkylation with alkyl iodides and C^2-substitution with amines were studied.

Figure 2: Direct arylation of polymer-supported trisubstituted purines.



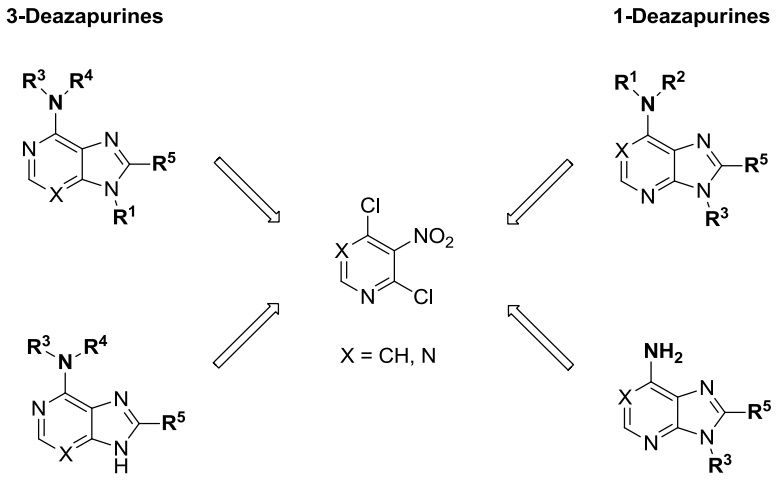
The main objective of this work was to develop and optimize Pd-catalyzed direct arylation of solid-supported purine scaffold. Various aryl iodides and aryl bromides were used for the reaction affording the target tetrasubstituted purines in very good purity. Additionally, trisubstituted purines with N^9 unsubstituted position were also synthesized. Applicability of the method was demonstrated by the preparation and characterization of 17 derivatives which were tested for their anticancer activity, the results are reported and discussed. The developed method represents an efficient tool for preparation and systematic biological studies of purine derivatives with use of combinatorial chemistry.

2.3 Synthetic Method 3: Solid-Phase Synthesis of Purines and Deazapurines

Based on: Barbora Lemrová and Miroslav Soural, unpublished results.

Another goal of this work was to develop solid-phase synthesis of some deazapurine derivatives by heterocyclization of suitable intermediates immobilized on three different types of polystyrene resin (Wang resin, Rink amide resin, aminomethylated resin equipped with backbone amide linker (BAL)). Synthesis was based on an attachment of suitable amino compound and its reaction with 4,6-dichloro-5-nitropyrimidine or 2,4-dichloro-3-nitropyridine. Depending on building blocks used, the target purines, 1-deazapurines or 3-deazapurines were prepared. For instance, use of Rink amide resin afforded modified derivatives of 9-substituted-1-deazaadenine or conversely 9-unsubstituted-3-deazaadenine.

Figure 3: 2,4-Dichloro-3-nitropyrimidine as a key synthon for deazapurines synthesis.



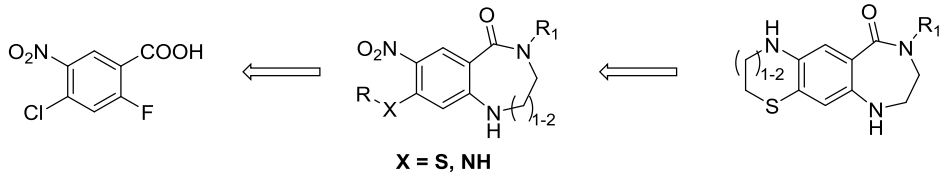
The main part of this chapter is a study of regioselectivity leading to different deazapurine isomers and their variously substituted derivatives.

2.4 Synthetic Method 4: Solid-Phase Synthesis of 4,7,8-Trisubstituted 1,2,3,4-Tetrahydro-benzo[*e*][1,4]diazepin-5-ones

Based on: Barbora Lemrová and Miroslav Soural, ACS Comb. Sci, 2012, 14, 645-650.[A4]

The last part of this thesis pays attention to expansion of diversity-oriented solid-phase synthesis of different heterocyclic scaffolds from 4-chloro-2-fluoro-5-nitrobenzoic acid. This starting material was previously used for solid-phase synthesis of 3-hydroxyquinolin-4(1*H*)-one derivatives and some other 5-7 membered nitrogenous heterocycles. In this case, an efficient method based on solid-phase synthesis was developed for the preparation of trisubstituted tetrahydrobenzodiazepinone derivatives.

Figure 4: Retrospective synthesis of tetrahydrobenzodiazepinones and some "benzene fused heterocycles"



The developed synthetic approach allows the preparation of derivatives with variable substitution at positions 4 and 8. Additionally, a skeletal diversity was increased when the nitro group was reduced and some "benzene fused heterocycles" were synthesized. Twenty model compounds were prepared, fully characterized and submitted to MTT biological screening.

3 State of the art

This chapter summarizes the most important known information about the target heterocyclic scaffolds, their preparation and biological/physical properties to give a brief introduction to each experimental topic mentioned in the previous chapter. Since one part of the presented work is based on application of direct C-H arylation strategy, we report also a brief review of this method. Due to large amount of available information, especially from the field of purine chemistry and direct arylation, a detailed review would go far beyond the scope of this thesis. Therefore all subchapters provide just a basic introduction in which only the most important examples are mentioned. With respect to the fact that the thesis is focused

on solid-supported chemistry the synthetic strategies discussed in this chapter are oriented particularly to this area.

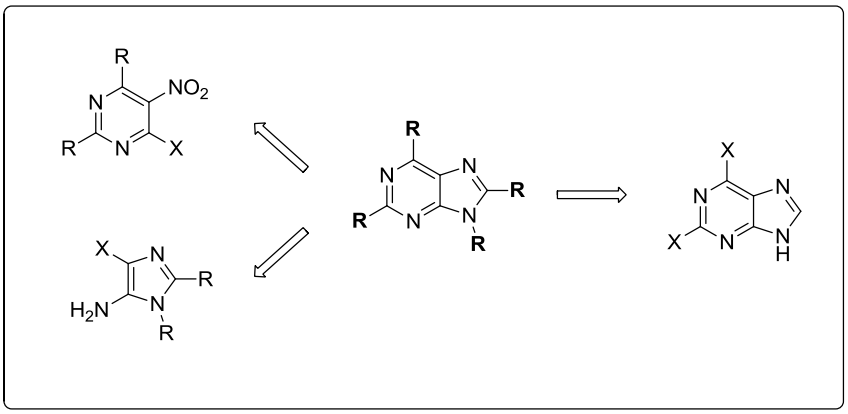
3.1 Purines

Substituted purines and their tautomers are the most often occurring heterocycles in nature.[7] The first synthesis of purine was carried out by German chemist Emil Fischer in 1899 from uric acid.[8] From that time, the purine scaffold has become one of the most widely studied structures by medicinal chemists.

3.1.1 Strategies for synthesis of purine derivatives

In general, three different approaches to the substituted purine derivatives synthesis have been developed: (i) heterocyclization from pyrimidine derivatives; (ii) heterocyclization from imidazole derivatives; (iii) decoration of the purine scaffold with suitable building blocks (Figure 5). The method of preparation is typically chosen with respect to substitution of the target compounds and required isomers.

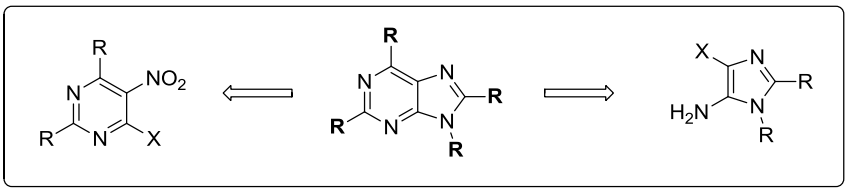
Figure 5: General strategies for purine syntheses



Synthesis by heterocyclization

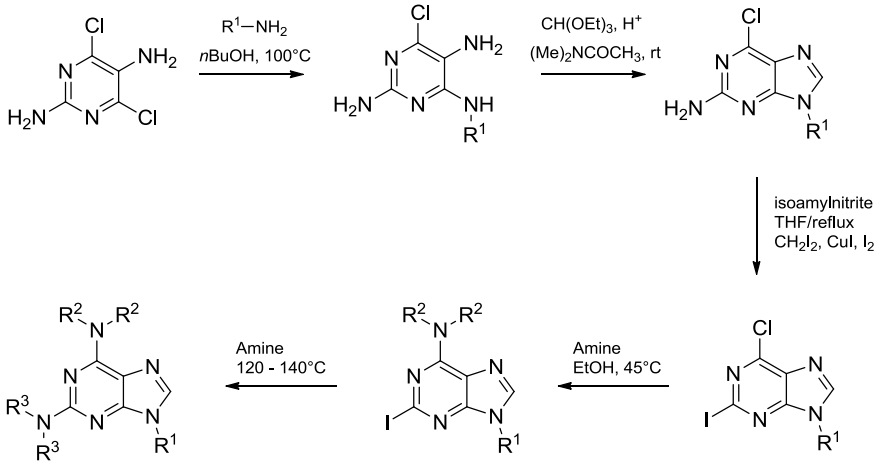
The purine scaffold can be created from suitable substituted pyrimidine or imidazole precursors (Figure 6). Advantages of heterocyclization are particularly an accurate preparation of appropriate isomers or simple modification of synthesis to obtain the corresponding deazapurine derivatives.

Figure 6: General synthesis of purine derivatives by the heterocycle assembly.



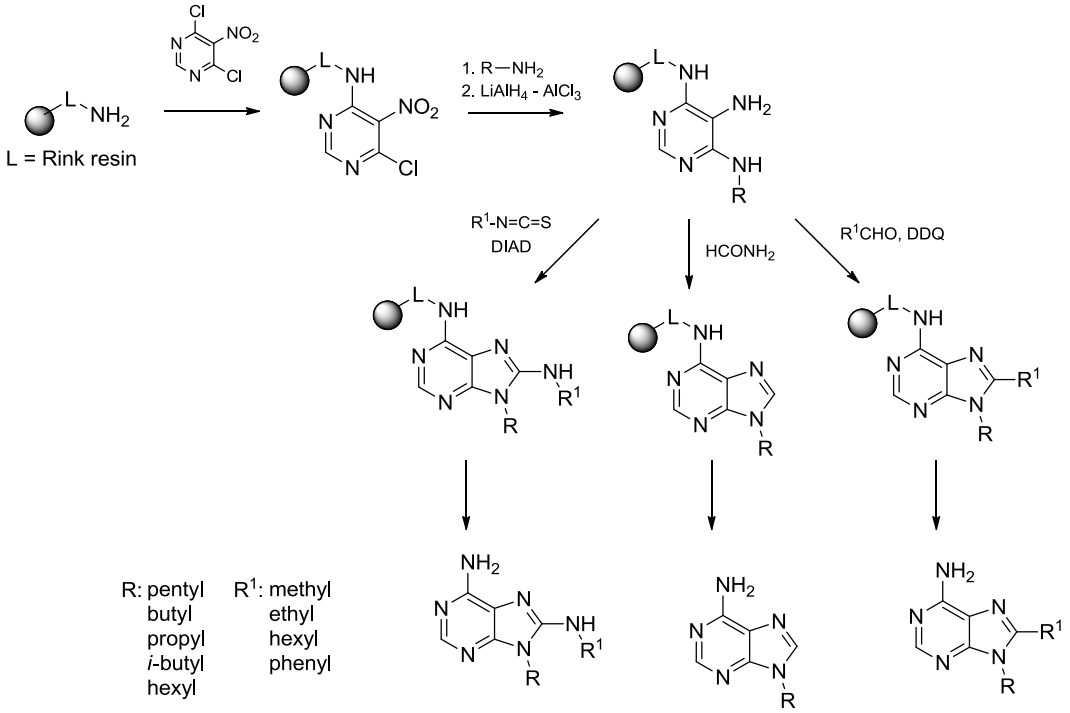
For instance, 2,5-diamino-4,6-dichloropyrimidine was used as a key building block for the preparation of 2-amino-6-chloro-9-substituted purines. This starting material was initially applied for synthesis of 9-substituted guanines.[9] In case of trisubstituted purines preparation, 2,5-diamino-4,6-dichloropyrimidine was selectively substituted by reaction with one equivalent of amine followed by acid-catalyzed cyclization in *N*,*N*-dimethylacetamide. 6-Chloro-2-iodopurine was subsequently obtained by diazotation and substitution of the resulting 2-amino-6-chloropurine with a mixture of CH_2I_2, I_2 and CuI. The final two-step reactions with amines provided the target purine derivatives.[10]

Scheme 1: Preparation of trisubstituted purines with use of traditional solution-phase synthesis.



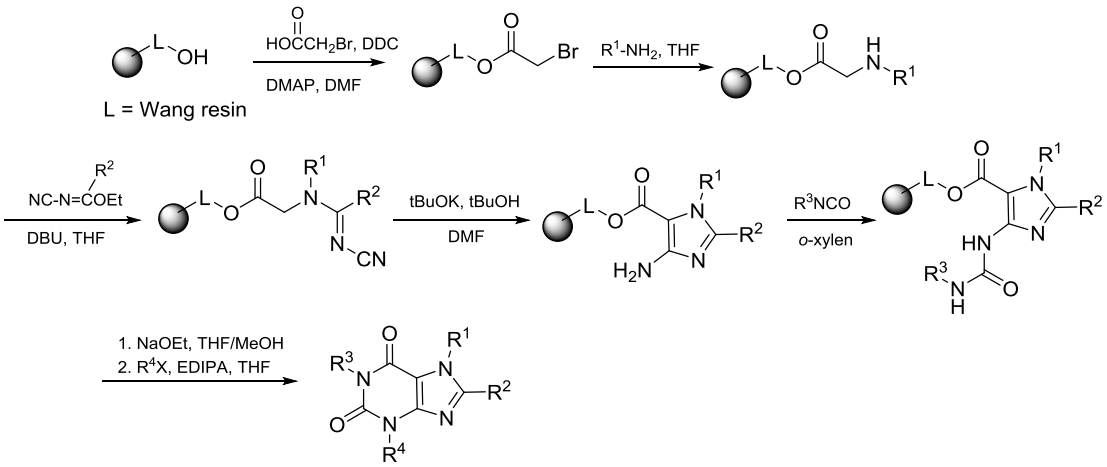
In 2000, a similar strategy was applied to solid-phase synthesis. 4,6-Dichloro-5-nitropyrimidine was attached to Rink resin followed by a substitution of the second chlorine atom with a primary amine and a subsequent reduction of the nitro group. A reagent for the final cyclization was chosen according to required C^8-substitution of target purine derivatives.[11]

Scheme 2: Solid-phase synthesis of purines from dichloronitropyrimidine.



The efficient solid-phase synthesis of di- tri- or tetra-substituted purines from pyrimidines initiated an intensive research in this area and several similar methodologies were reported.[12,13] An alternative method is based on a synthesis of imidazole ring followed by the cyclization to the purine scaffold. This strategy has been described many times with the use of solution-phase chemistry and usually it has been applied for a preparation of xanthine derivatives.[14-16]

Scheme 3: Solid-phase synthesis of xanthines from imidazoles.



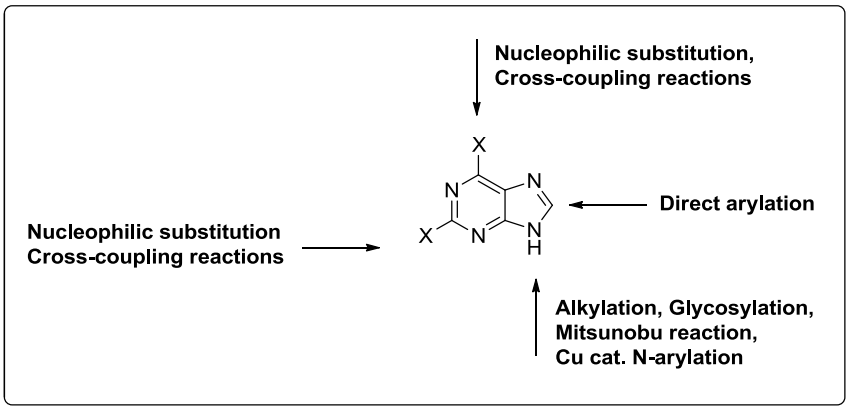
In its solid-phase alternative, He and co-workers reported a synthetic procedure to access di-, tri- and tetrasubstituted xanthines on Wang resin (Scheme 3).[17] Syntheses were carried out

with immobilized imidazole intermediate which reacted with isocyanate. Subsequent treatment with sodium ethoxide gave the target xanthines.

Synthesis by decoration of the purine scaffold

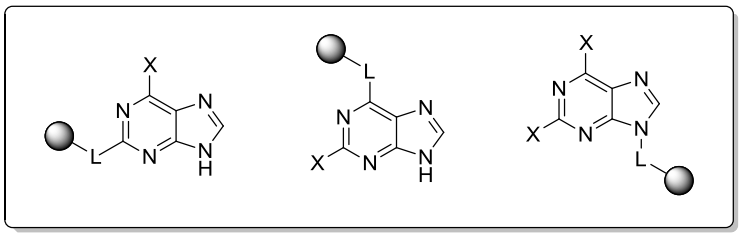
An alternative approach to the synthesis of purine derivatives by heterocyclization consists of modification of suitably substituted purine scaffold. Positions 2, 6, 8 and 9 were studied the most frequently in various combinations. A typical intermediate for this purpose is 2, 6-dihalopurine (Figure 7) that can be simply modified by suitable reagents. For instance, in traditional solution-phase chemistry, both halogens in position 2 and 6 were subjected to reaction with oxygenous[17,18] nitrogenous[19,20] or sulfurous[21] nucleophiles and organometal or metal catalyzed reactions.[22] N^9 position is generally modified by alkylation with alkyl iodides or Mitsunobu procedure with alcohols.[23] Among another reactions at N^9 position belong glycosylation or Cu-catalyzed arylation.[24] Additionally, also C^8 position can be modified and the typical reaction for this purpose is direct arylation.[25]

Figure 7: Possible modification of purine scaffold with use of traditional solution-phase chemistry.



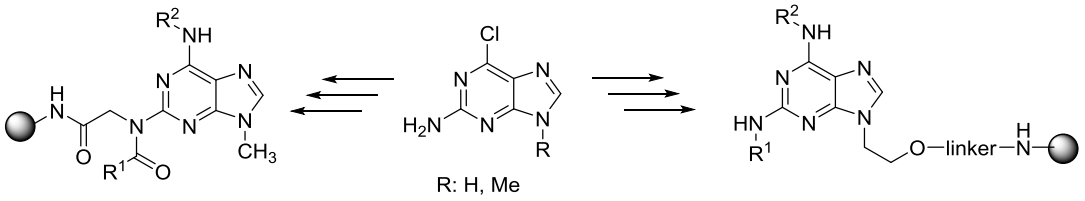
In case of solid-supported reactions, 2,6-dihalopurine can be smoothly immobilized *via* position 2, 6 or 9 depending on the type of resin and reagents used (Figure 8). Subsequently, other free positions can react with various nucleophiles and reagents as described for solution-phase chemistry.[26]

Figure 8: Possible immobilization of the purine scaffold to the resin.



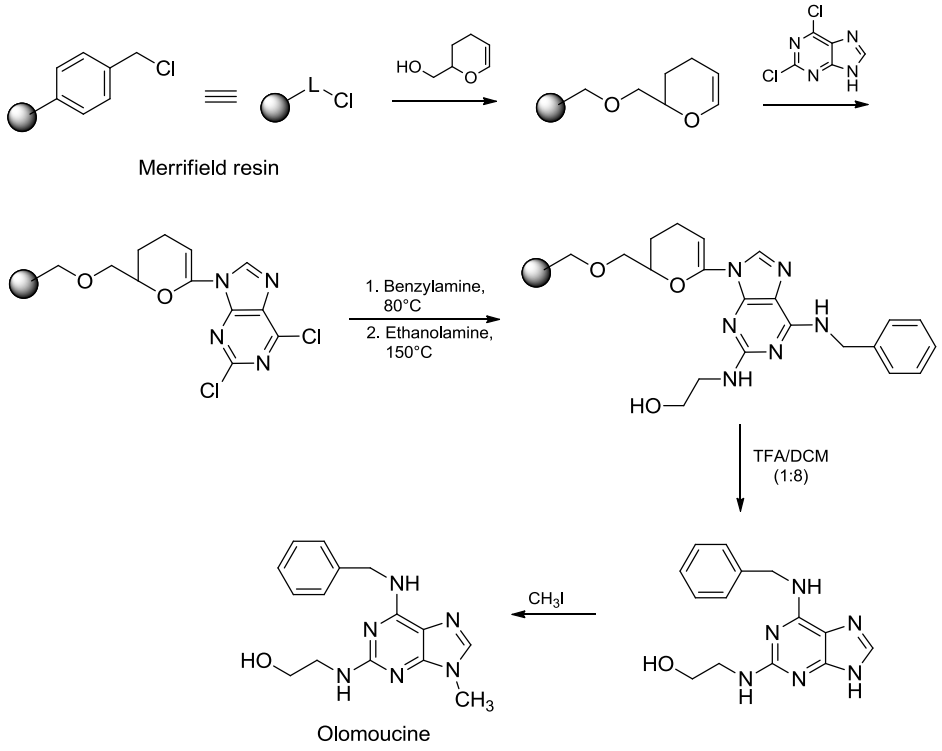
Interestingly, the first described solid-phase synthesis of purine derivatives was not based on heterocyclization but on the decoration method. It was carried out on Rink resin with use of 2-amino-6-chloropurine and two different types of anchoring. The purine scaffold was attached to the resin by either a glycinamide formed at C^2 or a hydroxyethyl chain located at N^9 (Scheme 4).[27]

Scheme 4: First solid-phase synthesis of purine derivatives.



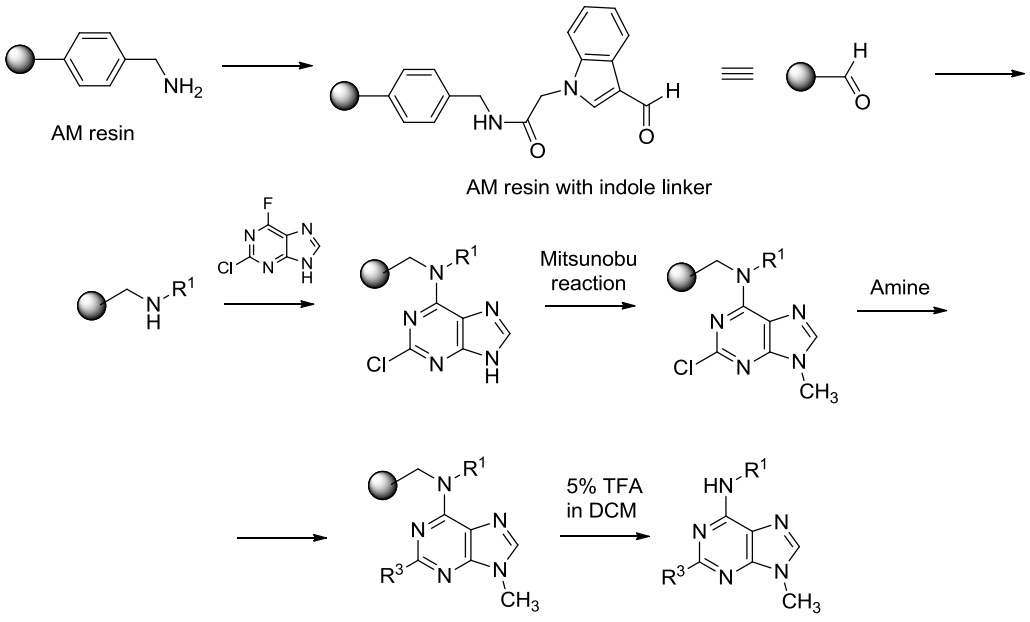
In 1997 Nugiel and co-workers, inspired by Olomoucine, introduced a solid-phase method for the preparation of 2,6-disubstituted purines. The described strategy consisted of attachment of tetrahydropyranyl linker (THP-linker) to Merrifield Resin followed by the reaction with 2,6-dichloropurine (Scheme 5). Subsequently the chlorine atoms in positions 2 and 6 reacted selectively with suitable amines and the resulting 2,6-disubstituted purines were cleaved from resin.[28]

Scheme 5: Described solid-phase synthesis of Olomoucine derivatives.



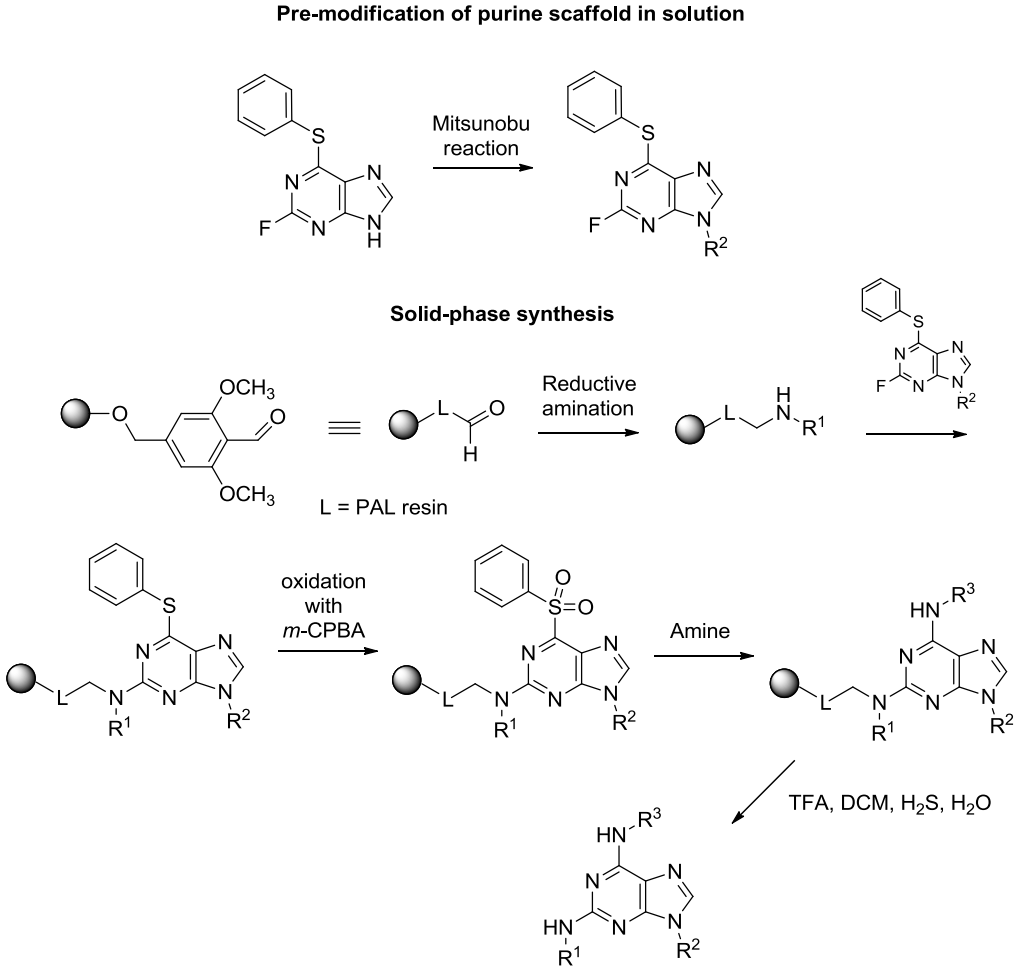
Unfortunately, the method did not allow a derivatization in N^9 position directly on solid phase. It was necessary to use already pre-modified purine or to perform alkylation in solution after cleavage from the solid support. The modification of N^9 position was possible only if the purine scaffold was attached through different position to the resin (Scheme 6).

Scheme 6: Solid-phase synthesis of trisubstituted purines.



2,6,9-Trisubstituted purines were prepared on AM resin equipped with indole linker. The strategy of the synthesis is based on attachment of 2-fluoro-6-chloropurine by amination in position C^6. The following steps are Mitsunobu alkylation[29,30] and substitution of the fluorine atom in C^2 position (Scheme 6).[31] This method was used only for the preparation of 9-methylderivatives. In a literature there is also described the solid-phase synthesis of 2,6,9-trisubstituted purines on 4-formyl-3,5-dimethoxyphenoxymethyl-functionalized polystyrene resin (PAL) (Scheme 7).

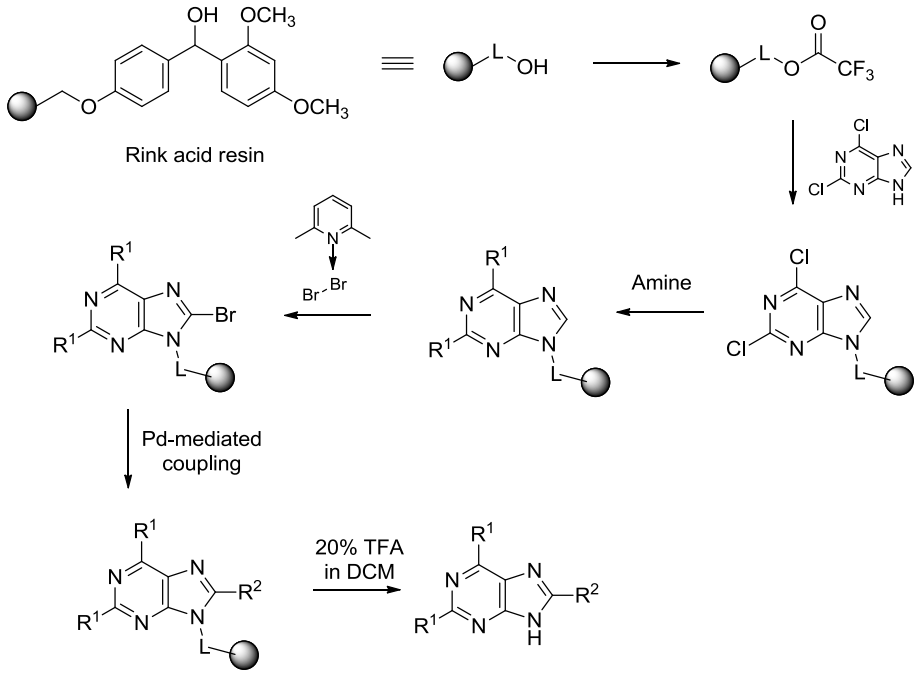
Scheme 7: Solid-phase synthesis of trisubstituted purines on PAL resin.



With use of this strategy, N^9 position was pre-derivatized in solution by the Mitsunobu procedure and the corresponding intermediate was immobilized on solid phase.[32] C^8-modified purines are usually prepared by heterocyclization but in the last decade a cross-coupling reactions from appropriate C^8-halogenated derivatives were described for both synthesis in solution[25,33] and solid-phase synthesis.[34,35] For instance, in 2001 Brill et al. reported such strategy with use of Rink acid resin (Scheme 8). 2,6-Dichloropurine was immobilized to the

resin after activating the linker as trifluoroacetate. Next reaction was the substitution of the both chlorine atoms with amines in one step. Final C^8 modification was performed by displacement of the hydrogen atom by bromine using bromine-lutidine complex followed by the coupling reaction[34,35]

Scheme 8: Solid-phase synthesis of C^8-substituted purines

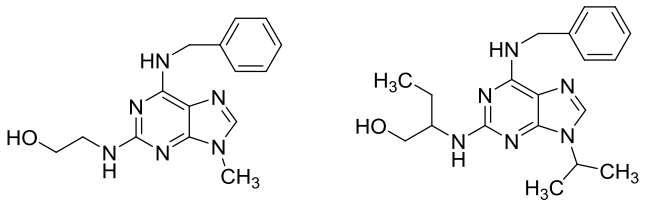


C^8 modified purines can be also obtained with use of direct arylation, this reaction is discussed in detail in chapter 3.3.

3.1.2 Biological properties of purines

Both natural and synthetic purine derivatives represent one of the most studied groups of compounds for their biological effects.[36,37] In 1986 Parker and co-workers described trisubstituted purine derivative as inhibitor of cytokinins glycosylation.[38] In a next decade, the same derivatives – later named Olomoucine and more successful Roscovitine (Figure 9) – exhibited inhibition of cyclin-dependent kinases (CDK).[39,40] Identification of these hits was an impulse for many research groups and various purine libraries were synthesized and subjected to biological screening.

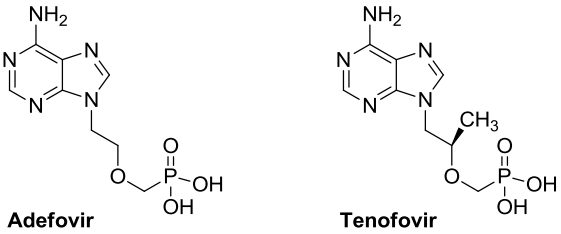
Figure 9: Olomoucine and Roscovitine: potent CDK inhibitors.



Olomoucine **Roscovitine**

One of the most important purine derivatives Adefovir and Tenofovir were synthesized in the Institute of Organic Chemistry and Biochemistry Academy of Science of the Czech republic by Antonín Holý.[41] From development of these acyclic nucleoside phosphonates which exhibited high antiviral activity,[42] they became an important template for other antiviral nucleosides.

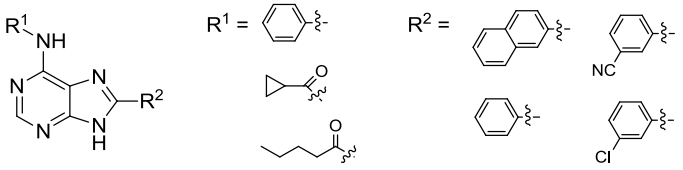
Figure 10: Adenine derivatives with significant antiviral activity.



Adefovir **Tenofovir**

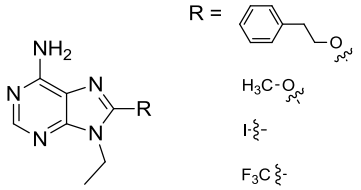
C^8-modified purine derivatives were reported as effective inhibitors of glycogen synthase kinase-3 (GSK-3). The structures of the most potent inhibitors are depicted in Fig 11. [43]

Figure 11: Strucrures of GSK-3 inhibitors.



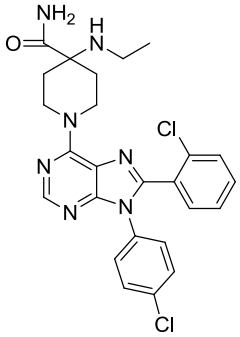
Some 8-substituted-9-ethyladenines were studied as adenosine A_{2A} receptor antagonists for the treatment of Parkinson´s disease.[44] This research was carried out *in vivo* in rat models and four derivatives (8-iodo; 8-methoxy; 8-phenethyloxy and 8-trifluoromethyl – see figure 12) exhibited excellent affinity and selectivity at rat $AA_{2A}R$.

Figure 12: Adenosine A_{2A} receptor antagonists



Research of other modified purines resulted in structure-activity relationships, design and synthesis of novel cannabinoid type 1 (CB_1) receptor antagonist. The compound with best results (Figure 13) exhibited subnanomolar potency at Human CB_1 receptors in binding and functional assays.[45]

Figure 13: 6,8,9-trisubstituted purine derivative with activity at human CB_1 receptors



In general, purines are significant cofactors associated with a number of enzymes and receptors.[7,36] For this reason, they have a key effect in fundamental biological processes.[46]

3.2 3-Hydroxyquinolin-4(1*H*)-ones

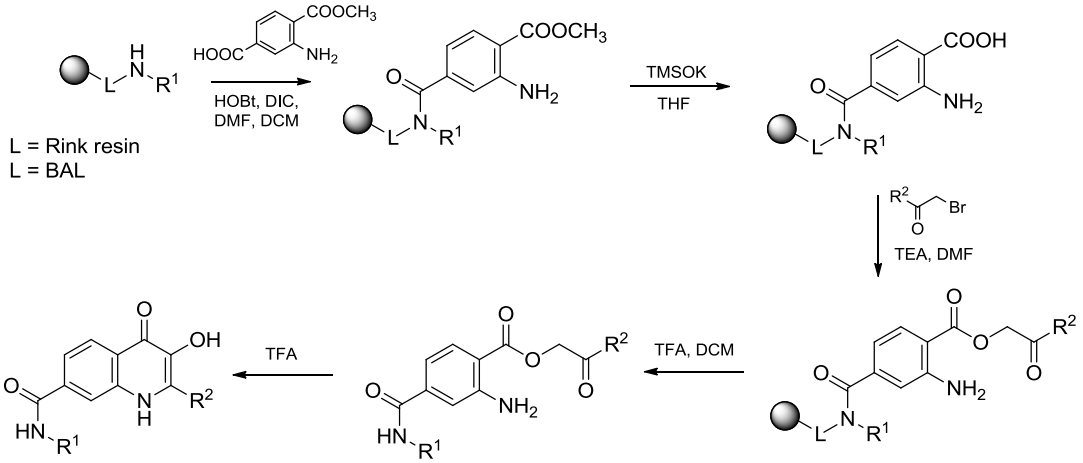
3-Hydroxyquinolin-4(1*H*)-ones are known as aza-analogs of natural flavones. This class of compounds contains number of significant substances like an Quercetin, Luteonin or semisynthetic drug Diosmin and Flavoxate.[47] For this reason, quinolinones and especially 3-hydroxyquinolin-4(1*H*)-ones became an interesting topic for organic synthesis and biological research in the last decade.

3.2.1 Synthesis of 3-hydroxyquinolin-4(1*H*)-ones

The first synthetic approaches leading to 3-hydroxyquinolin-4(1*H*)-ones included cyclization of 2-nitrochalcone epoxide,[48] Darzens reaction[49] or indol scaffold expansion.[50] However, derivatives of 3-hydroxyquinolin-4(1*H*)-one started to be intensively studied since 1995 when Hradil published a powerful synthetic method based on the cyclization of phenacylesters.[51] Use of Hradil´s reaction for hydroxyquinolinones synthesis was reviewed quite recently.[52] In a following decade, the synthetic pathway was used for the preparation of

hundreds of various derivatives for biological screening purposes. Herein we report in detail only solid-phase methodologies based on Hradil´s reaction which were recently described to access high throughput organic synthesis of hydroxyquinolinones. In 2007, the the preparation of 3-hydroxyquinolin-4(1*H*)-one carboxamides with two diversity positions was published (Scheme 9).[53]

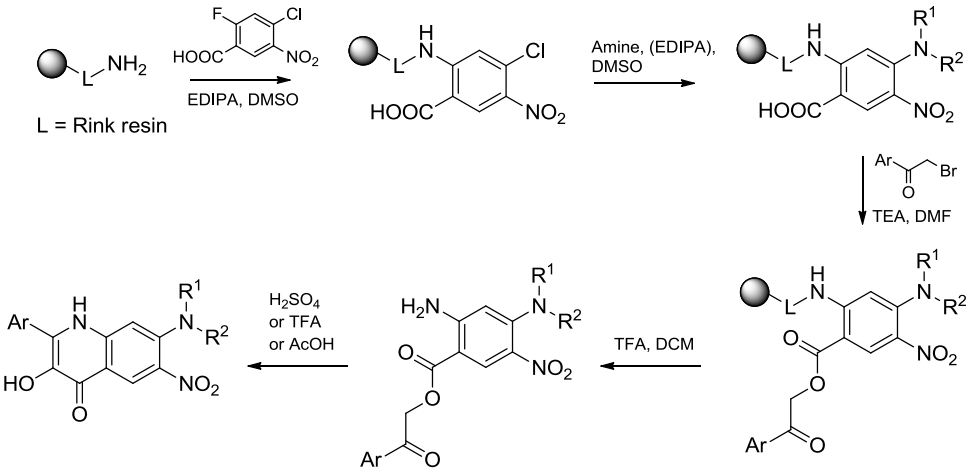
Scheme 9: First solid-phase synthesis of 3-hydroxyquinolin-4(1*H*)-ones.



The same solid-phase methodology was later applied for the preparation of 2-substituted 3-hydroxyquinolin-4(1*H*)-one carboxamides library with variable positioning of a carboxamide group (6-, 7- and 8-carboxamides were prepared).[54]

In 2009, 4-chloro-2-fluoro-5-nitrobenzoic acid was used as a starting material for solid-phase synthesis of some nitrohydroxyquinolinones with final cyclization performed in sulfuric acid.[55]

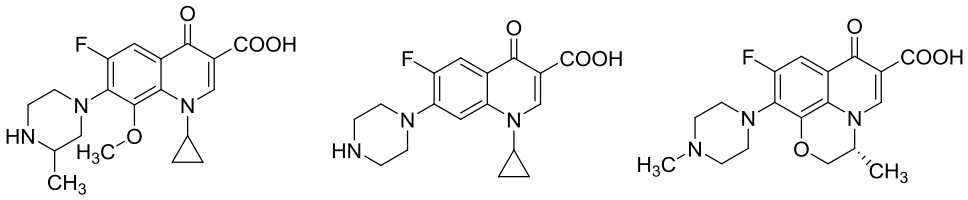
Scheme 10: Use of 4-chloro-2-fluoro-5-nitrobenzoic acid for the preparation of 3-hydroxyquinolin-4(1*H*)-ones.



3.2.2 Biological properties

Some quinolin-4(1*H*)-ones, especially 6-fluoroquinolin-4(1*H*)-one-3-carboxylic acids, are known as very potent antibiotics.[56] Research in this area resulted in four generations of marketed drugs with large effects on a broad spectrum of gram-positive and gram-negative bacteria.[57] Among the most frequently used products belong for instance Gatifloxacin, Ciprofloxacin or Levofloxacin (Figure 14).

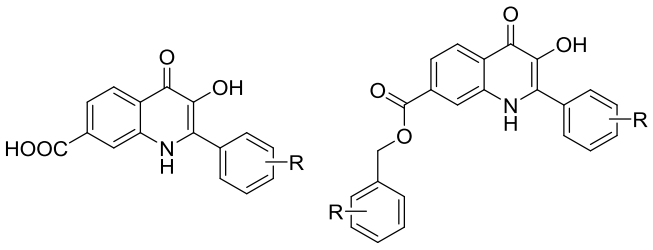
Figure 14: Known antibiotics derived from quinolin-4-one scaffold.



Gatifloxacin **Ciprofloxacin** **Levofloxacin**

Derivatives of 2-substituted-3-hydroxyquinolin-4(1*H*)-one were reported as enzyme inhibitors with effects on topoisomerase II, gyrase or inosinmonophosphate dehydrogenase resulting in significant cytotoxic and antibacterial activity.[58] Cytotoxic screening on several cancer cell lines derived from normal tissues and malignant tumors was performed in the first decade of 21st century.[59] Using the MTT cytotoxic test, the anticancer activity *in-vitro* was studied and some basic structure-activity relationship studies have been done. 3-Hydroxy-2-phenylquinolin-4(1*H*)-ones-7-carboxylic acids provided relatively low cytotoxicity except 4-amino-3,5-dichlorophenyl derivative. The anticancer activity was further increased when the carboxylic group was esterified by phenacyl moiety.[58]

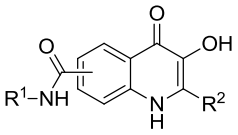
Figure 15: General structures of 3-hydroxyquinolin-4(1*H*)-one for MTT cytotoxic screening.



Relationship study between structure and cytotoxic activity was also reported for 2-phenylsubstituted-3-hydroxyquinolin-4-(1*H*)-one carboxamides. In this extensive research three generations of chemical libraries were studied. The first biological assay was performed with compounds containing polar/nonpolar or hydrophilic/lipophilic substituents at

carboxamide group: the carboxamide group was substituted with two hydrophilic ligands, two lipophilic ligands and two heterocyclic ligands of a different polarity. Results of the biological screening brought some trends: (i) *N*-unsubstituted carboxamide provided lower activity; (ii) the best cytotoxic activity was observed for derivatives with lipophilic carboxamide *N*-substitution; (iii) aromatic ligands in position 2 positively influenced the cytotoxic activity.

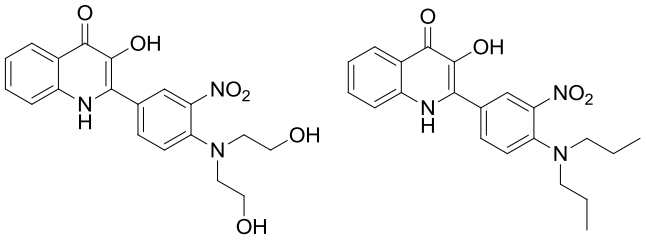
Figure 16: General structure of 3-hydroxyquinolin-4(1*H*)-one carboxamides for MTT cytotoxic screening.



For this reason the second generation of chemical library with different carboxamide lipophilic substitution was prepared and studied. The results indicated that the presence of longer carbon chains or larger carbocycles does not further significantly increase the cytotoxicity of the substrates. The last part of this research was dedicated to study of carboxamide group location-cytotoxic activity relationship. For this purpose 6-carboxamides, 7-carboxamides and 8-carboxamides were synthetized. It was found that the activity depends mainly on substitution of carboxamide, but in specific cases also the location of carboxamide group influences the biological properties.[60]

The anticancer activity was also extensively studied for derivatives bearing 3-nitrophenyl-4-substituted phenyl moiety in position 2. The compounds with the best results are portrayed at figure 17.

Figure 17: Nitrohydroxyquinolines with strong anticancer activity.



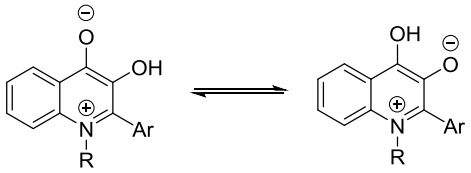
In addition, the derivatives containing hydrophobic substituent were active against the leukemia cell line resistant to daunorubicine (CEM-DNR-BULK).[61] 3-Hydroxyquinolin-4(1*H*)-one derivatives linked with oxazoline ring in position 6 were described as inhibitors of

inosin monophosphate dehydrogenase (IMPDH), the enzyme responsible for the regulation of cellular proliferation and differentiation.[62]

3.2.3 Fluorescence properties

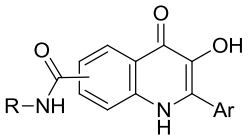
Besides a significant biological activity, the derivatives of 3-hydroxyquinolin-4(1*H*)-ones were also studied for their fluorescent properties, because they can be useful in the area of cell and tissue research as fluorescent probes. 3-Hydroxyquinolin-4(1*H*)-ones exhibit dual emission spectra caused by an excited state intramolecular proton transfer (ESIPT). Two tautomer forms are formed in an excited state (Scheme 11) and due to their different photophysical properties, these forms provide separated emission bands.[63]

Scheme 11: Excited state tautomeric forms in ESIPT.



Derivatives synthetized at our department were also subjected to fluorescent properties research. Motyka and co-workers performed fluorescent study of 2-aryl-3-hydroxyquinolin-4(1*H*)-one-carboxamides with different *N*-substituted carboxamide groups at positions 6, 7 and 8. In this research there was confirmed the hypothesis that not only substituent in position 2, but also ligands on the second aromatic ring influence the fluorescence activity.[64]

Figure 18: General structure of studied hydroxyquinolinone carboxamides.

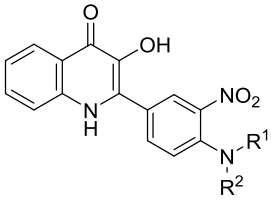


The compounds substituted at position 6 and 7 exhibited typical two-band fluorescence spectra. In contrast, the substitution in position 8 led to only single-band spectra with the emission maxima at lower wavelengths. Compounds with various substitutions of *N*-alkyl carboxamides were tested and no significant influences upon fluorescence properties were observed. This study also paid attention to pH effect on fluorescence properties. It was proved that the ratio of fluorescence intensities at local emission spectrum maxima decreased with increasing pH for selected compounds. The described fluorescent behavior shows a practical

potential of these compounds as fluorescent pH indicators.[65] The 3-hydroxyquinolin-4(1*H*)-ones can be appended to the biomolecule *via* a spacer attached to the carboxamide group at position 6 or preferably 7 without the loss of the characteristic two-band emission properties. Additionally, the spacer character has no influence on the fluorescence activity.

One year later, this research was followed by fluorescent evaluation of some 2-(4-amino-substituted-3-nitrophenyl)-3-hydroxyquinolin-4(1*H*)-ones. The modification of amino group was carried out with use of aliphatic diamines or aminoalcohols.

Figure 19: General structure of studied derivatives.

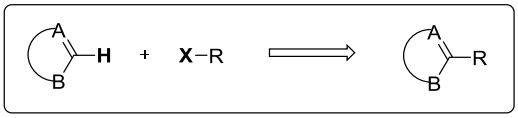


This study confirmed that the presence of substituted alkyl groups (with terminal amino or hydroxyl groups) mostly increased fluorescence quantum yields.[66]

3.3 Direct arylation

Palladium-catalyzed direct arylation is a method for an efficient formation of carbon-carbon bond. The traditional cross-coupling reactions are based on a principle in which both reaction components (heterocyclic/aromatic/aliphatic systems) contain functional groups.[67] In contrast, the direct arylation is used for functionalization of heterocycles with unreactive C-H bond which is substituted with some aryl halides (Figure 20).[68]

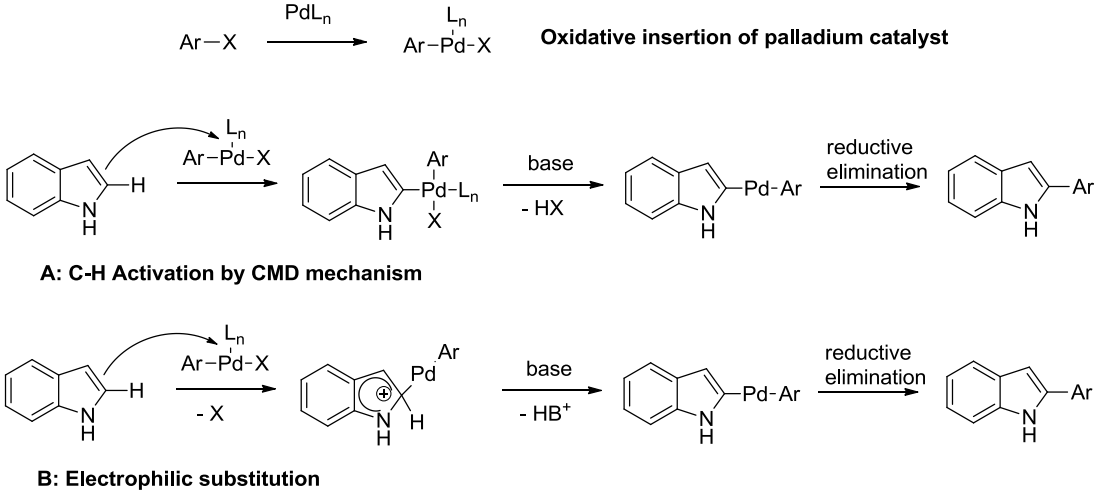
Figure 20: Direct C-H functionalization.



The first direct arylation was described in 1980s.[69,70]Since then there has been a boom in carbon - carbon bond formation chemistry and number of methodologies have been introduced with use of traditional synthesis in solution as well as solid-phase synthesis. Several research groups studied the C-H bond cleavage mechanism by theoretical[71] and experimental approaches and the most often obtained evidences confirmed two possible mechanisms: concerned metalation - deprotonation mechanism (CMD) and electrophilic aromatic substitution (S_EAr).[72-74] The activation of aryl halides by oxidative insertion of

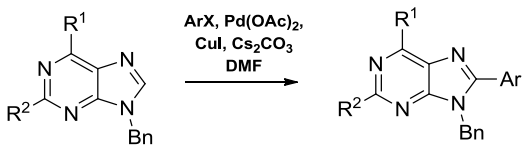
palladium catalyst is the first step of each mechanism. Path A in Scheme 12 describes the initiation by palladium-aryl complex followed by cleavage of halogen atom and reductive elimination. In the second path B, Pd-aryl complex reacts with C-H bond as an electrophile and halide atom is cleaved. After that, in a presence of a base, the proton is released and the final step is reductive elimination again. These mechanisms were described for several substrates, for example by Gevorgyan and co-workers on an indole scaffold.[75]

Scheme 12: Possible mechanism of C-H activation on indole substrate.



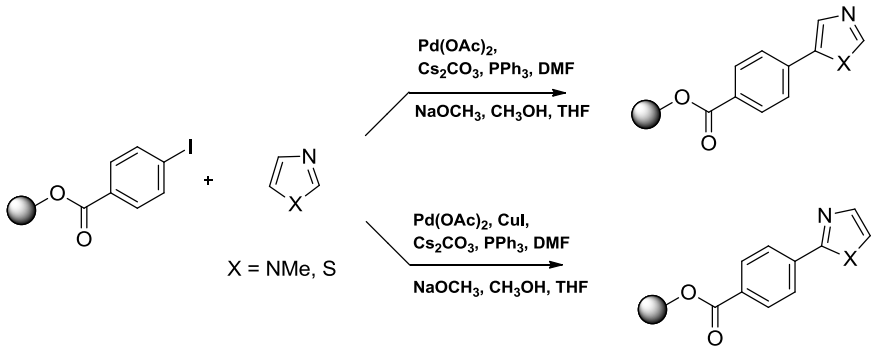
The exact mechanism distinctly depends on a type of heterocyclic system and it can be described separately for each structure. In general, C-H arylation of electron-rich heterocyclic systems has been studied more frequently than C-H arylation of electron-deficient heterocyclic systems.[68] Other factors influencing the reaction mechanism are type of aryl halide and catalyst, presence of metal ions and reaction conditions such as solvent, type of base or reaction temperature.[68,73,76] Direct arylation has been applied in various modifications to many different heterocyclic substrates including arenes, pyrrols, furans, indols, thiophenes, thiazoles, pyridines, pyridine-*N*-oxides and many others.[77-81] In 2006, Čerňa and co-workers developed methodology for direct arylation of purines and he used this method for a regioselective synthesis of 2,6,8 – trisubstituted purines (Scheme 13).[82]

Scheme 13: Synthesis of 2,6,8 –trisubstituted purines.



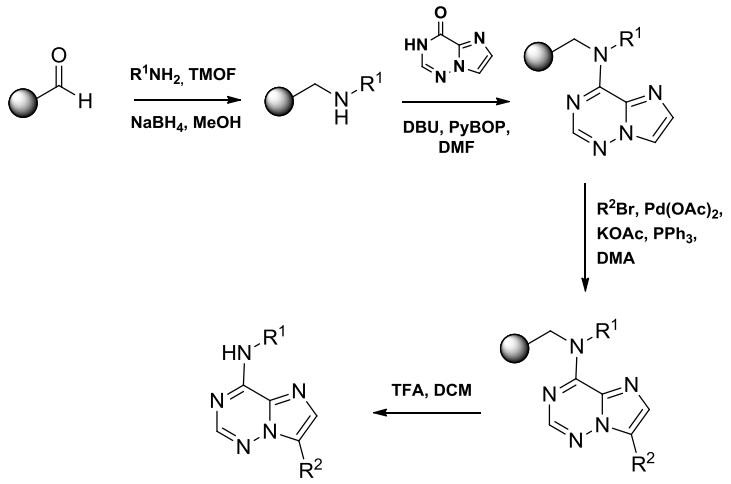
The same research group also performed the first C-H arylation of purine nucleosides[83,84] and they also expanded development of purine derivatives[25] by using intramolecular direct arylation to achieve the fused purine systems.[85] The direct arylation of purine nucleoside was also examined by Storr who described the C-H arylation of unprotected adenine nucleosides[86] and 2´-deoxyadenosines.[87] In area of solid-phase synthesis, only a few examples of palladium-catalyzed direct arylation are known. Although solid-phase synthesis can offer significant benefits especially in connection with combinatorial chemistry, the direct arylation was not largely studied. Selective functionalization of azoles using palladium-catalyzed reaction was reported in 2000 by Kondo et al.[88]

Scheme 14: Regioselective palladium-catalyzed monoarylation of azoles.



This mentioned work is an excellent example for comparison of solid-phase and solution-phase chemistry. Direct arylation of azoles employing palladium-catalyzed solution-phase chemistry was initially reported by Pivsa-Art and co-workers, but the reaction was not regioselective and except the target 4-aryl azols also 2,4-diaryl azols were formed.[89] In contrast, with use of solid-phase synthesis no side products were observed. In addition, the presence or absence of copper iodide influenced regioselectivity of arylation. Recently, also the direct arylation of imidazotriazines on solid support was reported.[90]

Scheme 15: Combinatorial synthesis of substituted imidazotriazines.

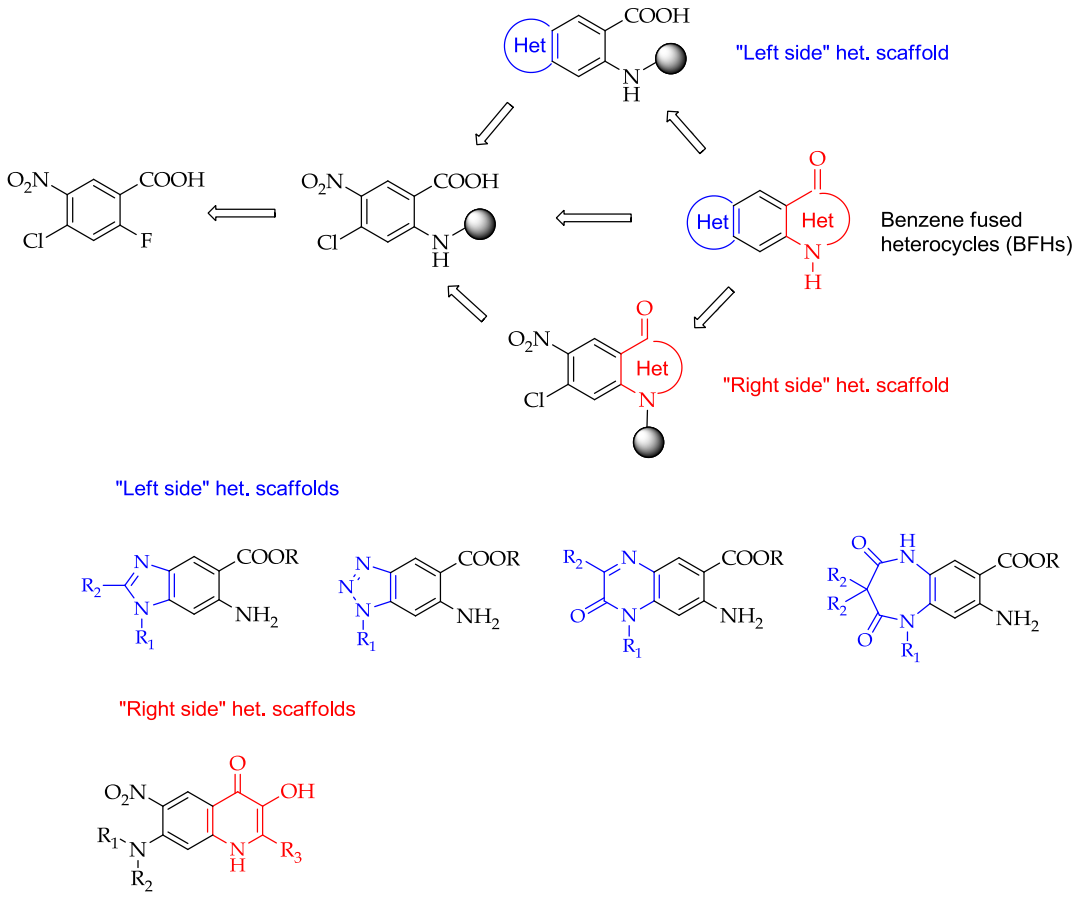


Maechling et al. synthetized 20-membered library of diverse target derivatives which proved the developed method to be applicable for combinatorial chemistry purposes.

3.4 4-chloro-2-fluoro-5-nitrobenzoic acid as a starting material for the synthesis of heterocycles

Use of polyfunctional building blocks (i.e. more than three different functional groups) in combination with solid-phase synthesis concepts enables quick access to a number of various organic substances, particularly when diversity oriented synthesis is applied. In an ongoing research at our department we have been focused on use of 4-chloro-2-fluoro-5-nitrobenzoic acid for the preparation of various heterocyclic scaffolds. Although the substance is commercially available and represents an excellent starting material for number of chemical transformations, its use in organic synthesis has been very rare so far. Our current effort aims to prepare benzene fused heterocycles (BFHs) that comprise two different heterocyclic scaffolds in various combinations located at opposite sides of a central benzene ring (Scheme 16).

Scheme 16: Applicability of 4-chloro-2-fluoro-5-nitrobenzoic acid in BFHs synthesis and heterocyclic scaffolds reported so far



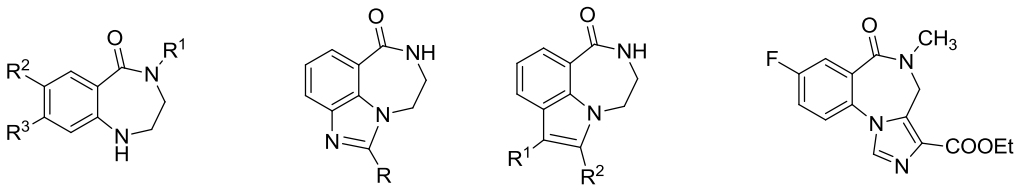
Quite recently, two articles dedicated to the mentioned area were published. In an initial work, 4-chloro-2-fluoro-5-nitrobenzoic acid was used for the "right side" heterocycle formation and 3-hydroxypyridin-4(1*H*)-one scaffold was introduced.[55] The second contribution was focused on the "left side" heterocycle formation and methodology for the preparation of various nitrogen-containing scaffolds was developed (Figure 21).[91]

As a subproject of this thesis, we focused on an expansion of the "right side" heterocycles group to increase the number of scaffolds available for future synthesis of BFHs library. We aimed at the 1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one derivatives **I** (Figure 21). The preparation of such diazepinones has been described several times with use of solution-phase synthesis. For instance, a regioselective reduction of 1,2,3,4-tetrahydro-5*H*-1,4-benzodiazepin-2,5-dione with $LiAlH_4$ was published.[92] Alternatively, a benzodiazepine scaffold was synthesized by a cyclization approach from anthranilamide derivatives,[93,94] 2-nitrobenzoylchloride[95] or 2-chloro-3-nitrobenzoic acid[96] being the starting material. Also an interesting synthetic pathway starting from *o*-nitroaniline and acrylonitrile was described[97]

taking advantage of the cyclization to a 6-membered scaffold with use of Eaton reagent[98] and subsequent ring expansion by Schmidt reaction.[99] Solid-phase synthesis of some 1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-ones has been published once with use of bromoacetal resin and Leuckart-Wallach reaction.[100]

Concerning biological activity of the discussed compounds, the amount of relevant information is limited. Some derivatives substituted with alkyl at N^4 position were tested as psychotropic agents but such activity was not detected.[101] On the other hand, interesting biological properties were observed at some bisheterocyclic derivatives (imidazobenzodiazepinones and diazepinoindol-1-ones) that act as inhibitors of poly(ADP ribosa)polymerase (PARP-1),[102,103] an enzyme responsible for apoptosis and DNA reparation.

Figure 21: Target general structure and already reported biologically active analogous derivatives



General structure PARP-1 inhibitors Flumazenil (Anexate)

Research in this area is quite intensive and number of dedicated articles and patents appeared recently. Except this, an imidazobenzodiazepinone scaffold can be found in a structure of Flumazenil (Anexate), a clinically used antidote for central nervous system (CNS) benzodiazepines intoxication.

4 Results and discussion

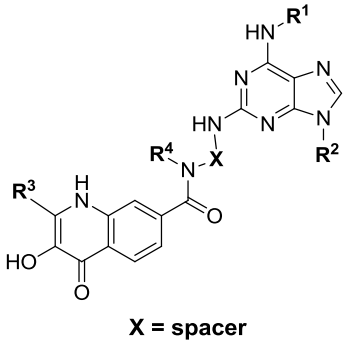
In this chapter we describe development of four solid-phase syntheses of target heterocyclic compounds, results of their biological activity and fluorescence properties. Except an ongoing research of deazapurines, all developed methods for the preparation of target molecules were published in impacted journals. Notation of all synthesized compounds "Final Compound (R^1,R^2, R^3,..)" is derived from used building blocks, which are shown in each subchapter.

4.1 Solid-phase Synthesis of Highly Diverse Purine-Hydroxyquinolinone Bisheterocycles

Based on: Barbora Vaňková, Jan Hlaváč and Miroslav Soural, J. Comb. Chem. 2010, 6, 890-894.[A1]

This chapter describes solid-phase synthesis of bisheterocyclic molecules in which 3-hydroxyquinolin-4(1*H*)-one derivatives (termed "hydroxyquinolinones" hereafter) are accompanied by purine scaffold. As we demonstrated in chapter 3.1.2, purine derivatives are well known for their ability to interact with both nucleic acids and enzymes. Similarly quinolin-4-ones are widely used as inhibitors of topoisomerase, enzyme responsible for cleavage and recombination of DNA (see chapter 3.2.2). Based on this fact, we can conclude that the combination of both scaffolds in one molecule could afford substances with interesting biological (and spectral) properties. In this part of the research, our intention was to develop a methodology for simple synthesis of a chemical library consisting of purine-hydroxyquinolinone bisheterocycles with five diversity positions:

Figure 22: Suggested general structure of the target bisheterocycles.

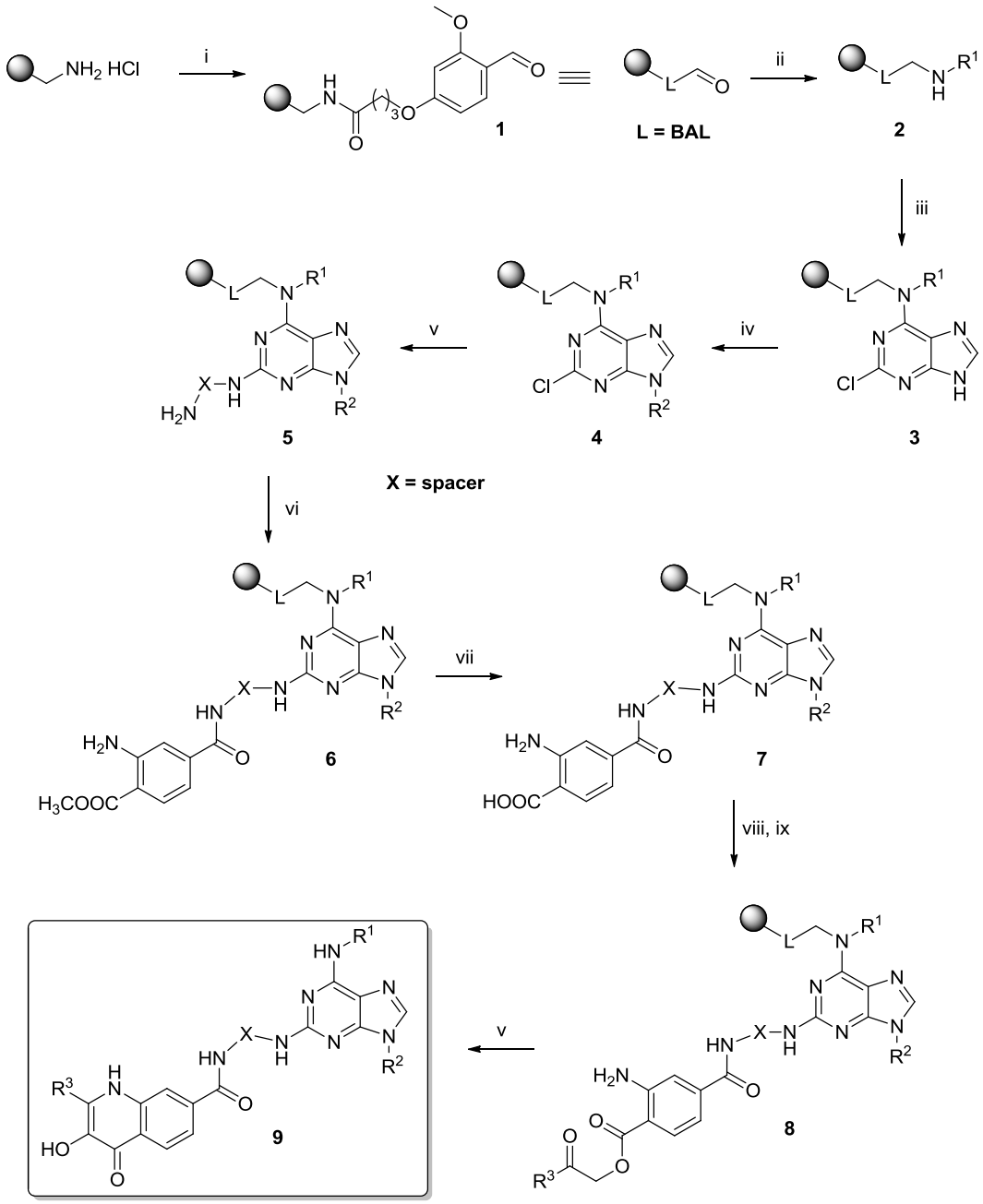


Two positions at purine (C^6 and N^9 substitution), two positions at hydroxyquinolinone (C^2 and the carboxamide C^7 *N*-substitution), and the fifth diversity position resulting from the constitution of the spacer between both heterocyclic systems. The connection between both heterocyclic systems was located via hydroxyquinolinone C^7 and purine C^2 position.

4.1.1 Synthesis

The synthesis was carried out on aminomethylated polystyrene resin equipped with an acid-labile BAL linker[104] (4-(4-formyl-3-methoxyphenoxy)butyric acid) according to Scheme 17. After the immobilization of the primary amines with use of the reductive amination[105] the resulting secondary amines (**2**) were regioselectively arylated with 2,6-dichloropurine to give the intermediates (**3**).

Scheme 17: Preparation of purine-hydroxyquinolinone bisheterocycles (9).[a]



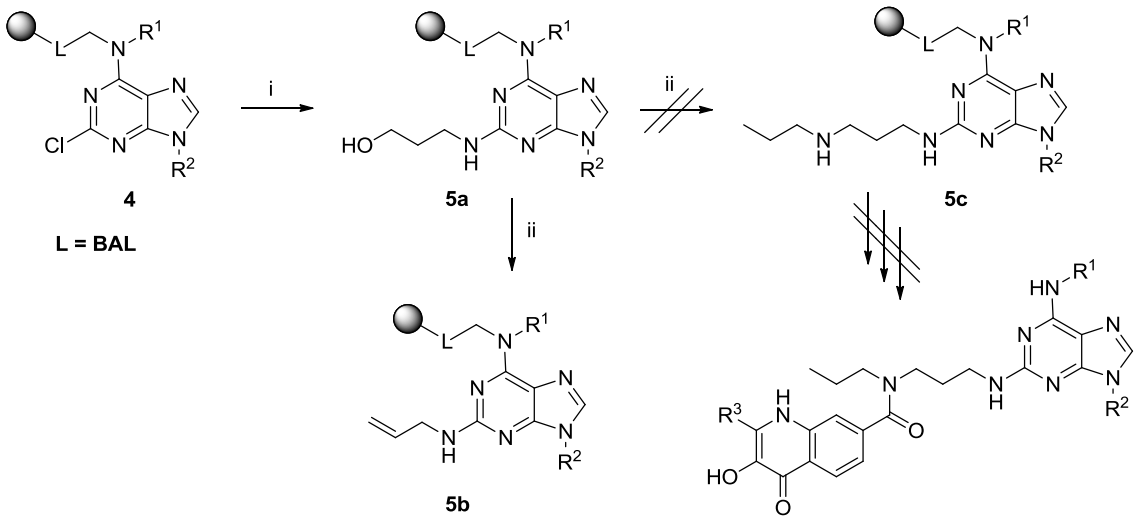
[a]Reagents and conditions: (i) 5% EDIPA, DMF, 10 min., rt. then 4-(4-formyl-3-methoxyphenoxy)butyric acid, HOBt, DIC, DMF, DCM, rt., on; (ii) amine, 10% AcOH/DMF, on, rt then $NaBH(OAc)_3$, 4 h; rt (iii) 2,6-dichloropurine, EDIPA, THF, 50°C, on; (iv) alkyl iodide, DBU, DMSO, 50°C, on, (repeated once in THF); (v) diamine, diethylene glycol diethyl ether, 150°C, 24 h; (vi) 1-methyl-2-aminoterephtalate, DIC, HOBt, DCM, DMF, rt, on; (viii) TMSOK, THF, rt, 24 h; (ix) haloketones, EDIPA, DMF, rt, 2 h; (x) TFA, DCM, rt., 30 min.; (xi) AcOH, reflux, 3 h or TFA, reflux, 2 h.

In the next step, the purine N^9 was alkylated. Alkylation of purine derivatives using solution-phase synthesis is usually performed with alkyl iodides in a presence of a suitable base whereas the N^9 modification of solid-supported purine derivatives takes advantage of the Mitsunobu protocol which allows alkylation with various alcohols under mild conditions.[31] In our case, when Mitsunobu alkylation of the intermediates (**3**) with ethanol or isopropanol was tested, the reaction did not furnish satisfactory yield although various conditions and reagents were tested. With use of triphenylphosphine or tributylphosphine in various concentration, different solvents (dry THF or NMP) and reaction times as well as with repeating of the reaction step we could not manage the quantitative conversion. Additionally, the purity of the products was diminished by appearance of number of unknown impurities when the alkylation step was repeated 2 or 3 times. With respect to such problems we turned our attention to the solution-phase method of alkylation with alkyl iodides and finally we successfully transferred this strategy to a solid-phase. The reaction was performed in DMSO at elevated temperature (50°C). Various bases were tested (such as EDIPA, NaH, Na_2CO_3, Cs_2CO_3) but the best results (70-80% of conversion, HPLC traces) were obtained when diazabicyclo[5.4.0]undec-7-ene (DBU) was used. For the quantitative yield, the reaction was repeated in THF giving intermediates (**4**) of high purity. When the reaction was repeated in DMSO instead of in THF, the yield did not go over 90%. The developed alkylation method was shown to be limited by the character of the alkyl iodide used: It did not work for methyl iodide and cyclohexyl iodide but it was successfully tested for ethyl iodide, *iso*-propyl iodide, *t*-butyl iodide and cyclopentyl iodide. In an absolute combination of all used amines and alkyl iodides (see Figure 30) only one exception was detected: The alkylation of 6-*N*-benzylamine intermediate (**3**) with *t*-butyl iodide furnished only 60% of conversion.

The subsequent substitution of the chlorine atom at purine C^2 required high temperature (150°C) to proceed and it was tested for various aliphatic diamines. First, the reaction was performed in solvents typically used for a nucleophillic substitution such as DMSO, DMF or NMP but the intermediates (**5**) were obtained in a limited purity. In each case, formation of unknown side-products (20-30%, HPLC traces) was observed which was probably caused by the reaction of the intermediates (**4**) with solvent under harsh conditions. Therefore selection of suitable solvent was crucial at this step - when the reaction was carried out in an inert diethylene glycol diethyl ether, the intermediates (**5**) were obtained in excellent purity. Surprisingly, the method worked well with propylenediamine, butylenediamine and 2,2´-oxybis(ethylamine), however the same reaction with ethylenediamine produced only a

mixture of unknown compounds. Compounds **5** were acylated with 1-methyl-2-aminoterephtalate to give intermediates (**6**). When aminopropanol was used for the reductive amination (step ii), its hydroxy group was also partially acylated (20%, HPLC traces), however, this side product was removed in the next step, when saponification of the intermediates (**6**) with potassium trimethylsilanolate leading to the carboxylic acids (**7**) was performed. Surprisingly, the saponification was very slow, at least 24 h was necessary for the quantitative hydrolysis. After the esterification of the carboxylic acids (**7**) with haloketones of aliphatic, aromatic or heterocyclic character, the precursors (**8**) were obtained in excellent purity over 90% (HPLC traces). The final cyclization to bisheterocycles (**9**) was performed after the acid-mediated cleavage of the precursors (**8**) from the resin. Two cyclization methods were tested: When trifluoroacetic acid (method A) was used as the cyclizing agent, the target products were usually obtained in excellent purity, only in several cases the partial acidic hydrolysis of the esters (**9**) resulting in a contamination of the products with derivatives (**7**) was observed. Thus we also tested the cyclization in acetic acid (method B) which did not cause the competitive hydrolysis and afforded the target products in very good purity, typically over 90% (HPLC traces). Nevertheless, when the method B was used for the cyclization of the hydroxyl group-containing intermediates (obtained by the reductive amination with aminopropanol, step ii), the final compounds were not obtained as hydroxyderivatives, corresponding *O*-acetylderivatives were isolated instead. Generally, TFA cyclization can be considered as more versatile method, however, the cyclization of derivatives (**8**) bearing nitro group must have been performed with use of the method B otherwise the cyclization was not observed. The final purification of the crude products was accomplished by simple sonification in diethylether and subsequent filtration of the precipitated material.

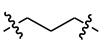
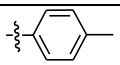
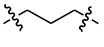
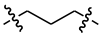
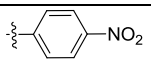
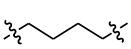
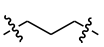
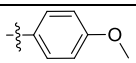
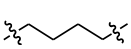
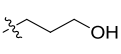
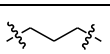
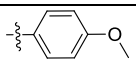
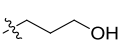
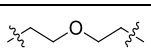
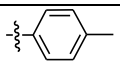
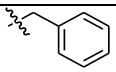
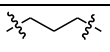
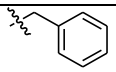
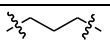
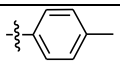
Scheme 18: Unsuccessful set of experiments to increase the diversity of the target structures.[a]



[a]Reagents and conditions: (i) aminopropanol, diethylene glycol diethyl ether, 150°C, 24 h; (ii) mesylchloride, pyridine, rt, 2 h. then propylamine, DMSO, rt, on.

Along with the substitution step (Scheme 17, v), we also made an attempt to increase the diversity of the target bisheterocycles. Instead of diamines, the intermediates **(4)** were subjected to a reaction with aminoalcohol (aminopropanol in our case, Scheme 18) and derivatives (**5a**) were obtained. Our intention was to modify the terminal hydroxyl group with use of mesylation and subsequent reaction with primary amines (propylamine in our case) resulting in possible formation of the secondary aminoderivatives (**5c**). Unfortunately, such modification was unsuccessful and afforded only mixture of compounds with major products of the molecular mass corresponding to the formation of alkene derivatives (**5b**).

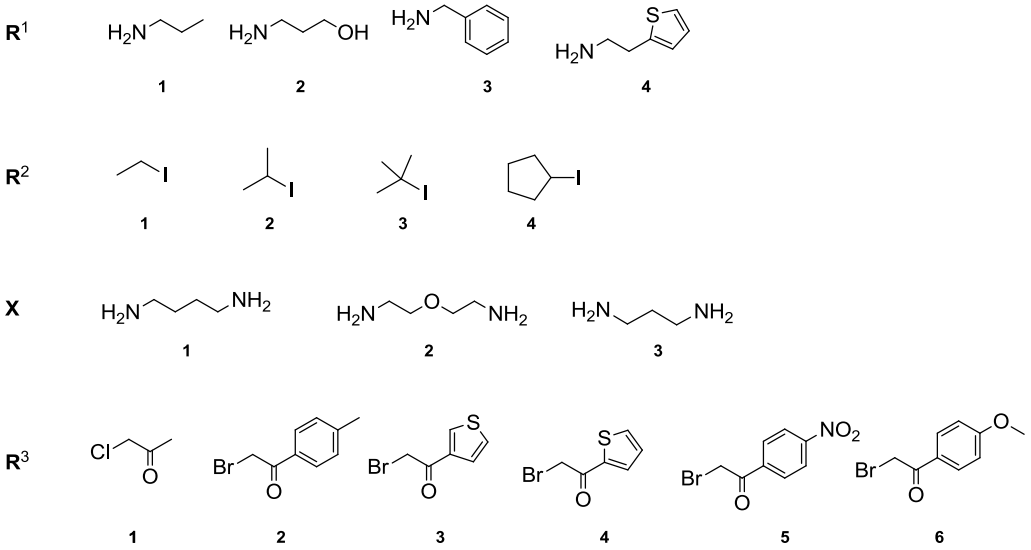
Table 1: Summary of the prepared products.

Compound	R^1	R^2	X	R^3	Purity (%)	Yield (%)
9(1,1,3,2)					90	80
9(1,2,3,3)					93	80
9(1,2,3,5)					98	43
9(1,2,1,1)					85	82
9(1,3,3,6)					90	79
9(1,4,1,1)					82	65
9(2,2,3,6)					95	67
9(2,2,2,2)					98	14
9(3,1,3,3)					86	52
9(3,1,3,2)					80	81
9(3,1,1,1)					88	89
9(3,2,2,1)					90	12
9(3,2,1,2)					93	45
9(3,2,1,4)					98	42
9(3,2,3,6)					95	97
9(4,1,1,2)					95	98
9(4,1,3,2)					99	95
9(4,2,1,3)					95	25
9(4,3,3,6)					65	98
9(4,3,1,5)					86	95

[a]Purity was determined on basis of HPLC-UV traces

The building blocks used for the synthesis were selected with respect to the highest diversity of the target substances as well as the commercial availability of the synthones. As can be seen in Figure 23, ligands with aliphatic, aromatic and heterocyclic character were introduced for R^1 and R^2, also substituted phenyls for R^3 were successfully tested (with both electron-donating and electron-withdrawing functional groups). Three different aliphatic spacers of various length or constitution were selected.

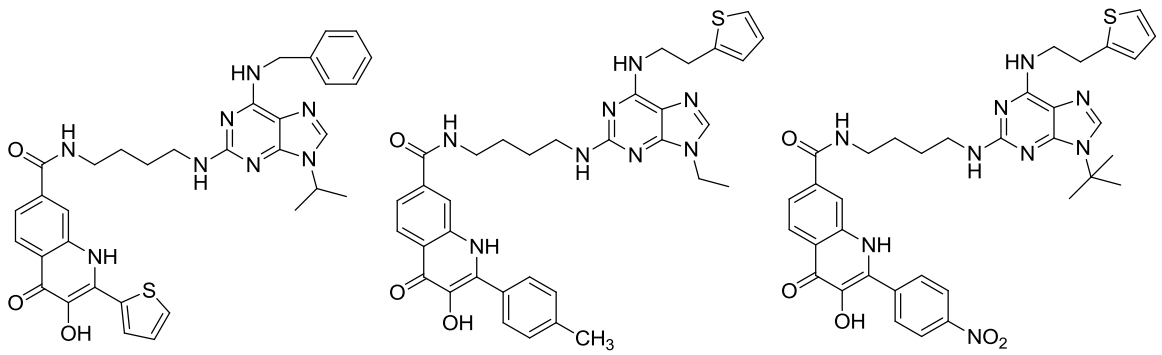
Figure 23: Building blocks successfully used for the evaluation of the synthetic route.



4.1.2 Biological screening

We reported the solid-phase synthesis of novel purine-hydroxyquinolinone derivatives. The versatility of the method was demonstrated by preparation and full characterization of 20 compounds. We used only commercially available and structurally versatile building blocks for each diversity position. For the first diversity position (**R^1**) 4 various amine were used. The alkylation (**R^2**) was carried out with 4 different alkyl iodides. The spacer (**X**) was created by 3 types of diamines and for the final diversity position (**R^3**) 6 different haloketones were used. Full combination of successfully tested building blocks can in theory afford 288 derivatives. ((R1)x(R^2)x(X)x(R^3) = 288 structures in an absolute combination). However, only smaller collection of compounds was synthesized and 18 model derivatives were fully characterized and subjected to biological screening to explore the cytotoxicity before the synthesis of a larger library.

Figure 24: Structures with significant results of biological activity.



9(3,2,1,4)
IC_{50} = 0.33 μM for CEM lines
IC_{50} = 0.86 μM for K562 lines

9(4,1,1,2)
IC_{50} = 0.58 μM for CEM lines

9(4,3,1,5)
IC_{50} = 0.69 μM for CEM lines

All prepared derivatives exhibited very good solubility in methanol, mixture methanol/water, MeCN and DMSO. Such properties were quite surprising because majority of single hydroxyquinolinones suffer from very poor solubility in alcohols and other common organic solvents. The cytotoxicity screening results are summarized in Table 2. From the whole set of compounds, no significant results were obtained for lung adenocarcinoma cell line A549. On the other hand, majority of derivatives exhibited promising activity against both myelogenous and lymhoblastic leukemia. Unfortunately, only cytotoxicity against chemotherapy sensitive cells has been observed. Although it is quite tricky to search for detailed structure-activity relationship due to limited size of the chemical library, some simple conclusions can be made. For instance, derivatives bearing alkyl (methyl) instead of aryl ligand at the R^3 position (entries 4, 8, 11) do not exhibit any cytotoxicity. However, a presence of aromatic ligand in the same position do not guarantee the overall cytotoxicity. Three compounds of such kind (entries 2, 7, 17) are also inactive. The length of the spacer (3 vs. 4 carbon atoms) probably does not have a large influence on the resulting activity.

Three derivatives (Figure 24) exhibited activity in submicromolar concentration on T-lymphoblastic leukemia cell lines (CEM). Additionally, all of the three compounds exhibited good therapeutic index ranging from 50-100 (see Table 2). On the other hand, the compounds are not selective towards individual cell lines and similar results have been observed for CEM, K562 and HCT116p53W. Similarly, no significant selectivity has been observed among all of the tested compounds.

Table 2: Result of MTT cytotoxicity screening.

Entry	Code of Compound	CEM	CEM/DNR	K562	K562 tax	A549	HCT116 p53mut	HCT116 p53WT	BJ	MRC-5	Substituents			
		x	*x*	*X*	*x*	*x*	*x*	*x*	*x*	*X*	R1	R2	X	R3
1	9(3,2,1,4)	0.3	86.2	0.9	34.4	19.0	1.0	1.0	143.8	82.9	Benzyl	i-Pr	$(CH_2)_4$	2-Thiofen
2	9(1,3,1,2)	34.8	98.9	25.5	195.8	206.8	186.2	58.7	190.7	237.7	Propyl	t-Bu	$(CH_2)_4$	Me-Ph
3	9(4,1,3,2)	1.9	148.0	2.3	14.6	19.7	2.8	2.1	23.9	3.4	Ethylthiofen	Et	$(CH_2)_3$	Me-Ph
4	9(3,1,1,1)	27.8	82.9	115.3	121.9	157.2	139.9	98.5	215.3	219.3	Benzyl	Et	$(CH_2)_4$	Me
5	9(1,3,3,6)	1.7	60.5	2.0	11.6	12.6	3.3	2.5	116.2	3.7	Propyl	t-Bu	$(CH_2)_3$	MeO-Ph
6	9(3,2,3,6)	1.8	66.6	2.0	13.1	9.4	3.0	4.6	105.0	3.3	Benzyl	i-Pr	$(CH_2)_3$	MeO-Ph
7	9(1,2,3,5)	27.8	79.3	4.1	102.8	47.1	11.7	7.3	205.1	22.2	Propyl	i-Pr	$(CH_2)_3$	NO_2-Ph
8	9(1,2,1,1)	35.3	59.6	68.4	113.0	170.8	83.7	47.2	186.9	233.5	Propyl	i-Pr	$(CH_2)_4$	Me
9	9(4,3,3,6)	2.2	89.0	2.5	17.5	14.8	3.1	2.6	39.3	19.9	Ethylthiofen	t-Bu	$(CH_2)_3$	MeO-Ph
10	9(4,1,1,2)	0.6	87.4	1.7	16.4	11.7	2.6	2.1	66.1	32.6	Ethylthiofen	Et	$(CH_2)_4$	Me-Ph
11	9(1,4,1,1)	34.7	63.1	39.0	139.6	147.3	172.1	43.4	179.0	236.7	Propyl	c-pentyl	$(CH_2)_4$	Me
12	9(4,2,1,3)	6.9	111.0	9.1	180.9	162.5	20.5	18.7	237.8	231.5	Ethylthiofen	i-Pr	$(CH_2)_4$	3-Thiofen
13	9(4,3,1,5)	0.7	93.4	4.6	13.4	28.9	3.3	3.2	215.4	58.9	Ethylthiofen	t-Bu	$(CH_2)_4$	NO_2-Ph
14	9(1,1,3,2)	3.4	80.6	5.7	25.9	25.7	11.7	8.8	52.9	14.1	Propyl	Et	$(CH_2)_3$	Me-Ph
15	9(3,1,3,2)	4.9	179.5	5.7	158.3	197.6	7.7	72.9	231.7	214.3	Benzyl	Et	$(CH_2)_3$	Me-Ph
16	9(3,1,3,3)	2.2	108.1	3.0	12.1	17.1	4.0	4.8	114.7	9.9	Benzyl	Et	$(CH_2)_3$	3-Thiofen
17	9(2,3,3,3)	43.4	147.2	148.2	154.5	181.9	92.9	165.2	191.9	54.2	Hydroxypropyl	t-Bu	$(CH_2)_3$	3-Thiofen
18	9(1,2,3,3)	1.7	125.7	5.6	12.2	10.6	4.1	10.0	23.3	16.8	Propyl	i-Pr	$(CH_2)_3$	3-Thiofen

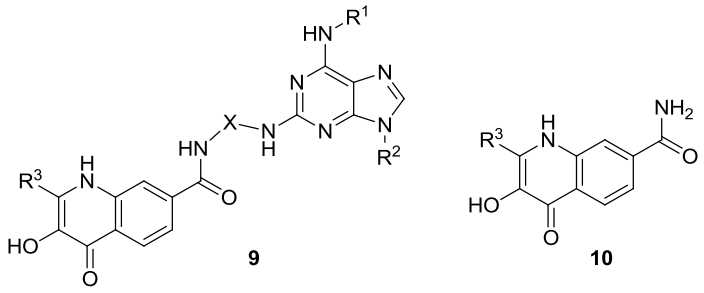
Average values of IC_{50} with SD ranging from 10 to 25%.

4.1.3 Fluorescent properties

Based on: Kamil Motyka, Barbora Vaňková, Jan Hlaváč, Miroslav Soural and Petr Funk, J. Fluoresc., 2011, 21, 2207-2212.[A2]

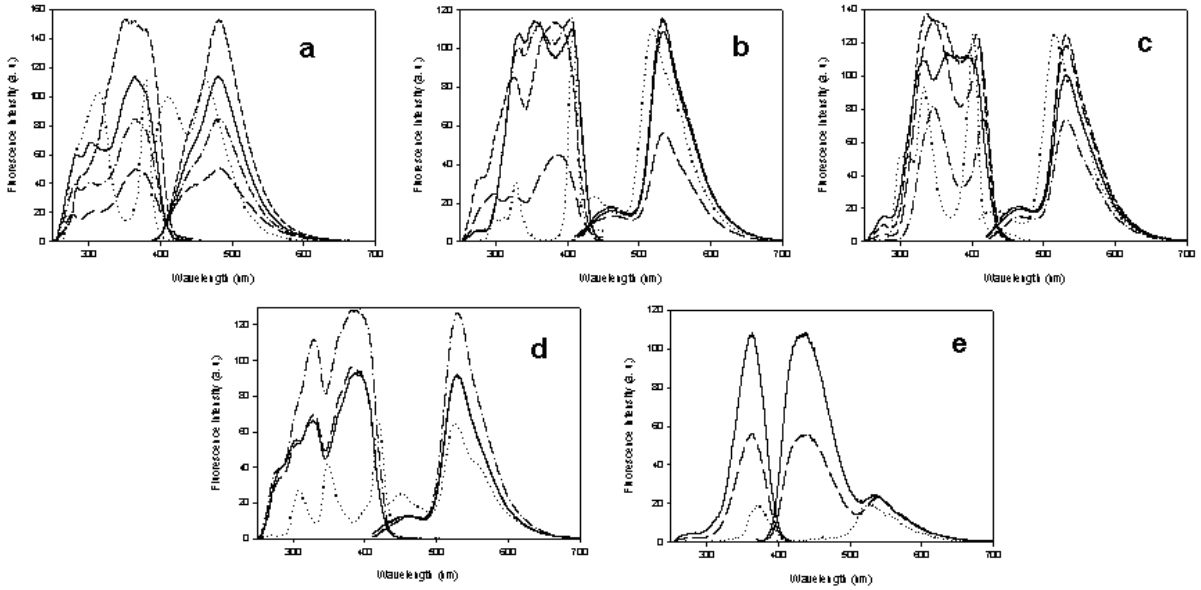
The prepared compounds were studied for their potential fluorescent properties. The emission spectra of hydroxyquinolinones usually exhibit two well-separated local maxima. For this reason, they are interesting as potential novel fluorescent labels with dual fluorescence. On the other hand, the fluorescent activity in a biomolecule system is affected by a presence of the spacer between the label and a biomolecule. In this subchapter, we briefly describe the first research of fluorescent properties of hydroxyquinolinones directly connected to natural heterocycle – purine. Recently it was found that the hydroxyquinolinones can be appended to a biomolecule *via* the spacer attached to the carboxamide group at position 6 or 7 without loss of two-band emission properties.[66] Single hydroxyquinolinones (**10**) with the same substitution in position 2 were compared with our bisheterocyclic molecules (**9**) (Figure 25). The significant comparative element was the substituent in position 2 of hydroxyquinolinone. For this research methyl-, 4-methylphenyl, 3-thienyl-, 4-nitrophenyl- and 4-methoxyphenyl- groups were used.

Figure 25: Structures for comparison of fluorescent properties.



Our synthetized bisheterocyclic molecules **9** exhibited excitation maximum in the higher range of wavelengths from 350 to 420 nm with narrow distinctive local maxima (Figure 26). In addition, some our prepared structures (compounds entry: **7**, **13** and **20**) could be excited at relatively high wavelengths around 400 nm. This information is very important for possible use in biological applications.

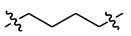
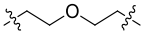
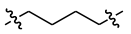
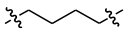
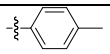
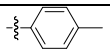
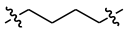
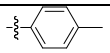
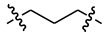
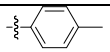
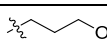
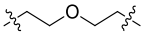
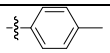
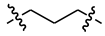
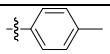
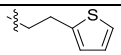
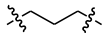
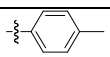
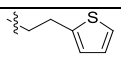
Figure 26: Excitation and emission spectra of 3HQPs in DMSO.



Each number demonstrates structure according to entry in table 1. Legend: a: (•••) 1; (—) 2; (– –) 3; (– • –) 4; (---) 5; b: (•••) 6; (– • –) 7; (---) 8; (– –) 9; (—) 10; c: (•••) 13; (– –) 14; (—) 15; (---) 16; (– • –) 17; d: (•••) 18; (—) 19; (– • –) 20; (– –) 21; e: (•••) 22; (– –) 23; (—) 24.

In general, we observed slight longer wavelength shift of dual emission spectra for hydroxyquinolinones connected with purine scaffold. In case of the 2-methyl derivatives (**9(R^1,R^2,1)** and **9(2,2,2,2)** the emission spectra lost the dual fluorescence character that means the lower wavelength maxima merged in the second local maxima and became extinguished (Figure 1a). The opposite effect was observed for 2-(4-nitrophenyl) quinolinones – the compound **10(5)** exhibited only single-band emission spectrum with the maximum at 528 nm whereas for the bisheterocycles **9(1,2,3,5)** and **9(4,3,1,5)** dual fluorescence were recorded (Figure 26e). Mostly, a decrease in the quantum yield after the introducing of the spacer-purine scaffold (with some exceptions such as compounds **9(1,2,1,1)**, **9(3,2,1,2)**, **9(3,2,2,6)**, **9(1,2,3,5)** and **9(4,3,1,5)**) was observed. An interesting positive influence of the connection of spacer-purine scaffold on the quantum yield was recorded for compounds with 2-(4-nitrophenyl) as R^3 (**9(R^1,R^2,1)**. Although the quantum yields of these 3 structures were more than 40 times higher, their value was still too low (up to 0.74 %). The ethoxyethyl as the spacer (**X = 2**) was used only for two studied compounds. However, this small tested group showed that its presence in the 3HQP molecule led to the decrease in quantum yield.

Table 3. List of investigated Structures **9** and **10**.

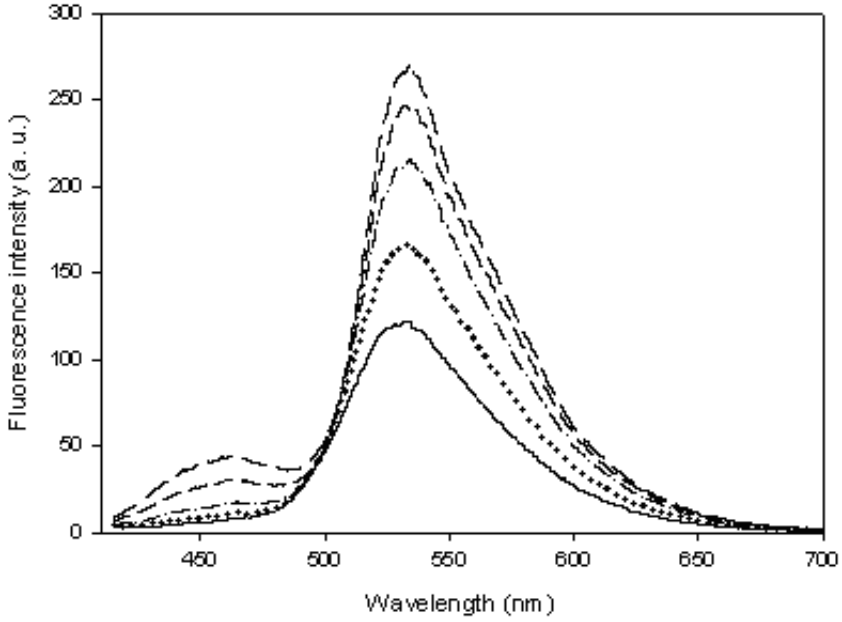
Entry	Comp.	R^3	R^1	R^2	X	φ (%)
1	10(1)		-	-	-	49.73
2	9(3,1,1,1)					22.98
3	9(3,2,2,1)					17.80
4	9(1,4,1,1)					22.93
5	9(1,2,1,1)					53.27
6	10(2)		-	-	-	35.97
7	9(3,2,1,2)					37.53
8	9(3,1,3,2)					20.94
9	9(2,2,2,2)					11.39
10	9(1,1,3,2)					34.28
11	9(4,1,3,2)					27.52
12	9(4,1,1,2)					33.19
13	10(6)		-	-	-	43.00
14	9(3,2,2,6)					47.53
15	9(2,2,3,6)					28.99
16	9(1,3,3,6)					39.77
17	9(4,3,3,6)					31.46
18	10(3)		-	-	-	24.75
19	9(3,1,3,3)					18.73
20	9(1,2,3,3)					18.60
21	9(4,2,1,3)					22.04
22	10(5)		-	-	-	0.01
23	9(1,2,3,5)					0.45
24	9(4,3,1,5)					0.74

The structures are ordered by substituent R^3.

[a]φ – fluorescence quantum yield determined with quinine sulphate in 0.5 M H_2SO_4 (φ=0.557),[106] taken as reference standard.

The effect of solvents on the emission spectra was investigated as well. Specifically, methanol, acetonitrile, dimethyl sulfoxide, ethylacetate and toluene had no significant influence to fluorescence intensity of compounds **9(1,1,3,2)**, **9(3,2,2,6)**, **9(1,2,3,3)** and **9(4,3,1,5)**. On the other hand, using of mixture DMSO/water in ratio 1:1 as a solvent significantly changed the character of emission spectra.

Figure 27. Fluorescence emission spectra of compound **10** in water-DMSO mixture of various compositions.



Legend: **(– –)** V_{aq}/V_{DMSO} = 0**; (---)** V_{aq}/V_{DMSO} = 0.07**; (– • –)** V_{aq}/V_{DMSO} = 0.23**; (•••)** V_{aq}/V_{DMSO} = 0.45**; (—)** V_{aq}/V_{DMSO} = 0.78.

In case of DMSO/water mixture as a solvent, the emission spectra lost the dual character and exhibited only one maximum at longer wavelengths. For this reason, the relationship between the fluorescence properties and water content in solvent was studied in detail. Generally, the quantum yield was decreased with increasing volume of water. Figure 27 shows the fluorescence intensity of compound **9(1,1,3,2)** in DMSO/water mixture of various composition. Possible explanations of described facts relating to the effect of the presence of water in solvent can consist of (i) hydrophobic interactions of the spacer resulting in the convolution of the hydroxyquinolinone-purines molecule and/or (ii) a protic disruption of intra as well as inter molecular hydrogen bonds of hydroxyquinolinones. In the past, the presence of hydrogen bonds was confirmed by X-ray diffraction analysis.[107]

4.2 Direct arylation of Purine on Solid Phase and Its Use for Chemical Libraries Synthesis

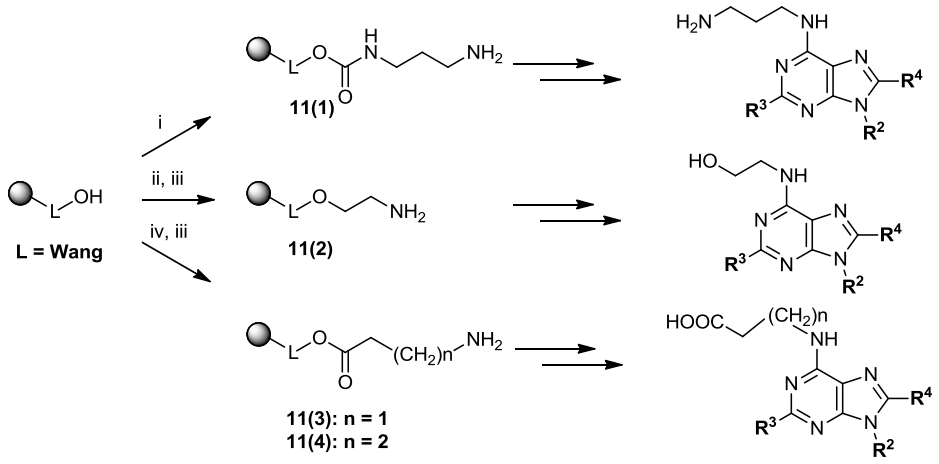
Based on: Barbora Vaňková, Viktor Krchňák, Miroslav Soural and Jan Hlaváč, ACS Comb. Sci. 2011, 12, 496-500.[A3]

This subchapter is dedicated to the preparation of tetrasubstituted purine derivatives by a decoration of the purine scaffold. The target molecules contain four diversity positions located at positions 2, 6, 8 and 9. The goal of this research was particularly to develop a method for direct arylation of the C^8 position on polymer supported purine derivatives. Our attention was not paid only to typically used aryl iodides but also the reactivity of more available aryl bromide derivatives was studied. Additionally, we focused on the preparation of trisubstituted purine derivatives with N^9-unsubstituted position. Previously, only direct arylation of N^9-substituted purines was described in literature with use of solution-phase synthesis.[82]

4.2.1 Synthesis on Wang resin

Building blocks used to introduce substituents R^1 – R^4 are portrayed in Figure 29. Notation of individual compounds is based on used building blocks. The synthesis was carried out on Wang resin using three different types of anchoring to demonstrate the resulting diversity in position 6 of the purine scaffold: 1,3-diaminopropane was attached via carbamate linkage using the carbonyldiimidazole (CDI) activation method.[108] 2-(Fmoc-amino)ethanol (Fmoc = 9-fluorenylmethyloxycarbonyl) was linked via an ether structure using trichloroacetimidate activation[109] and two protected Fmoc amino acids (Fmoc-β-Ala-OH and Fmoc-γ-ABU-OH) were immobilized via an ester bond by the Mitsunobu procedure[30] according to Scheme 19.

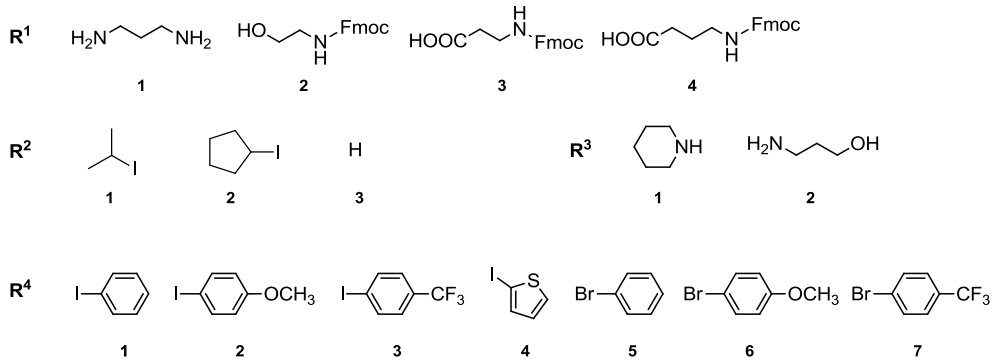
Scheme 19: Methods of amino derivative immobilization[a]



[a]Reagents and conditions: (i) CDI, pyridine, DCM, 2 h, rt, then 1,3-diaminopropane, DCM, 2 h, rt; (ii) trichloroacetonitrile, DBU, DCM, 2 h, rt, then Fmoc-aminoethanol, $BF_3 \cdot Et_2O$, THF 1 h, rt; (iii) 20% piperidine in DMF, 20 min, rt; (iv) Fmoc-amino acid, PPh_3, DIAD, anhydrous THF, 1 h, rt.

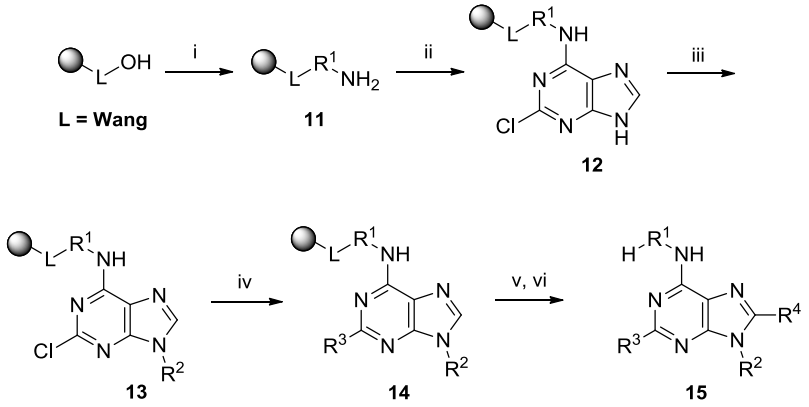
After the immobilization, the resin-bound primary amines **11** were regioselectively arylated with 2,6-dichloropurine to give the intermediates **12** (Scheme 20). In the next step, the purine N^9 was alkylated with alkyl iodide in DMSO at elevated temperature (50°C) in a presence of diazabicyclo[5.4.0]undec-7-ene (DBU). We used isopropyl iodide and cyclopentyl iodide as model compounds. We have already shown that this alkylation is compatible with other alkyl iodides[A1] and furnished alkyl intermediates **13** of high purity.

Figure 29: Building blocks used for the preparation of the final structures.



The subsequent substitution of the chlorine atom at purine C^2 required high temperature (150°C) to proceed. The reaction was carried out with 2 M solution of piperidine or 3-aminopropanol in diethylene glycol diethyl ether and the intermediates **14** were obtained in an excellent purity. Unfortunately, the harsh conditions needed for this reaction step led to decrease of the yield of derivates **14(3,R^2,1)** and **14(4,R^2,1)** due to aminolysis of ester bond and partial intermediates cleavage from the resin. For this reason, the final products **15(3,R^2,1,R^4)** and **15(4,R^2,1,R^4)** were isolated in yields only 7 - 25% (see Table 4).

Scheme 20: Preparation of the target purines[a].



[a]Reagents and conditions: (i) according Scheme 19 (ii) 2,6-dichloropurine, EDIPA, THF, 50°C, 14 h; (iii) alkyl iodide, DBU, DMSO, 50 °C, 14 h (iv) piperidine or 3-aminopropanol, diethylene glycol diethyl ether, 150°C, 24 h; (v) aryl halide, CuI, $Pd(OAc)_2$, piperidine, anhydrous DMF, 115°C, 24 h. (or 48 h for aryl bromides); (vi) 50% TFA in DCM, 1 h, rt.

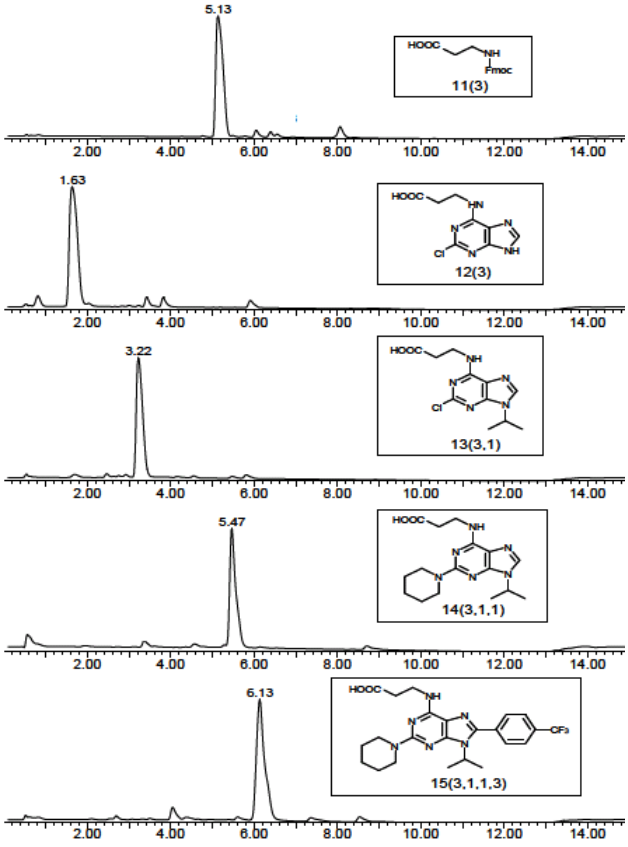
Direct C^8-H arylation of 2,6,9-trisubstituted purines

The C^8-H arylation was tested with use of phenyl iodide, palladium acetate and copper (I) iodide under various conditions (type of base, concentration of aryl iodide, temperature and solvent were varied). First, we tested the direct arylation of the intermediate **14(1,1,1)** in anhydrous DMF and anhydrous dioxane using Cs_2CO_3 as the base. However, the reaction did not furnish satisfactory conversion (max. about 60%, HPLC-UV traces) although temperatures from 90°C to 110°C and various concentrations of aryl iodide were tested. In addition, complete removal of Cs_2CO_3 from the resin was problematic. This problem was solved by addition of piperidine. The reaction was tested in anhydrous DMF and anhydrous dioxane at elevated temperature (90-115°C) and various concentrations of aryl iodides. While the direct arylation in anhydrous dioxane provided maximally 5% of product (HPLC-UV traces), substantially better results were obtained in anhydrous DMF with 0.8 M of PhI solution at 115°C after 24 hours, when the arylation was quantitative and we obtained product **15(1,1,1,1)** in very good purity (94%, HPLC-UV traces). Decreasing the concentration of aryl iodide to 0.2 M or 0.4 M under the same reaction conditions lowered the purity to 80%, and the reaction had to be repeated to drive it to completion.

The C^8-H arylation of 2,6,9-trisubstituted purines using the developed procedure (0.8 M of PhI solution, CuI, piperidine, $Pd(OAc)_2$, anhydrous DMF, 115°C, 24 h) was very effective. The protocol was successfully tested for unsubstituted phenyl iodide, and phenyl

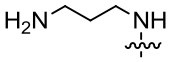
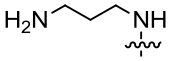
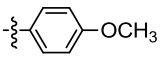
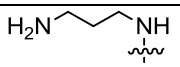
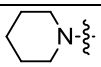
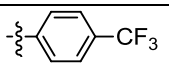
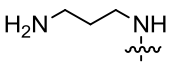
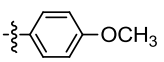
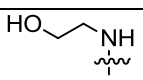
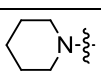
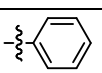
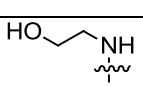
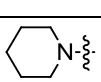
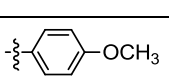
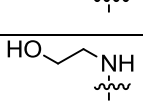
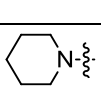
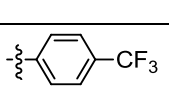
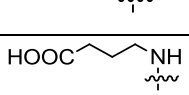
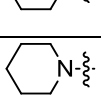
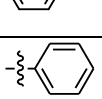
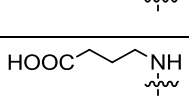
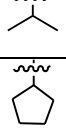
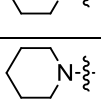
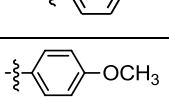
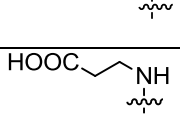
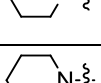
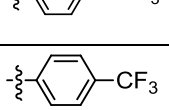
iodides substituted by electron-donating as well as electron-withdrawing functional groups (see Table 4, entries 1-10). Building blocks are depicted in Figure 29. The purity of crude products was excellent after each reaction step (see example in Figure 30), except for derivatives substituted on position 2 by 3-amino-propan-1-ol, where the reaction mixture contained a number of side products(see Table 4, entries 11 and 12).

Figure 30: Example of HPLC traces of crude intermediates leading to target compound **15(3,1,1,3)**. The analyses were performed after cleavage from resin with trifluoroacetic acid. The intermediate **11(3)** was analyzed before removing the Fmoc group.



The overall yields of the corresponding products **5** after cleavage from the resin and semipreparative HPLC purification varied from 7-70% (see Table 4). As mentioned before, derivatives **15(3,R^2,1,R^4)** and **15(4,R^2,1,R^4)** were obtained in lower yield due to partial cleavage from the resin during the displacement of chlorine with amines (Scheme 20, step iv) and arylation affording derivatives **15(R^1,R^2,2,R^4)** was accompanied with number of side products.

Table 4. Summary of the prepared products.

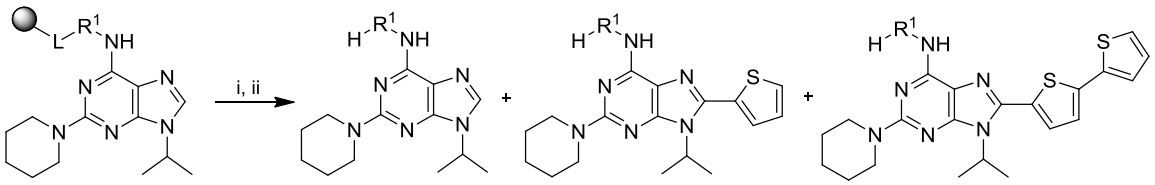
Entry	Code of compound	H-R[1]	R[2]	R[3]	R[4]	Crude purity[a] (%)	Yield[b] (%)
1	15(1,1,1,1)					94	61
2	15(1,1,1,2)					95	52
3	15(1,1,1,3)					90	70
4	15(1,2,1,2)					80	33
5	15(2,1,1,1)					90	42
6	15(2,1,1,2)					91	35
7	15(2,1,1,3)					91	52
8	15(4,1,1,1)					96	7
9	15(4,2,1,2)					91	15
10	15(3,1,1,3)					92	25
11	15(1,1,1,4)					42	15
12	15(1,1,1,bis4)					37	12
13	15(1,1,2,1)					61	16
14	15(1,1,2,3)					62	18
15	15(2,3,1,3)		H			71	21
16	15(2,3,1,1)		H			60	20
17	15(1,3,1,1)		H			58	14

[a]Purity of crude final product; integrated HPLC-UV traces.

[b]Overall yields of compounds 1**5** after the all reaction steps and HPLC purification.

Coupling reaction of derivative **14(1,1,1)** with 2-iodothiophene as representative heterocycle (0.8 M 2-iodothiophene; CuI, $Pd(OAc)_2$, piperidine, anhydrous DMF, 115 °C, 24 h.) yielded 35% of the product **15(1,1,1,4)** accompanied by 10% of a side product **15(1,1,1,bis4)** and 55% of starting material (HPLC-UV traces). Repeating the arylation increased the yield of the desired product **15(1,1,1,4)** as well as side product **15(1,1,1,bis4)** (see Table 4), but the starting material **14(1,1,1)** was still incompletely transformed. Optimization of the reaction conditions by varying 2-iodothiophene concentration and temperature did not improve the yield of the product **15(1,1,1,4)**. The mono **15(1,1,1,4)** and double **15(1,1,1,bis4)** arylated compounds were isolated and their structure was confirmed by NMR spectroscopy and HRMS. The arylation of a substrate **14(2,1,1)** with 2-iodothiophene yielded an analogous mixture of products (see Scheme 21).

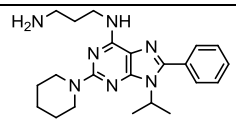
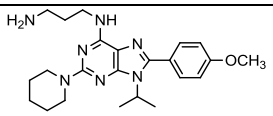
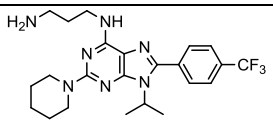
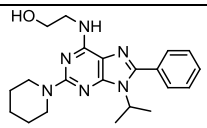
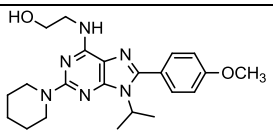
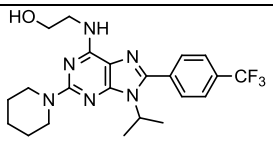
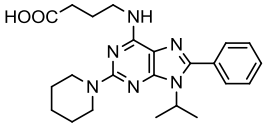
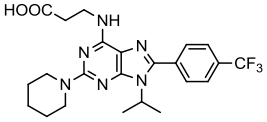
Scheme 21: C^8-H arylation with 2-iodothiophene[a].



[a]Reagents and conditions: (i) 2-iodothiophene, $Pd(OAc)_2$, CuI, piperidine, anhydrous DMF, 115°C, 24 h. (ii) 50% TFA in DCM, 1 h.

In addition to aryl iodides (method A) we also successfully tested commercially available cheaper aryl bromides (method B), such as 4-methoxyphenyl bromide, 4-trifluoromethylphenyl bromide and phenyl bromide. Due to their lower reactivity, the reaction time was prolonged to 48 hours but other reaction conditions were identical giving the final products in purity ranging from 90 to 95% according to HPLC-UV (see Table 5). Due to a longer reaction time for aryl bromides, versus aryl iodides, all preparative reactions in this study were performed with aryl iodides (method A).

Table 5: Comparison of preparation methods.

Entry	Code of structure	Target structure of purine	Method[a]	Purity[b]
1	15(1,1,1,1)		**A**	94%
2			**B**	98%
3	15(1,1,1,2)		**A**	95%
4			**B**	95%
5	15(1,1,1,3)		**A**	90%
6			**B**	98%
7	15(2,1,1,1)		**A**	90%
8			**B**	87%
9	15(2,1,1,2)		**A**	91%
10			**B**	80%
11	15(2,1,1,3)		**A**	91%
12			**B**	76%
13	15(4,1,1,1)		**A**	96%
14			**B**	90%
17	15(3,1,1,3)		**A**	92%
18			**B**	90%

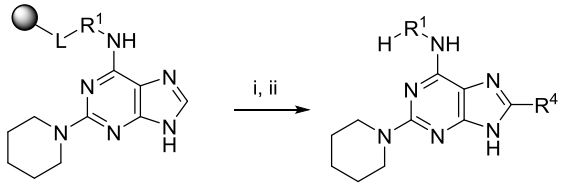
[a]Reaction time: Method A: 24 h.; Method B: 48 h.;

[b]Purity of crude final product, integrated HLPC-UV traces.

Direct C^8-H arylation of 2,6-disubstituted purines

We also tested C^8-H arylation of N^9-unsubstituted purines (Scheme 22), which can be subsequently converted to C^8-modified purine nucleosides. Unfortunately, the presence of the acidic hydrogen on purine N^9 decreased reactivity towards arylation. A very low conversion (about 10%, HPLC-UV traces) was observed under the previously-described conditions. Increasing the concentration of aryl iodide or piperidine or both did not improve the conversion (only 10% to 20%).

Scheme 22: Preparation of N^9-unsubstituted compounds[a].



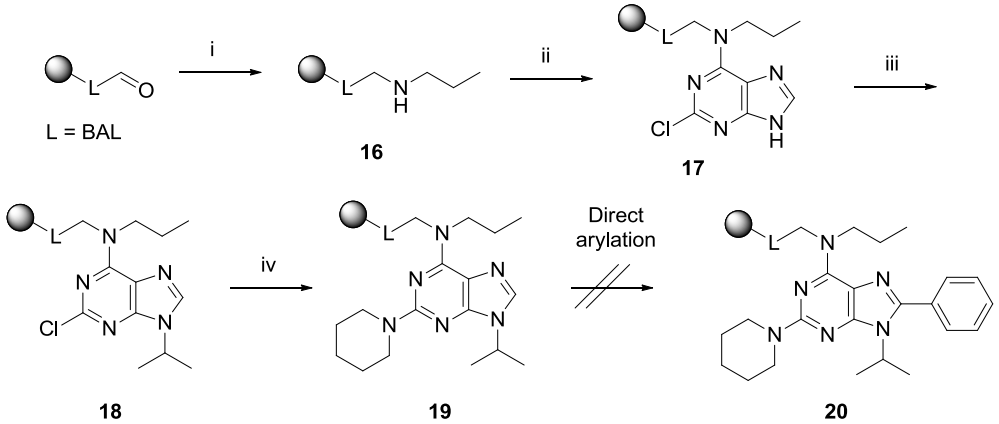
[a]Reagents and conditions: (i) aryl iodide, CuI, $Pd(OAc)_2$, piperidine, anhydrous DMF, 135 °C, 48 h; (ii) 50% TFA in DCM, 1 h, rt.

When higher temperature was used (135°C), the arylation proceeded significantly better. However, we did not observe quantitative reaction; the best purity (71%) was achieved for derivative **15(2,3,1,3)** (HPLC-UV traces). For this reason N^9-unsubstituted purines were isolated after HPLC purification in only 14 to 21% yield.

4.2.2 Synthesis on aminomethylated polystyrene resin

The C^8 direct arylation was simultaneously studied on different types of resin. For this purpose, the similar model of trisubstituted purine was prepared on aminomethyl polystyrene resin equipped with BAL linker according to scheme 23. The final step – a direct arylation - was performed under the same reaction conditions as for compound **14**. Unexpectedly, the arylation did not work.

Scheme 23: C^8 arylation on aminomethylated resin.



[a]Reagents and conditions: (i) propylamine, 10% AcOH/DMF, on, rt then $NaBH(OAc)_3$, 4 h, rt; (ii) 2,6-dichloropurine, EDIPA, THF, 50°C, on; (iii) *i*-propyl iodide, DBU, DMSO, 50°C, 14 h., (iv) piperidine, diethylene glycol diethyl ether, 150°C, 24 h.

As in the previous case, we tested various concentration of aryl iodide, type of base (piperidine or cesium carbonate), different reaction temperature, reaction time and type of solvent. It was very surprising that absolutely no reaction was observed. HPLC-MS analysis always indicated only starting material without any impurities or side products. Because of the lower reactivity, the aryl bromides were not tested.

4.2.3 Results of biological screening

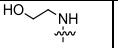
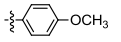
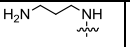
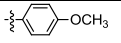
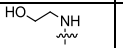
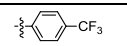
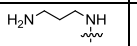
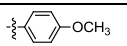
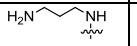
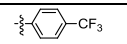
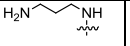
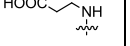
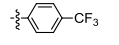
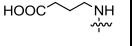
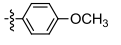
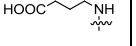
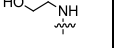
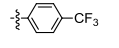
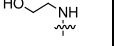
We developed method for direct arylation of purine scaffold on solid support suitable for chemical libraries synthesis. The target compounds contain 4 diversity positions. We used 4 different attachments to the Wang resin (**R^1**): 1,3-diaminopropane, two Fmoc protected amino acids and 2-(Fmoc-amino)ethanol. The N^9 position (**R^2**) was alkylated with use of 2 different alkyl iodides. Following substitution in position 2 (**R^3**) was performed with primary and secondary amines. The final position **R^4** was modified by unsubstituted phenyl and phenyl substituted by electron-withdrawing and electron-donating functional group. Full combination of all successfully tested building blocks would give 72 compounds. However, only small collection of 18 model compounds was prepared and 16 derivatives were subjected to the MTT cytotoxic test to explore biological properties of target molecules. Results of biological screening are summarized in table 6.

In general, significant anticancer activity has been detected for majority of tested derivatives. The results of biological screening indicate that cytotoxicity of target compounds is influenced particularly by C^6 purine substitution. Compounds with hydroxyethyl ligand (entries 1, 3, 10, 11, 15) exhibited medium or low activity with only one exception: compound **15(2,3,1,3)** gave IC_{50}~1mmol for CEM line. Two model compounds with terminal carboxylic group (entries 8 and 9) did not give any promising results. The best activity was detected in case of compounds with aminoalkyl C^6 substitution. Interestingly, no further structure-activity relationship was observed within this group of compounds, i.e. their activity was not influenced by neither N^9 or C^8 variable substitution (for demonstration, entries 4, 5 and 6 can be compared).

The only significant selectivity of individual compounds towards specific cell lines was observed in case of compound **15(2,3,1,3)**. Its cytotoxicity against CEM is approximately 10-20 times higher in comparison to other tested cells. In addition, the same compound exhibited the best therapeutic index (TI=50), but selectively only for CEM. Unfortunately, the other active derivatives showed high toxicity against normal tissues.

All active compounds were subjected to advanced biological tests. A detailed structure-activity relationship study could not be performed due to limited amount of data, also, larger chemical library would have to be synthesized.

Table 6: Results of MTT cytotoxic test.

Entry	Compound	CEM	CEM - DNR	K562	K562 - TAX	A549	HCT116 p53WT	HCT116 p53MUT	BJ	MRC-5	Substituents			
		x	X	x	*x*	x	*x*	x	x	*x*	R^1	R^2	R^3	R^4
1	15(2,1,1,2)	98.7	100.0	88.7	100	97.5	100.0	100.0	100.0	75.2				
2	15(1,1,1,2)	3.1	1.8	1.1	1.1	3.7	2.8	2.6	4.0	3.3				
3	15(2,1,1,3)	88.9	100.0	73.9	100.0	99.6	100.0	95.5	100.0	45.3				
4	15(1,2,1,2)	1.9	3.4	1.6	1.2	3.6	3.7	2.8	36.0	3.9				
5	15(1,1,1,3)	1.0	1.4	1.5	0.97	3.2	1.2	1.4	3.9	4.0				
6	15(1,1,1,1)	1.9	3.3	1.2	3.5	4.0	3.4	3.7	4.0	4.1				
7	15(3,1,1,3)	14.4	18.5	16.8	16.1	19.5	19.0	18.7	49.6	43.9				
8	15(4,2,1,2)	16.0	37.4	20.9	18.5	27.1	26.7	18.1	63.2	67.5				
9	15(4,1,1,1)	37.9	59.9	45.7	48.3	63.6	55.0	42.9	90.8	80.1				
10	15(2,3,1,3)	1.3	11.9	7.6	11.2	21.5	20.0	25.1	46.4	63.1		H		
11	15(2,3,1,1)	10.4	15.3	8.3	14.7	18.4	31.5	15.1	14.6	56.3		H		
12	15(1,3,1,1)	6.4	12.1	2.8	8.7	12.9	6.0	6.9	10.1	14.1		H		
13	15(1,1,1,4)	1.5	1.5	0.99	0.98	3.9	2.1	2.0	4.3	3.4				
14	15(1,1,1,bis4)	2.8	3.7	1.9	3.5	4.0	4.4	3.6	4.3	3.8				
15	15(2,1,1,1)	17.9	35.2	18.8	45.5	43.1	22.3	45.3	71.5	36.4				
16	15(1,3,1,2)	2.8	4.3	1.2	3.6	3.8	1.3	3.6	3.5	3.6		H		

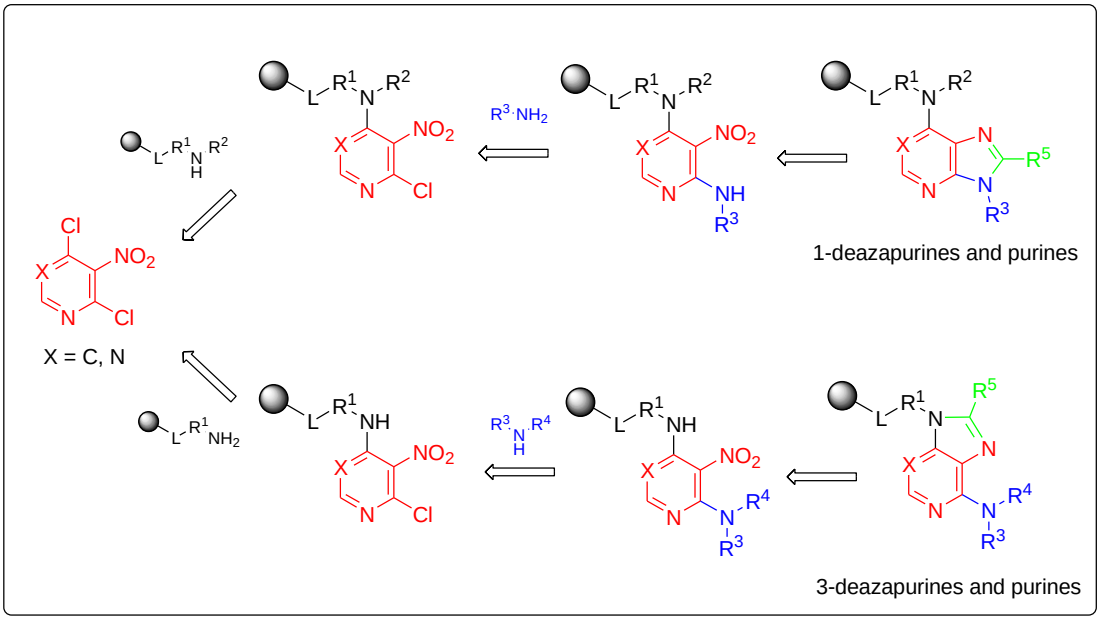
Average values of IC_{50} with SD ranging from 10 to 25%.

4.3 Solid-Phase Synthesis of Purines and Deazapurines

Based on: Barbora Lemrová and Miroslav Soural, unpublished results.

To demonstrate the versatility of solid-phase synthesis we were focused on the development of heterocyclization methods for the preparation of novel substituted purine and deazapurine derivatives. The key starting material for this purpose was 4,6-dichloro-5-nitropyrimidine for the preparation of purines and 2,4-dichloro-3-nitropyridine for the deazapurine synthesis (figure 31). The basic philosophy of our suggested approach consisted in simple possibility to prepare various isomers of target compounds by suitable and selective combination of building blocks (primary vs. secondary amines, polymer supported vs. solution-phase amines). A significant contribution consists in the case of target purines preparation: the method should allow very simple access to derivatives that contain substituted alkyl chain in position N^9. Preparation of N^9-alkylated purines is well known from both solid-phase and solution-phase synthesis.[13] In contrast, methods that enable incorporation of alkyl chain with terminal functional group in N^9 position of purine scaffold (such as carboxylic group, hydroxyl group or amino group) have been described very rarely probably due to the need of suitable protection of the appropriate reagents. In our case, the polymer support serves as the protective group thus, number of various functional groups can be chosen depended on well-known procedures from solid-phase peptide synthesis. General synthetic route leading to target compounds and their isomers is depicted in Figure 31.

Figure 31: Possible preparation of purine and dezapurine derivatives and their isomers



4.3.1 Synthesis

In this part of the research, three common polystyrene resins (Wang resin, Rink resin and aminomethyl resin) were used (Scheme 24). The starting material for target compounds were polymer supported primary or secondary amines (depending on the type and constitution of the target derivative or isomer, see Figure 31). For the preparation of resin-bound primary amines the following strategies can be used (some of them have already been described in previous chapters but we repeat them herein for better orientation of the reader):

A) Wang resin allows three different attachments of primary amines: (i) suitably protected aminoalcohols (such as 2-(Fmoc-amino)ethanol) can be linked *via* an ether bond; (ii) Fmoc amino acids (such as Fmoc-β-Ala-OH)) can be immobilized *via* an ester bond; (iii) diamines (e.g. 1,3-diaminopropane) can be attached via carbamate linkage. The target derivatives contain substituted alkyl chain in position N^9 or N^6 where the terminal functional group refers to the type of attachment used (ether~hydroxyl, ester~carboxyl and carbamate~amino group).

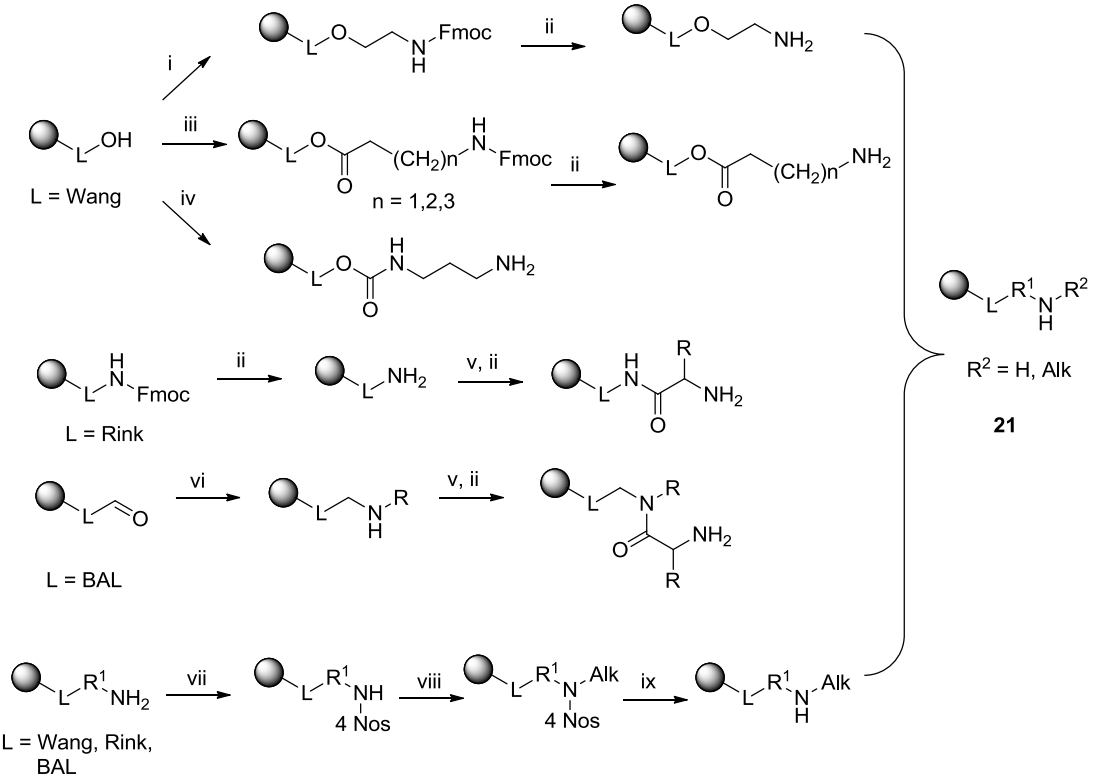
B) Rink amide resin can be used for direct reaction with 4,6-dichloro-5-nitropyrimidine or 2,4-dichloro-3-nitropyridine to give *N*-unsubstituted compounds. Alternatively, acylation with Fmoc-amino acids can be done to prepare appropriate purines or deazapurines with *N*-unsubstituted alkylcarboxamide in position N^9 or N^6.

C) Aminomethyl polystyrene resin equipped with benzaldehyde BAL linker can be after immobilization of primary amines acylated with Fmoc-amino acids which can lead to purines or 3-deazapurines with *N*-substituted alkylcarboxamides in N^9 or N^6 position.

Except polymer supported primary amines also resin-bound secondary amines can be applied for the preparation of selected target compounds. An example has been already given in the previous paragraph (entry C) in which preparation of polymer supported secondary amines with use of BAL linker is mentioned. Alternatively, variable resin-bound primary amines can react with 4-nitrobenzenesulfonyl chloride which followed by alkylation and deprotection of 4-nosyl group, gives polymer supported secondary amines.

All possible attachments described above are depicted in Scheme 24, the subsequent synthetic pathway leading to target molecules is demonstrated in Scheme 25.

Scheme 24: Various strategies and methods for the preparation of immobilized amines.[a]

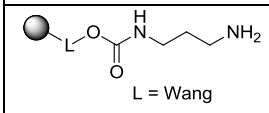
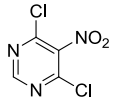
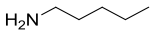
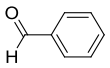
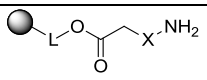
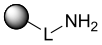
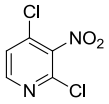
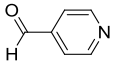
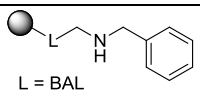


[a]Reagents and conditions: (i) trichloroacetonitrile, DBU, DCM, 2 h, rt, then Fmoc-aminoethanol, $BF_3 \cdot Et_2O$, THF 1 h, rt; (ii) 20% piperidine in DMF, 20 min, rt; (iii) Fmoc-amino acid, HOBt, DIC, DMAP, DMF, DCM, rt, on; (iv) CDI, pyridine, DCM, 2 h, rt, then 1,3-diaminopropane, DCM, 2 h, rt; (v) Fmoc-amino acid, HOBt, DIC, DMF, DCM, rt, on; (vi) amine, 10% AcOH/DMF, on, rt, then $NaBH(OAc)_3$, 4 h, rt.; (vii) 4-nitrobenzensulfonyl

chloride, lutidine, DCM, rt, on; (viii) alkyl iodide, DBU, DMF, rt, on; (ix) mercaptoethanol, DBU, DMF, rt, 5 min.

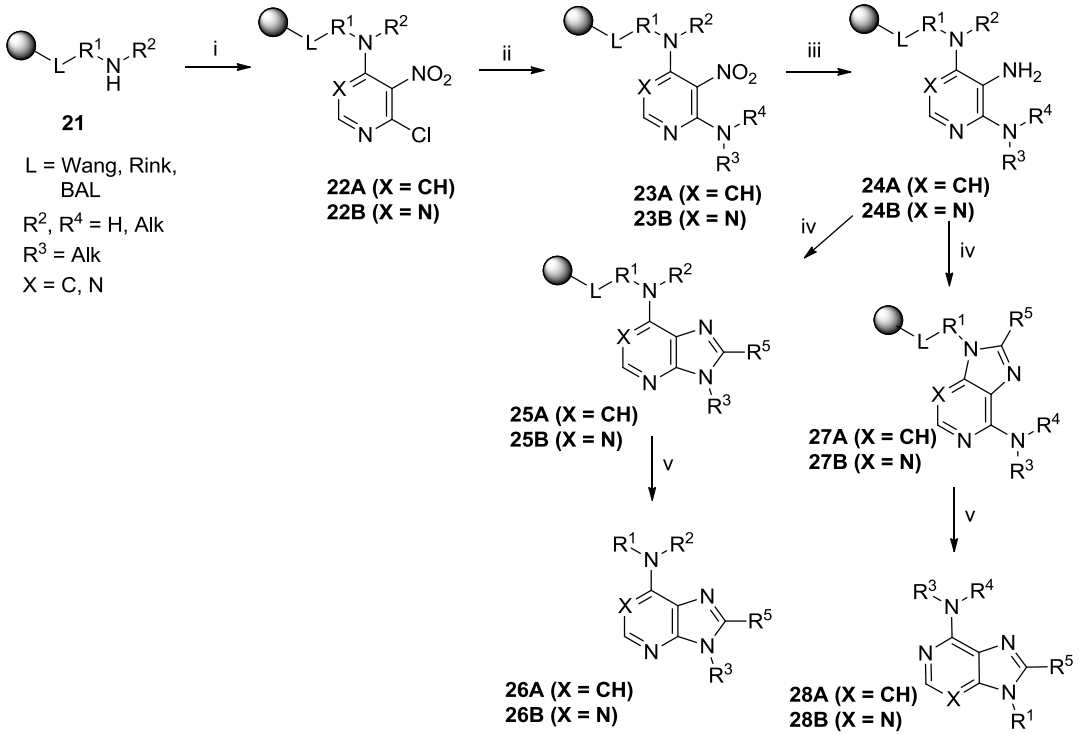
Such number of attachments allows the preparation of many diverse purine and deazapurine derivatives. To evaluate the applicability of our method we initially tested Wang resin with immobilized 1,3-diaminopropane and Fmoc-amino acids (Fmoc-Ala-OH and Fmoc-β-Ala-OH). Rink resin was used for direct arylation with 2,4-dichloro-3-nitropyridine followed by the preparation of 1-dezapurine and 3-deazapurine derivatives. Aminomethyl resin with BAL linker was also tested; in this case the starting secondary amine was formed by immobilization of benzylamine. All building blocks and resins for testing reactions are reported in table 7.

Table 7: Resins and reagents used for study of purine and deazapurine preparation.[a]

Immobilized amine	Arylation	Solution-phase amine	Aldehydes
 L = Wang **21(1)**		 **1**	 **1**
 L = Wang **21(2)**			
 L = Rink **21(3)**		 **2**	 **2**
 L = BAL **21(4)**			

[a]All prepared target structures **26** and **28** were termed according this table in order (attachment, used solution-phase amine, aldehyde).

Scheme 25: Solid-phase synthesis of target purine and deazapurine derivatives.[a]



[a]Reagents and conditions: (i) 4,6-dichloro-5-nitropyrimidine or 2,4-dichloro-3-nitropyridine, EDIPA, DMF, rt, on; (ii) 10% primary or secondary amine in DMSO, rt, 3 h.; (iii) tin (II) chloride dihydrate, EDIPA, DMF, rt, on; (iv) aldehyde, DMSO, 80°C, on; (v) 50% TFA in DCM, rt, 1 h.

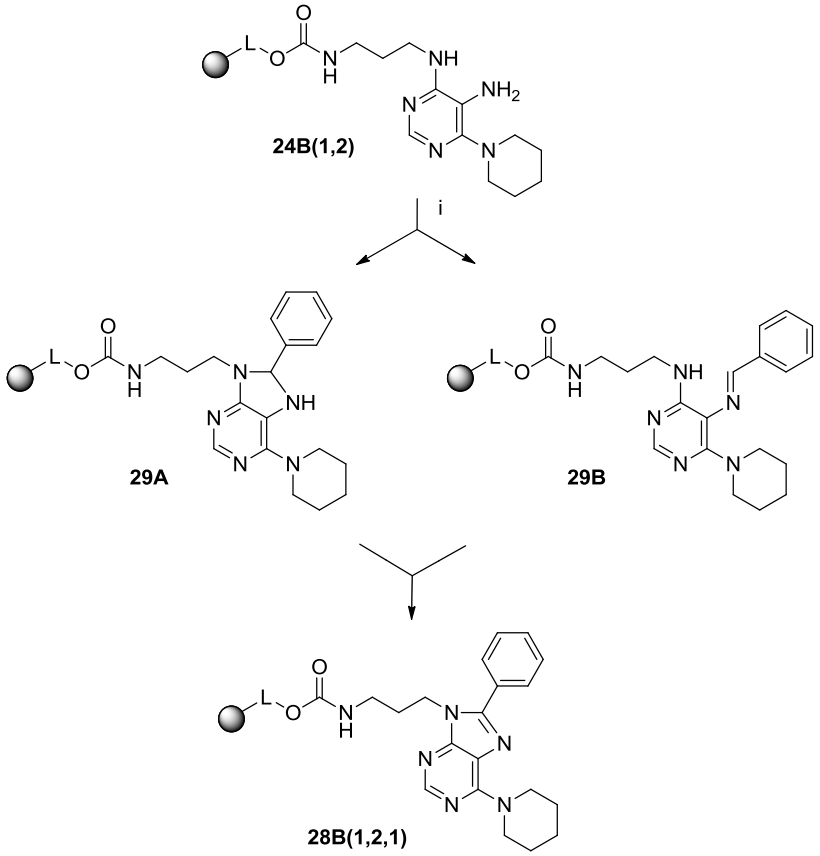
Preparation of purine derivatives

Solid-phase synthesis of purines was studied on Wang resin with immobilized 1,3-diaminopropane *via* carbamate linkage. After the preparation of the intermediate **21**, the resulting primary amine was arylated with 0.5 M solution of 4,6-dichloro-5-nitropyrimidine in a presence of base (EDIPA) in DMSO. The aryl reagent exhibited high reactivity and no starting material was observed after testing reaction with FmocOSu. Subsequently, the second chlorine atom was substituted by model secondary amine (piperidine in our case). The reaction was carried out with 10% solution of amine in DMSO for 3 hours at room temperature. In the next step, the nitro group was reduced by tin (II) chloride dihydrate.

The last step on solid support was cyclization reaction with aldehydes. We tested benzaldehyde under various reaction conditions. Firstly we used mild condition (0.2 M concentration of benzylaldehyde in DCM or DMF at room temperature) but the reaction was not completed and we observed some remaining starting material. In addition, the target product was accompanied by a compound of 2 units higher molecular weight.[110] Such

molecular weight corresponds either to dihydro-3-deazapurine (**29A**) or Schiff´s base intermediate (**29B**) (Scheme 26).

Scheme 26: Possible cyclization of 3-deazapurine.[a]



[a]Reagents and conditions: (i) benzaldehyde, DMSO, 80°C, on.

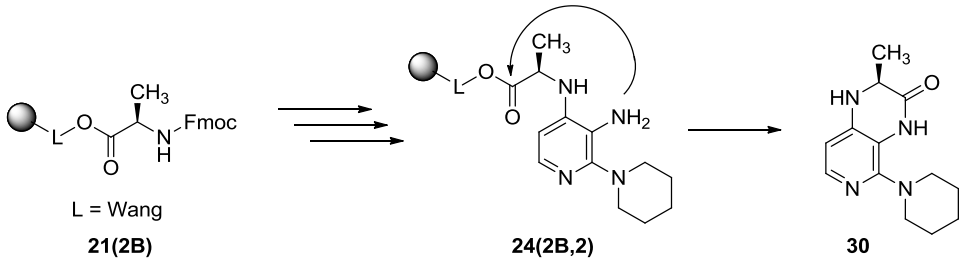
To obtain the final scaffold, we increased the concentration of benzaldehyde to 0.5 M solution and we performed the reaction at the same conditions (i.e. room temperature, overnight), however we obtained a mixture of product and the side product again. Subsequently we tested higher temperature from 40 – 80°C and also DMSO as an alternative solvent. Finally, the complete conversion and crude purity 90% of the product **28B(1,2,1)** was observed after the reaction with 0.5 M benzaldehyde in DMSO at 80°C for 16 hours (overnight). The compound **28B(1,2,1)** was prepared only in analytical amount and its structure was analyzed by HPLC-MS analysis. Subsequently, we used the same reaction conditions for the preparation of similar derivative **28B(1,2,2)** in a preparative amount (Table 8). The cyclization reaction was carried out with isonicotinaldehyde and we obtained the target compound with crude purity 92%. The compound **28B(1,2,2)** was isolated and the structure was confirmed by NMR spectra.

Preparation of deazapurine derivatives

For the preparation of 1-deazapurines and 3-deazapurines, Wang resin with immobilized Fmoc-amino acids was studied most intensively. We attached Fmoc-Ala-OH and Fmoc-β-Ala-OH to the Wang resin via ester bond and the entire reaction sequence was performed. After the deprotection of Fmoc protective group the primary amine was arylated with 2,4-dichloro-3-nitropyridine. Due to a similar reactivity of both chlorine atoms, the reaction was not fully regioselective and two isomers formed in a ratio approximately 8.5 : 1.5 (calculated from HPLC traces). This minor complication was also observed when other solid supports (aminomethyl resin with BAL linker and Rink resin) were used. According to the literature, the chlorine atom in position 4 should be more reactive, therefore we expected the major product to be immobilized via this position.[111,112]

Following reactions - substitution with piperidine, reduction step and cyclization with benzaldehyde - were performed under the same reaction conditions as for the purine derivatives. Unfortunately, in the case of Fmoc-Ala-OH, the resulting intermediate was released from the resin after the reduction step by the cyclative cleavage. This cyclization method on Wang resin was described for the preparation of dihydroquinoxalinone derivatives,[113]. For this reason, immobilization of α-amino acids via ester bond is not generally applicable for our purposes (Scheme 26).

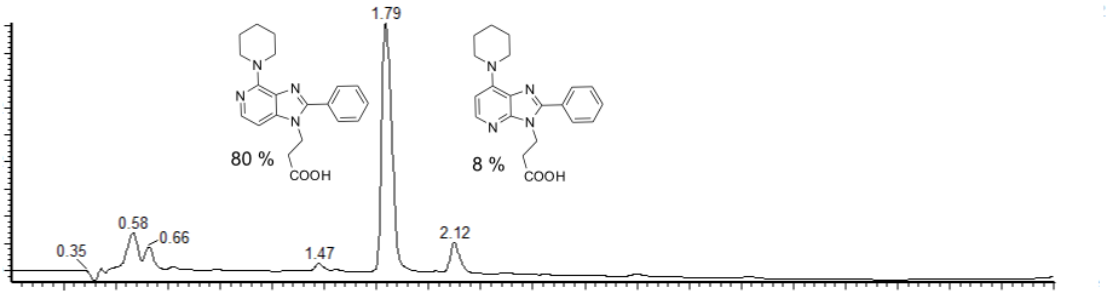
Scheme 26: Use of α-amino acids leading to cyclative cleavage.



When amino acid with a longer chain (Fmoc-β-Ala-OH) was used, the cyclative cleavage did not take place and the corresponding intermediate was obtained after subsequent acidic cleavage from the resin. In such case, the reaction sequence was performed in a preparative scale and the target product **28A(2,2,1)** was obtained in very good crude purity 80%. In addition, HPLC-UV traces of the crude mixture shown both isomers, which exhibited suffition difference of retention time suitable for their simple separation with semi-

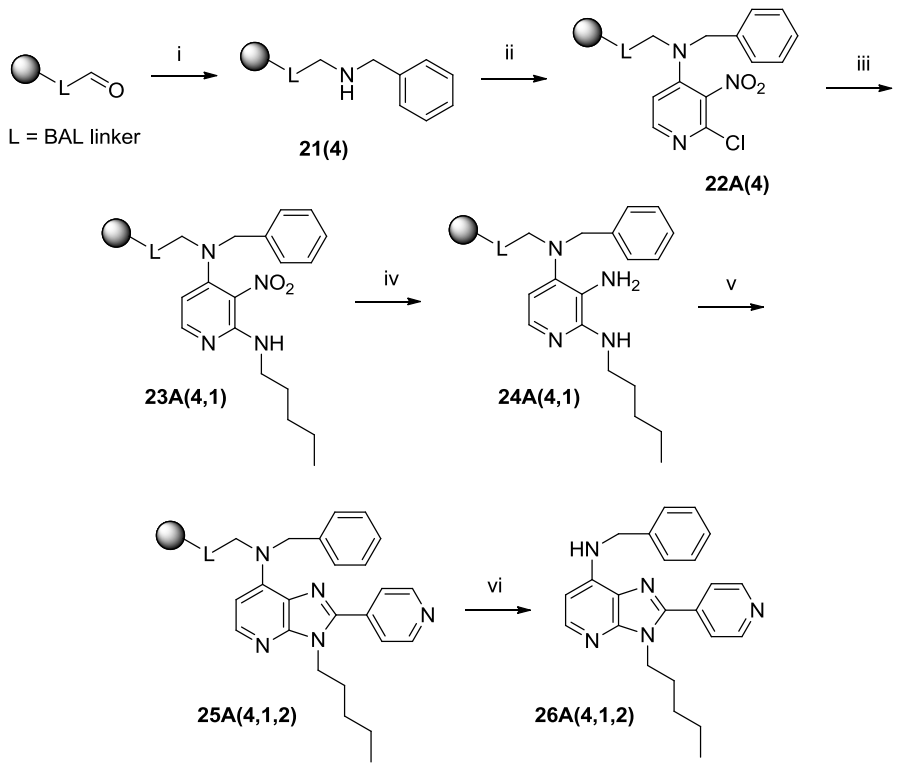
preparative reverse phase chromatography (Figure 32). Both of these compounds were isolated and characterized (Table 8).

Figure 32: HPLC-UV traces of compounds **28A(2,2,1)** and **26A(2,2,1)** after final cleavage from resin.



The use of aminomethyl resin equipped with BAL linker was also tested. In this case we used a combination of polymer-supported secondary amine (benzylamine) and solution-phase primary amine (pentylamine) to prepare the target 1-deazapurine. Surprisingly, in this case the substitution with pentylamine required higher temperature and longer reaction time to proceed (120°C, overnight) although the same raction on Rink resin was carried out under mild conditions (Scheme 28).

Scheme 27: Preparation of deazapurine derivatives with use of BAL linker.[a]



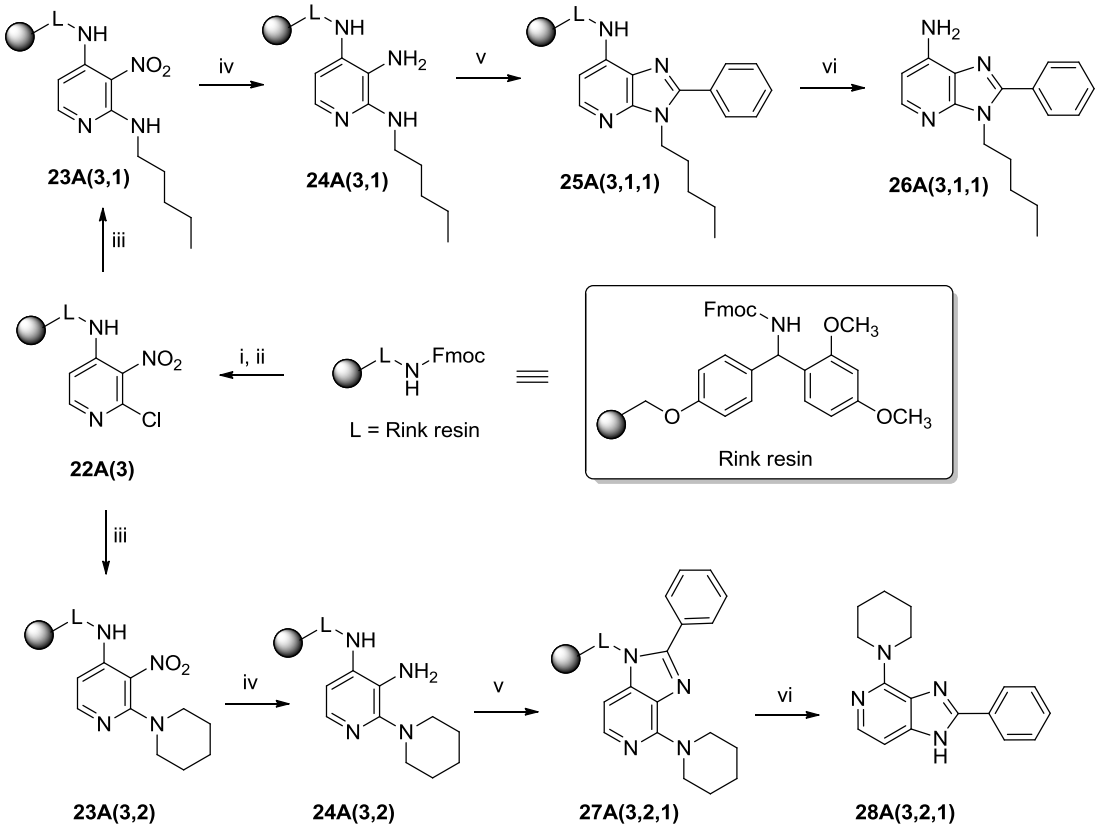
[a]Reagents and conditions: (i) benzylamine, 10% AcOH in anhydrous DMF, rt, on, then $NaBH(OAc)_3$, 5% AcOH in anhydrous DMF, rt, 4 h.; (ii) 2,4-dichloro-3-nitropyridine, EDIPA, DMF, rt, on; (iii) 10% pentylamine in DMSO, 120°C, on; (iv) tin (II) chloride dihydrate, EDIPA, DMF, rt, on; (v) isonicotinaldehyd, DMSO, 80°C, on; (vi) 50% TFA in DCM, rt, 1 h.

Compound **26A(4,1,2)** was prepared in an analytical amount with crude purity 79% accompanied by the isomer formed after non-regioselective arylation with 2,4-dichloro-3-nitropyridine (mentioned earlier in the text). At the end of the reaction sequence, both isomers exhibited sufficient distance in HPLC-UV traces which should allow their simple separation with semi-preparative reverse phase chromatography.

The Rink resin was tested for the preparation of deazapurines (Scheme 28). Initially, we followed the general strategy (Scheme 25) and after arylation with 2,4-dichloro-3-nitropyridine the second chlorine atom was substituted with secondary amine (piperidine). After finishing the reaction sequence with use of benzaldehyde the expected compound **28A(3,2,1)** was prepared with crude purity 77%, isolated and its structure was confirmed by advanced NMR experiments including COSY, DEPT, HMBC and HMQC spectra. The results of NMR analysis are in accordance with a literature.[111,112] Subsequently we replaced piperidine with primary amine (pentylamine) and the course of heterocyclization was tested.

Although the intermediate **24A(3,2)** can undergo the imidazole formation in two different directions, only one product was detected. Its crude purity was 66% (HPLC-UV traces) and no single side product was detected, only several minor impurities. The product **26A(3,1,1)** was isolated, purified and its structure was confirmed by NMR analysis.

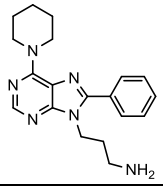
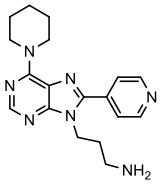
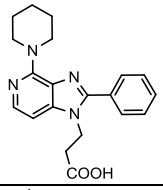
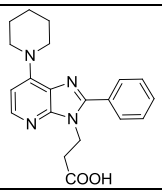
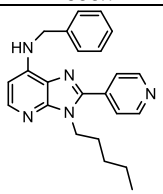
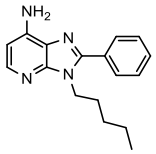
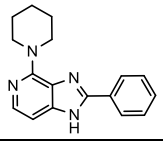
Scheme 28: Preparation of deazaadenine derivatives.[a]



[a]Reagents and conditions: (i) 20% piperidine in DMF, 20 min, rt; (ii) 2,4-dichloro-3-nitropyridine, EDIPA, DMF, rt, on; (iii) 10% amine in DMSO, rt, 3 h.; (iv) tin (II) chloride dihydrate, EDIPA, DMF, rt, on; (v) aldehyde, DMSO, 80°C, on; (vi) 50% TFA in DCM, rt, 1 h.

Experiments summarized in Scheme 28 showed that nucleophility of the benzhydrylamine ligand (i.e. body of Rink amide linker) is decreased probably due to sterical hindrance and in a presence of another suitable nucleophile the ring closure proceeds via the sterically more favorable direction (derivative **26A(3,1,1)**). On the other hand, when there is no alternative option, the cyclization proceeds the only possible way and benzhydrylamine nitrogen atom reacts. This means that the strategy could be generally applicable for simple preparation of diverse deazaadenines but the regioselectivity has to be tested with use of larger number of different intermediates.

Table 8: Prepared purine and deazapurine derivatives

Compound	Structure	Crude purity[a]	Yield[b]	Analysis[c]
28B(1,2,1)		75%	-	HPLC-MS
28B(1,2,2)		78%	51%	NMR
28A(2,2,1)		85%	76%	NMR
26A(2,2,1)		8%	8%	^{1}H NMR
26A(4,1,2)		79%	-	HPLC-MS
26A(3,1,1)		77%	71%	NMR
28A(3,2,1)		66%	57%	NMR

[a]Purity of crude final product after all reaction sequence; integrated HPLC-UV traces.

[b]Overall yields of compounds **26** or **28** after the all reaction steps and HPLC purification.

[c]HPLC-MS – the reaction was performed only as analytical experiment and the final product was verified by HPLC-MS traces. NMR – the final product was confirmed by NMR experiments.

The preliminary study of purines and deazapurines synthesis described in this subchapter was mainly focused on an optimization of each reaction step, study on

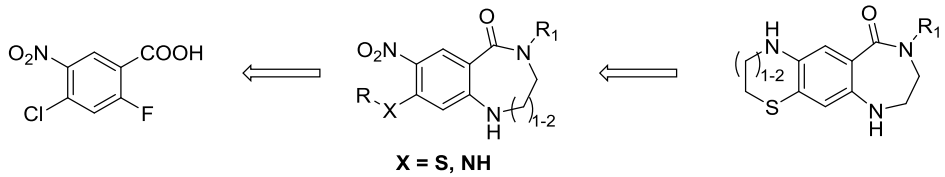
regioselectivity and development of purification conditions. Suggested synthetic strategies have been successfuly tested and several model compounds were prepared in a preparative scale (**table 8**). Due to its extensiveness this area still has not been completed and represents the ongoing research that will be published in a nearest future.

4.4 Solid-Phase Synthesis of 4,7,8-Trisubstituted 1,2,3,4-Tetrahydro-benzo[*e*][1,4]diazepin-5-ones

Based on: Barbora Lemrová and Miroslav Soural, ACS Comb. Sci, 2012, 14, 645-650.[A4]

The last experimental part of this thesis is focused on the expansion of diversity-oriented solid-phase synthesis of various heterocycles from 4-chloro-2-fluoro-5-nitrobenzoic acid.[55,91]. The strategy allowed the preparation of 1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-ones with 2 diversity positions and also ”benzene fused heterocycles” that consist of [1,4]thiazine or [1,4]thiazepine ring (figure 33). Similar strategy with use of 1,5-difluoro-2,4-dinitrobenzene as a key building block leading to imidazo[4,5-*g*]quinoxalinones has been previously reported by Mazurov.[114]

Figure 33: Retrospective synthesis of tetrahydrobenzodiazepinones and selected“benzene fused heterocycles”

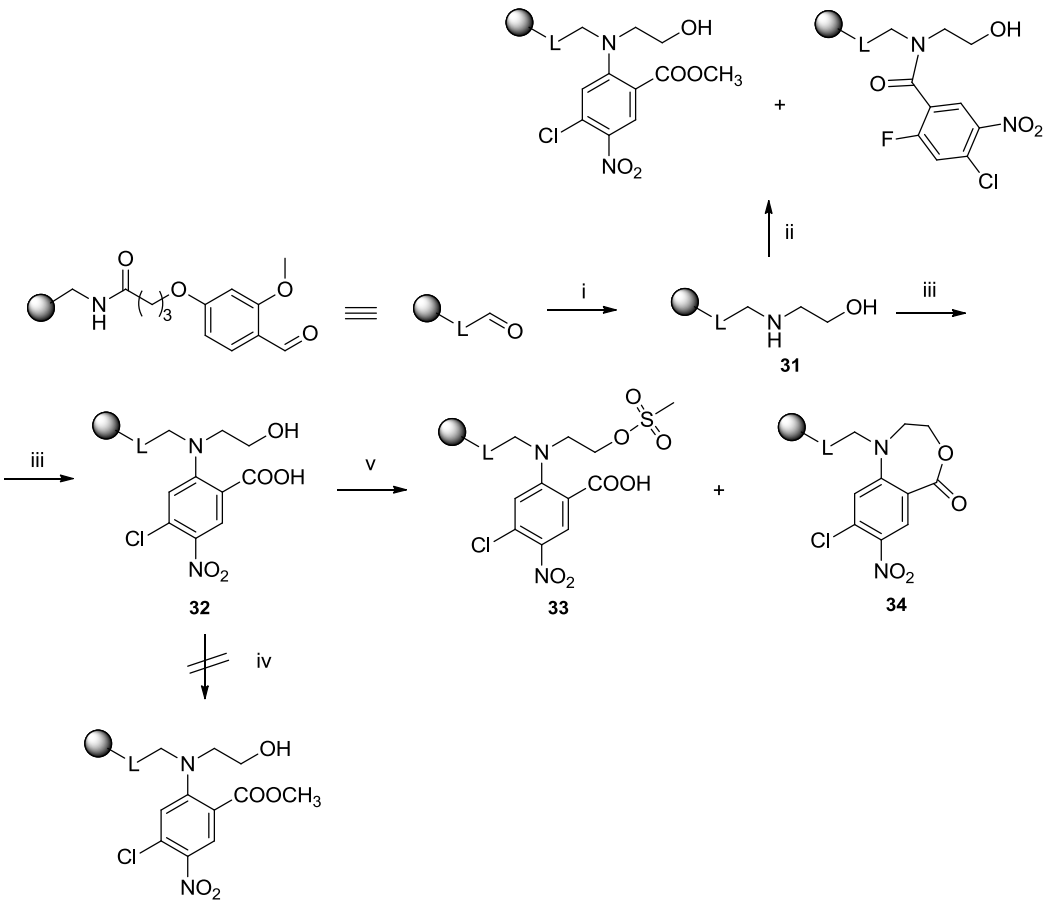


4.4.1 Synthesis

Our initial strategy for the preparation of the target compounds was based on immobilization of ethanolamine to a polystyrene resin equipped with an acid labile backbone amide linker (BAL). The polymer supported ethanolamine **31** was subsequently arylated with 4-chloro-2-fluoro-5-nitrobenzoic and intermediate **32** was obtained. In the next step, the terminal hydroxy group reacted with methanesulfonylchloride to obtain the reactive ester **33** that should enable replacement of the hydroxy group with primary amines resulting in N^4 substitution of the target scaffold (Scheme 29). Unfortunately, a presence of a base (pyridine) caused activation of the carboxylic group and azlactone **34** was detected as a side product (about 40%, HPLC traces). We tried to protect the carboxylate with help of methylester

formation, but the intermediate **32** exhibited an unexpected resistance towards various methods (Mitsunobu reaction with methanol, esterification with methyl iodide or diazomethane) and only up to 10% of the desired methylester was detected in each case. Alternatively, we also tried to use methylester of 4-chloro-2-fluoro-5-nitrobenzoic acid for immobilization on resin **31** (Scheme 2, step ii), but we did not obtain sufficiently pure product due to side reaction caused by the corresponding amide formation (30-40% of amide side product, HPLC-UV traces). Because we could not manage to prevent the unwanted lactonization, the strategy had to be changed.

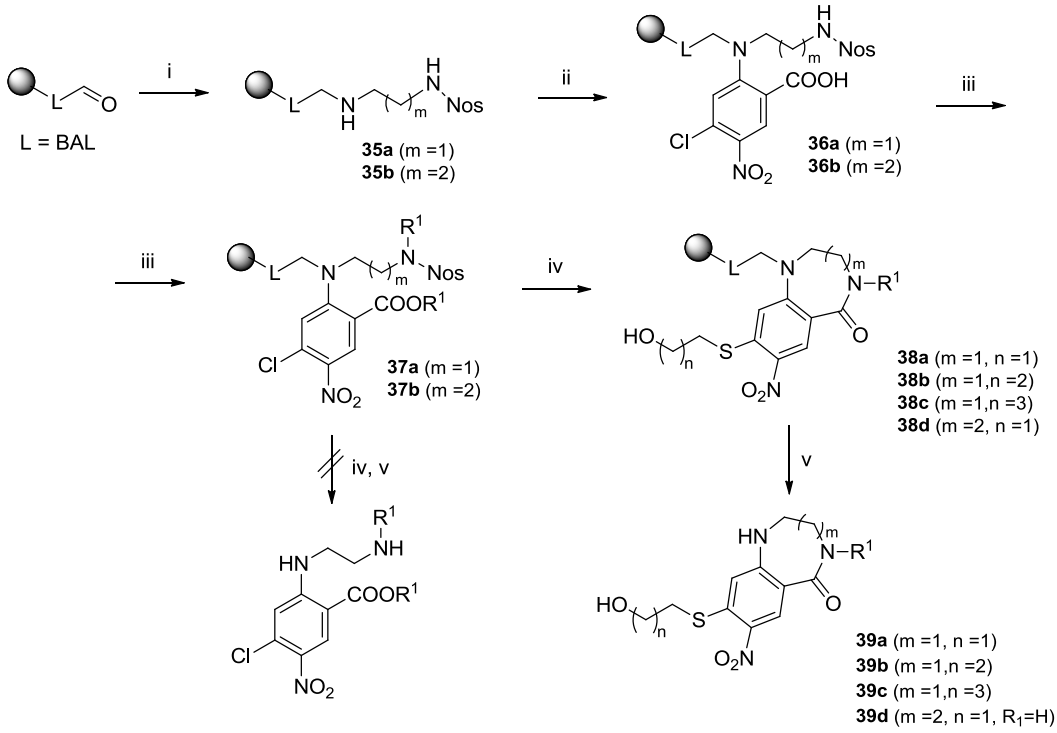
Scheme 29: An unsuccessful strategy for the preparation of target compounds[a]



[a]Reagents and conditions: (i) ethanolamine, 10% AcOH in DMF, on, rt then $NaBH(OAc)_3$, 5% AcOH in DMF, 4 h, rt; (ii) 4-chloro-2-fluoro-5-nitrobenzoic acid methyl ester, EDIPA, DMSO, 50°C, on; (iii) 4-chloro-2-fluoro-5-nitrobenzoic acid, EDIPA, DMSO, 50°C, on (iv) methyl iodide, EDIPA, DMSO, rt, on or diazomethane, ether, rt, on or methanol, PPh_3, DIAD, anhydrous THF, rt, on; (v) methansulfonylchloride, pyridine, 1 h, rt.

An alternative strategy consisted in a preparation of *N*-(2-amino-ethyl)-2-nitro-benzenesulfonamide with use of solution-phase synthesis and its subsequent immobilization to a polystyrene resin-BAL system (Scheme 30). The 2-nitrobenzensulfonyl (2-Nos) protected, polymer supported diamine **35a** was then arylated with 4-chloro-2-fluoro-5-nitrobenzoic acid to obtain intermediate **36a**.

Scheme 30: Successful strategy for the preparation of target compounds (see Figure 34 for R^1).[a]

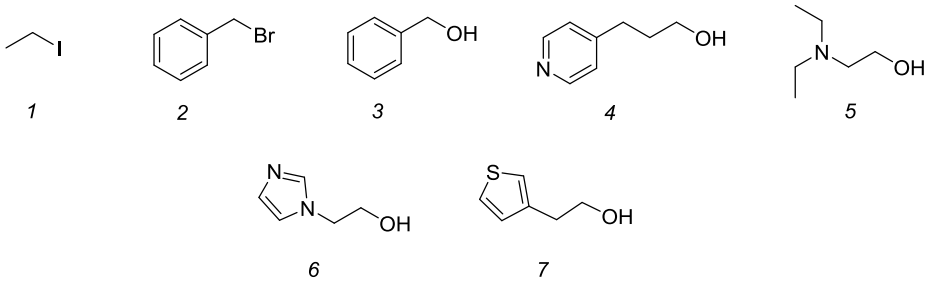


[a]Reagents and conditions: (i) *N*-(2-amino-ethyl)-2-nitrobenzenesulfonamide or *N*-(3-Amino-propyl)-2-nitro-benzenesulfonamide, 10% AcOH in DMF, on, rt, then $NaBH(OAc)_3$, 5% AcOH in DMF, 4 h, rt; (ii) 4-chloro-2-fluoro-5-nitrobenzoic acid, EDIPA, DMSO, 50°C, on; (iii) ethyl iodide or benzyl bromide, DBU, DMF, rt, on or alcohol, DIAD, PPh_3, anhydrous THF, rt, on; (iv) mercaptoalcohol, DBU, DMF, rt, on, then (only for **38d**) EDIPA, DMSO, 80°C, on; (v) 50% TFA in DCM, rt, 1 h.

Advantageously, the 2-Nos group enabled alkylation of the nitrogen atom to incorporate various ligands R^1 (see Figure 34) to a position 4 of the target diazepinones. Two alternative methods were tested: (i) alkylation with alkyl halides, (ii) alkylation with alcohols under Mitsunobu protocol. Reaction with ethyl iodide in a presence of a tertiary base such as TEA or EDIPA led to alkylation of N-H group but also partial esterification of a carboxylic

group was observed. When 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used instead, the esterification was quantitative and only double-alkylated intermediate **37a**(*1*) was detected. The same reaction conditions (i.e. 0.5 M solution of alkyl halide in DMF, 16 hours) were successfully tested for benzyl bromide. The Mitsunobu reaction was tested for various alcohols (see Figure 34) and in each case the double alkylated intermediates **37a**(*3-7*) in an excellent purity were obtained. From a combinatorial point of view, the modification based on Mitsunobu alkylation represents much more favorable alternative with respect to number of commercially available alcohols.

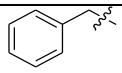
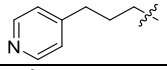
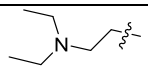
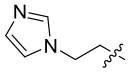
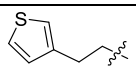
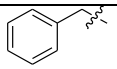
Figure 34: Building blocks used for the preparation intermediates **37a**(R^1)



1 *2* *3* *4* *5* *6* *7*

In the next step the 2-Nos protective group had to be removed. This cleavage is typically performed with 0.6 M solution of mercaptoethanol and 0.2 M DBU in DMF for 5 min.[115] In our case the 2-Nos group was not cleaved under usual conditions and additionally a partial substitution of the chlorine atom with mercaptoethanol was observed. When the reaction time was extended to 16 hours and the concentration of DBU was increased to 0.6 M, only diazepinone derivatives **39a**(*1-7*) were detected indicating the cyclization to a seven membered ring took place spontaneously after the cleavage of the protective group. The same course of reaction was observed when mercaptoethanol was replaced with mercaptopropanol and mercaptobutanol, respectively. The appropriate diazepinones were obtained in very good, crude purity (see Table 9).

Table 9: List of prepared diazepinones **39**.

Compound	R^1	n	Crude purity[a]	Yield[b]
39a(*1*)		1	83%	80%
39a(*2*)		1	81%	69%
39a(*4*)		1	73%	69%
39a(*5*)		1	82%	78%
39a(*6*)		1	82%	57%
39a(*7*)		1	70%	68%
39c(*1*)		3	73%	63%
39c(*2*)		3	67%	62%

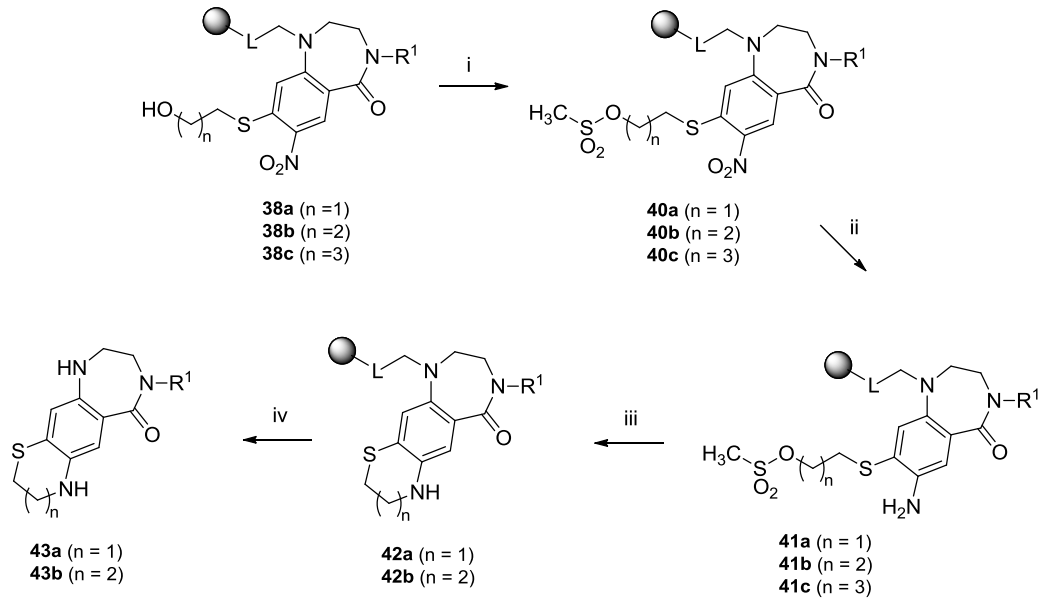
[a]Purity of crude product ; integrated HPLC-UV traces.

[b]Overall yields after preparative HPLC purification.

The smooth nucleophilic substitution of intermediates **37** with mercaptoalcohols is quite surprising as we know from our previous results that harsh conditions are needed for similar reaction with primary and secondary amines including strong nucleophiles (piperidine, pyrrolidine).[55,116] On the other hand, the formation of diazepinones **38** can be advantegously employed for the preparation of thiazine and thiazepine scaffold containing bisheterocycles which demonstrates a very first application of 4-chloro-2-fluoro-5-nitrobenzoic acid in a synthesis of bisheterocycles depicted in a Scheme 16. For this purpose, resins **38a**(*1*), **38a**(*2*), **38b**(*1*), **38b**(*2*)**, 38c**(*1*) and **38c**(*2*) were allowed to react with methansulfonyl chloride in pyridine followed by the reduction of the nitro group with tin(II) chloride dihydrate. After reduction we observed only about 10% of a tricyclic target products **43a**(*1*), **43a**(*2*), **43b**(*1*), **43b**(*2*), for a quantitative cyclization the appropriate resins **41a** and **41b** had to be treated with 0.2 M EDIPA in DMSO at 80°C for 16 hours. Thiazine and thiazepine rings were successfully formed giving the target compounds in an excellent crude purity (78-93%, HPLC traces). In contrast, the preparation of eight membered ring - [1,4]thiazocane – was unsuccessful. Although the mesylation gave appropriate intermediates **41c**(*1*) and **41c**(*2*) and

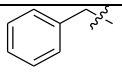
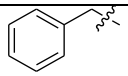
also their reduction proceeded well, the final ring closure did not take place even under harsh conditions (including a microwave heating).

Scheme 31: Preparation of [1,4]thiazine and [1,4]thiazepine containing bisheterocycles (for R^1 see Table 10).[a]



[a]Reagents and conditions: (i) MsCl, pyridine, rt, 30 min.; (ii) $SnCl_2 \cdot 2\ H_2O$, EDIPA, DMF, on, rt; (iii) EDIPA, DMSO, 80 ^oC, 16 h.; (iv) 50% TFA in DCM, rt, 1 h.

Table 10: List of prepared [1,4]thiazines **43a** and [1,4]thiazepines **43b**

Compound	R^1	n	Crude purity[a]	Yield[b]
43a(*1*)		1	78%	47%
43b(*1*)		2	73%	51%
43a(*2*)		1	93%	27%
43b(*2*)		2	82%	63%

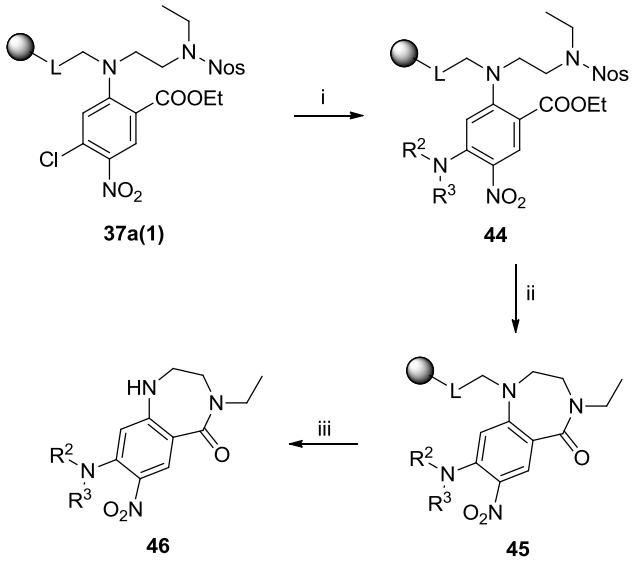
[a]Purity of crude product; integrated HPLC-UV traces.

[b]Overall yields after preparative HPLC purification.

To increase the diversity of diazepinones **39a-c**(*R^1*), we also tested a substitution of the chlorine atom in an intermediate **37a**(*1*) with a set of primary and secondary amines. For similar reaction, we have previously developed a procedure consisting in heating with amine

solution in DMSO to 120 °C.[116] In our case, the reaction had to be very carefully optimized due to relatively low reactivity of the starting material towards nucleophilic substitution at position 4 and simultaneous sensitivity of ester towards nucleophilic adition resulting in amide formation. We tested different reaction temperatures and solvents (such as DMSO, *N*-methylpyrrolidone (NMP), diethylene glycole diethyl ether) but the products were contaminated by the corresponding amides in each case in spite of the substitution being carried out at lower temperature 80°C (conventional heating). In contrast, use of 10% of amine in DMSO under microwave heating (200W, 150°C, 5 min.) surprisingly provided the target derivates **44**(*1-7*) without presence of the amide side products. This method was found to be suitable for both primary and secondary amines. Last step was the cyclization to derivatives **45**(*1-7*) according to previously discussed procedure. The final compounds **45**(*5*), **45**(*6*) and **45**(*7*) prepared from primary amines demonstrate applicability of the reaction sequence for the preparation of bisheterocycles that contain the "left side" heterocyclic scaffolds.

Scheme 32: Synthesis of 8-*N*-substituted 1,2,3,4-tetrahydro-benzo [*e*] [1,4]diazepin-5-ones (for $R^{2,3}$ see Figure 35).[a]



[a]Reagents and conditions: (i) Amine, DMSO, 150°C, 200 W, 5 min.; (ii) mercaptoethanol, DBU, DMF, rt, on; (iii) 50% TFA in DCM, rt, 1 h.

Figure 35: List of successfully tested amines

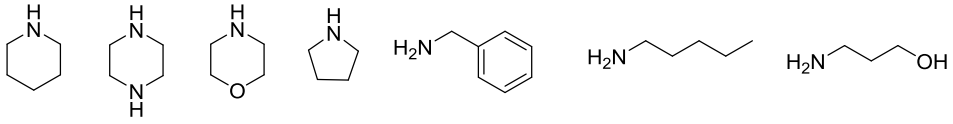


Table 11: List of prepared 8-*N*-(di)substituted diazepinones **16.**

Compound	Crude purity[a]	Yield[b]
46(*1*)	76%	61%
46(*2*)	60%	52%
46(*3*)	78%	60%
46(*4*)	65%	61%
46(*5*)	84%	68%
46(*6*)	85%	80%
46(*7*)	80%	75%

[a]Purity of crude product; integrated HPLC-UV traces.

[b]Overall yields after preparative HPLC purification.

As the synthesis of benzodiazepinones **39a-c** from the polymer supported 1,2-diaminoethane proved to be simple and quite versatile, we were also interested in use of a similar approach for the preparation of analogous benzodiazocines (Scheme 30, m = 2). For this purpose we prepared *N*-(3-amino-propyl)-2-nitro-benzenesulfonamide and immobilized such protected diamine to a polystyrene resin-BAL system to obtain appropriate intermediate **35b**.[117] Following the previously developed procedures, the cyclization of *N*-unsubstituted derivative **38d** (R^1 = H) was successfully performed. In contrast to a cyclization of derivatives **37a** the reaction did not take place simultaneously after the cleavage of the 2-Nos group but high temperature was necessary for completion. When the preparation of *N*-substituted intermediates **38d** (R^1 = ethyl or benzyl) was tested, the cyclization did not take place even under harsh conditions (including microwave heating to 150^{o}C) which indicates the synthesis of a benzodiazocine derivatives **38d** is limited only to N^4-unsubstituted derivatives in this case.

4.4.2 Biological screening

We developed an efficient method for solid-phase synthesis of diverse 1,2,3,4-tetrahydro-benzo [*e*][1,4]diazepin-5-ones from commercially available synthons (alcohols, amines). The target substances were isolated in very good crude purity and yields. The reaction sequence has been successfully tested for seven alcohols (giving N^4-substitution), three mercaptoalcohols and seven amines (giving C^8-substitution). A total combination of all used building blocks would give 7x10 = 70 compounds. As in the previous caces, we have synthesized only smaller set of 15 derivatives. Except the applicability of the developed method for simple preparation of diverse 4,7,8-substituted benzodiazepinones, our approach can be applied for combinatorial synthesis of libraries of benzene fused heterocycles, four examples were given. All prepared compounds were subjected to MTT cytotoxic test, unfortunately to the deadline submission of this thesis the results were not known.

5 Conclusion

Solid-supported synthesis can be an efficient strategy for the preparation of various chemical entities in combinatorial fashion. This thesis describes development of four original solid-phase organic syntheses using only commercially available building blocks and common coupling reagents and procedures. Three parts of the presented research share a common feature – the structure of purine. The last part of the thesis follows on previous study of reactivity of 4-chloro-2-fluoro-5-nitrobenzoic acid. The first part is dedicated to solid-phase synthesis of bisheterocyclic molecules that consist from purine and 3-hydroxyquinolin-4(1*H*)-ones. The versatility of the synthetic method was tested by the preparation and full characterization of 18 model compounds. In this small library a majority of compounds provided significantly high biological activity and three derivatives were active in submicromolar concentration of IC_{50} (subchapter 4.1.2). Additionally, the products were studied as potential fluorescent probes with promising results (subchapter 4.1.3.).

Another part of the thesis was focused on the preparation of tetrasubstituted purine derivatives. For the preparation of the key intermediates (2, 6 and 9 trisubstituted purines), we used experiences from the previous research. The major challenge of this part of our work was the application of Pd-catalyzed reaction to the area of solid-phase synthesis. The modification of C^8 position of purine scaffold was successfully performed by using of aryl iodides but also less reactive aryl bromides were reactive enough to afford the desired target

structures. We also prepared 2,6,8-trisubstituted purines. In this case, the arylation required higher reaction temperature to proceed. The efficacy of Pd-catalyzed direct arylation depended on used linker. We tested Wang resin and aminomethyl resin with BAL linker. Surprisingly, the Wang resin provided excellent results while a similar intermediate immobilized on aminomethyl resin did not undergo any reaction. Screening of biological activity (subchapter 4.2.2) afforded number of compounds with significant cytotoxic activity. Unfortunately, a majority of compounds also exhibited high toxicity against normal cell lines.

The subchapter 4.3 describes solid-phase synthesis of purine and deazapurine derivatives by heterocyclization from suitable precursors. This synthesis was designed for three types of commonly used resins (Wang, Rink and Aminomethyl resin with BAL linker). Using of numerous immobilizations on various resins allows simple preparation of large number of diverse purines and deazapurines. The described research is focused on optimization of each reaction step, study of regioselectivity and purification conditions. Some reactions were carried out only in analytical scale, but several model compounds were isolated, purified and characterized by NMR. Interesting results were obtained in case of the reactions on the Rink amide resin, because this pathway is applicable for the preparation of novel adenine/deazaadenine derivatives. In a following work we suppose to apply the developed procedures for synthesis of a small library of target compounds to evaluate their biological properties.

The last part of this thesis was dedicated to expansion of diversity-oriented solid-phase synthesis of various heterocycles from 4-chloro-2-fluoro-5-nitrobenzoic acid. In this case, we developed a method for preparation of trisubstituted benzodiazepinones and some related "benzene fused heterocycles". The synthesized compounds were subjected to biological screening but unfortunately, the results were not known before submission of this thesis.

In conclusion, we developed high throughput synthetic methods for the preparation of bisheterocyclic compounds consisting of purine and 3-hydroxyquinolin-4(1*H*)-one scaffold, tetrasubstituted and trisubstituted purine derivatives, deazapurine derivatives and trisubstituted benzodiazepinones. In each case, the multistep reaction pathways were successfully optimized to afford the final compounds in very good to excellent crude purity. The developed procedures allow semi-automated combinatorial synthesis of chemical libraries of target compounds using simple instrumentation, common coupling reagents and large number of commercially available building blocks.

6 Experimental part

6.1 Material and Methods

Solvents and chemicals were purchased from Sigma-Aldrich (Milwaukee, IL, www.sigmaaldrich.com), Acros (Geel, Belgium, www.acros.cz) and Fisher (Pittsburgh, PA, www.fishersci.com). The aminomethylene resin (100-200 mesh, 1% DVB, 0.98 mmol/g) and 4-(4-Formyl-3-methoxyphenoxy)butyric acid were obtained from AAPPTec (Louisville, KY, www.aapptec.com). Synthesis was carried out on Domino Blocks in disposable polypropylene reaction vessels (Torviq, Niles, MI, www.torviq.com). Labquake Tube Rotator (Thermolyne, Dubuque, IA, www.barnsteadthermolyne.com) was used for gentle but efficient tumbling of resin slurry.

All reactions were carried out at ambient temperature (21 ^{o}C) unless stated otherwise. The volume of wash solvent was 10 mL per 1 g of resin. For washing, resin slurry was shaken with the fresh solvent for at least 1 min before changing the solvent. After adding a reagent solution, the resin slurry was manually vigorously shaken to break any potential resin clumps. Resin-bound intermediates were dried by a stream of nitrogen for prolonged storage and/or quantitative analysis.

For the LC/MS analysis a sample of resin (~5 mg) was treated by TFA in DCM, the cleavage cocktail was evaporated by a stream of nitrogen, and cleaved compounds extracted into 1 mL of MeOH.

The LC/MS analyses were carried out on UHPLC-MS system consisting of UHPLC chromatograph Accela with photodiode array detector and triple quadrupole mass spectrometer TSQ Quantum Access (both Thermo Scientific, CA, USA), using Nucleodur Gravity C18 column at 30°C and flow rate of 800 µL/min (Macherey-Nagel, 1.8 µm, 2.1 x 50 mm, Germany). Mobile phase was (A) 0.01 M amonium acetate in water, and (B) acetonitrile, linearly programmed from 10 % to 80 % B over 2.5 min, kept for 1.5 min. The column was re-equilibrated with 10 % of solution B for 1 min. The APCI source operated at discharge current of 5 µA, vaporizer temperature of 400°C and capillary temperature of 200 ^{o}C.

Purification was carried out on C18 reverse phase column 19 x 100 mm, 5 µm particles; gradient was formed from 10 mM aqueous ammonium acetate and acetonitrile, flow rate 15 mL/min. Only a heart cut of correct mass peak was taken in order to obtain as high

purity as possible. The yield of final structure was derived from a loading of resin after the immobilization of the first building block.

NMR ^{1}H and ^{13}C spectra were obtained on a Bruker Avance (300 MHz) instrument. NMR spectra were recorded at ambient temperature (21 ^{o}C) in DMSO-d_6 solutions and referenced to the resonance signal of DMSO. Chemical shifts δ are reported in ppm and coupling constants *J* in Hz. All ^{1}H and ^{13}C NMR experiments of the final structures from subchapter 4.2 were performed at magnetic field strengths of 14.09 T corresponding to ^{1}H resonance frequencies of 599.89 MHz and at elevated temperature (~60 °C). The ^{13}C NMR analyses were measured for demonstration on four target structures. The other analogues were characterized only by ^{1}H NMR, LC-MS and HRMS.

6.2 Experimental procedures

6.2.1 Solid-phase Synthesis of Highly Diverse Purine-Hydroxyquinolinone Bisheterocycles

Barbora Vaňková, Jan Hlaváč and Miroslav Soural, J. Comb. Chem. 2010, 6, 890-894.[A1]

6.2.2 Experimental procedures

Acylation with BAL linker (1)

A polypropylene fritted syringe was charged with polystyrene aminomethylated resin (1 g, 0.98 mmol). The resin was washed 3 x with DCM, treated with 5% *N*,*N*-diisopropylethylamine (EDIPA) in DCM (2 mL) for 5 min. and washed 3 x with DCM. A solution of 4-(4-formyl-3-methoxyphenoxy)butyric acid (1.96 mmol, 524 mg), HOBt (1.96 mmol, 299 mg), and DIC (1.96 mmol, 300 μL) in 10 mL DCM/DMF (1:1) was added to the resin and the resin slurry was shaken overnight. After reaction the resin was washed 3 x with DMF and 3 x with DCM. Sample of resin (~10 mg) was checked for the presence of free amino group by 0.03 M solution of bromophenol blue (BB) in *N*-methylpyrrolidone (NMP).

Immobilization of primary amines (2)

Aminomethyl resin with BAL linker (1 g) was washed 3 x with DCM and 3x with anhydrous DMF. A solution of primary amine (5 mmol) in 10 mL 10% AcOH/anhydrous DMF and TFA (5 mmol; 384 μL) was added and the slurry was shaken overnight. The next day, $NaBH(OAc)_3$ (10 mmol, 2.2 g) was added in three portions and the syringe was punctured with a needle just below the plunger to enable hydrogen gas evolve. The slurry was shaken

for 4 hours. The resin was washed 3 x with 5% AcOH/DMF 3x with DMF, neutralized with 20% piperidine/DMF for five minutes, washed 3 x with DMF and 3 x with DCM.

*Quantification of resins **2**:*

Sample of resin **2** (10 mg) was reacted with Fmoc-OSu (0.5 mmol, 168 mg) in 1 mL DCM for 30 min at ambient temperature. The resin was washed 5 x with DCM and the product was cleaved from resin with 50% TFA in DCM for 30 min. The cleavage cocktail was evaporated by a stream of nitrogen and cleaved material was extracted into 1 mL of MeOH. The sample was analyzed by HPLC-UV-MS and the quantity was calculated with use of an external standard (Fmoc-Ala-OH; concentration 1 mg/ml).

Arylation with 2,6-dichloropurine (3)

A polypropylene fritted syringe was charged with resin **2** (1g, 0.5 mmol/g) and solution of 2,6-dichloropurine (2.5 mmol; 467 mg) and EDIPA (2.5 mmol; 435 μL) in THF (10 mL) was added. The resin was shaken at 50°C overnight and subsequently washed 3 x with THF and 3 x with DCM.

No Fmoc-starting amines were detected after the fmocylation of the resin sample (10 mg of the resin was shaken with solution of Fmoc-OSu (0.1 mmol; 33 mg) in DCM (1 mL) for 30 minutes) (LC-MS) indicating the reaction with 2,6-dichloropurine was quantitative.

Alkylation of purine N^9 (4)

A glass vial was charged with resin **3** (500 mg) and 0.6 M solution of the appropriate alkyl iodide (3 mmol) and eqv. DBU (3 mmol; 450 μL) in DMSO (5 mL) was added. The resin was shaken overnight at 50°C. Then the resin was transferred to a polypropylene fritted syringe, washed 3 x with DMF and 3 x with DCM. The reaction step was repeated using THF as a solvent.

Substitution of the chlorine atom (5)

A glass vial was charged with resin **4** (500 mg) and 2 M solution of the appropriate diamine (10 mol) in diethylene glycol diethyl ether (5 mL) was added. The resin was shaken for 24 hours at 150°C. Then the resin was transferred to the polypropylene fritted syringe and washed 3 x with DMF and 3 x with DCM.

Acylation with 1-methyl-2-aminoterephtalate (6)

A polypropylene fritted syringe was charged with resin **5** (500 mg). A 0.2 M solution of 1-methyl-2-aminoterephtalate (1.0 mmol; 195 mg), HOBt (1.0 mmol; 153 mg) and DIC (1.0 mmol; 153 μL) in 2.5 mL DMF and 2.5 mL DCM was added. Resin was kept on a tumbler overnight, and then washed 3 x with DMF and 3 x with DCM.

Saponification of methylester (7)

A polypropylene fritted syringe was charged with resin **6** (500 mg) and 0.2 M solution of TMSOK (1.0 mmol; 128.3 mg) in THF (5 mL) was added. The resin was shaken at room temperature for 24 hours, washed 3 x with THF and 3 x with DCM.

Reaction with haloketones (8)

A polypropylene fritted syringe was charged with resin **7** (300 mg) and 0.2 M solution of appropriate haloketone (0.6 mmol) with equivalent of EDIPA (0.6 mmol; 105 μL) in DMF (3 mL) was added. The resin was shaken at room temperature for 3 hours, washed 3 x with DMF and 3 x with DCM.

Cleavage from the resin and final cyclization to bisheterocycles (9)

A polypropylene fritted syringe was charged with resin **8** (300 mg) and a cleavage cocktail (50% TFA in DCM, 3 mL) was added. The resin was shaken at room temperature for 30 minutes. The cleavage cocktail was removed and the resin was additionally washed 2 x with 50% TFA in DCM. The washes were collected and evaporated in a stream of the nitrogen. The cleaved intermediates **7** were cyclized using the following procedures:

Method A: TFA

The evaporated material was dissolved in TFA (2 mL) and the solution was refluxed for 4 hours. TFA was evaporated in a stream of nitrogen and the resulting oil was sonified in diethyl ether for 5 minutes. The precipitated solid was filtrated, washed with diethyl ether and dried.

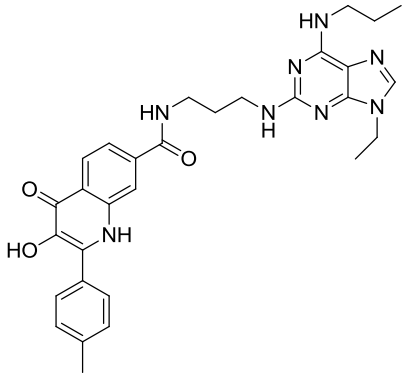
The oily residue obtained after cyclization of derivatives **9(1,2,3,5); 9(3,2,1,2); 9(3,2,1,4)** was dissolved in MeOH to hydrolyze the *O*-trifluoroacetyl side-products. Then MeOH was evaporated and the final oil was sonified in diethyl ether to precipitate the final compound.

Method B: CH_3COOH

The evaporated material was dissolved in acetic acid (2 mL) and the solution was refluxed for 4 hours. CH_3COOH was evaporated in a stream of nitrogen and the oil obtained was sonified in diethyl ether for 5 minutes. The precipitated material was filtrated, washed with diethyl ether and dried.

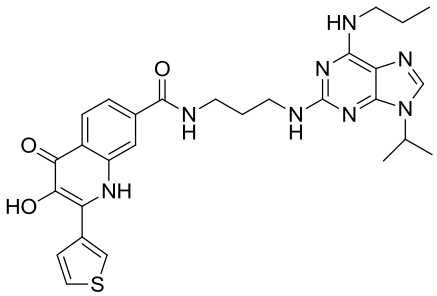
6.2.3 Analytical data

3-Hydroxy-4-oxo-2-p-tolyl-1,4-dihydro-quinoline-7-carboxylic acid [3-(9-ethyl-6-propylamino-9*H*-purin-2-ylamino)-propyl]-amide 9(1,1,3,2)



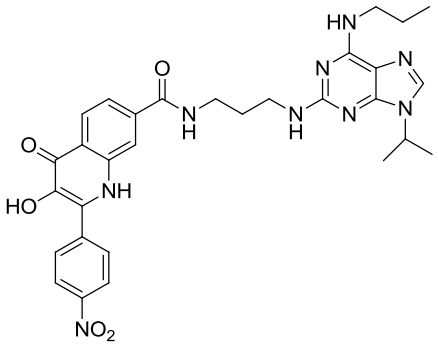
Prepared with use of method A Yield 74 mg (80%). Purity of the crude product 90%; MS $[M+H]^+$= 555.28. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77 - 0.97 (m, 3 H) 1.32 (d, *J*=5.85 Hz, 3 H) 1.48 - 1.64 (m, 2 H) 1.78 - 1.93 (m, 2 H) 2.40 (s, 3 H) 3.40 (d, *J*=5.31 Hz, 4 H) 3.42 - 3.51 (m, 2 H) 4.01 - 4.12 (m, 2 H) 7.38 (d, *J*=7.87 Hz, 2 H) 7.67 (d, *J*=8.60 Hz, 1 H) 7.73 (d, *J*=7.87 Hz, 2 H) 7.95 (s, 1 H) 8.19 (d, *J*=8.42 Hz, 1 H) 8.25 (s, 1 H) 8.73 (t, *J*=4.76 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.0, 14.8, 21.0, 21.9, 28.8, 30.8, 35.8, 37.1, 42.2, 114.4, 118.3, 118.7, 119.7, 122.9, 124.6, 128.9, 129.1, 129.2, 132.5, 136.3, 137.5, 138.5, 139.1, 158.6, 159.0, 162.3, 165.9, 169.4.

3-Hydroxy-4-oxo-2-thiophen-3-yl-1,4-dihydro-quinoline-7-carboxylic acid [3-(9-isopropyl-6-propylamino-9*H*-purin-2-ylamino)-propyl]-amide 9(1,2,3,3)



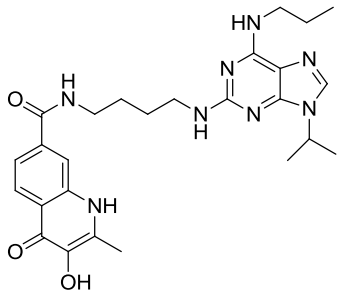
Prepared with use of method A. Yield 40 mg (80%). Purity of the crude product 93%; MS $[M+H]^+$= 561.23. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 - 0.98 (m, 3 H) 1.43 (br. s., 6 H) 1.52 - 1.69 (m, 2 H) 1.87 (quin, *J*=6.45 Hz, 2 H) 3.32 - 3.53 (m, 6 H) 4.52 - 4.64 (m, 1 H) 7.68 (d, *J*=7.50 Hz, 1 H) 7.77 (dd, *J*=5.12, 3.11 Hz, 1 H) 7.86 (d, *J*=5.12 Hz, 1 H) 8.18 (d, *J*=8.42 Hz, 2 H) 8.27 - 8.40 (m, 2 H) 8.75 (t, *J*=5.31 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.6, 21.7, 25.8, 28.7, 30.8, 31.8, 35.8, 118.7, 119.7, 122.7,124.5, 127.7, 132.4, 136.4, 137.4, 138.5, 142.7, 145.1, 148.8, 152.3, 158.0, 158.5, 159.0, 162.3, 165.9, 169.5.

3-Hydroxy-2-(4-nitro-phenyl)-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [3-(9-isopropyl-6-propylamino-9*H*-purin-2-ylamino)-propyl]-amide 9(1,2,3,5)



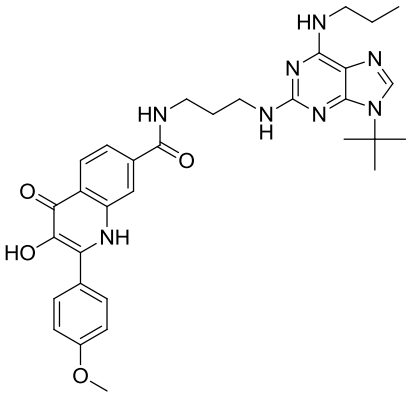
Prepared with use of method B. Yield 34 mg (43%). Purity of the crude product 98%; MS $[M+H]^+$= 600.26. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (t, *J*=6.95 Hz, 3 H) 1.43 (d, *J*=5.85 Hz, 6 H) 1.52 - 1.68 (m, 2 H) 1.77 - 1.97 (m, 2 H) 2.55 (t, *J*=5.49 Hz, 2 H) 3.31 - 3.51 (m, 4 H) 4.43 - 4.68 (m, 1 H) 7.71 (d, *J*=8.60 Hz, 1 H) 8.11 (d, *J*=8.97 Hz, 2 H) 8.21 (d, *J*=8.60 Hz, 1 H) 8.25 (s, 1 H) 8.41 (d, *J*=8.96 Hz, 2 H) 8.77 (t, *J*=5.67 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.1, 18.3, 21.8, 28.8, 34.3, 37.2, 110.9, 114.9, 118.8, 119.9, 121.5, 123.0, 123.3, 124.7, 130.7, 136.7, 137.8, 138.5, 139.2, 147.5, 157.9, 158.4, 158.8, 159.2, 162.3, 165.7, 184.1.

3-Hydroxy-2-methyl-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [4-(9-isopropyl-6-propylamino-9*H*-purin-2-ylamino)-butyl]-amide 9(1,2,1,1)



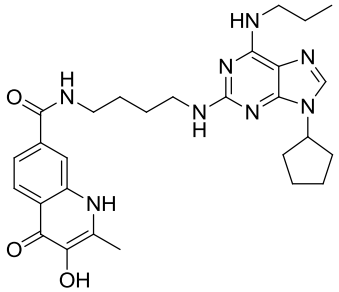
Prepared with use of method A. Yield 25 mg (82%). Purity of the crude product 85%; MS $[M+H]^+$= 507.28. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89 (t, *J*=7.23 Hz, 3 H) 1.46 (d, *J*=6.40 Hz, 6 H) 1.53 - 1.73 (m, 6 H) 2.40 (s, 3 H) 3.26 - 3.41 (m, 4 H) 3.61 (t, *J*=5.12 Hz, 1 H) 4.48 - 4.71 (m, 2 H) 7.63 (d, *J*=8.60 Hz, 1 H) 8.01 (s, 1 H) 8.13 (d, *J*=8.42 Hz, 1 H) 8.30 (br. s., 1 H) 8.68 (t, *J*=5.40 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.1, 14.3, 21.8, 26.6,117.8,118.5, 119.6, 123.1, 123.4, 124.6, 125.8, 133.5, 135.9, 136.6, 138.8, 139.5, 155.7, 156.7, 158.1, 158.7, 159.1, 163.5, 165.6, 167.6, 167.8.

3-Hydroxy-2-(4-methoxy-phenyl)-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [3-(9-tert-butyl-6-propylamino-9*H*-purin-2-ylamino)-propyl]-amide 9(1,3,3,6)



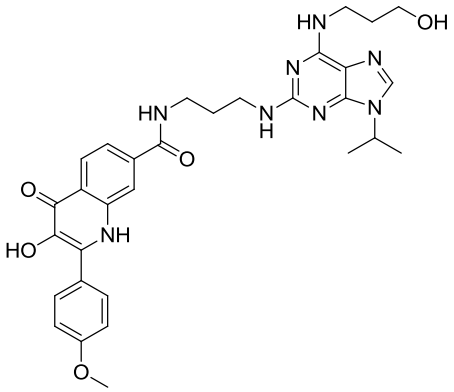
Prepared with use of method A. Yield 75 mg (79%). Purity of the crude product 90%; MS $[M+H]^+$= 599.30. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.70 - 1.00 (m, 9 H) 1.60 (d, *J*=4.39 Hz, 2 H) 1.76 - 1.95 (m, 3 H) 2.07 (dt, *J*=13.72, 6.86 Hz, 2 H) 2.54 (s, 3 H) 3.30 - 3.55 (m, 4 H) 3.77 - 3.96 (m, 2 H) 7.13 (d, *J*=8.78 Hz, 2 H) 7.67 (d, *J*=8.42 Hz, 1 H) 7.80 (d, *J*=8.78 Hz, 2 H) 8.13 (br. s., 1 H) 8.18 (d, *J*=8.60 Hz, 1 H) 8.15 - 8.16 (m, 1 H) 8.25 (s, 1 H) 8.73 (t, *J*=5.12 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.2, 19.4, 23.1, 24.6, 26.9, 28.4, 28.8, 37.1, 55.4, 113.8, 114.1, 118.0, 118.7, 119.7, 122.9, 124.2, 124.6, 130.7, 132.6, 136.2, 137.4, 138.3, 158.6, 159.0, 160.1, 165.9, 167.1,169.2.

3-Hydroxy-2-methyl-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [4-(9-cyclopentyl-6-propylamino-9*H*-purin-2-ylamino)-butyl]-amide 9(1,4,1,1)



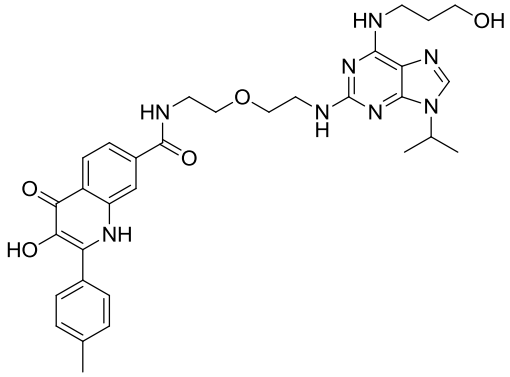
Prepared with use of method A. Yield 16 mg (65%). Purity of the crude product 82%, purity of the purified product 91%; MS $[M+H]^+$= 533.29. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, *J*=7.14 Hz, 3 H) 1.62 (br. s., 8 H) 1.89 (s, 6 H) 2.10 (br. s., 2 H) 2.40 (s, 3 H) 3.23 - 3.52 (m, 4 H) 4.70 (t, *J*=6.86 Hz, 1 H) 7.62 (d, *J*=8.42 Hz, 1 H) 8.01 (s, 1 H) 8.13 (d, *J*=8.60 Hz, 1 H) 8.67 (t, *J*=4.48 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 6.8, 11.1, 14.2, 15.7, 21.9, 23.6, 26.2, 26.5, 31.7, 55.9, 114.6,115.6, 117.7,118.5, 119.5, 123.4, 124.6, 131.8, 133.4,135.9, 136.6, 138.7, 158.3, 158.7, 165.6, 167.6.

3-Hydroxy-2-(4-methoxy-phenyl)-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [3-[6-(3-hydroxy-propylamino)-9-isopropyl-9*H*-purin-2-ylamino]-propyl]-amide 9(2,2,3,6)



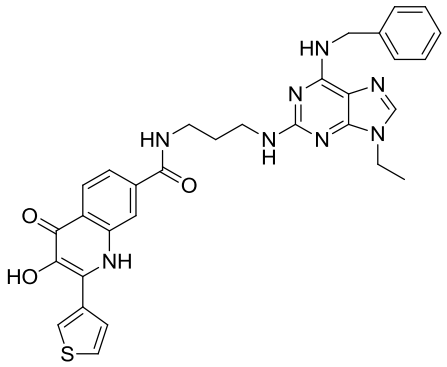
Prepared with use of method A. Yield 47 mg (67%). Purity of the crude product 95%; MS $[M+H]^+$= 591.28. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (d, *J*=5.67 Hz, 6 H) 1.69 - 1.80 (m, 2 H) 1.80 - 1.90 (m, 2 H) 3.30 - 3.56 (m, 8 H) 3.84 (s, 3 H) 4.55 (t, *J*=6.40 Hz, 1 H) 7.13 (d, *J*=8.78 Hz, 2 H) 7.67 (d, *J*=8.96 Hz, 1 H) 7.80 (d, *J*=8.60 Hz, 2 H) 7.95 (s, 1 H) 8.18 (d, *J*=8.42 Hz, 1 H) 8.25 (s, 1 H) 8.73 (t, *J*=5.21 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.7, 18.0, 21.8, 28.8, 37.2, 53.5, 55.4, 58.3, 113.8, 115.0, 118.6, 119.0, 119.7, 122.8, 124.21, 124.6, 130.7, 132.4, 136.3, 137.4, 138.3, 157.7, 158.1, 158.6, 159.0, 160.1, 165.9, 169.3.

3-Hydroxy-4-oxo-2-p-tolyl-1,4-dihydro-quinoline-7-carboxylic acid (2-{2-[6-(3-hydroxy-propylamino)-9-isopropyl-9*H*-purin-2-ylamino]-ethoxy}-ethyl)-amide 9(2,2,2,2)



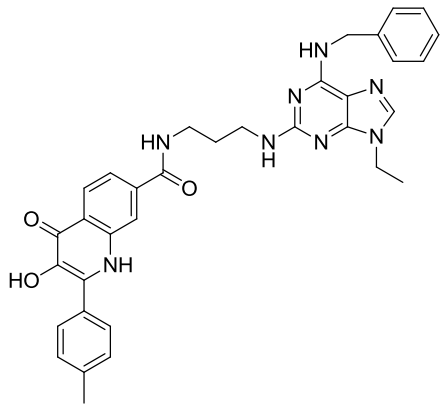
Prepared with use of method A. Yield 13 mg (14%). Purity of the crude product 98%; MS $[M+H]^+$= 615.30. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.44 (d, *J*=6.59 Hz, 6 H) 1.72 (t, *J*=6.04 Hz, 2 H) 2.40 (s, 3 H) 2.55 (br. s., 4 H) 3.25 - 3.66 (m, 7 H) 7.01 (s, 1 H) 7.18 (s, 1 H) 7.38 (d, *J*=7.50 Hz, 2 H) 7.66 (br. s., 1 H) 7.69 - 7.76 (m, 2 H) 8.12 - 8.20 (m, 1 H) 8.23 (br. s., 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.5, 22.5, 34.8, 46.6, 59.1, 69.1, 89.1, 91.3, 111.8, 113.3,115.8, 119.2, 119.8, 120.2, 123.4, 125.1, 129.4, 129.6, 132.9, 136.7, 138.0, 139.1, 139.6, 157.8, 158.2, 158.7, 159.1, 166.5, 170.1.

3-Hydroxy-4-oxo-2-thiophen-3-yl-1,4-dihydro-quinoline-7-carboxylic acid [3-(6-benzylamino-9-ethyl-9*H*-purin-2-ylamino)-propyl]-amide 9(3,1,3,3)



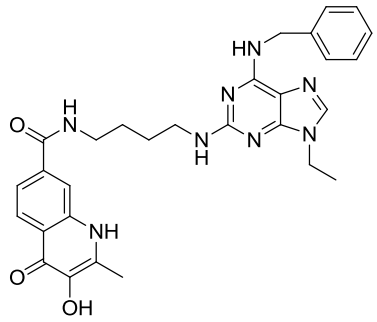
Prepared with use of method A. Yield 46 mg (52%). Purity of the crude product 86%; MS $[M+H]^+$= 595.22. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (t, *J*=6.86 Hz, 3 H) 1.74 - 1.92 (m, 2 H) 2.54 (s, 2 H) 3.26 - 3.49 (m, 4 H) 4.07 (d, *J*=5.49 Hz, 2 H) 7.16 - 7.40 (m, 6 H) 7.67 (d, *J*=8.60 Hz, 1 H) 7.73 - 7.80 (m, 1 H) 7.85 (d, *J*=6.40 Hz, 1 H) 8.16 (d, *J*=8.42 Hz, 2 H) 8.32 (s, 1 H) 8.61 - 8.80 (m, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9, 20.1, 25.5, 28.9, 35.9, 37.2, 115.5, 118.6, 119.6, 122.7, 124.6, 126.4, 127.0, 127.5, 127.7, 128.4, 130.4, 132.4, 136.4, 137.4, 138.5, 139.0, 148.7, 158.4, 158.8, 165.9, 167.8, 169.6, 170.3.

3-Hydroxy-4-oxo-2-p-tolyl-1,4-dihydro-quinoline-7-carboxylic acid [3-(6-benzylamino-9-ethyl-9*H*-purin-2-ylamino)-propyl]-amide 9(3,1,3,2)



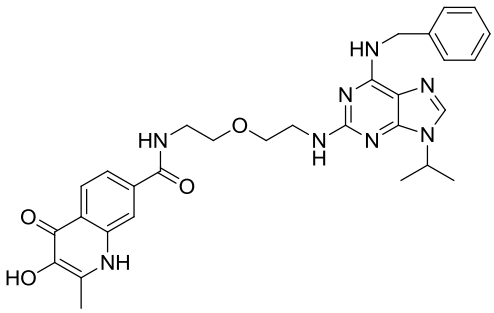
Prepared with use of method A. Yield 86 mg (81%). Purity of the crude product 90%; MS $[M+H]^+$= 603.28. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.34 (t, *J*=6.50 Hz, 3 H) 1.73 - 1.93 (m, 2 H) 2.40 (s, 3 H) 3.30 - 3.51 (m, 4 H) 3.99 - 4.19 (m, 2 H) 4.56 - 4.76 (m, 2 H) 7.14 - 7.34 (m, 4 H) 7.22 - 7.22 (m, 0 H) 7.38 (d, *J*=8.05 Hz, 3 H) 7.67 (d, *J*=8.42 Hz, 1 H) 7.73 (d, *J*=8.05 Hz, 2 H) 7.95 (s, 1 H) 8.18 (d, *J*=8.42 Hz, 1 H) 8.25 (s, 1 H) 8.72 (t, *J*=5.40 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.8, 21.0, 28.8, 30.8,35.8, 37.1, 114.1, 118.0, 118.7, 119.7, 122.9, 124.6, 127.1, 127.4, 128.4, 128.8, 129.1, 132.6, 136.3, 137.4, 138.4, 139.1, 158.0, 158.4, 158.9, 159.3, 162.3, 165.9, 166.7, 169.3.

3-Hydroxy-2-methyl-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [4-(6-benzylamino-9-ethyl-9*H*-purin-2-ylamino)-butyl]-amide 9(3,1,1,1)



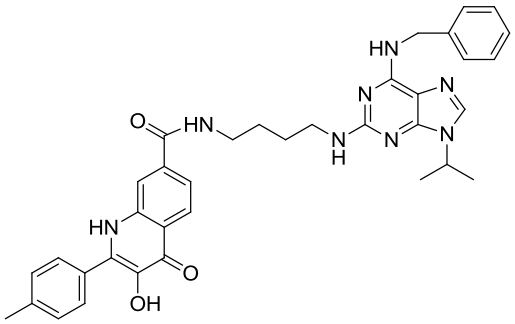
Prepared with use of method A. Yield 60 mg (89%). Purity of the crude product 88%; MS $[M+H]^+$= 541.26. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (t, *J*=7.04 Hz, 3 H) 1.59 (br. s., 4 H) 2.42 (s, 3 H) 3.22 - 3.35 (m, 2 H) 4.02 - 4.19 (m, 4 H) 4.57 - 4.75 (m, 2 H) 7.14 - 7.27 (m, 2 H) 7.28 - 7.42 (m, 4 H) 7.65 (d, *J*=8.78 Hz, 1 H) 8.03 (s, 1 H) 8.14 (d, *J*=8.60 Hz, 1 H) 8.68 (t, *J*=5.40 Hz, 1 H);^{13}C NMR (75 MHz, DMSO-d_6) δ 14.3, 14.8, 26.3, 26.5, 110.4, 114.3, 115.6, 117.8, 118.2, 119.8, 122.1, 123.3, 124.5, 127.1, 128.4, 134.1, 136.0, 136.5, 138.7, 151.2, 157.9, 158.4, 158.8, 159.3, 165.6, 167.2.

3-Hydroxy-2-methyl-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid {2-[2-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino)-ethoxy]-ethyl}-amide 9(3,2,2,1)



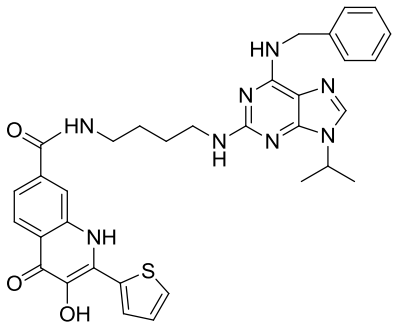
Prepared with use of method A. Yield 13 mg (12%). Purity of the crude product 90%; MS $[M+H]^+$= 571.27. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.17 - 1.31 (m, 2 H) 1.44 (d, *J*=6.59 Hz, 6 H) 2.38 (s, 3 H) 7.19 (d, *J*=3.84 Hz, 2 H) 7.23 - 7.39 (m, 3 H) 7.62 (d, *J*=8.78 Hz, 1 H) 7.98 (d, *J*=17.20 Hz, 2 H) 8.12 (d, *J*=8.23 Hz, 1 H) 8.68 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.2, 22.0, 46.1, 68.6, 68.9, 108.4, 115.2, 117.8, 119.2, 119.4, 123.6, 124.7, 126.6, 127.3, 128.1, 132.9, 135.7, 136.7, 138.9, 140.1, 157.3, 157.7, 158.1, 158.6, 165.9, 166.2.

3-Hydroxy-4-oxo-2-p-tolyl-1,4-dihydro-quinoline-7-carboxylic acid [4-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino)-butyl]-amide 9(3,2,1,2)



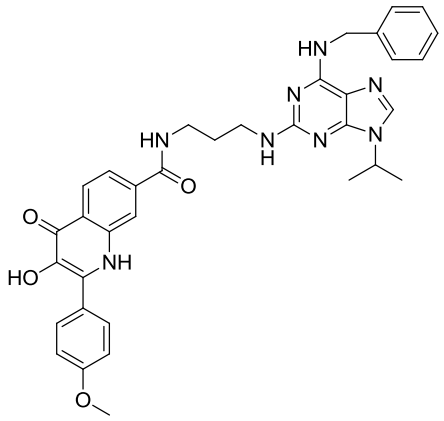
Prepared with use of method B. Yield 42 mg (45%). Purity of the crude product 93%; MS $[M+H]^+$= 631.31. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48 (d, *J*=6.59 Hz, 6 H) 1.61 (br. s., 4 H) 2.40 (s, 3 H) 2.56 (t, *J*=5.49 Hz, 1 H) 3.19 - 3.50 (m, 4 H) 4.44 - 4.82 (m, 2 H) 7.24 (d, *J*=6.77 Hz, 1 H) 7.28 - 7.33 (m, 2 H) 7.37 (d, *J*=8.23 Hz, 5 H) 7.66 (d, *J*=8.42 Hz, 1 H) 7.73 (d, *J*=7.87 Hz, 2 H) 8.18 (d, *J*=8.60 Hz, 1 H) 8.24 (s, 1 H) 8.64 (br. s., 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.9, 21.7, 26.4, 26.5, 47.1, 71.6, 116.1, 118.6, 119.7, 122.8, 124.5, 126.9, 127.4, 128.3, 128.8, 129.0, 129.2, 129.5, 132.4, 136.5, 137.4, 138.4, 139.0, 145.8, 157.5, 158.3, 165.7, 169.4, 173.7, 177.0, 177.7.

2-Hydroxy-4-oxo-3-thiophen-2-yl-1,4-dihydro-quinoline-7-carboxylic acid [4-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino)-butyl]-amide 9(3,2,1,4)



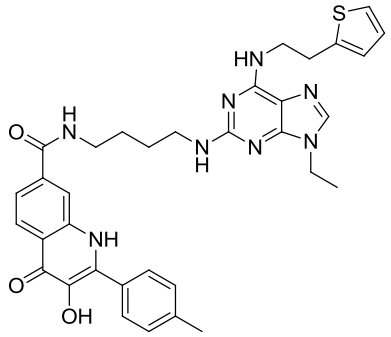
Prepared with use of method B. Yield 22 mg (42%). Purity of the crude product 98%; MS $[M+H]^+$= 623.25. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48 (d, *J*=6.59 Hz, 6 H) 1.61 (br. s., 2 H) 1.91 (s, 2 H) 3.25 - 3.47 (m, 4 H) 4.51 - 4.78 (m, 3 H) 7.25 (d, *J*=6.22 Hz, 1 H) 7.28 - 7.34 (m, 2 H) 7.34 - 7.42 (m, 4 H) 7.65 (d, *J*=8.23 Hz, 1 H) 7.87 (d, *J*=5.12 Hz, 1 H) 8.10 (d, *J*=3.66 Hz, 1 H) 8.15 (d, *J*=8.60 Hz, 1 H) 8.36 (s, 1 H) 8.68 (t, *J*=5.21 Hz, 1 H); (75 MHz, DMSO-d_6) δ 21.1, 21.7, 26.6, 40.8, 64.9, 112.1, 114.2, 116.2, 118.1, 119.7,122.0, 122.8, 124.4, 125.9, 126.9, 127.1, 127.4, 127.5, 128.4, 130.7, 132.8, 136.6, 137.6, 158.1, 158.5, 159.0, 159.5, 165.7, 169.8, 172.0.

3-Hydroxy-2-(4-methoxy-phenyl)-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid [3-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino)-propyl]-amide 9(3,2,3,2)



Prepared with use of method A. Yield 95 mg (95%). Purity of the crude product 97%; MS $[M+H]^+$= 633.29. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.45 (d, *J*=4.39 Hz, 6 H) 1.82 (br. s., 2 H) 2.88 (s, 3 H) 3.29 - 3.50 (m, 4 H) 4.49 - 4.75 (m, 3 H) 7.13 (d, *J*=8.60 Hz, 2 H) 7.19 - 7.41 (m, 5 H) 7.67 (d, *J*=8.78 Hz, 1 H) 7.80 (d, *J*=8.60 Hz, 2 H) 7.95 (s, 1 H) 8.18 (d, *J*=8.42 Hz, 1 H) 8.25 (s, 1 H) 8.72 (t, *J*=4.94 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.0, 21.7, 30.8, 32.1, 35.8, 37.1, 55.4, 113.8, 114.0, 118.7, 119.7, 122.8, 124.2, 124.6, 127.1, 127.4, 128.4, 130.7, 134.4, 136.2, 137.4, 138.3, 144.1, 158.4, 160.1, 162.3, 165.9, 170.1, 176.6.

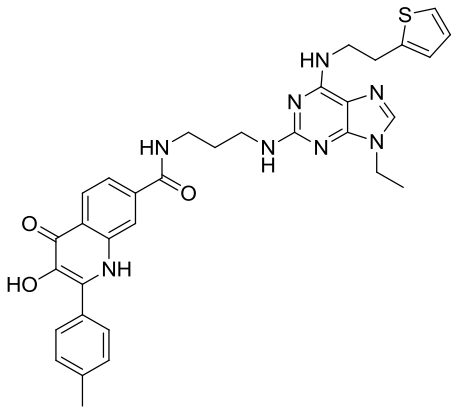
3-Hydroxy-4-oxo-2-*p*-tolyl-1,4-dihydro-quinoline-7-carboxylic acid {4-[9-ethyl-6-(2-thiophen-2-yl-ethylamino)-9*H*-purin-2-ylamino]-butyl}-amide 9(4,1,1,2)



Prepared with use of method A. Yield 90 mg (95%). Purity of the crude product 98%; MS $[M+H]^+$= 637.26. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (t, *J*=6.95 Hz, 3 H) 1.64 (br. s., 4 H) 2.40 (s, 3 H) 3.15 (t, *J*=6.86 Hz, 2 H) 3.28 - 3.47 (m, 4 H) 3.72 (d, *J*=2.56 Hz, 2 H) 3.99 - 4.18 (m, 2 H) 6.93 (br. s., 2 H) 7.33 (d, *J*=3.48 Hz, 2 H) 7.38 (d, *J*=8.05 Hz, 2 H) 7.65 (d, *J*=8.60 Hz, 1 H) 7.72 (d, *J*=7.87 Hz, 2 H) 8.17 (d, *J*=8.60 Hz, 1 H) 8.23 (s, 1 H) 8.61 - 8.73 (m, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9, 21.0, 23.9, 26.5, 28.2, 30.1, 35.8, 40.8, 108.3, 114.4,

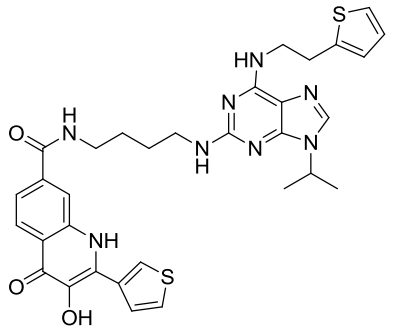
118.7, 119.7, 122.9, 124.3, 124.6, 125.5, 127.0, 128.9, 129.1, 132.5, 133.9, 136.4, 137.5, 138.5, 139.1, 145.9, 158.6, 159.1, 165.7, 169.4, 174.0, 179.1.

3-Hydroxy-4-oxo-2-p-tolyl-1,4-dihydro-quinoline-7-carboxylic acid {3-[9-ethyl-6-(2-thiophen-2-yl-ethylamino)-9*H*-purin-2-ylamino]-propyl}-amide 9(4,1,3,2)



Prepared with use of method A. Yield 93 mg (95%). Purity of the crude product 99%; MS $[M+H]^+$= 623.25. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (t, *J*=6.22 Hz, 3 H) 1.86 (t, *J*=6.40 Hz, 2 H) 2.40 (s, 3 H) 3.04 - 3.20 (m, 2 H) 3.29 - 3.53 (m, 4 H) 3.69 (br. s., 2 H) 4.08 (br. s., 2 H) 6.91 (br. s., 2 H) 7.32 (br. s., 1 H) 7.38 (d, *J*=8.05 Hz, 2 H) 7.67 (d, *J*=8.78 Hz, 1 H) 7.73 (d, *J*=8.05 Hz, 2 H) 8.18 (d, *J*=8.42 Hz, 1 H) 8.25 (s, 1 H) 8.72 (t, *J*=4.48 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9, 20.1, 26.7, 28.9, 37.2, 47.0, 52.7, 76.2, 112.4, 114.6, 118.7, 119.7, 122.9, 124.2, 124.6, 125.4, 127.0, 128.8, 129.1, 129.2, 132.5, 136.3, 137.5, 138.5, 139.1, 144.8, 151.1, 158.4, 158.8, 165.9, 180.7.

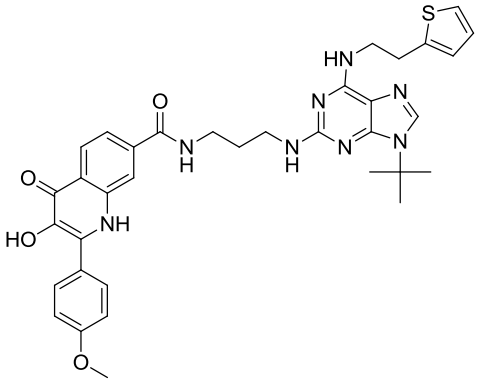
3-Hydroxy-4-oxo-2-thiophen-3-yl-1,4-dihydro-quinoline-7-carboxylic acid {4-[9-isopropyl-6-(2-thiophen-2-yl-ethylamino)-9*H*-purin-2-ylamino]-butyl}-amide 9(4,2,1,4)



Prepared with use of method A. Yield 20 mg (25%). Purity of the crude product 95%; MS $[M+H]^+$= 643.22. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.47 (d, *J*=6.40 Hz, 6 H) 1.64 (br. s., 4 H) 3.16 (t, *J*=7.04 Hz, 4 H) 3.28 - 3.48 (m, 4 H) 4.54 - 4.71 (m, 1 H) 6.86 - 7.01 (m, 2 H) 7.33 (d, *J*=4.03 Hz, 1 H) 7.64 (d, *J*=8.60 Hz, 1 H) 7.73 - 7.80 (m, 1 H) 7.85 (d, *J*=5.31 Hz, 1 H) 8.15 (d, *J*=8.60 Hz, 1 H) 8.30 (br. s., 2 H) 8.68 (t, *J*=5.12 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6)

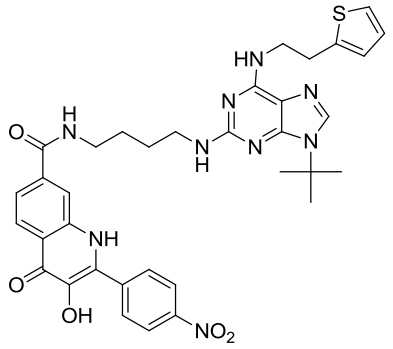
δ 11.2, 19.4, 21.8, 22.7, 28.4, 28.7, 37.1, 55.4, 113.8, 114.1, 118.0, 118.7, 119.7, 121.9, 122.9, 124.2, 124.6, 130.7, 132.6, 136.2, 137.4, 138.3, 146.4, 158.6, 159.0, 159.5, 160.1, 163.0, 165.9, 169.2.

3-Hydroxy-2-(4-methoxy-phenyl)-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid {3-[9-tert-butyl-6-(2-thiophen-2-yl-ethylamino)-9*H*-purin-2-ylamino]-propyl}-amide 9(4,3,3,6)



Prepared with use of method A. Yield 19 mg (65%). Purity of the crude product 98%; MS $[M+H]^+$= 667.27. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (br. s., 9 H) 1.73 - 2.01 (m, 2 H) 3.14 (br. s., 1 H) 3.32 - 3.53 (m, 6 H) 3.70 (br. s., 1 H) 3.78 - 3.96 (m, 2 H) 3.85 (s, 3 H) 6.93 (br. s., 2 H) 7.13 (d, *J*=8.78 Hz, 2 H) 7.33 (br. s., 1 H) 7.68 (d, *J*=7.87 Hz, 1 H) 7.79 (d, *J*=8.42 Hz, 2 H) 7.95 (s, 1 H) 8.18 (d, *J*=8.60 Hz, 1 H) 8.25 (s, 1 H) 8.73 (t, *J*=5.31 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.3, 21.6, 26.9, 28.3, 30.8, 32.8, 35.8, 55.4, 91.1, 95.1, 100.1, 101.8, 103.3, 113.8, 117.7, 118.7, 119.8, 122.8, 124.2, 124.6, 125.5, 127.0, 130.8, 136.2, 137.4, 138.3, 140.2, 160.2, 162.3, 165.9.

3-Hydroxy-2-(4-nitro-phenyl)-4-oxo-1,4-dihydro-quinoline-7-carboxylic acid {4-[9-tert-butyl-6-(2-thiophen-2-yl-ethylamino)-9*H*-purin-2-ylamino]-butyl}-amide 9(4,3,1,5)



Prepared with use of method B. Yield 92 mg (95%). Purity of the crude product 86%; MS $[M+H]^+$= 696.26. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84 (d, *J*=6.59 Hz, 9 H) 1.64 (br. s., 4 H) 2.55 (t, *J*=5.49 Hz, 2 H) 3.07 - 3.21 (m, 2 H) 3.29 - 3.37 (m, 2 H) 3.88 (d, *J*=2.56 Hz, 2 H) 6.86 - 6.98 (m, 2 H) 7.32 (d, *J*=4.21 Hz, 1 H) 7.68 (d, *J*=9.70 Hz, 1 H) 8.04 (br. s., 1 H) 8.11

(d, *J*=8.78 Hz, 2 H) 8.18 (s, 1 H) 8.22 (d, *J*=5.49 Hz, 1 H) 8.41 (d, *J*=8.96 Hz, 2 H) 8.70 (t, *J*=5.58 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.4, 26.6, 28.3, 34.3, 114.7, 115.9, 118.7, 119.9, 120.8, 121.3, 123.0, 123.3, 124.2, 124.7, 125.4, 127.0, 128.6, 129.5, 130.7, 136.8, 137.8, 138.3, 138.6, 139.2, 141.1, 147.5, 158.6, 159.1, 163.2, 165.5, 169.5.

6.3 Direct arylation of Purine on Solid Phase and Its Use for Chemical Libraries Synthesis

Barbora Vaňková, Viktor Krchňák, Miroslav Soural and Jan Hlaváč, ACS Comb. Sci. 2011, 12, 496-500.[A3]

6.3.1 Experimental procedures

Reaction with CDI and 1,3-diaminopropane (11(1)).

Wang resin (1 mmol/g; 1g) was washed 3x with DCM and a solution of CDI (5 mmol; 810 mg) and pyridine (5 mmol; 400 μL) in 10 ml DCM was added. Resin slurry was shaken for 2 h, the resin was washed 3x with DCM and a solution of 1,3-diaminopropane (5 mmol; 417 μL) in 10 ml DCM was added. Resin slurry was shaken for 3 h, washed 5 x with DCM. A sample of resin was dried by a stream of nitrogen and product was quantified.

Quantification of resin loading: a 10 mg sample of dried resin was reacted with Fmoc-OSu (160 mg) in 1 mL DCM for 30 min at ambient temperature. The resin was washed 5 x with DCM and the product was cleaved from resin with 50% TFA in DCM for 30 min. The cleavage cocktail was evaporated by a stream of nitrogen, and cleaved compounds extracted into 1 mL of MeOH. This sample of Fmoc derivatives of the structures **11(1)** was analyzed by LC-MS and the quantity were compared with analysis of the standard (Fmoc-Ala-OH; concentration 1 mg/ml). The loading of the resin was determined by external standard method.

Reaction with 2-(fmoc-amino)ethanol (11(2)).

Wang resin (1 mmol/g; 1g) was washed 3 x with anhydrous DCM and a solution of trichloroacetonitrile (15 mmol; 1.5 mL) in 10 mL anhydrous DCM was added. The resin was kept in a freezer for 30 min. Then solution of DBU (0.67 mmol; 100 μL) in 2 mL anhydrous DCM was added, the resin slurry was shaken for 1 h and washed 3 x with anhydrous DCM and 3x with anhydrous THF. Solution of 2-(fmoc-amino)ethanol (3 mmol; 849 mg) in 10 mL anhydrous THF was added to the resin followed by drop wise addition of $BF_3 \cdot Et_2O$ (0.3 mmol; 63 μL) and the resin slurry was shaken for 30 min. Resin was washed 3x with THF, 3x

with MeOH and 3x with DCM. The quantification was carried out with a sample of resin as describe above. After the quantification, the resin was washed 3x with DMF, solution of 20% piperidine in DMF was added for fmoc deprotection and resin slurry was shaken 20 min. The resin was washed 3x with DMF and 3x with DCM.

Reaction with Fmoc-amino acids (11(3), 11(4)).

Resin **11(3)** and **11(4)** were performed according a published procedure.[30] Wang resin (1 mmol/g; 1 g) was washed 3 x with DCM and 3 x with THF and 3 x with anhydrous THF. A solution of Fmoc-β-Ala-OH (2 mmol, 620 mg) and triphenylphosphine (2 mmol, 524 mg) in 8 mL of anhydrous THF was added. The resin slurry was kept in freezer for 30 minutes. Diisopropylazocarboxylate (DIAD) (2 mmol, 390 μL) was diluted in 2 mL of anhydrous THF and resulted solution was also kept in freezer for 30 minutes. After this time the solution of DIAD was slowly sucked into the syringe with resin and resin slurry was shaken for 1 hour. Then the resin was washed 3 x with THF, 3 x with DCM. The product was quantified and then Fmoc protecting group was removed as described above.

Reaction with 2,6-dichloropurine (12(R^1)).

1 g of the resin **11(R^1)** was washed 3x with THF and a solution of 2,6-dichloropurine (2.5 mmol; 467 mg) and DIEA (2.5 mmol; 435 μl) in 10 mL THF was added. Resin slurry was shaken at 50 °C overnight and subsequently the resin was washed 3 x with THF, 3 x with DCM. No Fmoc-starting amines were detected after the fmocylation of the resin sample (10 mg of the resin was shaken with solution of Fmoc-OSu (0.1 mmol; 33 mg) in 1 mL DCM for 30 min.) indicating the reaction with 2,6-dichloropurine was quantitative.

Alkylation with alkyl iodide (13(R^1,R^2).

500 mg of resin **12(R^1)** was washed 3 x with DCM, 3 x with DMSO and a 0.6 M solution of appropriate alkyl iodide (3 mmol) and eqv. DBU (3 mmol; 440 μL) in DMSO (5 mL) was added. Resin slurry was shaken overnight at 50 °C, then the resin was washed 3 x with DMF and 3x with DCM.

Reaction with piperidine or 3-aminopropanol (14(R^1,R^2,R^3)).

250 mg of resin **13(R^1,R^2)** was washed 3x with DCM and 3x with DMSO and a 2 M solution of piperidine or 3-aminopropanol in diethylene glycol diethyl ether (3 mL) was added and resin slurry was shaken at 150 °C for 24 h. The resin was washed 3 x with DMF and 3x with DCM.

Coupling reaction with aryl iodide (15(R^1,R^2,R^3,R^4)).

200 mg of resin **14(R^1,R^2,R^3)** was washed 3x with anhydrous THF and 3 x with anhydrous DMF, then 2 mL of anhydrous DMF was added and the reaction mixture was saturated with Argon for 2 min. Subsequently the mixture was added to a glass vial and aryl iodide (1.6 mmol), CuI (1.1 mmol; 210 mg), piperidine (1.8 mmol; 180 μL) and $Pd(OAc)_2$ (18 μmol; 4 mg) was added. The resin slurry was shaken at 115 °C for 24 h. Then the resin was transferred back to polypropylene fritted vessel and it was washed 5 x with DMF and 3x with DCM.

Coupling reaction with aryl bromide (15(R^1,R^2,R^3,R^4)).

200 mg of resin **14(R^1,R^2,R^3)** was washed 3x with anhydrous THF and 3 x with anhydrous DMF, then 2 mL of anhydrous DMF was added and the reaction mixture was saturated with Argon for 2 min. Subsequently the mixture was added to a glass vial and aryl bromide (1.6 mmol), CuI (1.1 mmol; 210 mg), piperidine (1.8 mmol; 180 μL;) and $Pd(OAc)_2$ (18 μmol; 4 mg) was added. The resin slurry was shaken at 115 °C for 48 h. Then the resin was transferred back to polypropylene fritted vessel and it was washed 5 x with DMF and 3x with DCM.

Coupling reaction of N^9 unsubstituted purine (15(R^1,H,R^3,R^4)).

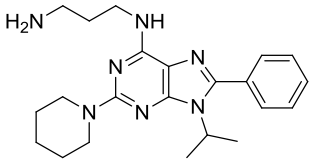
200 mg of resin **14(R^1,H,R^3)** was washed 3x with anhydrous THF and 3x with anhydrous DMF, then 2 mL of anhydrous DMF was added and the reaction mixture was saturated with Argon for 2 min. Subsequently a mixture was added to a glass vial and aryl iodide (1.6 mmol), CuI (1.1 mmol; 210 mg), piperidine (1.8 mmol; 180 μL;) and $Pd(OAc)_2$ (18 μmol; 4 mg) was added. The resin slurry was shaken at 135 °C for 48 h. Then the resin was transferred back to polypropylene fritted vessel and it was washed 5 x with DMF and 3x with DCM.

Cleavage and Isolation (15(R^1,R^2,R^3,R^4)).

After washing, the resin **15** was treated with 50% TFA in DCM for 1 h. The cleavage cocktail was separated and the resin was washed 2 x with 50% TFA in DCM. The liquid phases were collected and evaporated by a stream of nitrogen. The evaporated material was dissolved in MeOH and purified by semipreparative HPLC.

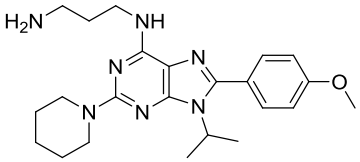
6.3.2 Analytical data

N^1-(9-Isopropyl-8-phenyl-2-piperidin-1-yl-9*H*-purin-6-yl)-propane-1,3-diamine 15(1,1,1,1)



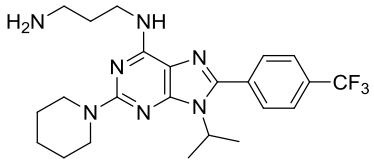
Yield 19.2 mg (61%). Purity of the crude product 94%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.56 - 7.60 (m, 2 H) 7.49 - 7.55 (m, 3 H) 4.46 - 4.54 (m, 1 H) 3.68 - 3.74 (m, 4 H) 3.34 - 3.52 (m, 2 H) 2.82 - 2.88 (m, 2 H) 1.85 - 1.90 (m, 2 H) 1.59 (d, *J*=6.7 Hz, 8 H) 1.50 - 1.55 (m, 4 H). ^{13}C NMR (151 MHz, DMSO-*d6*): δ 158.4, 158.2, 157.9, 146.7, 130.9, 129.2, 129.1, 128.7, 104.6, 48.3, 45.1, 37.1, 36.6, 27.4, 25.2, 24.6, 20.7. HRMS (ESI) *m/z* calcd for $C_{22}H_{31}N_7$ $[M + H]^+$ 394.2641 found 394.2708.

N^1-[9-Isopropyl-8-(4-methoxy-phenyl)-2-piperidin-1-yl-9*H*-purin-6-yl]-propane-1,3-diamine 15(1,1,1,2)



Yield 11.7 mg (52%). Purity of the crude product 95%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.49 - 7.52 (m, 2 H) 7.07 - 7.09 (m, 2 H) 4.48 (quin, *J*=6.7 Hz, 1 H) 3.82 (s, 3 H) 3.70 (t, *J*=5.3 Hz, 4 H) 1.85 (br. s., 6 H) 1.57 - 1.62 (m, 9 H) 1.52 (d, *J*=3.5 Hz, 4 H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 160.9, 158.2, 152.8, 151.7, 146.1, 131.1, 119.9, 114.5, 109.2, 55.5, 49.3, 45.2, 42.2, 36.9, 27.3, 25.3, 24.6, 20.5. HRMS (ESI) *m/z* calcd for $C_{23}H_{33}N_7O$ $[M + H]^+$ 424.2743; found 424.2809.

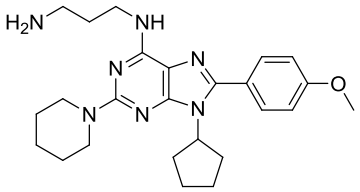
N^1-[9-Isopropyl-2-piperidin-1-yl-8-(4-trifluoromethyl-phenyl)-9*H*-purin-6-yl]-propane-1,3-diamine 15(1,1,1,3)



Yield 26 mg (70%). Purity of the crude product 90%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (d, *J*=8.2 Hz, 2 H) 7.82 (m, *J*=8.2 Hz, 3 H) 4.52 (quin, *J*=6.7 Hz, 1 H) 3.70 - 3.76 (m, 4 H) 2.82 - 2.89 (m, 2 H) 2.54 (s, 3 H) 1.85 - 1.91 (m, 2 H) 1.61 (d, *J*=6.7 Hz, 8 H) 1.53 (d, *J*=3.8 Hz, 4 H). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 158.5, 158.1, 154.5, 152.5, 145.2, 134.8, 129.9,

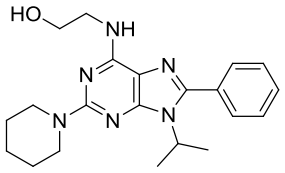
129.3 (q, *J* = 31.9 Hz), 125.7 (q, *J* = 3.5 Hz), 124.2 (q, *J* = 272.3 Hz), 48.7, 45.2, 42.2, 40.4, 37.0, 27.4, 25.3, 24.7. HRMS (ESI) *m/z* calcd for $C_{23}H_{30}F_3N_7$ $[M + H]^+$ 462.2515; found 462.2573.

N^1-[9-Cyclopentyl-8-(4-methoxy-phenyl)-2-piperidin-1-yl-9*H*-purin-6-yl]-propane-1,3-diamine 15(1,2,1,2)



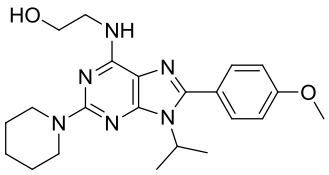
Yield 9 mg (33%). Purity of the crude product 80%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.58 (d, *J*=8.5 Hz, 2 H) 7.13 (d, *J*=8.8 Hz, 2 H) 4.65 (quin, *J*=8.3 Hz, 1 H) 3.84 (s, 3 H) 3.71 (br. s., 6 H) 2.85 (t, *J*=7.6 Hz, 2 H) 2.33 - 2.44 (m, 2 H) 2.08 (s, 1 H) 1.98 (br. s., 4 H) 1.82 - 1.91 (m, 2 H) 1.57 - 1.66 (m, 4 H) 1.53 (d, *J*=3.5 Hz, 4 H). ^{13}C NMR (151 MHz, DMSO-*d*6): δ 160.4, 158.4, 158.2, 157.9, 153.4, 151.4, 146.9, 130.7, 114.3, 56.7, 55.4, 45.2, 40.1, 37.1, 36.7, 30.2, 27.3, 25.1, 24.6 HRMS (ESI) *m/z* calcd for $C_{25}H_{35}N_7O$ $[M + H]^+$ 450.2923; found 450.2978.

2-(9-Isopropyl-8-phenyl-2-piperidin-1-yl-9*H*-purin-6-ylamino)-ethanol 15(2,1,1,1)



Yield 12.7 mg (42%). Purity of the crude product 90%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.58 - 7.61 (m, 2 H) 7.51 - 7.57 (m, 3 H) 4.51 (quin, *J*=6.7 Hz, 1 H) 3.69 - 3.74 (m, 4 H) 3.56 - 3.60 (m, 2 H) 2.54 (s, 2 H) 1.60 (d, *J*=6.7 Hz, 8 H) 1.50 - 1.55 (m, 4 H). HRMS (ESI) *m/z* calcd for $C_{21}H_{28}N_6O$ $[M + H]^+$ 381.2325; found 381.2369.

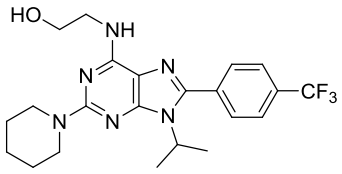
2-[9-Isopropyl-8-(4-methoxy-phenyl)-2-piperidin-1-yl-9*H*-purin-6-ylamino]-ethanol 15(2,1,1,2)



Yield 8.5 mg (35%). Purity of the crude product 91%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.50 (d, *J*=8.2 Hz, 2 H) 7.08 (d, *J*=8.2 Hz, 2 H) 4.72 (t, *J*=5.3 Hz, 1 H) 4.48 (dt, *J*=13.3, 6.7 Hz, 1 H) 3.82 (s, 3 H) 3.70 (t, *J*=5.0 Hz, 4 H) 3.52 - 3.61 (m, 2 H) 1.58 (d, *J*=6.7 Hz, 8 H) 1.52 (br. s., 4 H). ^{13}C NMR (151 MHz, DMSO-*d*6): δ 159.8, 157.8, 154.3, 152.3, 146.7, 130.5, 123.2,

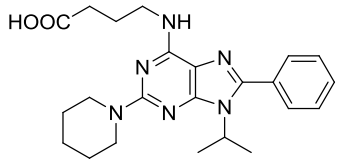
114.1, 113.4, 60.0, 55.3, 48.2, 45.1, 42.5, 25.2, 24.6, 20.7. HRMS (ESI) *m/z* calcd for $C_{22}H_{30}N_6O_2$ $[M + H]^+$ 411.2430; found 411.2477.

2-[9-Isopropyl-2-piperidin-1-yl-8-(4-trifluoromethyl-phenyl)-9*H*-purin-6-ylamino]-ethanol 15(2,1,1,3)



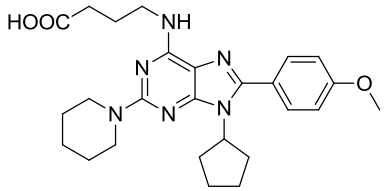
Yield 13.9 mg (52%). Purity of the crude product 91%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.89 (d, *J*=7.9 Hz, 2 H) 7.81 (d, *J*=8.2 Hz, 2 H) 4.73 (t, *J*=5.3 Hz, 1 H) 4.51 (quin, *J*=6.7 Hz, 1 H) 3.68 - 3.76 (m, 4 H) 3.54 - 3.62 (m, 2 H) 3.49 (br. s., 1 H) 1.61 (d, *J*=6.7 Hz, 8 H) 1.52 (br. s., 4 H). ^{13}C NMR (151 MHz, DMSO-*d*6): δ 158.0, 154.6, 145.0, 134.9, 129.9, 129.1 (q, *J* = 31.7 Hz), 127.4, 125.6 (q; *J* = 3.83 Hz), 124.2 (q; *J* = 272.3 Hz), 113.9, 59.9, 48.6, 45.1, 42.4, 25.2, 24.6, 20.7. HRMS (ESI) *m/z* calcd for $C_{22}H_{27}F_3N_6O$ $[M + H]^+$ 449.2198; found 449.2254.

4-(9-Isopropyl-8-phenyl-2-piperidin-1-yl-9*H*-purin-6-ylamino)-butyric acid 15(4,1,1,1)



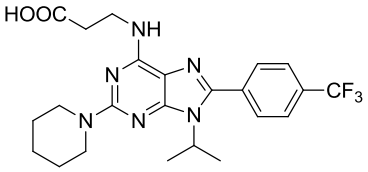
Yield 2.5 mg (7%). Purity of the crude product 96%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.59 - 7.62 (m, 2 H) 7.52 - 7.57 (m, 3 H) 4.53 (quin, *J*=6.7 Hz, 1 H) 3.71 - 3.75 (m, 4 H) 2.29 (t, *J*=7.5 Hz, 2 H) 1.86 (quin, *J*=7.1 Hz, 2 H) 1.62 - 1.65 (m, 2 H) 1.61 (d, *J*=6.7 Hz, 7 H) 1.52 - 1.57 (m, 4 H). HRMS (ESI) *m/z* calcd for $C_{23}H_{30}N_6O_2$ $[M + H]^+$ 423.2430; found 423.2503.

4-[9-Cyclopentyl-8-(4-methoxy-phenyl)-2-piperidin-1-yl-9*H*-purin-6-ylamino]-butyric acid 15(4,2,1,2)



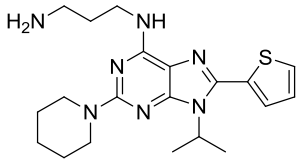
Yield 5.7 mg (15 %). Purity of the crude product 91%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.57 (d, *J*=8.8 Hz, 2 H) 7.12 (d, *J*=8.8 Hz, 2 H) 4.66 (quin, *J*=8.2 Hz, 1 H) 3.86 (s, 3 H) 3.70 - 3.74 (m, 4 H) 2.39 - 2.45 (m, 2 H) 2.30 (t, *J*=7.3 Hz, 2 H) 1.93 - 2.02 (m, 4 H) 1.86 (quin, *J*=7.1 Hz, 2 H) 1.58 - 1.66 (m, 4 H) 1.51 - 1.57 (m, 4 H). HRMS (ESI) *m/z* calcd for $C_{26}H_{34}N_6O_3$ $[M + H]^+$ 479,2692; found 479.2765.

3-[9-Isopropyl-2-piperidin-1-yl-8-(4-trifluoromethyl-phenyl)-9*H*-purin-6-ylamino]-propionic acid 15(3,1,1,3)



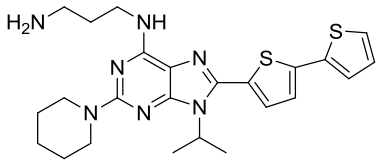
Yield 9.4 mg (25%). Purity of the crude product 92%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 - 7.91 (m, 2 H) 7.81 - 7.85 (m, 2 H) 4.54 (quin, *J*=6.7 Hz, 1 H) 3.71 - 3.77 (m, 4 H) 2.61 (t, *J*=7.0 Hz, 2 H) 1.60 - 1.67 (m, 9 H) 1.52 - 1.59 (m, 4 H). ^{13}C NMR (151 MHz, DMSO-*d*6): δ 173.2, 158.0, 154.0, 152.9, 144.9, 134.3, 129.9, 125.6, 124.1 (q, *J* = 271.1 Hz), 113.1, 48.8, 45.1, 36.0, 33.8, 25.2, 24.5, 20.7 (Due to low concentration the other quartets were not detected).. HRMS (ESI) *m/z* calcd for $C_{23}H_{27}F_3N_6O_2$ $[M + H]^+$ 477.2148; found 477.2220.

N^1-(9-Isopropyl-2-piperidin-1-yl-8-thiophen-2-yl-9*H*-purin-6-yl)-propane-1,3-diamine 15(1,1,1,4)



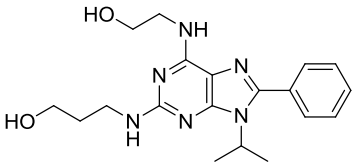
Yield 3.6 mg (15%). Purity of the crude product 42%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.71 (dd, *J*=5.1, 1.0 Hz, 1 H) 7.41 (dd, *J*=3.7, 1.0 Hz, 1 H) 7.22 (dd, *J*=5.0, 3.5 Hz, 1 H) 4.82 (quin, *J*=6.7 Hz, 1 H) 3.71 - 3.75 (m, 4 H) 2.85 - 2.92 (m, 2 H) 1.88 - 1.94 (m, 2 H) 1.61 - 1.66 (m, 9 H) 1.52 - 1.58 (m, 4 H). HRMS (ESI) *m/z* calcd for $C_{20}H_{29}N_7S$ $[M + H]^+$ 400.2205; found 400.2278.

N^1-(8-[2,2']Bithiophenyl-5-yl-9-isopropyl-2-piperidin-1-yl-9*H*-purin-6-yl)-propane-1,3-diamine 15(1,1,1,bis4)



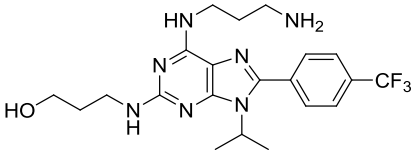
Yield 2.8 mg (12%). Purity of the crude product 37%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 (dd, *J*=5.0, 1.2 Hz, 1 H) 7.37 (dd, *J*=3.5, 1.2 Hz, 1 H) 7.33 - 7.35 (m, 2 H) 7.12 (dd, *J*=5.1, 3.7 Hz, 1 H) 4.87 (quin, *J*=6.8 Hz, 1 H) 3.69 - 3.73 (m, 4 H) 2.85 - 2.90 (m, 2 H) 1.90 (quin, *J*=7.0 Hz, 2 H) 1.65 (d, *J*=6.7 Hz, 7 H) 1.59 - 1.63 (m, 1 H) 1.51 - 1.56 (m, 5 H). HRMS (ESI) *m/z* calcd for $C_{24}H_{31}N_7S_2$ $[M + H]^+$ 482.2082; found 482.2155.

3-((6-((2-hydroxyethyl)amino)-9-isopropyl-8-phenyl-9*H*-purin-2-yl)amino)propan-1-ol 15(1,1,2,1)



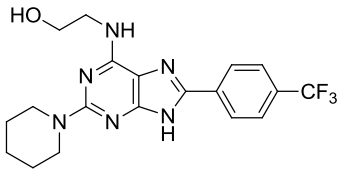
Yield 4.5 mg (16%). Purity of crude product 61%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.52 - 7.65 (m, 5 H) 4.52 (dt, *J*=13.63, 6.91 Hz, 1 H) 3.57 - 3.65 (m, 2 H) 3.51 (t, *J*=6.22 Hz, 4 H) 3.40 (t, *J*=6.68 Hz, 2 H) 1.75 (quin, *J*=6.50 Hz, 2 H) 1.59 (d, *J*=6.77 Hz, 6 H). MS $[M+H]^+$= 371.2117.

3-((6-((3-aminopropyl)amino)-9-isopropyl-8-(4-(trifluoromethyl)phenyl)-9*H*-purin-2-yl)amino)propan-1-ol 15(1,1,2,3)



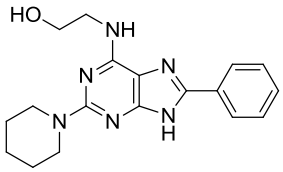
Yield 6 mg (18 %). Purity of the crude product 62%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.78 - 7.98 (m, 4 H) 4.53 (dt, *J*=13.13, 6.52 Hz, 1 H) 3.51 (m, *J*=6.22 Hz, 4 H) 3.38 (t, *J*=6.68 Hz, 2 H) 2.85 (d, *J*=4.21 Hz, 2 H) 1.89 (d, *J*=5.85 Hz, 2 H) 1.75 (quin, *J*=6.50 Hz, 2 H) 1.61 (d, *J*=6.77 Hz, 6 H). ^{13}C NMR (75 MHz, DMSO-*d*6): δ 20.7, 27.3, 32.2, 36.6, 38.5, 49.0, 58.8, 120.2 (q, *J* = 31.7 Hz), 121.8, 122.3, 124.1 (q, *J* = 272.4 Hz), 125.7 (q, *J* = 3.7 Hz), 125.9, 128.2, 130.0. MS $[M+H]^+$= 451.2307.

2-[2-Piperidin-1-yl-8-(4-trifluoromethyl-phenyl)-9*H*-purin-6-ylamino]-ethanol 15(2,3,1,3)



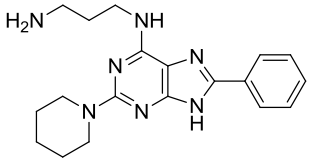
Yield 6.7 mg (21%). Purity of the crude product 71%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.02 (br. s., 1 H) 8.22 (d, *J*=8.2 Hz, 2 H) 7.84 (d, *J*=8.2 Hz, 2 H) 4.75 (t, *J*=5.6 Hz, 1 H) 3.69 - 3.75 (m, 4 H) 3.60 (q, *J*=5.8 Hz, 2 H) 1.58 - 1.64 (m, 2 H) 1.51 (d, *J*=3.5 Hz, 4 H). ^{13}C NMR (151 MHz, DMSO-*d*6): δ 158.9, 154.3, 143.2, 134.2, 128.4 (q; *J* = 32.0 Hz), 125.9 (q; *J* = 3.8 Hz), 125.7, 125.6, 124.3 (q; *J* = 271.6), 114.5, 59.8, 45.0, 42.5, 25.3, 24.6. HRMS (ESI) *m/z* calcd for $C_{19}H_{21}F_3N_6O$ $[M + H]^+$ 407.1729; found 407.1802.

2-(8-Phenyl-2-piperidin-1-yl-9*H*-purin-6-ylamino)-ethanol 15(2,3,1,1)



Yield 4 mg (20%). Purity of the crude product 60%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.04 (d, *J*=7.3 Hz, 2 H) 7.48 - 7.54 (m, 2 H) 7.42 - 7.47 (m, 1 H) 3.71 - 3.76 (m, 4 H) 3.64 - 3.68 (m, 2 H) 3.63 (br. s., 2 H) 1.61 - 1.67 (m, 2 H) 1.53 - 1.59 (m, 4 H). HRMS (ESI) *m/z* calcd for $C_{18}H_{22}N_6O$ $[M + H]^+$ 339.1855; found 339.1928.

***N*^{1}-(8-Phenyl-2-piperidin-1-yl-9*H*-purin-6-yl)-propane-1,3-diamine 15(1,3,1,1)**



Yield 3 mg (14%). Purity of the crude product 58%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (d, *J*=7.0 Hz, 2 H) 7.50 - 7.54 (m, 2 H) 7.45 - 7.49 (m, 1 H) 3.71 - 3.77 (m, 4 H) 2.92 (br. s., 1 H) 1.94 (quin, *J*=7.2 Hz, 2 H) 1.61 - 1.67 (m, 2 H) 1.54 - 1.60 (m, 5 H). HRMS (ESI) *m/z* calcd for $C_{19}H_{25}N_7$ $[M + H]^+$ 352.2171; found 352.2244.

6.4 Solid-Phase Synthesis of Purines and Deazapurines

Lemrová, B.; Soural M. unpublished results

6.4.1 Experimental procedures:

Reaction with CDI and 1,3-diaminopropane (21(1))

Wang resin (1 mmol/g; 1g) was washed 3x with DCM and a solution of CDI (5 mmol; 810 mg) and pyridine (5 mmol; 400 μL) in 10 ml DCM was added. Resin slurry was shaken for 2 h, the resin was washed 3x with DCM and a solution of 1,3-diaminopropane (5 mmol; 417 μL) in 10 ml DCM was added. Resin slurry was shaken for 3 h, washed 5 x with DCM. A sample of resin was dried by a stream of nitrogen and product was quantified as described above.

Acylation of Wang resin with Fmoc-β-Ala-OH (21(2))

Wang resin (1 mmol/g; 1 g) was washed 3 x with DCM and a solution of Fmoc-β-Ala-OH (2 mmol; 622 mg), HOBt (2 mmol; 306 mg), DIC (2 mmol; 310 μL) and DMAP (0.5 mmol; 60 mg) in 5 mL DMF and 5 mL DCM was added. Resin slurry was shaken for 16 hours and then

the resin was washed 3 x with DMF and 3 x with DCM. Sample of resin was quantified as described above.

Acylation with BAL linker and immobilization of benzylamine (21(4))

Aminomethyl resin (0.98 mmol; 1 g) was acylated with BAL linker according above described procedure (compound **1**). Subsequently resin **1** (1 g) was washed 3 x with DCM and 3x with anhydrous DMF. A solution of benzylamine (5 mmol; 547 μL) in 10 mL 10% AcOH/anhydrous DMF and TFA (5 mmol; 384 μL) was added and the slurry was shaken overnight. The next day, $NaBH(OAc)_3$ (10 mmol, 2.2 g) was added in three portions and the syringe was punctured with a needle just below the plunger to enable hydrogen gas evolve. The slurry was shaken for 4 hours. The resin was washed 3 x with 5% AcOH/DMF 3x with DMF, neutralized with 20% piperidine/DMF for five minutes, washed 3 x with DMF and 3 x with DCM. Sample of resin was quantified according procedure described above.

Reaction with 4,6-dichloro-5-nitropyrimidine or 2,4-dichloro-3-nitropyridine (22A, 22B)

Resin **21** (1 g) was washed 3 x with DCM, 3 x with DMF and a solution of aryl reagent (4,6-dichloro-5-nitropyrimidine (5 mmol; 970 mg) or 2,4-dichloro-3-nitropyridine (5 mmol; 960 mg)) and eqv. EDIPA (5 mmol; 870 μL) in 10 mL DMF was added. The resin slurry was shaken for 16 hours. After the reaction, the resin was washed 3 x with DMF and 3 x with DCM. Sample of resin was fmocylated with 0.5 M solution of FmocOSu and analyzed. No fmoc starting material confirmed quantitative arylation.

Substitution with amine (23A, 23B)

Resin **22A** or **22B** (250 mg) was washed 3 x with DCM and 3 x with DMSO. 10% solution of amine (pentylamine, piperidine) in DMF was added to the resin. The resin slurry was shaken at ambient temperature overnight besides resin **22A(4)** where the reaction conditions were 120°C overnight. Subsequently the resins were washed 3 x with DMSO and 3 x with DCM.

Reduction with tin(II) chloride dehydrate (24A, 24B)

Resin **23A** or **23B** (250 mg) was washed 3 x with DCM and 3x with DMF. A solution of tin(II) chloride dihydrate (3 mmol; 675 mg) and EDIPA (6 mmol; 1044 μL) in 3 mL of deoxygenated DMF was added to the resin and resin slurry was shaken overnight. Then the resin was washed 3x with DMF and 3 x with DCM.

Cyclization (25A, 25B, 27A, 27B)

Resin **24A** or **24B** (250 mg) was washed 3 x with DCM, 3 x with DMSO and transferred to reaction vial. A solution of aldehyde (benzaldehyde (1.5 mmol; 159 μL), isonicotinaldehyde

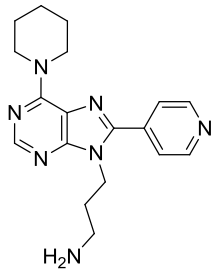
(1.5 mmol; 141 μL) in 3 mL DMSO was added. The resin was mixed at 80°C overnight. After the reaction, the resin was transferred back to the polystyrene fritted syringe and the resin was washed 3 x with DMSO and 3 x with DCM.

Cleavage and isolation (26A, 26B, 28A, 28B)

Resins **25A, 25B, 27A** or **27B** (200 mg) were treated with 50% TFA in DCM for 1 h. The cleavage cocktail was collected and the resin was washed 3 x with 50% TFA in DCM. The liquid phases were combined and evaporated by a stream of nitrogen. Removing of inorganic salts: evaporated material was dissolved in 1 mL DMSO and the solution was diluted with 9 mL of 10 mM solution of ammonium acetate. Resulting suspension was added to a reverse phase C18 cartridge (2 g) pre-washed with 10 mL of AcCN and 10 mL of 10 mM solution of ammonium acetate. After loading the sample, the cartridge was washed by 10 mL of 10 mM solution of ammonium acetate. Finally, the cartridge was washed with 5 mL of AcCN and the solution was purified by semipreparative HPLC.

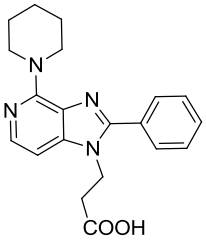
6.4.2 Analytical data

3-(6-(piperidin-1-yl)-8-(pyridin-4-yl)-9*H*-purin-9-yl)propan-1-amine 28B(1,2,2)



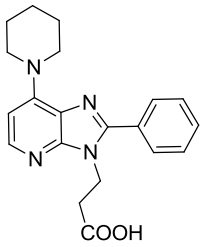
Yield 13 mg (51%). Purity of the crude product 78%. MS $[M+H]^+$ = 337.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59 (br. s., 6 H) 1.94 (t, *J*=6.68 Hz, 2 H) 2.63 (br. s., 2 H) 3.20 (s, 1 H) 3.40 (s, 1 H) 3.86 (s, 1 H) 4.23 (br. s., 3 H) 4.39 (t, *J*=7.14 Hz, 2 H) 7.82 (d, *J*=5.67 Hz, 2 H) 8.27 (s, 1 H) 8.77 (d, *J*=5.67 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.1, 152.5, 152.4, 150.4, 145.4, 142.9, 137.1, 122.9, 41.0, 36.9, 33.2, 29.4, 25.7, 24.3.

3-(2-phenyl-4-(piperidin-1-yl)-1*H*-imidazo[4,5-*c*]pyridin-1-yl)propanoic acid 28A(2,2,1)



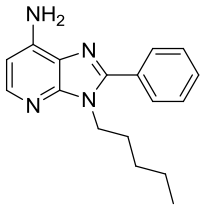
Yield 20 mg (76%). Purity of the crude product 85%. MS $[M+H]^+$ = 351.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (d, *J*=5.70 Hz, 1 H) 7.71 - 7.75 (m, 2 H) 7.55 - 7.58 (m, 3 H) 6.97 (d, *J*=5.70 Hz, 1 H) 4.42 (t, *J*=7.24 Hz, 2 H) 4.04 - 4.11 (m, 4 H) 2.67 (t, *J*=7.45 Hz, 2 H) 1.60 - 1.66 (m, 2 H) 1.58 (d, *J*=3.95 Hz, 4 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 151.3, 149.4, 141.3, 140.0, 130.1, 129.7, 129.2, 128.8, 127.1, 97.3, 46.8, 33.8, 25.6, 2 4.7, 21.1.

3-(2-phenyl-7-(piperidin-1-yl)-3H-imidazo[4,5-b]pyridin-3-yl)propanoic acid 26A(2,2,1)



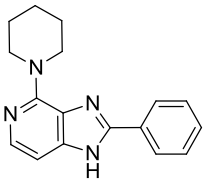
Yield 2 mg (8%). Purity of crude product 8% - side product of compound 28A(2,2,1). MS $[M+H]^+$ = 351.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, *J*=5.70 Hz, 1 H) 7.75 - 7.78 (m, 2 H) 7.55 - 7.58 (m, 3 H) 6.55 (d, *J*=6.14 Hz, 1 H) 4.40 - 4.47 (m, 2 H) 3.92 (d, *J*=5.26 Hz, 4 H) 2.73 - 2.80 (m, 2 H) 1.63 (br. s., 6 H).

3-pentyl-2-phenyl-3*H*-imidazo[4,5-*b*]pyridin-7-amine 26A(3,1,1)



Yield 15 mg (71%). Purity of the crude product 77%. MS $[M+H]^+$ = 280.17. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.72 (t, *J*=6.86 Hz, 3 H) 1.02 - 1.19 (m, 4 H) 1.63 (quin, *J*=7.18 Hz, 2 H) 4.25 (t, *J*=7.41 Hz, 2 H) 6.34 - 6.42 (m, 2 H) 7.50 - 7.60 (m, 2 H) 7.72 - 7.79 (m, 2 H) 7.85 (d, *J*=5.49 Hz, 1 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.2, 148.8, 146.5, 144.5, 131.0, 129.4, 128.8, 128.7, 122.6, 102.3, 42.6, 40.4, 28.6, 28.04, 21.4.

2-phenyl-4-(piperidin-1-yl)-1*H*-imidazo[4,5-*c*]pyridine 28A(3,2,1)



Yield 12 mg (57%). Purity of the crude product 66%. MS $[M+H]^+$ = 278.15. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.94 (br. s., 1 H) 8.12 (d, *J*=7.34 Hz, 2 H) 7.79 (d, *J*=5.50 Hz, 1 H)

7.51 - 7.57 (m, 2 H) 7.44 - 7.50 (m, 1 H) 6.83 (d, *J*=5.50 Hz, 1 H) 4.12 (br. s., 4 H) 1.57 - 1.69 (m, 6 H) ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.2, 147.7, 140.8, 139.9, 129.8, 129.6, 128.9, 128.4, 126.2, 97.9, 46.8, 25.6, 24.7.

6.5 Efficient Solid-Phase Synthesis of Some 1,2,3,4-Tetrahydro-benzo[e][1,4]diazepin-5-ones

Barbora Lemrová and Miroslav Soural, ACS Comb. Sci, 2012, 14, 645-650.[A4]

6.5.1 Experimental procedures:

Acylation with BAL linker (1)

Aminomethyl resin (0.98 mmol; 1 g) was acylated with BAL linker according above described procedure (compound **1**).

Immobilization of primary amines

Preparation of *N*-(2-aminoethyl)-2-nitrobenzenesulfonamide and *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide:[105]

Ethane-1,2-diamine or propane-1,3-diamine (41 mmol) was dissolved in DCM (22.5 mL) and the solution was stirred at -5°C. Solution of 2-Nos-Cl (13.5 mmol, 3 g) in 90 mL DCM was added during 45 minutes. The reaction mixture was stirred for 60 minutes. After this, the precipitated di-2-Nos-diamine was filtered off and the mixture was concentrated *in vacuo*. The residue was dissolved in 15 mL solution of *i*PrOH:NH_3:H_2O in the ratio 5:1:1 and purified by column chromatography (mobile phase: *i*PrOH:NH_3:H_2O = 5:1:1). The solvent was evaporated and the product was dried under vacuum.

Reductive amination with *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide or *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide or ethanolamine (resins 31, 35a, 35b):[117]

Aminomethyl resin with BAL linker (1 g) was washed 3 x with DCM and 3x with dry DMF. A solution of *N*-(2-aminoethyl)-2-nitrobenzenesulfonamide or *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide or ethanolamine (5 mmol) in 10 mL 10% AcOH/anhydrous DMF and TFA (5 mmol; 384 μL) was added and the slurry was shaken overnight. The next day, $NaBH(OAc)_3$ (10 mmol, 2.2 g) was added in two portions and the syringe was punctured with a needle just below the plunger to enable hydrogen gas evolve. The slurry was shaken for 4 hours. The resin was washed 3 x with 5% AcOH/DMF 3 x with DMF, neutralized with 20% piperidine/DMF for five minutes, washed 3x with DMF and 3 x with DCM.

Quantification of resins **35a,35b***:*

Sample of resin (10 mg) was reacted with Fmoc-OSu (0.5 mmol, 168 mg) in 1 mL DCM for 30 min at ambient temperature. The resin was washed 5 x with DCM and the product was cleaved from resin with 50% TFA in DCM for 30 min. The cleavage cocktail was evaporated by a stream of nitrogen and cleaved material was extracted into 1 mL of MeOH. The sample was analyzed by LC-UV-MS and the quantity was calculated with use of an external standard (Fmoc-Ala-OH; concentration 1 mg/ml).

Reaction with 4-chloro-2-fluoro-5-nitrobenzoic acid (32, 36)

A polypropylene fritted syringe was charged with resin **31** or **35** (1g) and 0.2 M solution of 4-chloro- 2-fluoro-5-nitrobenzoic acid (2 mmol; 420 mg) and EDIPA (5 mmol; 870 μl) in 10 mL of DMSO was added. The resin slurry was shaken at 50°C overnight and subsequently washed 3 x with DMF and 3 x with DCM. Sample of resin **32** or **36** was reacted with 0.5 M solution of FmocOSu in DCM for 30 min. No Fmoc-starting amine was detected after the reaction (LC-MS) indicating the reaction was quantitative.

Reaction with methansulfonyl chloride (mixture of compounds 33 and 34)

Resin **32** (250 mg) was washed 3 x with DCM. 1 M solution of methansulfonyl chloride (3 mmol; 231 μL) in 3 mL of pyridine was added to the resin. The resin slurry was shaken for 60 min. Then the resin was washed 3 x with anhydrous THF.

Esterification of resin 32 (unsuccessful)

a) Resin **32** (50 mg) was washed 3x with DCM. 0.5 M solution of methyl iodide (0.5 mmol; 31 μL) with equivalent of DBU (0.5 mmol; 75 μL) in 1 mL of DMF was added to the resin. The resin slurry was shaken overnight. Then the resin was washed 3 x DMF and 3 x with DCM.
b) Resin **32** (50 mg) was charged with 1 M solution of diazomethane in diethyl ether (1 mL). The resin slurry was shaken overnight. Then the resin was washed 3x THF and 3 x with DCM.
c) Resin **32** (50 mg) was washed 3x with DCM, 3x with THF and 3x with anhydrous THF. A solution of alcohol (0.75 mmol) and triphenylphosphine (0.375 mmol; 98 mg) in 1 mL of anhydrous THF was added to the resin. The resin slurry was kept in a freezer for 30 minutes. DIAD (0.375 mmol; 74μL) was dissolved in 1 mL of anhydrous THF and resulted solution was also kept in a freezer for 30 minutes. After this time the solution of DIAD was slowly added into the syringe with resin **2** and the

resin slurry was shaken overnight. Then the resin was washed 3x with THF, 3x with DCM.

Alkylation with ethyl iodide (37(1))

Resin **36** (250 mg) was washed 3x with DCM and 3x with DMF. A 0.5M solution of ethyl iodide (1.5 mmol; 120 μl), and eqv. DBU (1.5 mmol; 225μl) in 3 mL of DMF was added to the resin. The resin slurry was shaken overnight. Then the resin was washed 3 x with DMF and 3x with DCM.

Mitsunobu Alkylation (37(*3-7*))

Resin **36** (250 mg) was washed 3x with DCM, 3x with THF and 3x with anhydrous THF. A solution of alcohol (0.75 mmol) and triphenylphosphine (0.75 mmol, 198 mg) in 2 mL of anhydrous THF was added to the resin. The resin slurry was kept in a freezer for 30 minutes. DIAD (0.75 mmol; 144 μL) was dissolved in 1 mL of anhydrous THF and resulted solution was also kept in a freezer for 30 minutes. After this time the solution of DIAD was slowly added into the syringe with resin **6** and the resin slurry was shaken overnight. Then the resin was washed 3x with THF, 3x with DCM.

Reaction with amines (44(*1-7*))

Resin **37a(*1*)** (250 mg) was washed 3x with DCM and 3x with DMF. Then the resin was transferred to a microwave tube and a 10% solution of amine in DMSO (3 mL) was added. The resin slurry was reacted at 150°C (200 W) for 5 min. After then the resin was transferred to a polypropylene fritted syringe and the resin was washed 3x with DMF and 3x with DCM.

Denosylation (reaction with mercaptoalcohols) (38, 45)

Resins **37, 44** (250 mg) were washed 3x with DCM and 3x with DMF. 0.6 M solution of mercaptoalcohol (1.8 mmol) and eqv. DBU (1.8 mmol; 270 μL) in 3 mL of DMF was added to the resin. The resin slurry was shaken overnight, then the resin was washed 3 x with DMF and 3 x with DCM.

Reaction with methansulfonyl chloride (40)

Resin **38** (250 mg) was washed 3 x with DCM. 1 M solution of methansulfonyl chloride (3 mmol; 231 μL) in 3 mL of pyridine was added to the resin. The resin slurry was shaken for 30 min. Then the resin was washed 3 x with anhydrous THF.

Reduction with tin(II) chloride dihydrate (41)

Resin **40** (250 mg) was washed 3 x with DCM and 3x with DMF. A solution of tin(II) chloride dihydrate (3 mmol; 675 mg) and EDIPA (6 mmol; 1044 μL) in 3 mL of

deoxygenated DMF was added to the resin and resin slurry was shaken overnight. Then the resin was washed 3x with DMF and 3 x with DCM.

Cyclization (42)

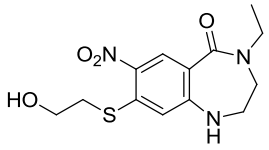
Resins **41** (250 mg) were washed 3 x with DCM, 3x with DMF and transferred to a glass vial. A 0.2 M solution of EDIPA in DMSO (3 mL) was added and the resin slurry was shaken at 80°C overnight. After reaction the resin was transferred to a polypropylene fritted syringe and the resin was washed 3 x with DMF and 3x with DCM.

Cleavage and isolation (39, 43, 46)

Resins **38**, **42**, **45**, were treated with 50% TFA in DCM for 1 h. The cleavage cocktail was collected and the resin was washed 3 x with 50% TFA in DCM. The liquid phases were combined and evaporated by a stream of nitrogen. The evaporated material (except **13**) was dissolved in MeOH and purified directly by semipreparative reverse phase HPLC. Removing of inorganic salts: evaporated material **(43)** was dissolved in 1 mL DMSO and the solution was diluted with 9 mL of 10 mM solution of ammonium acetate. Resulting suspension was added to a reverse phase C18 cartridge (2 g) pre-washed with 10 mL of AcCN and 10 mL of 10 mM solution of ammonium acetate. After loading the sample, the cartridge was washed by 10 mL of 10 mM solution of ammonium acetate. Finally, the cartridge was washed with 5 mL of AcCN and the solution was purified by semipreparative HPLC.

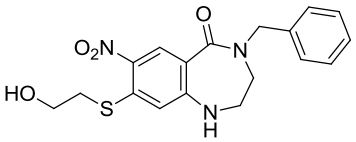
6.5.2 Analytical data

4-Ethyl-8-(2-hydroxy-ethylsulfanyl)-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39a(1)



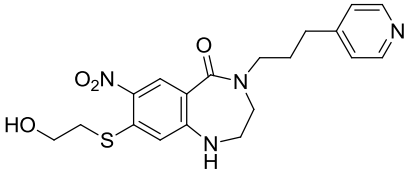
Yield 19.1 mg (80%). Purity of the crude product 83%. MS $[M+H]^+$ = 312.09. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 1 H) 7.99 (br. s., 1 H) 6.69 (s, 1 H) 5.08 (br. s., 1 H) 3.71 (d, *J*=4.8 Hz, 2 H) 3.42 - 3.62 (m, 6 H) 2.99 (t, *J*=6.4 Hz, 2 H) 1.09 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 150.3, 140.9, 134.4, 134.0, 113.8, 112.7, 58.8, 47.5, 46.4, 44.0, 34.8, 12.7.

4-Benzyl-8-(2-hydroxy-ethylsulfanyl)-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39a(2)



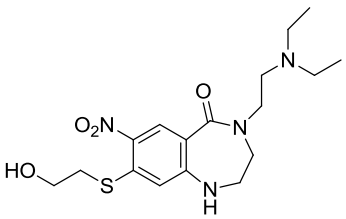
Yield 19.4 mg (69%). Purity of the crude product 81%. MS $[M+H]^+$ = 374.11. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 1 H) 8.01 (br. s., 1 H) 7.17 - 7.41 (m, 5 H) 6.71 (s, 1 H) 5.08 (br. s., 1 H) 4.71 (s, 2 H) 3.64 - 3.82 (m, 2 H) 3.28 - 3.59 (m, 4 H) 3.00 (t, *J*=6.3 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.1, 150.1, 140.7, 137.4, 134.4, 133.7, 128.6, 127.8, 127.2, 113.1, 112.5, 58.5, 51.9, 46.6, 46.4, 34.5.

8-(2-Hydroxy-ethylsulfanyl)-7-nitro-4-(3-pyridin-4-yl-propyl)-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39a(4)



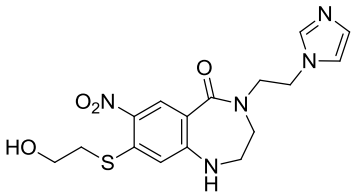
Yield 39.9 mg (69%). Purity of the crude product 73%. MS $[M+H]^+$ = 403.14. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73 (s, 1 H) 8.44 (d, *J*=4.2 Hz, 2 H) 7.97 (br. s., 1 H) 7.27 (d, *J*=4.6 Hz, 2 H) 6.69 (s, 1 H) 5.06 (br. s., 1 H) 3.72 (br. s., 2 H) 3.54 (br. s., 4 H,) 3.34 (br. s., 2 H) 2.99 (t, *J*=6.2 Hz, 2 H) 2.61 (t, *J*=7.5 Hz, 2 H) 1.77 - 1.95 (m, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 150.5, 150.0, 149.4, 140.6, 134.3, 133.6, 123.8, 113.4, 112.4, 58.5, 48.5, 46.9, 46.5, 34.5, 31.7, 27.6.

4-(2-Diethylamino-ethyl)-8-(2-hydroxy-ethylsulfanyl)-7-nitro-1,2,3,4-tetrahydro-benzo [*e*][1,4]diazepin-5-one 39a(5)



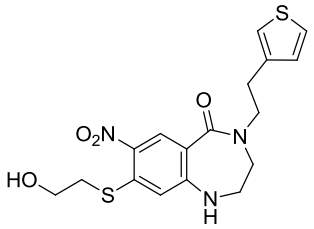
Yield 59.7 mg (78%). Purity of the crude product 82%. MS $[M+H]^+$ = 383.17. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 1 H) 7.99 (br. s., 1 H) 6.68 (s, 1 H) 3.70 (t, *J*=6.3 Hz, 2 H) 3.54 (d, *J*=7.0 Hz, 6 H) 2.98 (t, *J*=6.2 Hz, 2 H) 2.46 - 2.64 (m, 6 H) 0.94 (t, *J*=7.0 Hz, 6 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 150.1, 140.6, 134.3, 133.6, 113.4, 112.4, 58.6, 50.1, 47.6, 47.5, 46.8, 46.6, 34.5, 11.7.

8-(2-Hydroxy-ethylsulfanyl)-4-(2-imidazol-1-yl-ethyl)-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39a(6)



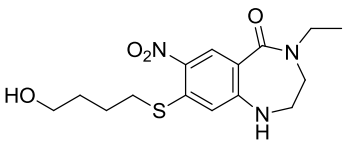
Yield 21.6 mg (57%). Purity of the crude product 82%. MS $[M+H]^+$ = 378.12. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 1 H) 7.95 (br. s., 1 H) 7.69 (br. s., 1 H) 7.23 (br. s., 1 H) 6.93 (br. s., 1 H) 6.67 (s, 1 H) 4.20 (br. s., 2 H) 3.82 (t, *J*=5.7 Hz, 2 H) 3.70 (t, *J*=6.2 Hz, 3 H) 3.22 - 3.44 (m, 4 H) 2.98 (t, *J*=6.3 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.1, 150.1, 140.8, 137.4, 134.3, 133.6, 127.9, 119.8, 112.7, 112.5, 58.5, 50.5, 47.5, 47.0, 46.4, 44.2, 34.5.

8-(2-Hydroxy-ethylsulfanyl)-7-nitro-4-(2-thiophen-3-yl-ethyl)-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39a(7)



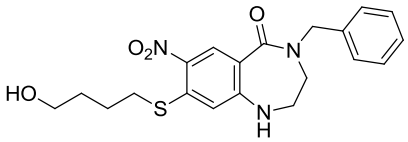
Yield 38.6 mg (68%). Purity of the crude product 70%. MS $[M+H]^+$ = 394.08. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73 (s, 1 H) 7.97 (br. s., 1 H) 7.47 (dd, *J*=4.8, 2.9 Hz, 1 H) 7.24 (d, *J*=1.6 Hz, 1 H) 7.05 (d, *J*=4.8 Hz, 1 H) 6.68 (s, 1 H) 3.71 (t, *J*=6.4 Hz, 4 H) 3.53 (br. s., 2 H) 3.42 (br. s., 2 H) 2.99 (t, *J*=6.2 Hz, 2 H) 2.87 (t, *J*=7.2 Hz, 2 H) 2.55 (t, *J*=5.5 Hz, 1 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.7, 150.1, 140.6, 139.3, 134.3, 133.6, 128.4, 126.0, 121.5, 113.3, 112.4, 58.5, 50.0, 47.0, 46.8, 34.5, 27.8.

4-Ethyl-8-(4-hydroxy-butylsulfanyl)-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39c(1)



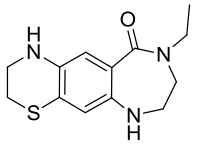
Yield 21.2 mg (63%). Purity of the crude product 73%. MS $[M+H]^+$ = 340.13. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 1 H) 7.97 (br. s., 1 H) 6.64 (s, 1 H) 4.49 (br. s., 1 H) 3.41 - 3.64 (m, 8 H) 2.86 (t, *J*=7.0 Hz, 2 H) 1.51 - 1.79 (m, 4 H) 1.09 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.4, 150.0, 140.7, 134.2, 133.5, 113.5, 112.4, 60.1, 47.1, 46.1, 43.6, 31.8, 31.1, 23.8, 12.6.

4-Benzyl-8-(4-hydroxy-butylsulfanyl)-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 39c(2)



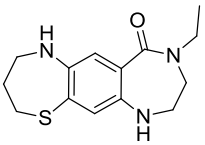
Yield 25 mg (62%). Purity of the crude product 67%. MS $[M+H]^+$ = 402.14. ^{1}H NMR (300 MHz, DMSO-d_6) □ 8.80 (s, 1 H) 8.01 (br. s., 1 H) 7.23 - 7.41 (m, 5 H) 6.67 (s, 1 H) 4.71 (s, 2 H) 4.49 (br. s., 1 H) 3.38 - 3.59 (m, 6 H) 2.87 (t, *J*=7.0 Hz, 2 H) 1.51 - 1.80 (m, 4 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.1, 150.1, 140.9, 137.3, 134.4, 133.5, 128.5, 127.8, 127.2, 113.1, 112.6, 60.1, 51.8, 46.5, 46.4, 31.8, 31.1, 23.8.

9-Ethyl-2,3,6,7,8,9-hexahydro-1*H*-4-thia-1,6,9-triaza-cyclohepta[*b*]naphthalen-10-one 43a(1)



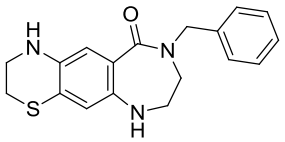
Yield 9.3 mg (47%). Purity of the crude product 78%. MS $[M+H]^+$ = 264.11. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.77 (s, 1 H) 6.35 (s, 1 H) 5.54 (br. s., 1 H) 3.26 - 3.50 (m, 8 H) 2.96 - 3.02 (m, 3 H) 1.08 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.0, 137.7, 135.9,121.8, 121.4, 117.4, 116.7, 49.9, 46.0, 42.4, 41.7, 26.8, 21.6.

4-Ethyl-1,2,3,4,7,8,9,10-octahydro-11-thia-1,4,7-triaza-benzo[1,2;4,5]dicyclohepten-5-one 43b(1)



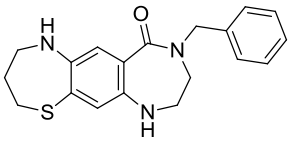
Yield 10.7 mg (51%). Purity of the crude product 73%. MS $[M+H]^+$ = 278.12. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (s, 1 H) 6.72 (s, 1 H) 5.48 (br. s., 1 H) 5.10 - 5.25 (m, 1 H) 3.35 - 3.49 (m, 6 H) 2.94 - 3.02 (m, 2 H) 2.71 - 2.78 (m, 2 H) 1.84 - 1.93 (m, 2 H) 1.08 (t, *J*=7.1 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.1, 143.7, 139.2, 129.7, 126.8, 122.0, 121.1, 49.0, 46.9, 45.6, 42.2, 33.0, 31.3, 13.1.

9-Benzyl-2,3,6,7,8,9-hexahydro-1*H*-4-thia-1,6,9-triaza-cyclohepta[*b*]naphthalen-10-one 43a(2)



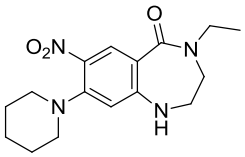
Yield 6.5 mg (27%). Purity of the crude product 93%. MS $[M+H]^+$ = 326.12. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.18 - 7.43 (m, 5 H) 6.85 (s, 1 H) 6.37 (s, 1 H) 5.59 (br. s., 1 H) 5.12 (br. s., 1 H) 4.65 (s, 2 H) 3.28 - 3.49 (m, 4 H) 3.20 (br. s., 2 H) 3.01 (d, *J*=4.8 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1, 138.2, 137.3, 135.4, 128.4, 127.6, 127.0, 121.2, 120.7, 116.9, 116.2, 50.3, 48.7, 45.9, 41.2, 26.3.

4-Benzyl-1,2,3,4,7,8,9,10-octahydro-11-thia-1,4,7-triaza-benzo[1,2;4,5]dicycloheptен-5-one 43b(2)



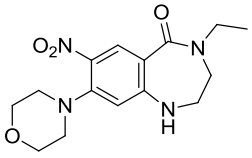
Yield 21.7 mg (63%). Purity of the crude product 82%. MS $[M+H]^+$ = 340.14. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.23 - 7.41 (m, 5 H) 7.20 (s, 1 H) 6.75 (s, 1 H) 5.52 (br. s., 1 H) 5.23 (br. s., 1 H) 4.66 (s, 2 H) 3.37 (br. s., 2 H,) 3.25 (br. s., 2 H) 3.00 (br. s., 2 H) 2.70 - 2.82 (m, 2 H) 1.89 (d, *J*=3.1 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.8, 143.8, 139.3, 138.0, 130.1, 128.4, 127.6, 127.0, 122.0, 121.2, 120.6, 50.5, 48.4, 46.9, 46.0, 33.0, 31.3.

4-Ethyl-7-nitro-8-piperidin-1-yl-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 46(1)



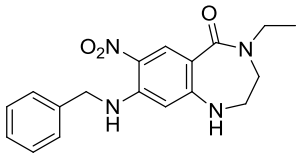
Yield 19.2 mg (61%). Purity of the crude product 76%. MS $[M+H]^+$ = 319.17. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (s, 1 H) 7.61 (br. s., 1 H) 6.24 (s, 1 H) 3.41 - 3.58 (m, 6 H) 2.88 (br. s., 4 H) 1.62 (br. s., 4 H) 1.56 (d, *J*=3.3 Hz, 2 H) 0.99 - 1.15 (m, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.7, 150.9, 149.8, 135.4, 130.5, 110.1, 105.0, 52.1, 47.2, 46.2, 43.5, 25.3, 23.6, 12.6.

4-Ethyl-8-morpholin-4-yl-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 46(3)



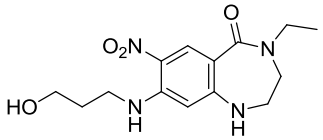
Yield 24 mg (60%). Purity of the crude product 78%. MS $[M+H]^+$ = 321.15. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 1 H) 7.70 (br. s., 1 H) 6.27 (s, 1 H) 3.66 - 3.77 (m, 4 H) 3.42 - 3.58 (m, 6 H) 2.92 (m, 4 H) 1.08 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.6, 151.0, 149.0, 135.6, 130.4, 110.7, 105.2, 65.9, 51.3, 47.2, 46.2, 43.5, 12.6.

8-Benzylamino-4-ethyl-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 46(5)



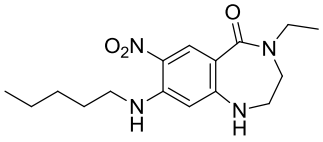
Yield 23.3 mg (68%). Purity of the crude product 84%. MS $[M+H]^+$ = 341.15 ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.68 (s, 1 H) 8.47 (t, *J*=5.9 Hz, 1 H) 7.57 (br. s., 1 H) 7.36 (d, *J*=4.4 Hz, 4 H) 7.27 (d, *J*=4.2 Hz, 1 H) 5.85 (s, 1 H) 4.46 (d, *J*=6.0 Hz, 2 H) 3.38 - 3.54 (m, 6 H) 1.06 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.6, 152.0, 146.0, 138.3, 135.3, 128.6, 127.1, 125.5, 124.2, 110.0, 95.4, 46.9, 46.3, 45.8, 43.4, 12.7.

4-Ethyl-8-(3-hydroxy-propylamino)-7-nitro-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 46(6)



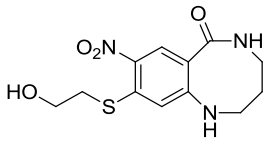
Yield 25 mg (80%). Purity of the crude product 85%. MS $[M+H]^+$ = 309.15. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1 H) 8.04 (t, *J*=4.9 Hz, 1 H) 7.59 (br. s., 1 H) 5.92 (s, 1 H) 4.67 (br. s., 1 H) 3.49 (dd, *J*=14.4, 7.2 Hz, 8 H) 3.19 - 3.29 (m, 2 H) 1.78 (quin, *J*=6.3 Hz, 2 H) 1.07 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.7, 152.2, 146.2, 135.2, 124.1, 109.0, 94.8, 58.6, 47.0, 46.3, 43.3, 40.0, 31.0, 12.7.

4-Ethyl-7-nitro-8-pentylamino-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 46(7)



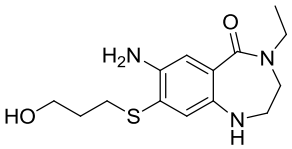
Yield 36 mg (75%). Purity of the crude product 80%. MS $[M+H]^+$ = 321.18. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1 H) 7.89 (br. s., 1 H) 7.58 (br. s., 1 H) 5.92 (s, 1 H) 3.40 - 3.57 (m, 6 H) 3.11 - 3.22 (m, 2 H) 1.61 (d, *J*=6.4 Hz, 2 H) 1.33 (d, *J*=3.3 Hz, 4 H) 1.08 (t, *J*=7.0 Hz, 3 H) 0.84 - 0.94 (m, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.6, 152.2, 146.1, 135.2, 124.0, 110.0, 94.8, 47.0, 46.3, 43.3, 42.3, 28.7, 27.7, 21.9, 13.9, 12.7.

9-(2-Hydroxy-ethylsulfanyl)-8-nitro-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,5]diazocin-6-one 41



Yield 12 mg (54%). Purity of the crude product 72%. MS $[M+H]^+$ = 298.08. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (s, 1 H) 8.11 (br. s., 1 H) 7.66 (t, *J*=6.1 Hz, 1 H) 6.73 (s, 1 H) 5.35 (s, 1 H,) 3.67 (t, *J*=6.5 Hz, 2 H) 3.32 (br. s., 2 H) 3.16 (br. s., 2 H) 2.98 (t, *J*=6.5 Hz, 2 H) 1.58 (br. s., 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.3, 170.2, 152.4, 139.9, 133.0, 113.7, 110.8, 58.6, 38.0, 37.8, 34.2, 29.2.

7-Amino-4-ethyl-8-(3-hydroxy-propylsulfanyl)-1,2,3,4-tetrahydro-benzo[*e*][1,4]diazepin-5-one 42



Yield 62.2 mg (84%). Purity of the crude product 74%. MS $[M+H]^+$ = 296.14. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.92 (s, 1 H) 6.70 (s, 1 H) 4.61 (v.br.s., 3 H) 3.27 - 3.51 (m, 9 H) 2.82 (t, *J*=7.3 Hz, 2 H) 1.66 (quin, *J*=6.7 Hz, 2 H) 1.09 (t, *J*=7.0 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.5, 139.8, 137.1, 123.6, 123.2, 121.5, 116.2, 59.3, 49.5, 45.4, 41.9, 32.1, 29.2, 13.2.

7 List of abbreviations

3HQPs	3-hydroxyquinolin-4(1*H*)-one - purine bisheterocycles
3HQs	3-hydroxyquinolin-4(1*H*)-ones
A549	lung adenocarcinoma cell line
AcOH	acetic acid
AM resin	aminomethyl polystyrene resin
BAL	backbone amide linker
BFHs	benzene fused heterocycles
CB_1	cannabinoid type 1 receptor
CDI	carbonyldiimidazole
CDK	cyclin-dependent kinase
CEM	T-lymphoblastic leukemia cell lines
CEM-DNR-bulk	T-lymphoblastic leukemia resistant to dorubicine
CMD	metalation - deprotonation mechanism
CNS	central nervous system
CSPOS	combinatorial solid-phase organic synthesis
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	diisopropyl azodicarboxylate
DIC	*N*,*N*-diisopropylcarbodiimide
DMA	*N*,*N*-dimethylacetamid
DMAP	4-*(N,N)*-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
EDIPA	*N*,*N*-diisopropylethylamine
ESIPT	excited state intramolecular proton transfer
Fmoc	9-fluorenylmethyloxycarbonyl
FmocOSu	9-Fluorenylmethyl *N*-succinimidyl carbonate
GSK-3	glycogen synthase kinase
HCT116 p53 MUT	colorectal cancer cells_12 Gy gamma radiation

HCT116 p53 WT	colorectal cancer cells_12 Gy gamma radiation
HOBt	1-hydroxybenzotriazole
HPLC-MS	high performance liquid chromatography - mass spectrometry
IMPDH	inosine-monophosphate dehydrogenase
K562	human myeloid leukemia lines
K562-tax	human myeloid leukemia resistant to paclitaxel
KOAc	potassium acetate
m-CPBA	3-chloroperoxybenzoic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltatrazilium bromide
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
Nos	nitrobenzensulfonyl
on	overnight
PAL resin	4-formyl-3,5-dimethoxyphenoxymethyl-functionalized polystyrene resin
PARP-1	poly(ADP ribosa)polymerase
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
rt	room temperature
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP linker	tetrahydropyranyl linker
TMOF	trimethyl orthoformate
TMSOK	potassium trimethylsilanolate

8 Author´s publications

A1 Vankova, B.; Hlavac, J.; Soural, M. Solid-phase synthesis of Highly Diverse Purine-Hydroxyquinolinone bisheterocycles *J. Comb. Chem.* **2010,** *6,* 890-894.

A2 Motyka, M.; Vankova, B.; Hlavac, J.; Soural, M.; Funk, P. Purine Scaffold Effect on Fluorescence Properties of Purine-Hydroxyquinolinone Bisheterocycles *J. Fluoresc.* **2011,** *21,* 2207-2212.

A3 Vankova, B.; Krchnak, V.; Soural, M.; Hlavac, J. Direct C – H Arylation of Purine on Solid Phase and Its Use for Chemical Libraries *ACS. Comb. Sci.* **2011,** *13,* 496-500.

A4 Lemrova, B.; Soural, M. Solid-Phase Synthesis of 4,7,8-Trisubstituted 1,2,3,4-Tetrahydro-benzo[*e*][1,4]diazepin-5-ones *ACS. Comb. Sci.* **2012,** *14,* 645-650.

A5 Vankova, B.; Brulikova, L.; Wu, B.; Krchnak, V. Synthesis of Piperazinones, Piperazines, Tetrahydropyrazines and Dihydropyrazinones from Polymer-Supported Acyclic Intermediates via N-Alkyl- and N-Acyliminiums. *Eur. J. Org. Chem.* **2012,** *26,* 5075-5084.

A6 La Venia, A.; Lemrova, B.; Krchnak, V. Regioselective Incorporation of Backbone Constraints Compatible with Traditional Solid-Phase Peptide Synthesis *ACS Comb. Sci.* **2013,** *15,* 59-72.

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