PALACKÝ UNIVERSITY IN OLOMOUC

Faculty of Science

Department of Organic Chemistry

Development of Solid-Phase Synthesis for Preparation of Chemical Libraries Containing Various Condensed Nitrogenous Heterocycles

Vývoj syntézy na pevné fázi pro přípravu chemických knihoven obsahujících kondenzované dusíkaté heterocykly

Ph.D. Thesis

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Declaration of the author

I hereby declare that this Ph.D. thesis has been written by me and using sources quoted in “References” part. Neither the thesis nor any of its substantial parts were used previously for obtaining any academic degree.

In Olomouc, 25.6. 2012 ----------

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Abstract

The searching for new pharmaceutical substances is a complex process in which a large number of chemical compounds are tested for their biological activity. With the development of high throughput screening (HTS) a demand for the access to a higher number of compounds emerged. The solid-phase synthesis and combinatorial chemistry constitute a powerful tool for the production of numerous chemical libraries.

In this project, the solid-phase synthesis approach was utilized in development of synthetic methods leading to various condensed nitrogenous heterocycles, a group of compounds that have been taking an important position in pharmaceutical chemistry for many years.

First part of the work was focused on use of 4-chloro-2-fluoro-5-nitrobenzoic acid which served as a starting building block for the preparation of immobilized anthranilic acid derivatives. These were converted either to a set of derivatives including diverse nitrogenous heterocycles or to a set of derivatives focused on a single target structure. The set of compounds synthesized by "diversity oriented strategy" contained benzimidazole, benzotriazole, succinimide, quinoxaline and benzodiazepine scaffolds. The "target oriented strategy" was used for the preparation of 3-hydroxyquinolinone derivatives.

In the second part of the work a pathway for the synthesis of quinazoline derivatives was developed. The target heterocycles were prepared *via* based-catalyzed rearrangement of 2*H*-indazoles 1-oxides obtained from easily accesible *N*-alkyl-2-nitro-*N*-(2-oxo-2-aryl-ethyl)-benzenesulfonamides.

Souhrn

Hledání nových farmaceutik představuje náročný proces, ve kterém je testováno velké množství chemických sloučenin za účelem najít molekulu s patřičnou biologickou aktivitou. Jako všechny vědní obory i tato oblast zaznamenala v průběhu let značný vývoj zajišťující efektivní a hlavně rychlý screening rozsáhlých chemických knihoven (high throughput screening HTS). Logicky tak vznikl požadavek na organické chemiky zajistit rychlý přísun velkého počtu chemických látek pro biologické testování. V tomto ohledu bylo velkým přínosem zavedení syntézy na pevné fázi a kombinatoriální chemie, díky kterým lze v porovnání s klasickými přístupy velmi rychle dosáhnout početných chemických knihoven.

Tato práce byla zaměřena na vývoj syntetických metod vedoucích ke kondenzovaným dusíkatým heterocyklům s využitím syntézy na pevné fázi.

V první části práce byla klíčovou sloučeninou 4-chloro-2-fluoro-5-nitrobenzoová kyselina sloužící jako výchozí materiál pro přípravu imobilizovaných derivátů anthranilové kyseliny. Ty byly následně převedeny na sérii látek obsahující různé dusíkaté cykly lišící se velikostí kruhu nebo na sérii látek se stejným heterocyklem a různou kombinaci substituentů. V rámci první zmíněné skupiny látek byly připraveny sloučeniny s následujícími heterocykly: benzimidazol, benzotriazol, chinoxalinon, benzodiazepindion a sukcinimid. Druhou skupinu látek tvořily deriváty 3-hydroxychinolonů s variabilní substitucí v poloze 2 a 7. Dalším záměrem bylo rozšířit řadu 3-hydroxychinolonů o bisheterocyklické deriváty. Pokus o spojení thiazolového cyklu s hydroxychinolonovým byl ale neuspěšný.

Druhá část práce byla zaměřena na přípravu derivátů chinazolinu. Ty byly syntetizovány s využitím přesmyku 2*H*-indazol-1-oxidů připravených jednoduchou nedávno popsanou metodou.

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1. Introduction

The beginning of the medicines discovery can be dated already in ancient time when people used various natural materials for a treatment of different diseases and lesions. In the course of history, this discipline has been significantly developed and sources of the medicine substances have been changed. Searching for new pharmaceutical substances nowadays take advantage of new knowledge in biological sciences and study of drug-protein interactions. New approach using small molecules to modulate protein functions demands supply of a large number of compounds which are screened for the biological activity. Despite numerous databases of synthesized compounds vast areas of chemical space comprising all possible small chemical molecules remain unexplored. As the known drugs cover only small percent of the chemical space the relevance of its complete probing is frequently discussed question. With the tendency to achieve wide region of chemical space a novel approach in chemical synthesis emerged. This diversity-oriented synthesis (DOS) aim at reaching of maximum diversity in the rising chemical library in contrast to target-oriented synthesis (TOS) which is focused on a particular molecular structure.

As a synthesis of a large chemical library for high throughput screening (HTS) represented demanding process the introduction of the combinatorial strategy to the synthesis constituted a significant asset in drug discovery. Development of this time- and money-saving method was closely related to the development of solid-phase synthesis. Easy performing of polymer-supported synthesis without necessity to isolate and purify intermediates allows numerous chemical libraries of different derivatives.

2. Aims of the work

This work is focused on solid-phase synthesis of various nitrogenous heterocycles. The key compound in most of the synthetic methods is 4-chloro-2-fluoro-5-nitrobenzoic acid which serves as an expediential starting building block allowing a large number of chemical modifications. Convenient distribution of functional groups enables formation of condensed cycle on both side of the molecule (carboxy-fluoro and nitro-chloro side).

One part of this study focused on utility of 4-chloro-2-fluoro-5-nitrobenzoic acid is dedicated to synthesis of nitrogenous heterocyclic scaffolds varying in size of cycle. Benzimidazole, benzotriazole, quinoxaline, benzodiazepine and benzodiazocine scaffolds represent heterocycles with a significant importance in drug research. Development of suitable methods for the preparation of these scaffolds *via* polymer-bounded *o*-phenylenediamines was a goal of the reported work.

Besides a diversity-oriented synthesis preparing various heterocycles we considered using of the acid in a target oriented synthesis. 3-Hydroxyquinolinones that were selected as target molecules in this task are another interesting group of compounds intensively studied for their significant biological activity. We decided to use the known synthetic route in which anthranilates are thermally cyclized to 3-hydroxyquinolin-4(*1H*)-ones and to apply this strategy in solid-phase synthesis of 3-hydroxy-6-nitroquinolin-4(1*H*)-ones with two diversity positions where 4-chloro-2-fluoro-5-nitrobenzoic acid is utilized for the preparation of esters of anthranilic acids as polymer-supported intermediates.

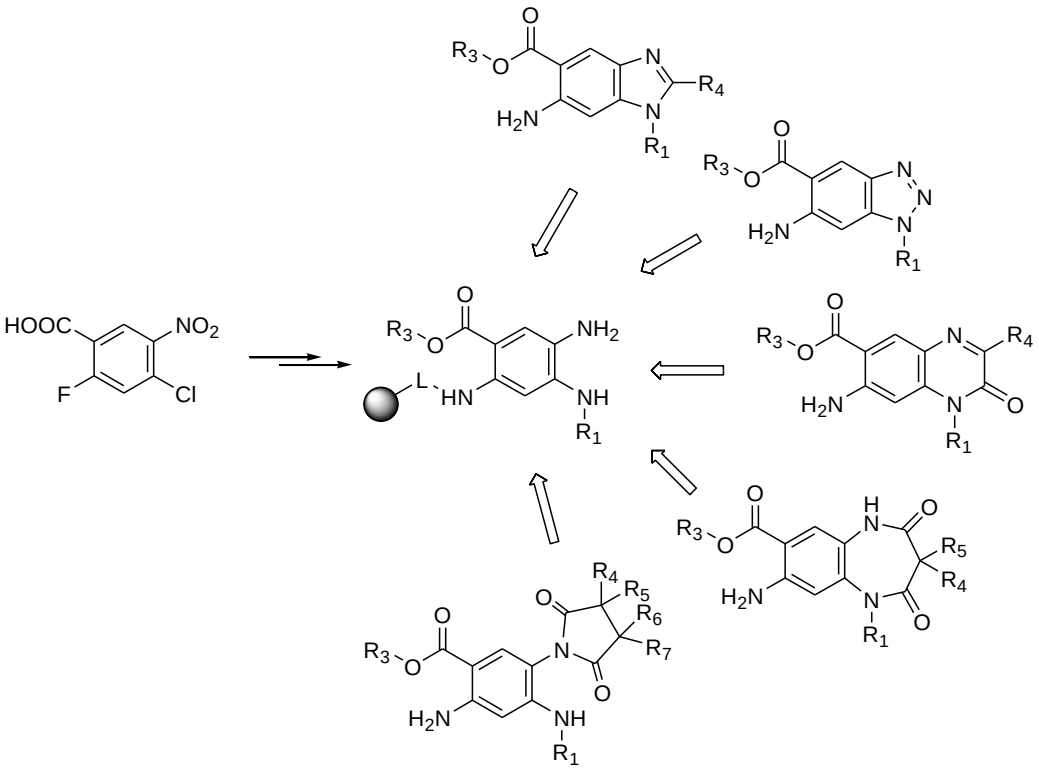
Subsequently, we paid attention to the synthesis of bisheterocycles. Since thiazoles belong to compounds with a great biological potency, attachment of this cycle to hydroxyquinolinone scaffold promised potentially active compounds. Thus, we focused on synthesis of 3-hydroxy-2,7-disubstituted-6-(4-substitutedthiazol-2-ylamino)-4(*1H*)-quinolinones - the quinolone-thiazol bisheterocyclic system with three diverse positions.

Quinazoline derivatives were the object of the last part of our work. We focused on development of synthetic method leading to the target heterocycles which utilizes a

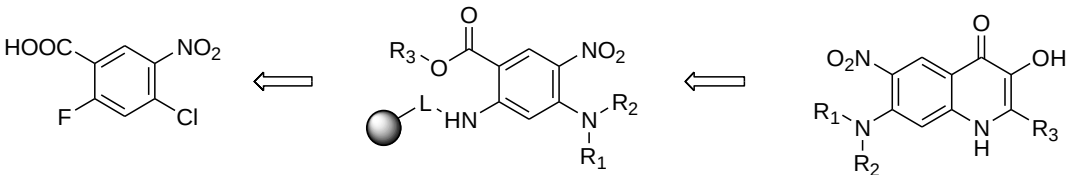
rearrangement of indazole 1-oxides prepared from easily accessible *N*-alkyl-2-nitro-*N*-(2-oxo-2-aryl-ethyl)-benzenesulfonamides.

Summary of the presented aims:

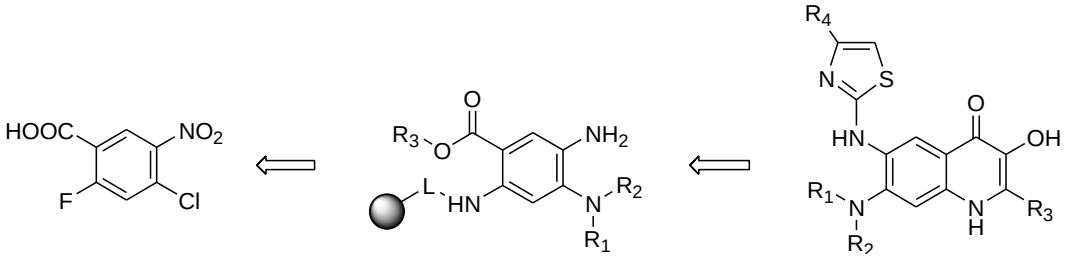
- a development of a solid-phase synthetic methods for the preparation of benzimidazole, benzotriazole, quinoxaline, benzodiazepine and benzodiazocine scaffolds with utility of polymer-supported *o*-phenylenediamines



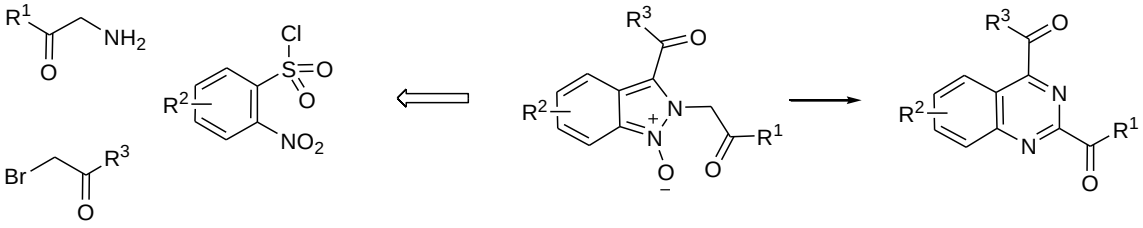
- a development of a solid-phase synthetic method for the preparation of 3-hydroxy-6-nitroquinolin-4(1*H*)-ones *via* anthranilic acid esters



- a development of a solid-phase synthetic method for the preparation of 3-hydroxy-2,7-disubstituted-6-(4-substitutedthiazol-2-ylamino)-4(*1H*)-quinolinones *via* anthranilic acid esters



- a development of a solid-phase synthetic method for the preparation of quinazoline derivatives



In this project, the solid-phase synthesis approach was utilized in development of synthetic methodologies which can be applied for chemical library synthesis affording new derivatives for high throughput synthesis.

3. Literature retrieval

3.1. Solid-phase synthesis and combinatorial chemistry

In 1963, an American biochemist Robert Bruce Merrifield reported a method for preparation of peptides.[1] In this paper he laid the foundations of solid-phase synthesis what was awarded by Nobel Prize in 1984.

Solid-phase synthesis is a method in which organic molecules are bounded to the insoluble support. Single building blocks are step by step attached to a support-bounded starting material until the aimed molecule is achieved. After the final step the target compound is cleaved from the resin. In this method intermediates are simply isolated from an excess of reagents. This advantage allows a rapid synthesis of a large number of compounds in the same time what is greatly utilized in combinatorial chemistry aimed at a preparation of chemical libraries for the drug research.

Different forms and materials are used as a solid support. The support has to satisfy the requirements such as a mechanical resistance and a chemical inertness towards chosen reaction conditions. The most common supports are beads of polystyrene cross-linked with 1-2% of divinylbenzen. This resin is insoluble in organic solvents, in which possess different swelling properties. The most effective swelling provide polar aprotic solvents, like dichloromethane, tetrahydrofurane, dimethylformamide, etc. Since reagents have to penetrate to functional groups inside of the beads the extent of swelling is an important factor. Resins with various functional groups (chloromethyl, hydroxymethyl and aminomethyl) are commercially available. Another type of the styrene-divinylbenzene support is a macroporous polystyrene with high cross-linking (more than 10%). In contrast to previous resin this macroporous polystyrene has a low ability to swell. Larger pores allow a facile reagent access to functional groups and the choice of solvents is not so important. Tentagel is the most used representative of poly(ethylene glycol)-polystyrene graft polymers. Long PEG chains are flexible and mobile and thus ensure the similar reaction environment as in solution reactions. The drawbacks of this type of the resin are a low loading of functional groups and cleavage of PEG in presence of acid causing a product contamination. This support can also become sticky and be difficult to dry.

From other materials employed in the solid-phase chemistry polyacrylamides, polysacharides or silica can be mentioned. These supports are not so used as polystyrene but also found application in some methodologies. Besides the insoluble supports solid-phase chemistry utilize soluble supports (for example poly(ethylene glycol)) as well. These polymers are soluble in water and organic solvents except several selected, which are used for getting the polymer supported compounds back. These supports enable the using of insoluble catalysts or NMR analysis of resin-bounded compounds. Despite these advantageous soluble polymers have a lot of drawbacks including especially the difficult synthesis automation or the product isolation.

Synthesized compounds are anchored to the support *via* a linker which has to also fulfill specific criteria.[2] The most important are following three:

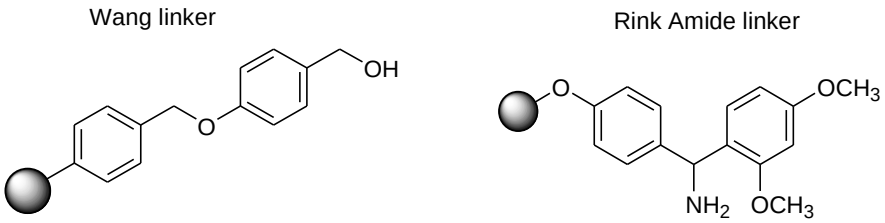
- the attachment of the starting component must be achieved in high yield
- linker has to be inert toward reaction conditions used during the synthesis
- linker has to allow a releasing of the target compound from the polymer without its modification or destruction

In some cases, the first of these above mentioned points can be problematic. Then, the starting material is attached to the linker in solution and resulting linker-starting component substrate is bounded to the solid support.[3]

Solid-phase chemistry has an access to various types of linker. These can be sorted according to several features. As regards to the method of cleavage, linkers can be classified as acid labile, base labile, photolabile, cleavable after cyclization and safety catch linkers. The acid labile and base labile linkers cleavable under corresponding conditions are the most common. In special cases, an intramolecular cyclization of polymer-bound compound can caused a simultaneous releasing of resulting compound from the polymer. This method called “cyclative cleavage” usually provides products of a high purity but with a reduced yield. Safety catch linkers are cleaved from the resin in two steps. First activating step is followed by a proper cleavage of the compound from the polymer. Photolabile linkers allow a release of compounds from the resin under mild conditions when the light is utilized as the cleavage agent.[3]

Wang and Rink linkers (Figure 1) represent the most used acid-labile linkers.

Figure 1: Wang and Rink Amide resins



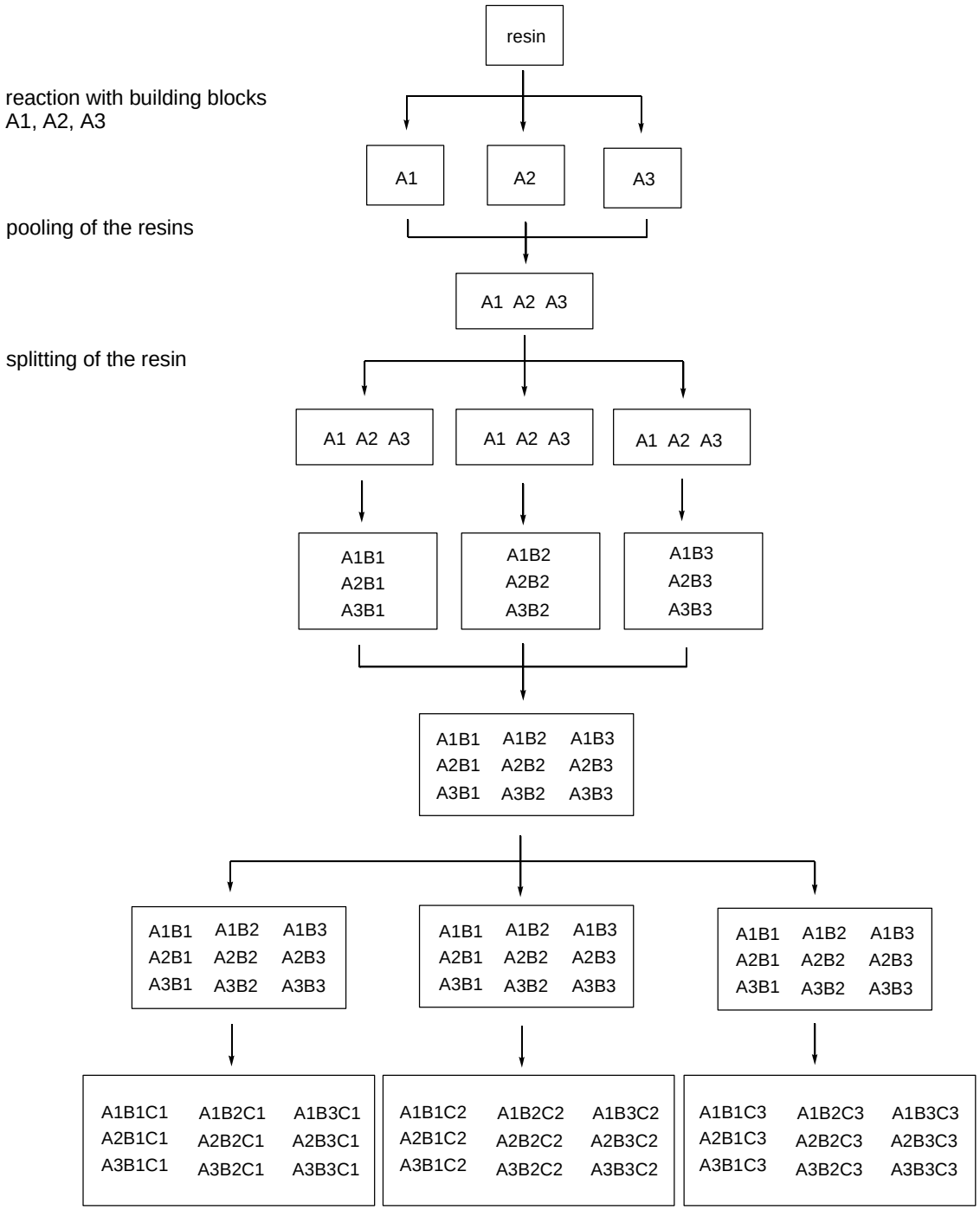
Usually, trifluoroacetic acid in mixture with dichloromethane is used for its cleavage. Initially, these linkers were developed to provide carboxylic acids or amides but with extension of solid-phase applications the number of functional groups greatly increased.

From the text above some limitations of solid-phase synthesis such as a limited range of solvents and reagents (concerning the resin and the linker) possible to use for the synthesis, follow. The reaction monitoring is more difficult and the intermediate analysis demands more time in contrast to the solution chemistry. Despite these disadvantages solid-phase synthesis is the convenient method for a synthesis of sizable chemical libraries especially in the connection with the combinatorial chemistry.

In contrast to the classical chemistry performing a synthetic route to prepare one single compound the combinatorial chemistry can produce a large number of different compounds. Primarily, only peptide and polynucleotide libraries were synthesized by means of a combinatorial approach. For example Geysen prepared a set of peptides with use of polypropylene pins[4] or Frank used cellulose rings for the synthesis of polynucleotides.[5] Later the combinatorial strategy was utilized also in a synthesis of small organic molecules. In 1992, Ellman described the synthesis of 1,4-benzodiazepines with use of pins method.[6] Hydantoine library prepared by DeWitt[7] or the synthesis of peptoides by Zuckermann[8] also represent the beginning of the combinatorial approach in the synthesis of small organic molecules.

Besides the parallel synthesis in which all members of library are prepared in the same time but each of the compounds separately, solid-phase synthesis in combinatorial fashion utilized the split-and-mix method developed by Furka.[9] In this efficient method resins from all reaction vessels are mixed together after the each step and split again into equal parts for the next reaction (process is depicted in Figure 2).

Figure 2: The split and mix method



In a final mixture of compounds the biologically active one is usually searched by the deconvolution method. The fact that each bead of the resin possesses only one compound utilizes another screening method. In this way compounds are tested directly on the polymer support and the active structure is identified by means of the biochemical color test. This method called OBOC (one bead one compound) was used

for the preparation of huge chemical libraries, but lucks the utility nowadays. In order to increase the practical utility various formulations of the solid support[10] combining features of split-and-mix and parallel method were developed. Houghten described method[11] called "tea bags" in which the resin is enclosed in permeable polypropylene bags labeled with a predetermined reaction sequence. Thus, although all bags are pooled into one vessel each of them contains after last step a compound of a known structure. Similarly, "Kans method" uses containers with a polypropylene mesh with pore size to be permeable for reagents and solvents but not for the resins. Another formulation was introduced by Beattie and Frost[12] who used porous wafers for a multiple synthesis of oligonucleotides and peptides. The porous wafer consists of a Teflon ring with both sides covered by a porous Teflon membrane. An alternative method uses resin capsules,[13] polyethylene rings sealed with a peek mesh on both sides which were designed for the multiple/combinatorial solid-phase organic synthesis in manual manner. As another option SynPhase Lanterns were developed.[14] In contrast to the previous methods used to compartmentalize resin beads SynPhase Lanterns serve directly as the solid support. The support consists of a rigid polypropylene mold of shape resembling a Chinese lantern which is grafted with a layer of polystyrene. The solid-phase synthesis is performed on the polystyrene graft. Resin plugs[15] represent another form of the solid support. The mixture of resin beads and powdered ultra-high molecular mass polyethylene is heated to provide the polyolefin matrix for a formation of the resin capsules.

3.2. Target oriented synthesis and diversity oriented synthesis as a valuable source of small organic molecules

Chemotherapy of various diseases is used from the beginning of a human life and has been developing for the millenniums from the treatment with various natural products through the preparation of various medicine made of herbs, roots and fruits to less or more effective isolation of an active substance from the natural matrix or highly sophisticated synthesis of natural compounds. The importance of natural compounds has been obvious since immemorial. A great number of organic molecules obtained from living organisms are still applied in a treatment of various diseases. This is described for example in the review by Newman[16] which monitored the utility of the

natural compounds or their derivatives as a source for novel structures in drug research in the period 1981-2002. However, natural compounds are not the only source of the active substances. Since the sources of natural compounds are not bottomless and the gain of pure active structures usually involves difficult isolation from compounds mixtures where they are usually present in a very low concentration,[17] synthetic procedures represent another valuable access to small molecules. Despite the fact that the comparison of natural compounds with synthetic drugs displays several general differences[18] (especially natural compounds are more complex, often include more stereogenic centers and less nitrogen atoms and their molecular weights and polarities are very often high) nature has served as a valuable inspiration and efficient tool in searching for novel potential lead structures.[19] And although complexity and stereochemical diversity of natural structures make the synthesis of natural mimics very complicated, it is an accomplishable challenge for organic chemists with the current state of knowledge in the synthesis and the analyses of organic compounds.

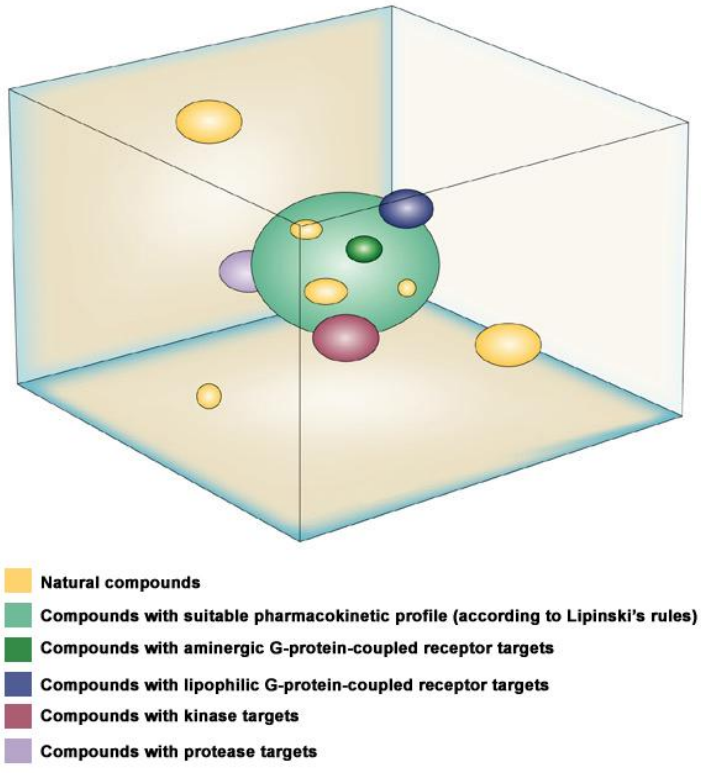
Searching for new biologically active compounds, the objective of the medicinal chemistry is a very lengthy, time- and money-consuming process. Last years a great effort was devoted to its facilitation and acceleration. Progress was made in synthetic techniques enabling rapid and efficient synthesis of libraries with a large number of compounds-high throughput organic synthesis (HTOS) and in the testing phase with the development of high-throughput screening (HTS) as well. Advancements in biological field such as better understanding of cell processes together with new approaches in medicinal chemistry research like the chemical genetics also contributed to achieve the goal of more efficient pathway to novel therapeutic agents. The chemical genetics represents a research method considered to create a bridge between the two adjacent sciences biology and chemistry. The main idea of this approach is to study biological systems by means of small organic molecules which can influence or alter protein functions. Thus the process is similar to approach when gene codes are changed to get this effect.[20] The first-fruit of this concept can be noted even in the nineteenth century when the first active organic compound (morphine) was isolated from the natural material and the earlier presumption that the biological activity of opium is caused by a single pure active substance was confirmed. Later, with respect of this, Paul Ehrlich

developed the concept of the receptor and then small molecules started to be discovering as a power tool for the study of biological systems.[21]

With the development of the chemical genetics approaches the demand for access to a number of small organic molecules was increasing with the aim to find more efficient synthetic methodologies as well as to develop high throughput automatic synthesizers. The invention of solid-phase synthesis and combinatorial chemistry facilitated the production of chemical compounds and enabled the rapid access to a numerous chemical libraries. The great importance has especially the split-and-mix technique as a valuable tool in the combinatorial chemistry (see chapter 3.1.).

There exist two different approaches to a production of chemical compounds. First of them, target-oriented synthesis (TOS) is based on a synthesis of predefined structures. The other one, diversity-oriented synthesis (DOS) is a method without specific target and the aim of this method is to create a large scale of different structural scaffolds.[22-24] Different purpose of these approaches is apparently demonstrated by the distribution of produced organic molecules in chemical space, the region including all possible small organic molecules (Figure 3).

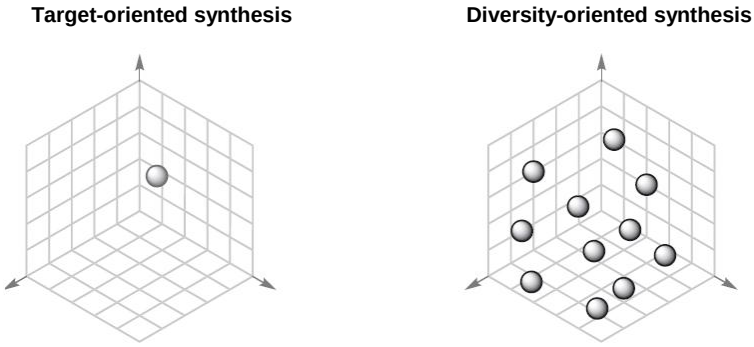
Figure 3: Distribution of chemical compounds in the chemical space[25]



Chemical compounds occupy various regions in chemical space according to their properties (in dependence on selected descriptors); compounds with similar properties occupy the same or close region. Since the natural molecules and known synthetic drug molecules fill only small part of chemical space the task to achieve the unexplored regions have arisen. This is the goal of diversity oriented synthesis.

The chemical space can be defined by various descriptors and when for example the molecular weight of less than 500 daltons is taken in account the calculated predictions of all possible molecules reach the number 10^{60}.[26] The limitation of the molecular weight follows from Lipinsky´s rules including five basic characteristics of small molecules which should be considered when the small molecules are synthesized with the aim of oral administration.[27] Probing of the chemical space facilitate understanding of biological systems and has an important role in the searching for lead structures in the drug discovery. When using TOS the organic molecules covered the particular point in the chemical space. The aim of DOS is to distribute the molecules to as large as possible region of the chemical space (Figure 4).[24]

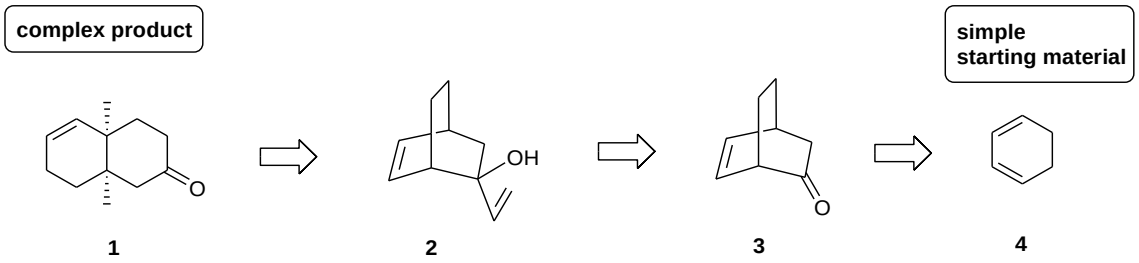
Figure 4: Distribution of organic compounds achieved by different approaches



Despite the diversity originating from various sources providing the natural compounds these molecules cover only small percent of chemical space (although products of combinatorial synthesis cover significantly smaller area of the chemical space). Detailed exploration of the chemical space can make significant advances in the pharmaceutical chemistry, brings the stately progress in a treatment of various diseases and finds modulators for all human proteins functions, what is the challenge for multidisciplinar science.

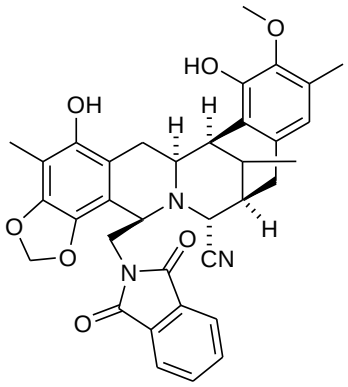
Different aims of these two synthetic approaches require different planning strategy. Planning in TOS was in the past facilitated by development of retrosynthetic analysis which proceeds from a complex target structure to a simple substrate. An example of this reverse process when the product is step by step simplified until the starting material is identified is shown in Scheme 1.[22]

Scheme 1: An example of finding the starting material by means of retrosynthetic analysis



The retrosynthetic analysis has a great value in a synthesis of particular target (TOS) or optimization of lead structures (combinatorial chemistry). It has been used in this field of drug discovery for a long time and a lot of synthetic target molecules were achieved by using this planning method. Such an example is depicted in Figure 5.[22,28]

Figure 5: Phthalascidin, potential anticancer agent, as an example of successful retrosynthetic planning strategy.



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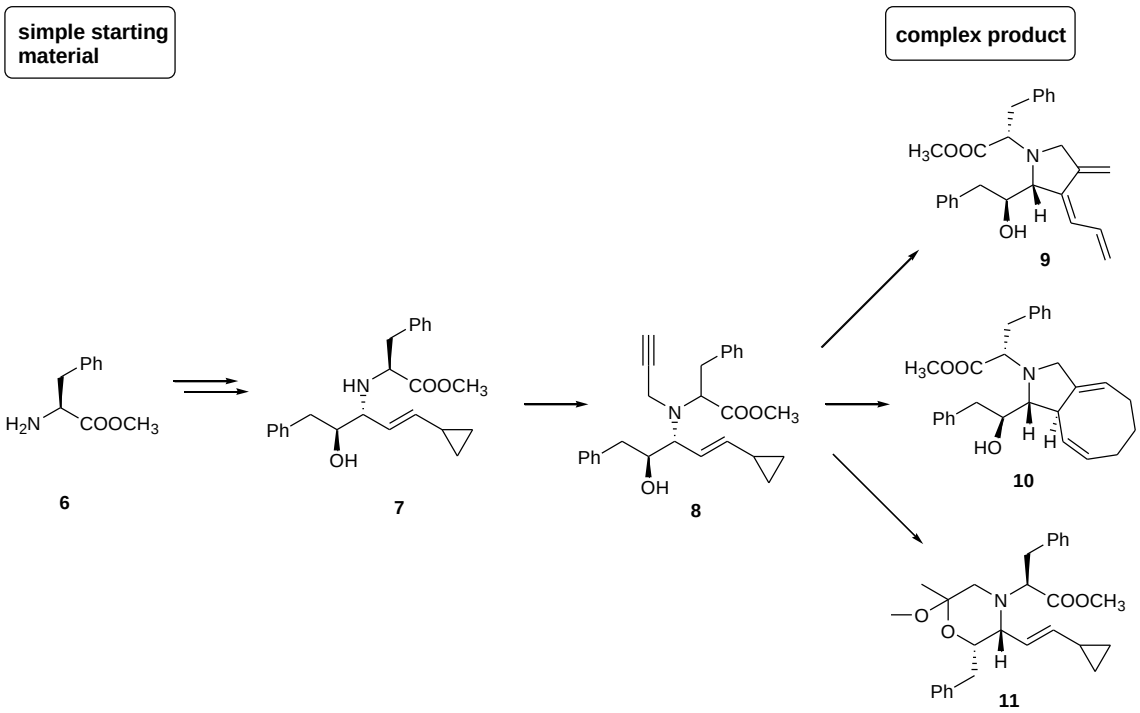
Considering the fact that the aim of DOS is to provide collection of structurally complex and diverse molecules without a specific target the retrosynthetic analysis is not applicable in this synthetic approach. The demand for the strategy enabling

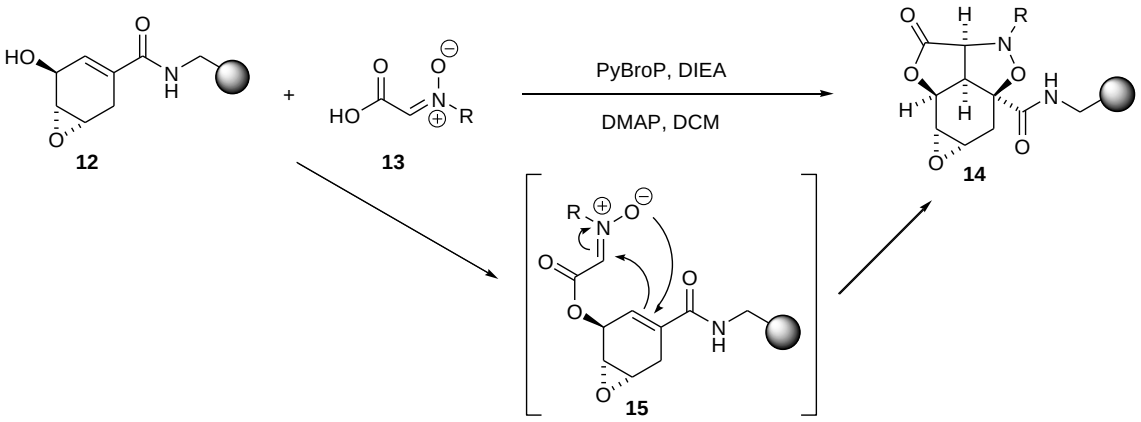
diversity-oriented synthesis resulted in development of the forward synthetic analysis[22] by means of which synthesis is planned in the direction from reactants to complex products (Scheme 2).

Scheme 2: Forward synthetic analysis from simple starting material to complex product



Since many of the molecules which are known for their ability to bind to proteins or to disrupt protein-protein interactions are complex natural compounds the complexity represents an important feature of the nascent chemical library.[29] Influence of the molecules complexity on their interaction with proteins and their biological activity was studied in some works.[30,31] An efficient accomplishment of the structural complexity can be achieved e.g. by using of pairs of complexity-generating reactions where the product of the first one is the substrate for the second reaction.[29,32-33] For example Schreiber utilized this kind of reactions in the synthesis of tetracyclic compounds where the complex products were achieved via tandem acylation/1,3-dipolar cycloadition with complete regio- and stereoselectivity (Scheme 3).[34] The employment of this reaction pairs in iterative manner has the special value for the reach of a high degree of complexity. Then the planning of a synthesis is based on the identification

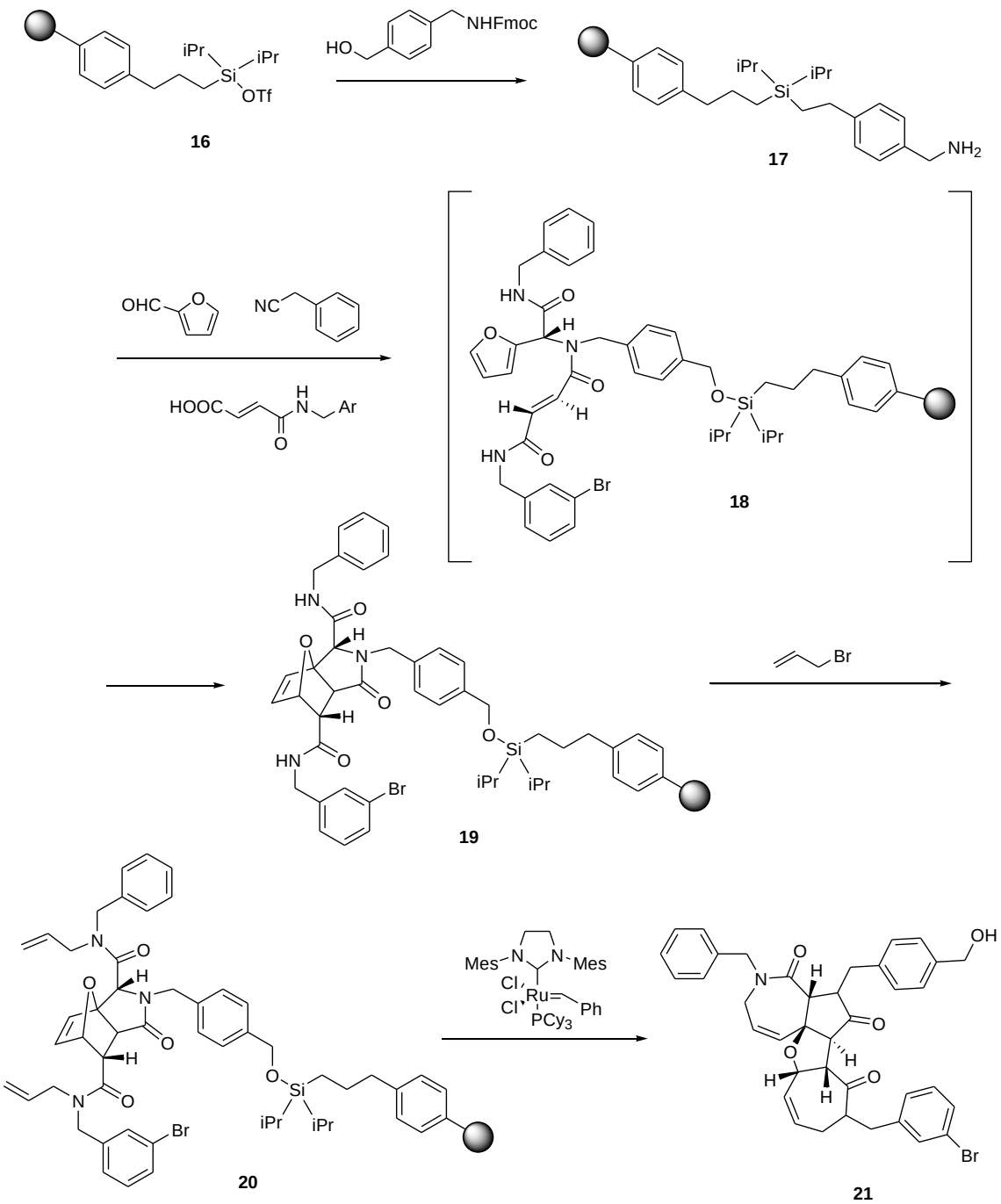
Scheme 3: An example of using tandem reaction to achieve complex molecules



of this product-substrate relationship through the all synthetic pathway in direct from the simple starting material to the complex product (forward planning strategy).[22] The strategy can be demonstrated by the synthesis employing Ugi four-component reaction, Diels-Alder reaction and ring-closing/ring-opening metathesis reaction to achieve complex molecules including five-, six- and seven-membered rings (Scheme 4).[29]

The main goal of the diversity-oriented synthesis is a high degree of diversity.[23] Three diversity elements can be distinguished in DOS libraries: a skeletal diversity, an appendage diversity and a stereochemical diversity. The appendage diversity represents the simplest access to the compound diversity which is based on a various substitution of the one concrete scaffold. For example, the numerous library including isoxazolidine derivatives[34]or library based upon galanthamine[35] were completed by using different building blocks. The possessing of stereocenters within small molecules can substantially impact the interaction of these ligands with chiral macromolecules in living organism. Aditionally, one of the natural compounds characteristic is a large number of stereocenters.[18] Therefore, production of stereoisomers and achievement of relevant stereochemical diversity through the chemical library by this way is an important point in the diversity-oriented synthesis. As an example of recently described stereoselective reactions the synthesis of glycosides with Au catalysis[36] or the synthesis of bicyclic lactones using microwave-assisted tandem Wittig /Diels-Alder intramolecular cycloadition[37] can be mentioned.

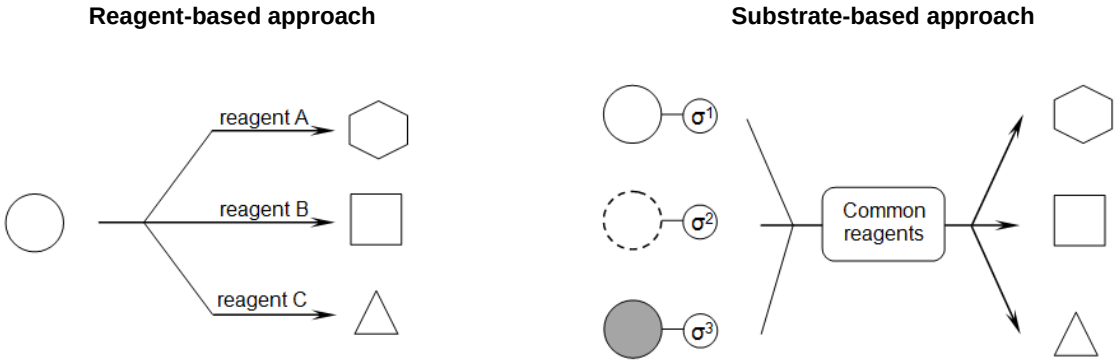
Scheme 4: An example of using tandem reactions in iterative manner



The last type of the diversity is presented as a particularly challenging area of DOS.[24] Synthetic pathways generating compounds with diverse skeletons utilize two different approaches.[23] In the reagent-based strategy (also termed as branching pathway) a common substrate is converted into collection of products with diverse skeletons by using various reagents. When using second strategy, the substrate-based strategy (also termed as folding pathway) a substrate is firstly equipped with various appendages

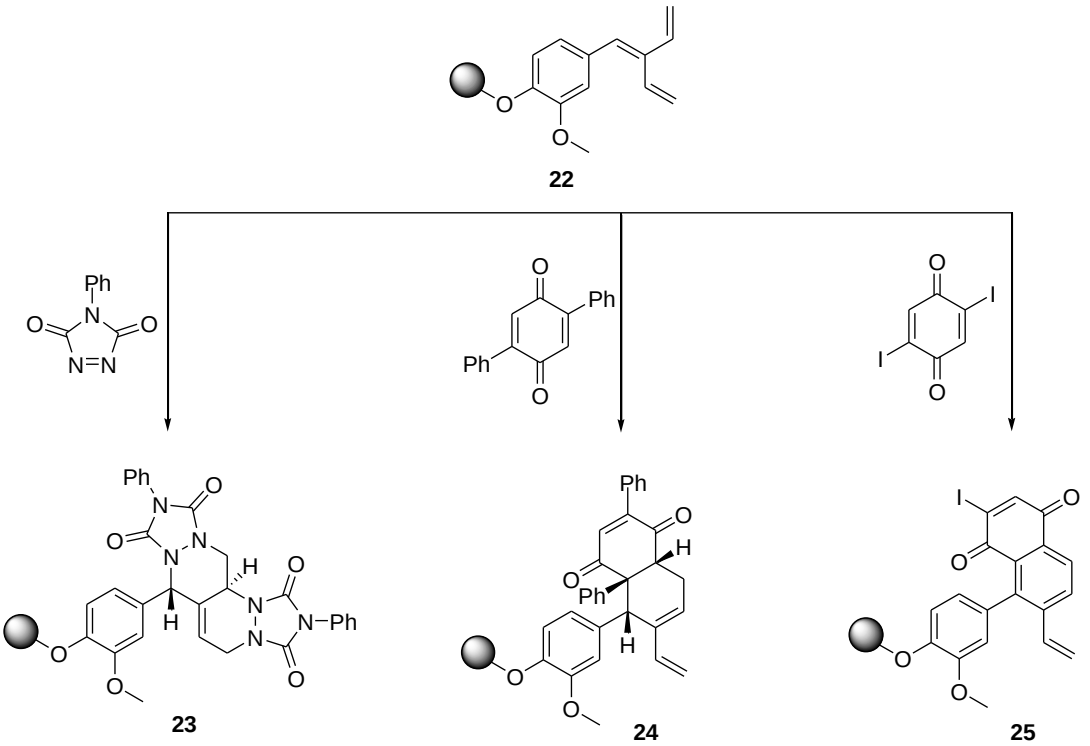
called σ-elements which pre-encoded the skeletal information. So modified substrates are then transformed to a collection of products with diverse skeletons by using common reagents (Figure 6).

Figure 6: Reagent-based and substrate-based strategies



The first strategy is exemplified by the synthesis of condensed aromates (Scheme 5).[23,38]

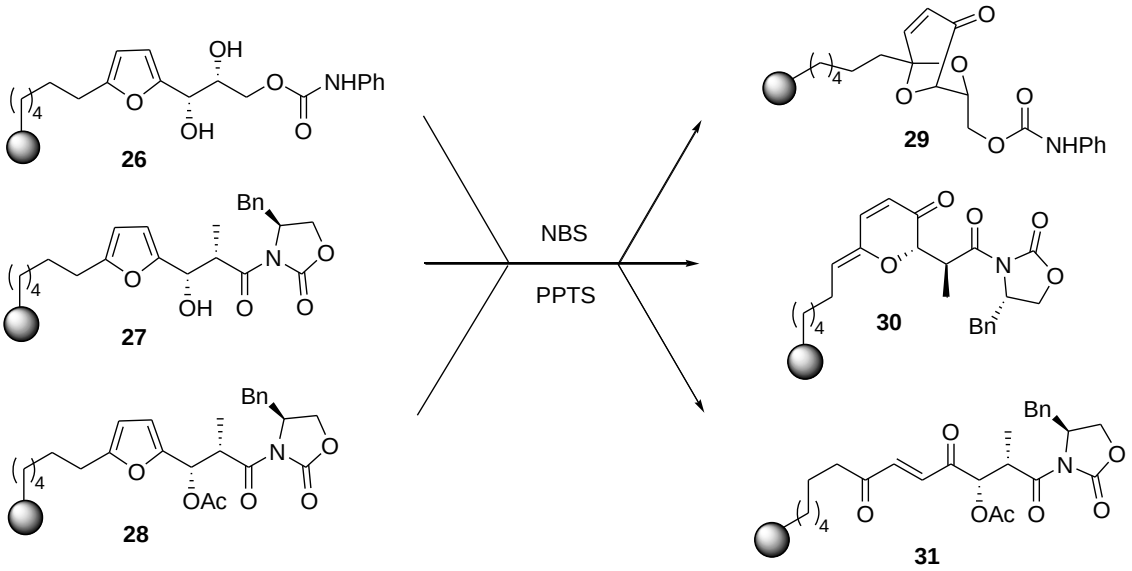
Scheme 5: An example of the reagent-based approach



A substrate is transformed into different scaffolds using various reagents.

The second strategy was applied for the synthesis of various heterocycles from substituted furane derivatives (Scheme 6).[23,39]

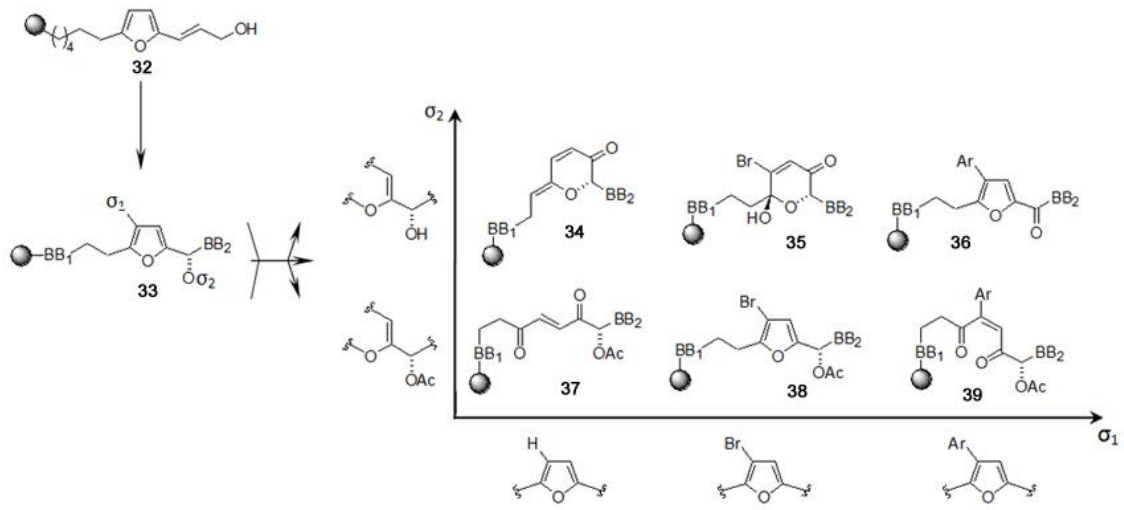
Scheme 6: An example of the substrate-based approach



Substrates with different σ-elements are transformed into different scaffolds using common reagents

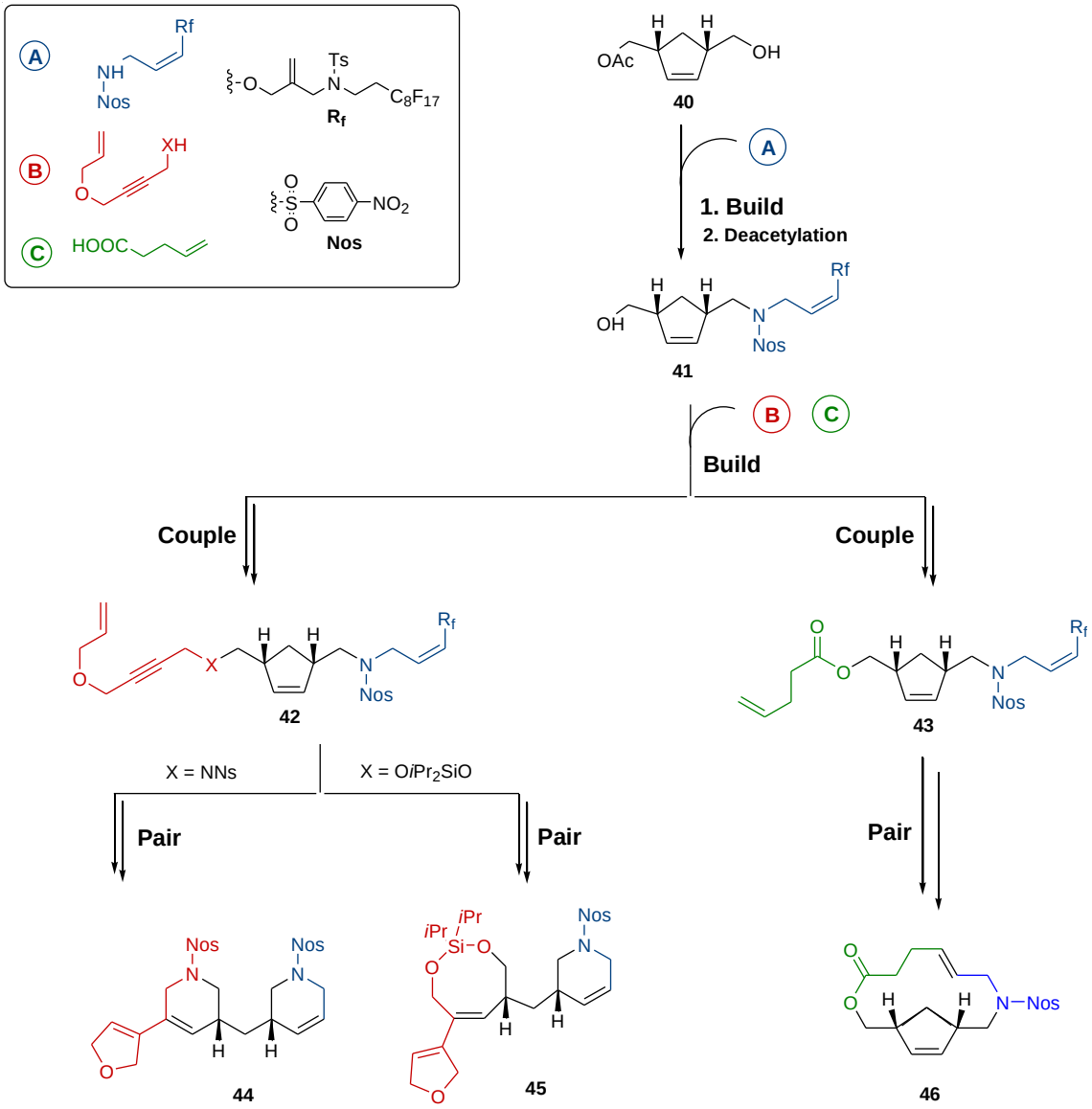
The combinatorial approach can be utilized in the access to the skeletal diversity what is reported e.g. in work by Burke who used the folding process to achieve a diversity-oriented chemical library.[40] In addition this paper demonstrated an utility of the split-and-pool method ensuring the skeletal diversity (σ-elements) as well as the appendage diversity (various building blocks). The described synthetic pathway started from a common alkoxy furan core which was transformed using two sets of σ-elements (influencing the formation of various scaffolds) and two sets of building blocks (deriving the final structures) into a collection of substrates which under oxidative and acidic reaction conditions yielded a chemical library of more than 1000 compounds of three different scaffolds variously substituted within the skeleton (Scheme 7).

Scheme 7: An example of the substrate-based strategy with utility of the combinatorial method



Since DOS is still evolving field of organic chemistry and a demand for small molecules with suitable properties is increasing a great endeavor to meet these demands is driving force in progress of the synthesis of small molecules.[41] Recently, Schreiber and co-workers introduced a new synthesis strategy generating skeletal and stereochemical diversity. Ready access to the diversity which is achieved just in a few steps is a remarkable advantage of this build/couple/pair strategy (B/C/P strategy).[42] The first phase (build phase) involves a preparation of chiral building blocks with convenient functionalities allowing coupling reactions in the subsequent couple and pair phases. The building blocks are joined in the couple phase *via* intermolecular coupling reactions and yield precursors entering to the following step. Despite the merit of this method consisting in a short reaction sequence further steps can be added after the coupling phase to attach new functional groups useful in the next phase. In this pair phase various scaffolds are created *via* intramolecular coupling reactions. Since the potency to accomplish all possible product stereoisomers during the couple and the pair phase is still limited it is expediential to perform these processes without new stereogenic centers. Thus the stereochemical diversity is introduced in the build phase whereas the skeletal diversity is provided by the pair phase. A practical example of the utility of B/C/P strategy is depicted in Scheme 8 where using this strategy the collection

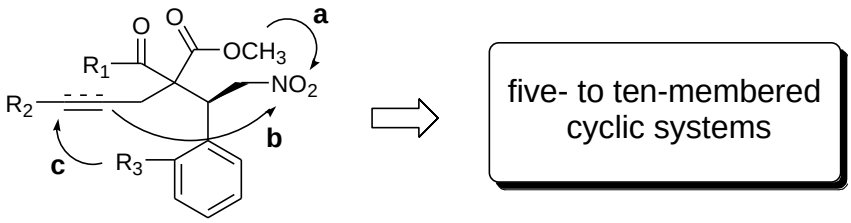
Scheme 8: Synthesis of structuraly diverse library using B/C/P strategy



of various scaffolds consisting of three type of cycle: azacycles, oxacycles and carbocycles was obtained.[43,44]

The formation of various scaffolds in the pair phase is based on using functional-group-pairing reactions. This kind of reactions where two selected functional groups are coupled was studied in the work by Porco and co-workers (who also developed the term functional-group-pairing reactions).[45] They performed Cinchona alkaloid-catalyzed Michael addition of dicarbonyls to nitrostyrenes to achieve multifunctional aduct **47**. The pairing of appropriate functional groups upon this compound resulting in various-size complex systems (five- to ten-membered rings) is outlined in Figure 7.

Figure 7: An example of functional-group pairing yielding a collection of various-size complex systems



47

a - pairing of ester and nitro group
b - pairing of nitro and alkyne group
c - pairing of alkyne and allyl group

Further advances in DOS noted in last years are discussed for example in the review by Dandapani.[46]Author describes developments of the synthetic pathways involving the types of reactions which have a substantial value in the question of diversity and complexity. The power of multi-component reactions[47] can be demonstrated for example by the recently developed synthesis employing a four-component one-pot reaction of amines, maleic anhydrides, aldehydes and thiols yielding the inhibitor of HOXA13, a transcription factor of certain kinds of cancer.[48]

Other classes of reactions often utilized in DOS are cycloaddition reactions and ring-closing metathesis (RCM). Snyder and co-workers described the synthesis of naphtyridines library using intramolecular cobalt-catalyzed [2+2+2] cycloaddition which included three compounds with anti-tuberculosis activity.[49] From the recent works involving RCM, synthesis of aminoalcohol-derived macrocycles library containing 12-14-membered systems can be mentioned. The screening of the library revealed an inhibitor of Sonic hedgehog (Shh) signaling pathway which is linked to development of specific kinds of cancers.[50]

Despite the pronounced advances and knowledge extension in the field of chemistry, biology and medicine the rate of the drug development is languid and there remain extensive gaps in the drug discovery especially with contribution of target specified as undruggable.[51-54] One of the possibility considered to accelerate the drug discovery is using privileged structures as a basic framework of created chemical library.[55,56] This term was first introduced by Evans in 1988 in the connection with 1,4-benzodiazepine-2-ones as structures that are able to provide ligands for more than one

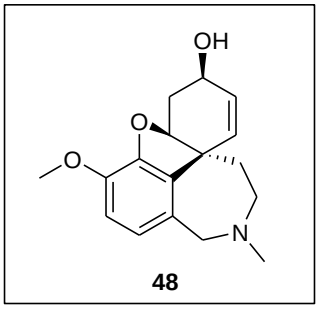
type of receptors.[57] Later, compound collections based on further privileged scaffolds and having a high hit rate were synthesized.[55,56,58] The concept of privileged structures started to be considered also in the DOS approach. For example Tan and co-workers synthesized a library of polyketides containing six different structural classes with the stereochemical diversity by means of the highly efficient rout employing propargylic alcohol precursors.[59] Park and co-workers described an approach to series of compounds holding benzopyranyl ring. The reported library comprised four different heterocycles as a combination of benzopyran with pyridine, pyrazole, pyrazolopyrimidine and pyrimidine.[60]

Despite the undoubted importance of natural compounds in the exploration of biological functions and drug discovery, the access to these compounds is burdened with difficulties connected with their isolation. This fact supported by expansion of combinatorial chemistry initiated the change of strategy in searching for new lead structures. Many of the pharmaceutical companies started to focus on synthetic compounds instead of previously studied natural compounds.[61] However, this move induced some doubts, moreover when the expected acceleration in producing of new therapeutics did not come.[16,51]

The biologically relevant structural features of natural compounds[19] are still an attractive element in the drug research and also for the realm of DOS.[62] Various strategies providing natural product-like libraries were developed. These can be classified into three groups differing in a degree of structural diversity. The first group includes libraries based on a core scaffolds of individual natural products, the second group includes libraries based on specific substructures from the classes of natural products and the third group consists of libraries possessing structural features of the natural compounds in a more general sense.[61,62] All three approaches provided a relevant source of biologically active compounds (Figure 8). The first example of

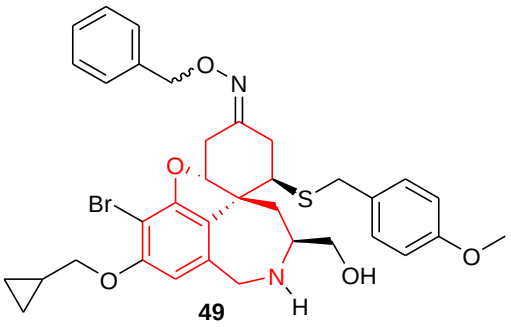
Figure 8: Examples of the active natural product-like compounds

Library based on core scaffold



48

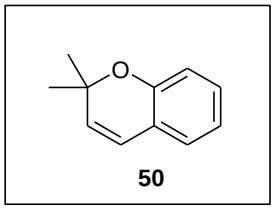
galanthamine
acetylcholinesterase inhibitor



49

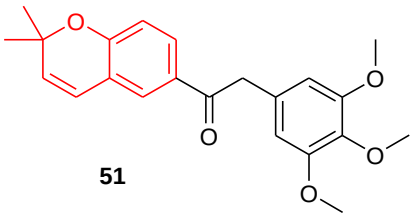
secramine
secretory pathway inhibitor

Library based on specific substructures from classes of natural compounds



50

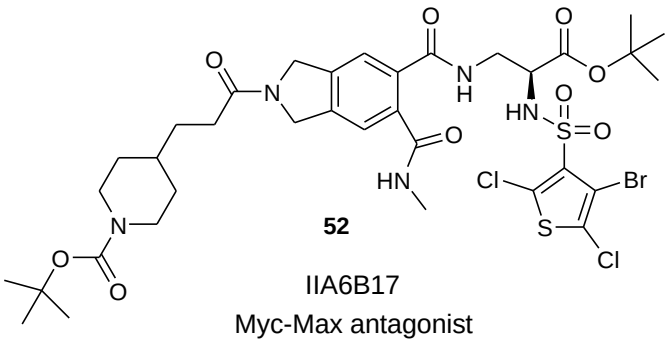
2,2-dimethylbenzopyran
substructure



51

NADH:ubiquinone oxidoreductase inhibitor

Library having general structural features of natural compounds



52

IIA6B17
Myc-Max antagonist

an active compound, secramine originated from a library of galanthamine analogs. Interestingly, both compounds exhibit different biological effects. Whereas synthetic secramine acts as a secretory pathway inhibitor, the natural alkaloid inhibits acetylcholinesterase and does not affect the secretory pathway.[63] The second strategy is depicted by molecules in a library based on 2,2-dimethyl-2*H*-benzopyran moiety. This

represents a subunit contained in a number of natural compounds with wide range of biological effects, e.g. inhibition of cyclooxygenase, inhibition of tyrosinase, inhibition of biosynthesis of juvenile hormone and other. In addition, analysis of structural features of known drugs showed benzopyran as a preferential structure for the drug design.[64] Thus, the benzopyran substructure was selected as a relevant template for a natural product-like library including novel NADH:ubiquinone oxidoreductase inhibitor.[65] The last approach is represented by a chemical library which was performed using reactions providing amide bonds. The resulting peptidomimetic molecules were supposed to modulate protein-protein interactions. Compound IIA6B17 displayed in Figure 8 is one of the compounds which were identified as Myc-Max antagonist.[66,67]

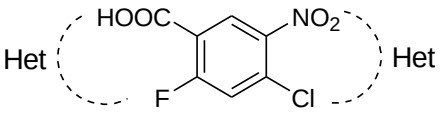
Despite all advancements in the concerning disciplines the searching for novel leads structures still represents a demanding process which renders many challenging questions waiting for a solution. The noteworthy contribution in the searching for novel leads brought along the development of computational methods termed as "virtual screening" (VS).[68] Literature describes two approaches; one of these is based on a molecular docking working with the known structure of a receptor.[69] The other one often called ligand-based is used when the receptor structure is not given and comprises methods such as clustering, similarity searching or statistical methods.[70] The principle of these techniques is not content of this text, we can refer interested readers to other literature.[71-74] This computer-based approach is a still evolving area and its power is increasing despite any drawbacks and limitations which especially at the beginning of VS made the experimental high-throughput screening (HTS) preferred method in the drug discovery. Today with the progress in genomics and number of known protein structures virtual screening becomes a valuable tool which can significantly facilitate the finding on novel hits and decrease the sum of money which is needed when using HTS.[75]

3.3. 2-Fluoro-4-chloro-5-nitrobenzoic acid

A great number of solid-phase synthetic methods utilize 2-fluoro-4-chlorobenzoic acid as a starting building block. Concerning this fact we decided to investigate the reactivity and application of similar component enriched of a one functional group, 2-fluoro-4-chloro-5-nitrobenzoic acid, in a development of simple synthetic methods for

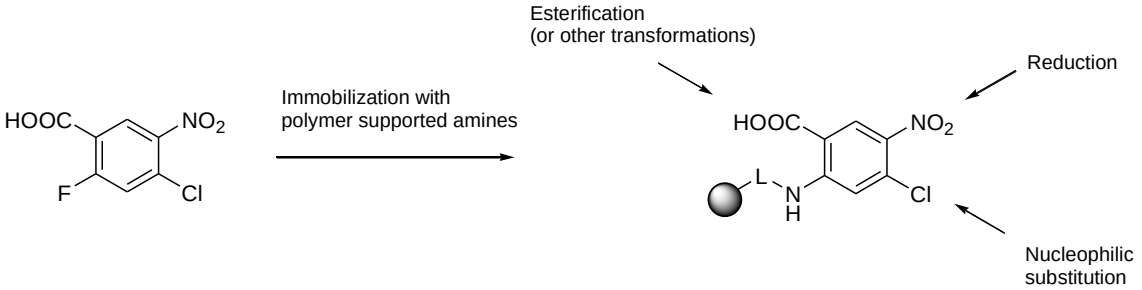
preparation of diverse heterocycles. 2-Fluoro-4-chloro-5-nitrobenzoic acid is a commercially available building block offering wide range of chemical transformations. Due to convenient arrangement of functional groups the heterocyclic scaffolds can be formed on two side of the molecule, nitro-chloro or carboxy-fluoro side. After suitable modification of both sides of the acid also tricyclic bisheterocycles situated on the opposite side of the benzene ring of the molecule can be theoretically created as well (Figure 9). Reduction of the nitro group or substitution of halogens allows a facile

Figure 9: Advantageous arrangement of functional groups



introduction of amino group into the molecule what enables another possibility to modify the molecule or conversion to different heterocycles (Scheme 9).

Scheme 9: Immobilized 4-chloro-5-nitroanthranilic acid offering various chemical transformations



Despite all advantageous this component has found applications only in a few patents so far. One of these describes the preparation of benzenecarboxamide derivatives as Gonadotropin-releasing hormone receptor antagonists using the acid.[76] Another patent reports utilizing of the compound in synthesis of benzoic acid derivatives useful as diuretics or saluretics.[77] Concerning nitrobenzoic acid was also employed in the synthesis of heterocyclic peptides used in a treatment of hepatitis C virus infection[78] or indazole sulfonamides as 11β –hydroxysteroid dehydrogenase inhibitors.[79] Only solid-phase methods employing the acid was the synthesis of hydroxyquinolinones[80] and the synthesis of various condensed nitrogen heterocycles recently described by our team.[81]

3.4. Condensed nitrogenous heterocycles

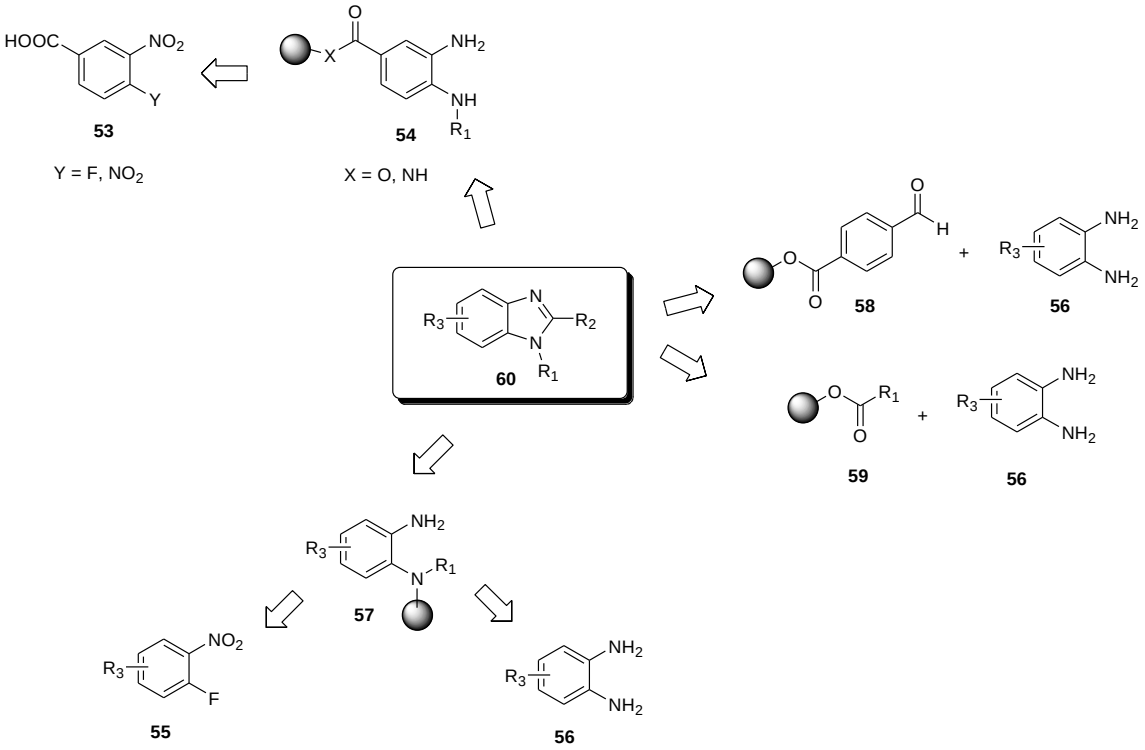
Condensed nitrogenous heterocycles represent a large group of compounds that have been taking an important position in pharmaceutical chemistry for many years. Significant biological and pharmacological properties of such molecules suggested this kind of derivatives to be in the spotlight of many investigations. Benzimidazole, benzotriazole, quinoxaline, quinolinone, quinazoline, benzodiazepine and benzodiazocine scaffolds that were selected as target structures in this work represent heterocycles with various biological effects occurring in a large number of market drugs. The following brief insight contains examples of described synthetic methods with use of solid-phase synthesis and biological activity relating to the mentioned frameworks.

3.4.1. Benzimidazoles

Solid-phase synthesis of benzimidazoles became the object of many investigations. A lot of synthetic method utilizes the polymer supported *o*-phenylenediamines **54** originating from the attachment of 4-fluro-3-nitrobenzoic **53** acid or dinitrobenzoic acid **53** to the resin *via* carboxylic group.[82-84] In another method the diamine precursors **57** are attached to the resin through the one of the amino groups.[85-87] Different strategy was exposed in works using reaction of the phenylenediamines **56** with polymer-bound aldehyde **58** or esters **59** (Scheme 10).[88,89] All of these approaches are summarized in a review by Kamal.[90]

Benzimidazoles exhibit a wide range of biological activities such as anticancer,[91-93] antiviral including human immunodeficiency (HIV),[91-93] antibacterial,[91,92] anthelmintic (Albendazole, Mebendazole),[91-93] antifungal[92] and also perform as structural isosters of naturally occurring nucleotides.[92]

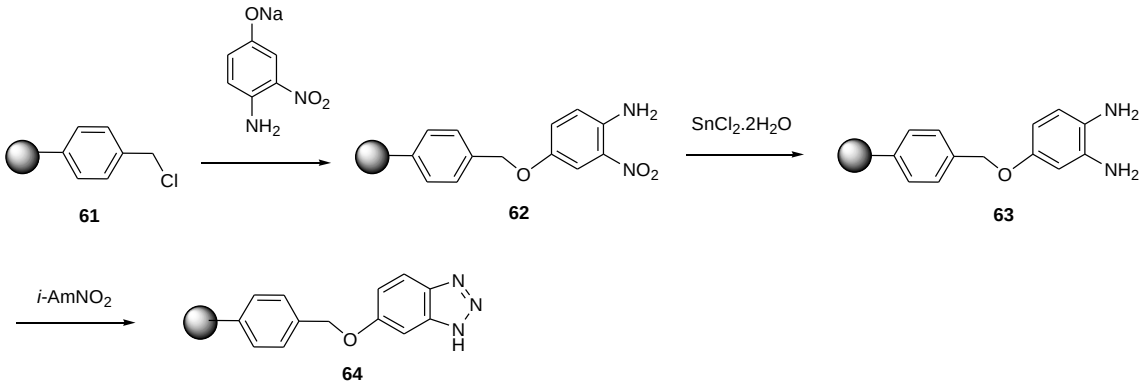
Scheme 10: Synthetic approaches to benzimidazoles



3.4.2. Benzotriazoles

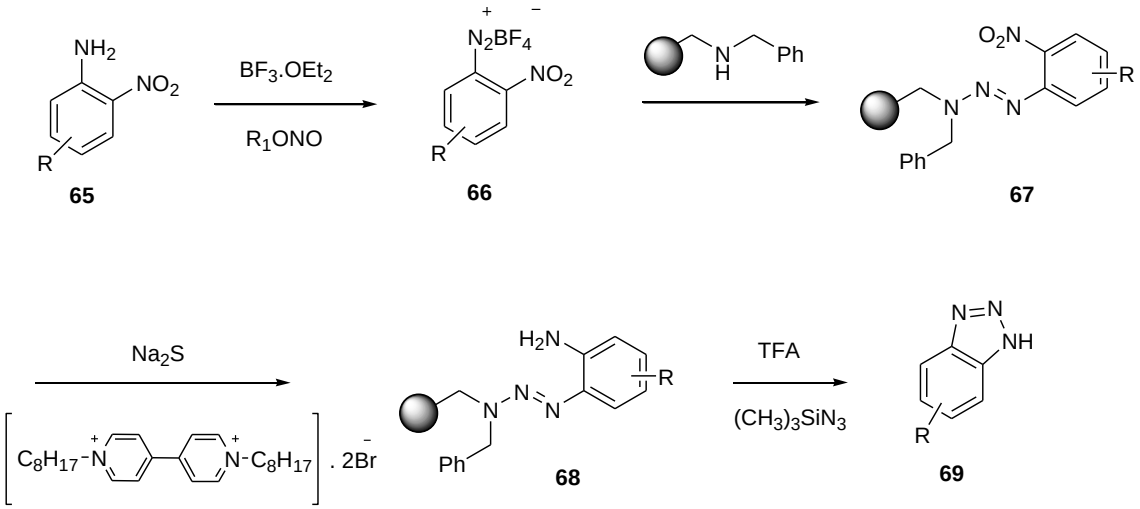
Since benzotriazole moiety performs in organic synthesis as auxiliary agent in a number of molecular transformations its application in the solid phase combinatorial chemistry contributes to diverse heterocyclic scaffolds.[94] Katritzky et al. reported method based on the reaction of polymer-supported phenylenediamine **63** with isoamyl nitrite (Scheme 11).[95]

Scheme 11: Synthesis of polymer-supported benzotriazoles using isoamyl nitrite



Another publication describes achieving of benzotriazoles **69** from the resin-bound triazene precursors **68** (Scheme 12).[96,97]

Scheme 12: Synthesis of benzotriazoles from resin-bound triazenes

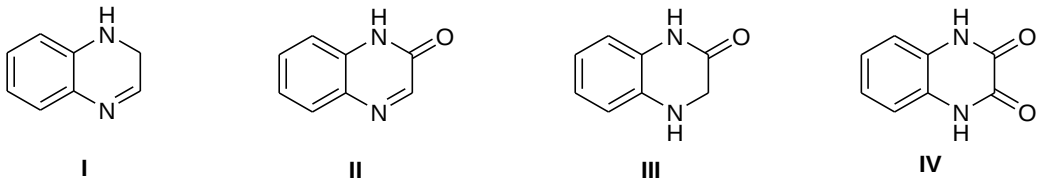


Concerning biological activity, benzotriazole derivatives act e.g. as inactivators of the severe acute respiratory syndrome 3CL Protease[98] and promising agents in the treatment of dyslipidemia and prevention of atherosclerosis.[99]

3.4.3. Quinoxalinones

Quinoxaline derivatives have been attracting attention of many researches and literature offers a lot of synthetic pathways carried out on polymer support and providing various derivatives. In our study we tested various methods leading to structures **I** - **IV** (Figure 10). In solid-phase chemistry immobilized *o*-fluro-nitrobenzene derivatives are involved

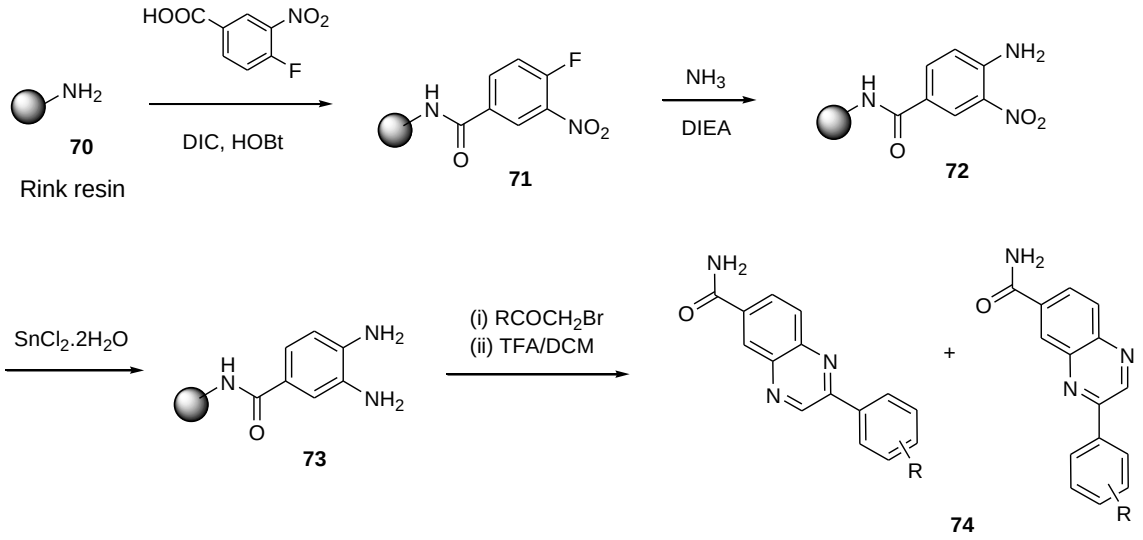
Figure 10: Structures of the aimed quinoxaline derivatives



in most of the strategies. For example Wu described method for the preparation of quinoxaline derivatives **74** utilizing reaction of polymer-supported *o*-phenylenediamine

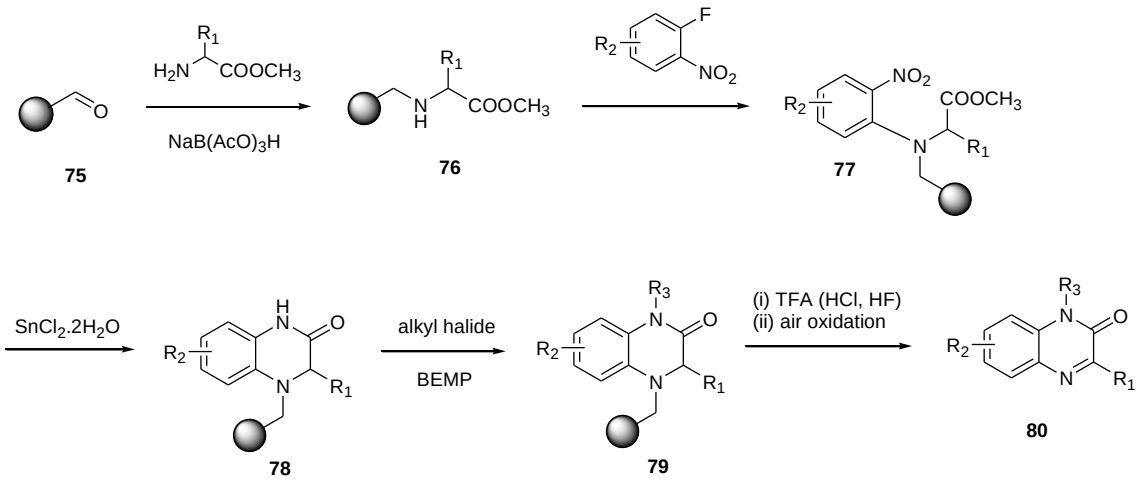
73 with α-bromoketones (Scheme 13).[100] The derivatives of the scaffold **II** were prepared using a method based on the substitution of *o*-fluoronitrobenzene with

Scheme 13: Solid-phase synthesis of quinoxalines using α-bromoketones



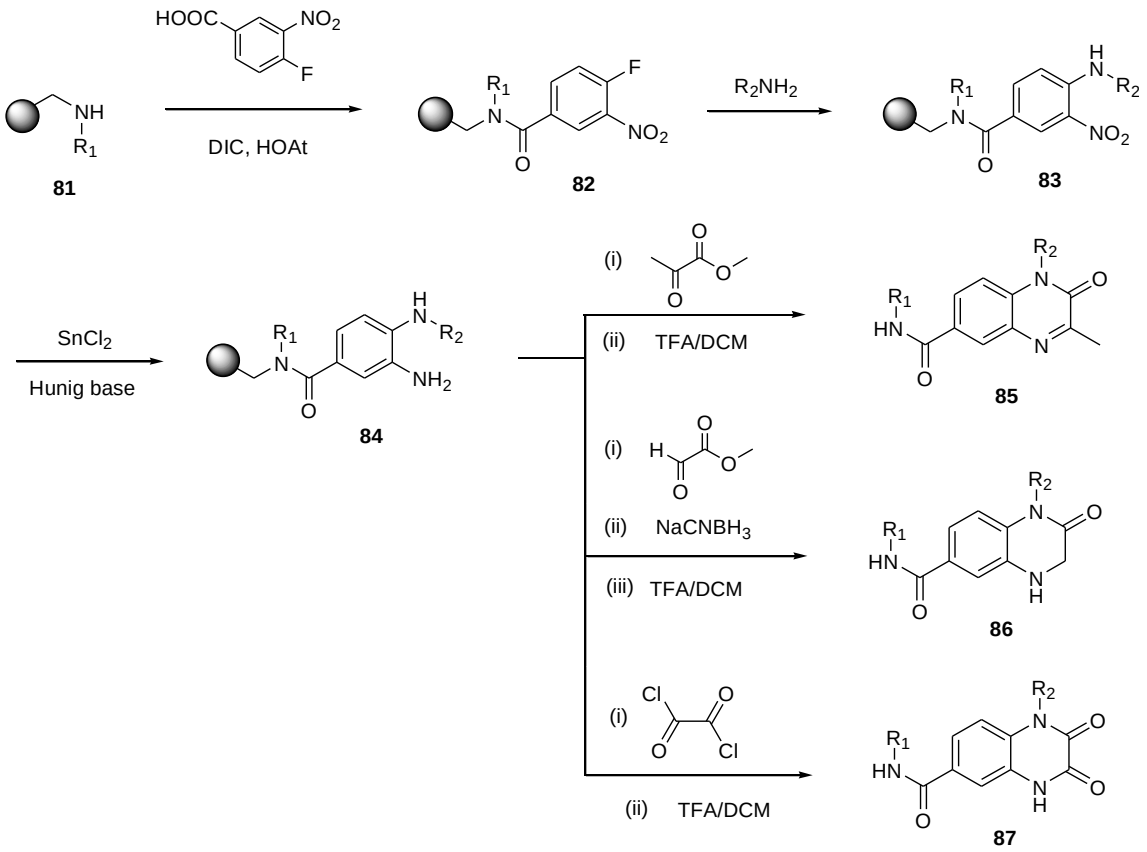
polymer-bound amino acid esters **76**, subsequent reduction of the resulting nitroanilines **77** yielding dihydroquinoxalinones **78** which were after cleavage from the resin air-oxidized to quinoxalinones **80** (Scheme 14).[101]

Scheme 14: A traceless synthesis of quinoxalinones



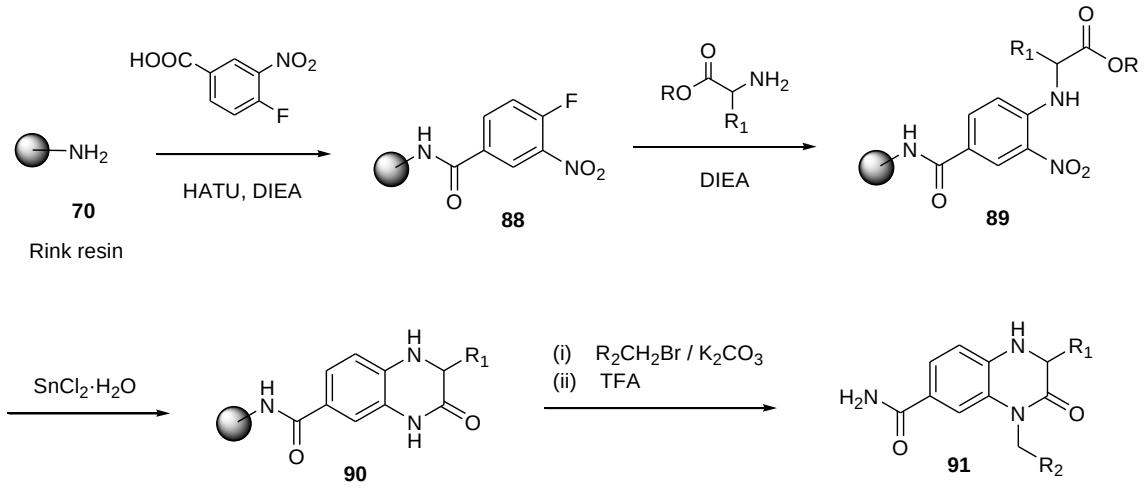
Purandare developed a method based on the reaction of the resin-bound diamine **84** with carbonyl compounds leading to various heterocycles (Scheme 15).[102] Besides quinoxalinones **II** author utilized the synthetic strategy for the preparation of derivatives possessing scaffolds **III** and **IV**.

Scheme 15: Synthesis of 3-methyl-quinoxalin-2-ones



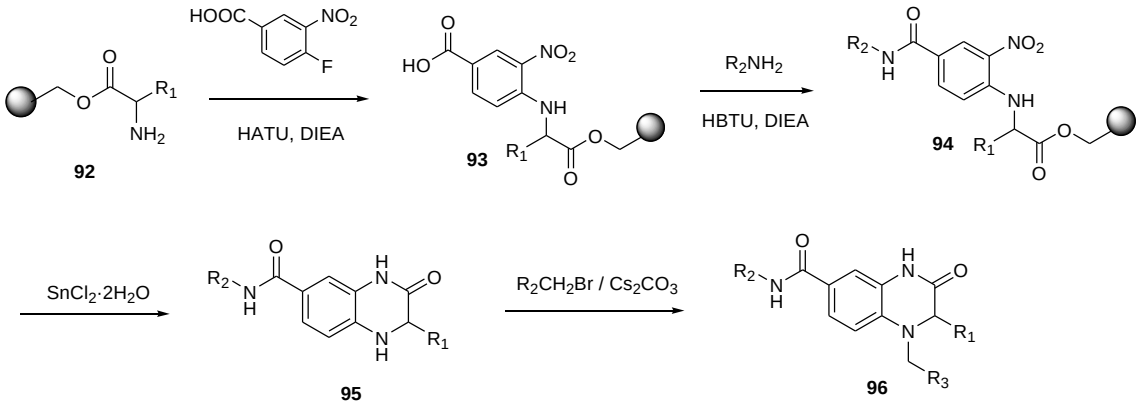
Lee acquired the set of dihydroquinoxalinones **91** by reaction sequence including replacement of the fluorine atom with α-amino esters **89** followed by reduction of the nitro group and subsequent intramolecular cyclization to target heterocycles **90** (Scheme 16).[103]

Scheme 16: Synthesis of dihydroquinoxalin-2-ones



Analogical compounds were achieved *via* reaction of α-amino acids preloaded onto Wang resin with 4-fluoro-3-nitrobenzoic acid and subsequent reduction of the nitro group (Scheme 17).[104]

Scheme 17: A traceless, self-cleaving synthesis of dihydroquinoxalin-2-ones



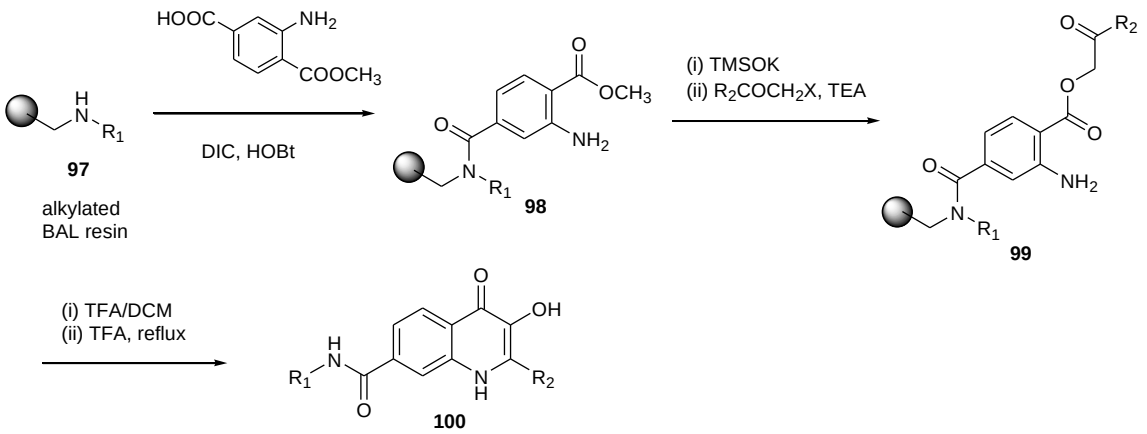
The biological testing of compounds containing quinoxaline cycle **I** revealed an activity against parasitic disease caused by genus Leishmania.[105] Oxygenous derivatives **II** were identified as inhibitors of P-glycoprotein which has an important role in multidrug resistance in the cancer chemotherapy.[106] Quinoxalinones exhibit also antimicrobial and anti-inflammatory activity.[107]

3.4.4. 3-Hydroxyquinolin-4(1H)-ones

Since our department have been interested in this type of compounds for many years more detailed description is paid to these heterocycles in comparison to others.

Literature describes various synthetic pathways of 3-hydroxyquinolin-4(1*H*)-ones and particularly these leading to derivatives with phenyl moiety in position 2 are summarized in review by Hradil and co-workers.[108] One of this solution-phase method which is widely applicable and which utilizes a thermal cyclization of anthranilates[109] was in 2007 applied in a synthesis of the hydroxyquinolinone derivatives on polymer support.[110] The method started with an immobilization of anthranilic acid achieved *via* acylation of the resin-bounded amines **97** with 1-methyl-2-aminoterephtalate. After hydrolysis of methylesters **98** the obtained acids were subsequently esterificated using haloketones and the resulting immobilized intermediates **99** were cleaved from the resin and cyclized in solution to final quinolinone-carboxamides **100** (Scheme 18).

Scheme 18: Solid-phase synthesis of hydroxyquinolinone-carboxamides

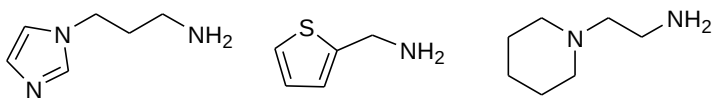


The development of solid-phase synthesis of 3-hydroxyquinolinones as an efficient tool to ready access to these compounds can significantly accelerate the probe of their biological effects, especially in the combination with combinatorial chemistry.

Last years, attention is paid also to molecules containing besides hydroxyquinolonone core another heterocycle. Since bisheterocycles have an important role in the drug research[111]an incorporation of hydroxyquinolinones with another heterocycle suggests compounds with a promising biological activity. Thus, the building of hydroxyquinolinone derivatives on the polymer support was expanded to this area as well. Already mentioned method leading to hydroxyquinolinone-carboxamides **100**[111] (Scheme 18) yields bisheterocycle structures when suitable building blocks are used (Table 1).

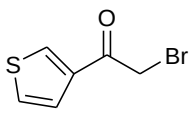
Table 1: The building blocks used for the preparation of hydroxyquinolinone-carboxamides substituted with another heterocycle.

amines providing substituent R



(1) (2) (3)

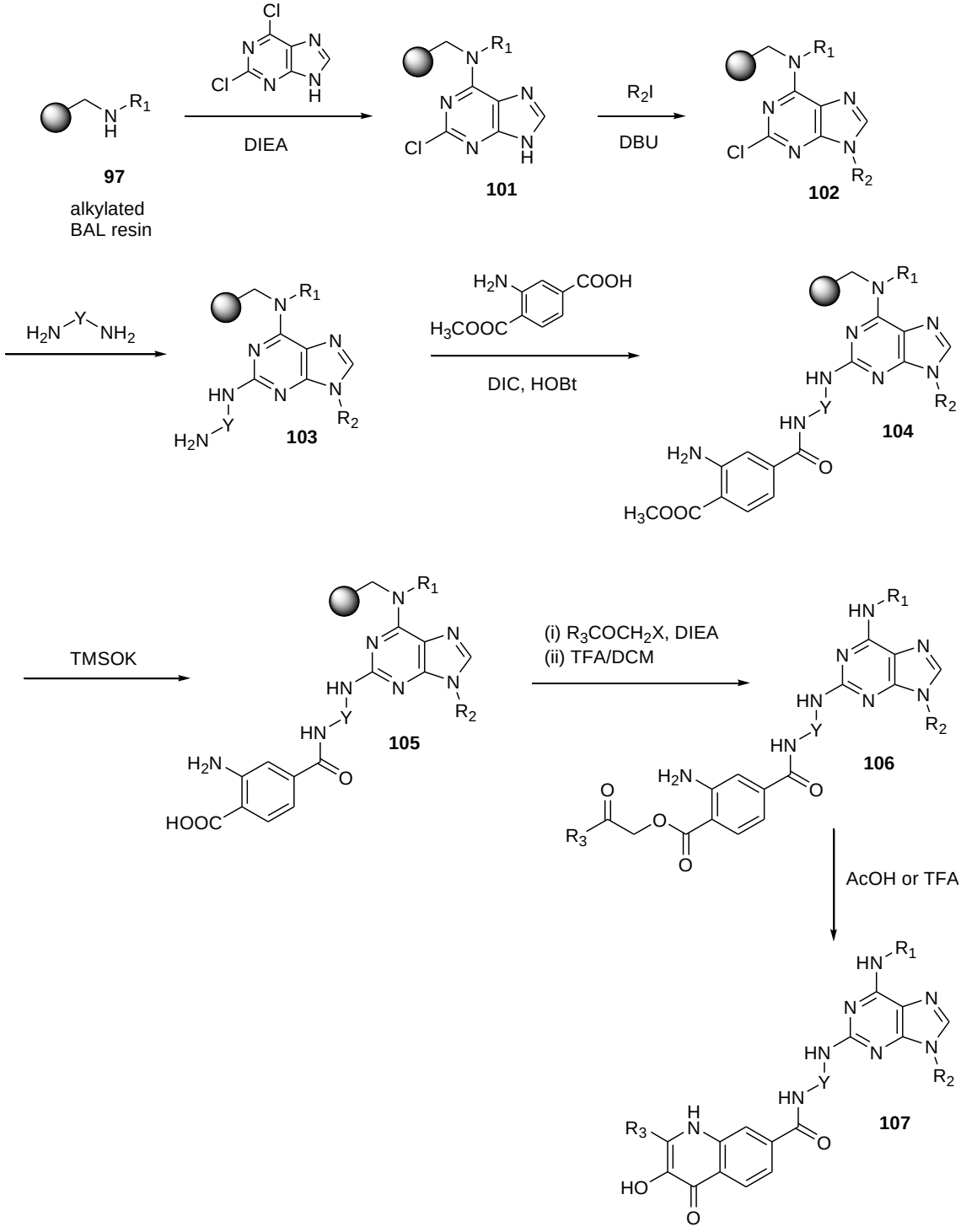
bromoketone providing substituent R´



(1)

Another recently reported example is the synthesis of purine-hydroxyquinolinone bisheterocycles **107** (Scheme 19).[112]

Scheme 19: Solid-phase synthesis of purine-hydroxyquinolinone derivatives

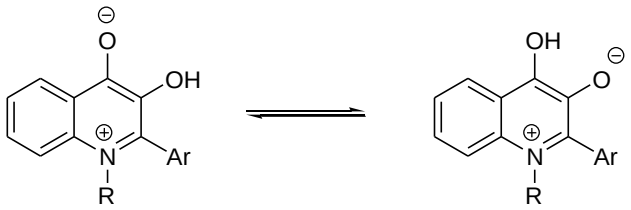


3-Hydroxyquinolin-4(1*H*)-ones became the intensely studied group of compounds because of their promising biological potency. The key biological effect showed by

these compounds is the anticancer activity.[113-115] The activity was tested towards selected cell lines (A549, K562, K562-Tax, CEM, CEM-DNRB) using MTT cytotoxic test. The mechanism of this effect is not iluminated and the exploration of this field including searching for the molecular target is still in proccess. Among other biological effects of 3-hydroxyquinolinones belongs the inhibiton of inosin monophosphate dehydrogenase[114] (IMPDH, enzym responsible for disorders such a cancer or allograft rejections) or immunosuppressive activity.[116] In addition, inhibition effects on bacterial DNA-gyrase or topoisomerase II have also been described.[117]

Apart from biological effects 3-hydroxyquinolinones dispose with another worthwhile quality, namely fluorescence properties. Fluorescence techniques serve as a valuable tool for the probing of biosystems and biological processeses. Fluorescent labels have to possess suitable properties and for this purpose 3-hydroxyquinolinones were studied as a convenient candidates due to their dual fluorescence ability. In comparison to 3-hydroxyflavones, one of the dual fluorescence label representative, structurally similar 3-hydroxyquinolinones have a higher photostability and a quantum yield.[118,119] The fluorescence spectrum with two separated emission bands are caused by a formation of two tautomeric forms of molecule in the excited state (Figure 11). In recent , the relationship between fluorescence properties and various

Figure 11: Excited state tautomeric forms of 3-hydroxyquinolinones

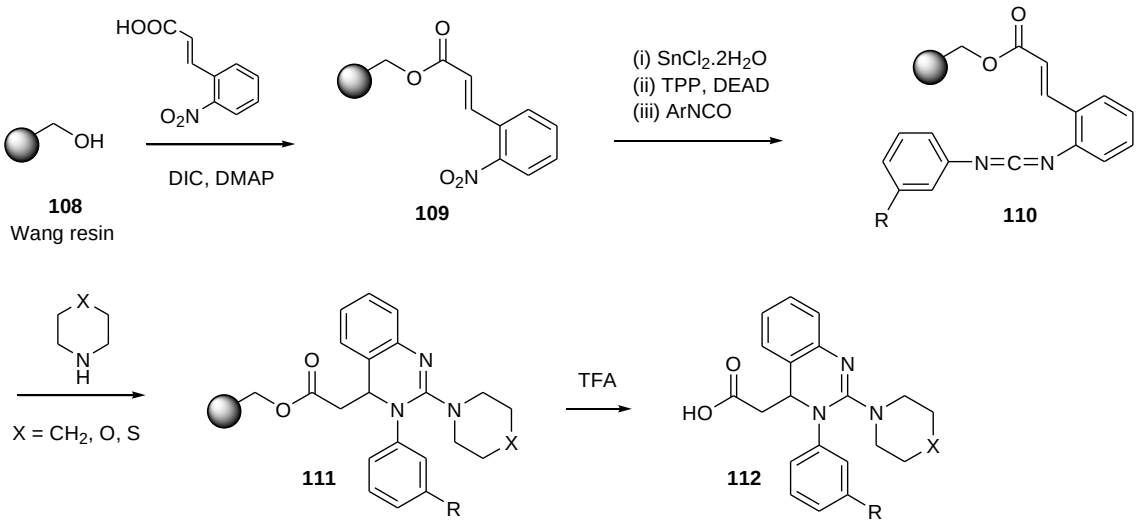


works substitution of 3-hydroxyquinolinone derivatives was studied with a view to evaluate the suitable derivatives as fluorescent probes.[118,119]

3.4.5. Quinazolines

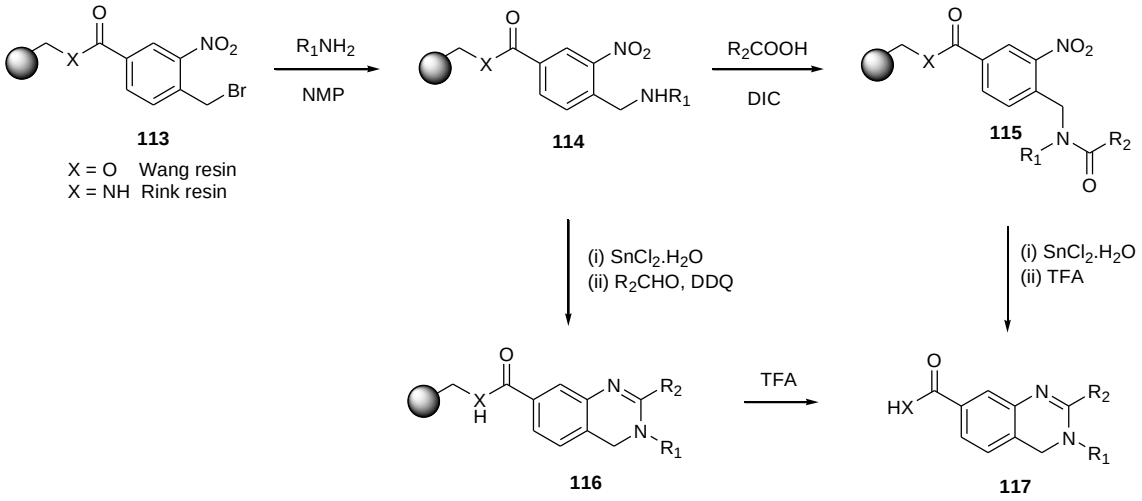
This class of heterocycles became a centre of attention in many research studies and a substantial effort was dedicated to devise synthetic approaches to these molecules.[120] Various synthetic methods were developed in the solid-phase chemistry as well. Wang and Hauske developed the first solid-phase method for 3,4-dihydroquinazoline utilizing 2-nitrocinnamic acid to form polymer-supported iminophosphorane derivative which was treated with *N*-aryl isocyanate to generate carbodiimide intermediate **110.** After exposure to a secondary amine carbodiimide compound provided 3,4-dihydroquinazolines **111** (Scheme 20).[121] Lou described a synthesis starting from 4-bromomethyl-3-nitrobenzoate or corresponding amide

Scheme 20: Solid-phase synthesis of 3,4-dihydroquinazoline



bounded to the solid phase **113**. After substitution of the bromide atom with amine nuclophiles **114**, acylation with carboxylic acids **115** and subsequent reduction of the nitro group the resulting precursors underwent cyclocondensation to quinazoline derivates **117** (Scheme 21).[122] These and other approaches carrying out on solid phase were summarized in the review article by Vögtle and Marzinzik[123] and Kamal and co-workers.[90]

Scheme 21: Solid-phase synthesis of disubstituted dihydroquinazolines

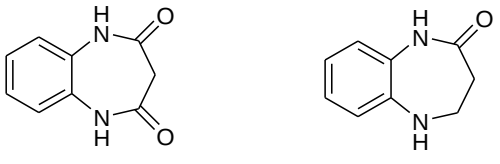


Quinazolines represent a group of pharmacologically very important compounds exhibiting a wide range of biological activities including antitumoral,[90] antibacterial,[124] antimalarial,[125] and anticonvulsant[126] activities. FDA approved drug Tarceva, a tyrosine kinase inhibitor, is a quinazoline derivative. Significant biological effects was also found in naturally occurring alkaloids with quinazoline moiety.[126]

3.4.6. 1,5-Benzodiazepine-2,4-diones

Benzodiazepine framework belongs to prominent representative of compounds termed as privileged structures. Benzodiazepine scaffold occurs in various modification one of which was synthesized in our work. Although a number of benzodiazepine substructures were prepared on polymer support,[127] solid-phase synthetic method leading to 1,5-benzodiazepine-2,4-diones **V** (Figure 12) was not reported so far. As an

Figure 12: 1,5-Benzodiazepine scaffolds

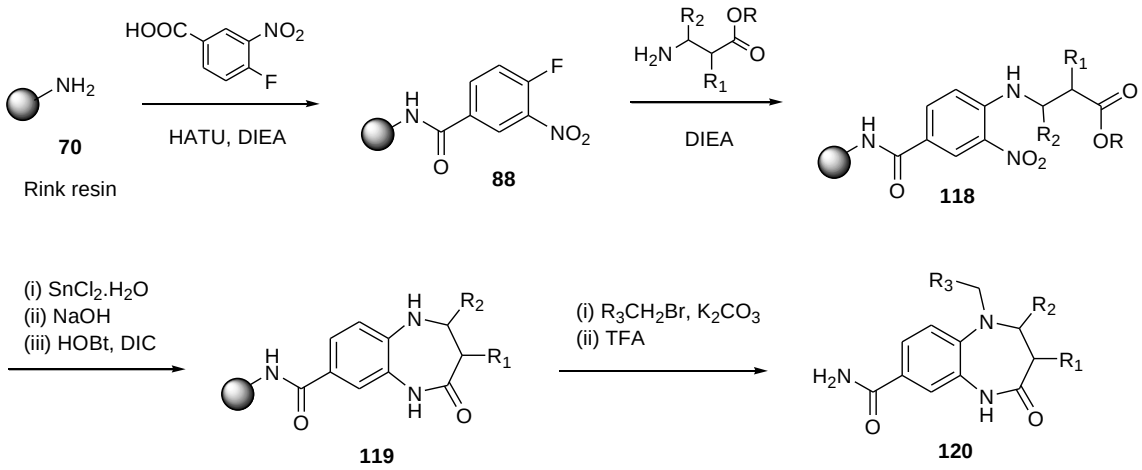


V **VI**

instance of solid-phase method affording a 7-membered cycle can be mentioned a preparation of closely related benzodiazepine-2-ones **VI** which were achieved using polymer supported 4-fluoro-3-nitrobenzoic acid **88**. Substitution with β-aminoacids **118**

followed by reduction of the nitro group gave the corresponding 7-membered heterocycles **119** (Scheme 22).[128] Similar strategy was employed in work by Lee.[129]

Scheme 22: Solid-phase synthesis of 1,5-benzodiazepin-2-ones

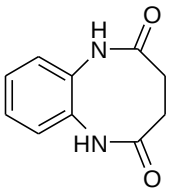


Compared to 1,4-benzodiazepine compounds with well-known biological effects employed especially in the treatment of epilepsy[130] and anxiety[131] 1,5-benzodiazepine-2,4-diones have been not so frequently studied, although some effective drugs, such e.g. clobazam are used as neurotherapeutics.[132] They exhibit antiviral[133] and anthelmintic activity[134] and also act as cholecystokinin-2 receptor associated with anxiety and panic.[135,136]

3.4.7. Benzodiazocinediones

In literature, benzodiazocinediones **VII** are not so frequent object of an interest. Despite various published approaches leading to condensed nitrogenous 8-membered

Figure 13: Benzodiazocinedione scaffold



VII

ring derivatives[137-139] no solid-phase method for the preparation of such compounds has been described. From biological effects can be mentioned for instance amoebicidal activity.[137]

4. Results and discussion

4.1. SYNTHESIS OF NITROGENOUS HETEROCYCLES UTILIZING 4-CHLORO-2-FLUORO-5-NITROBENZOIC ACID

This work is focused on the preparation of nitrogenous heterocycles with the utility of solid-phase synthesis and combinatorial chemistry. In following text we describe the utility of 4-chloro-2-fluoro-5-nitrobenzoic acid which served as a convenient starting building block in most of the methods developed.

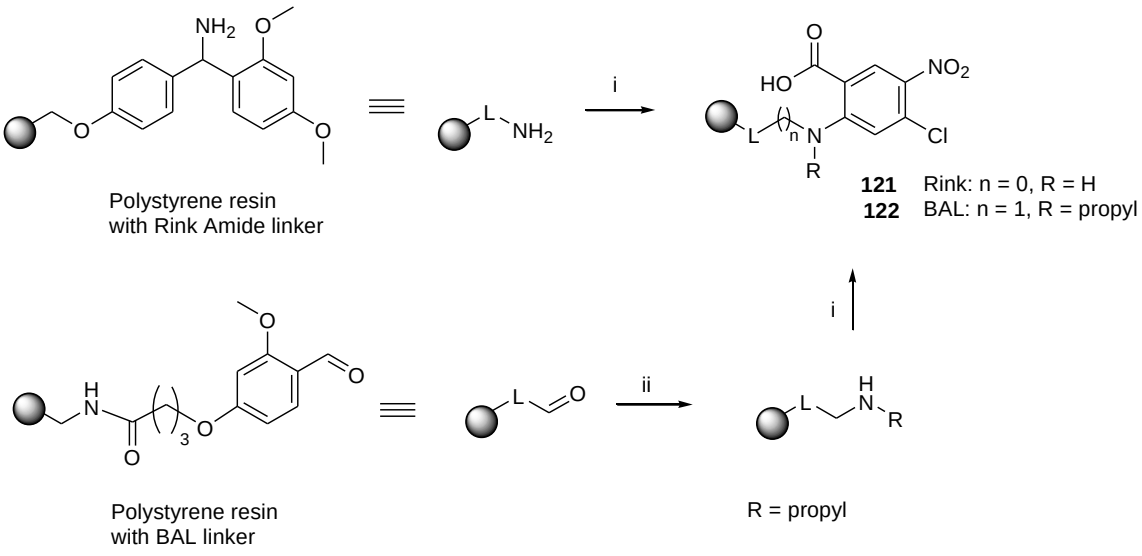
4.1.1. Synthesis of immobilized anthranilic acid intermediates

Several steps were common for the synthetic pathways utilizing 4-chloro-5-nitroanthranilic acid. All these steps are described in particular chapters (4.1.1 – 4.1.3) which are followed by the description of respective methods.

4.1.1.1. Immobilization of 4-chloro-5-nitroanthranilic acid

The difference in reactivity of chlorine and fluorine atom towards nucleophiles at moderately elevated temperature allowed a selective preparation of immobilized 4-chloro-5-nitroanthranilic acid *via* nucleophilic substitution of the fluorine atom with a polymer-supported amine (Scheme 23).

Scheme 23: Synthesis of immobilized 4-chloro-5-nitroanthranilic acids **121** and **122**[a]



[a]Reagents and conditions: (i) 4-chloro-2-fluoro-5-nitrobenzoic acid, DMSO, DIEA, 50 °C, overnight; (ii) propylamine, 10% AcOH in DMF, rt, overnight, then $NaBH(OAc)_3$, 5% AcOH in DMF, rt, 3h.

The resulting polymer-supported anthranilic acid served as a starting material for further transformation consisting of the nucleophilic substitution of the chlorine atom, esterification of the carboxylic group or reduction of the nitro group which were utilized for synthesis of target heterocycle compounds.

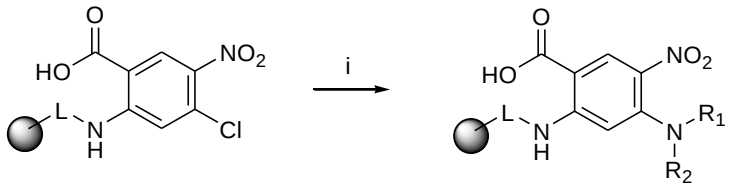
Two types of the resin were used to immobilize the acid, Rink Amide Resin and aminomethyled polystyrene resin equipped with BAL linker which after the reductive amination with propylamine provided *N*-substituted derivative **122**.

The washing process had an significant role in the reaction. It was revealed that the rest of solvent such a DMF or DCM remaining in the resin caused formation of side products in subsequent reaction step. Thus, it was very important to completely remove the solvents from the resin (the resin was shrunk and dried) or to use different solvents like DMSO and THF for washing the resin.

4.1.1.2. Nucleophilic substitution of the chlorine atom

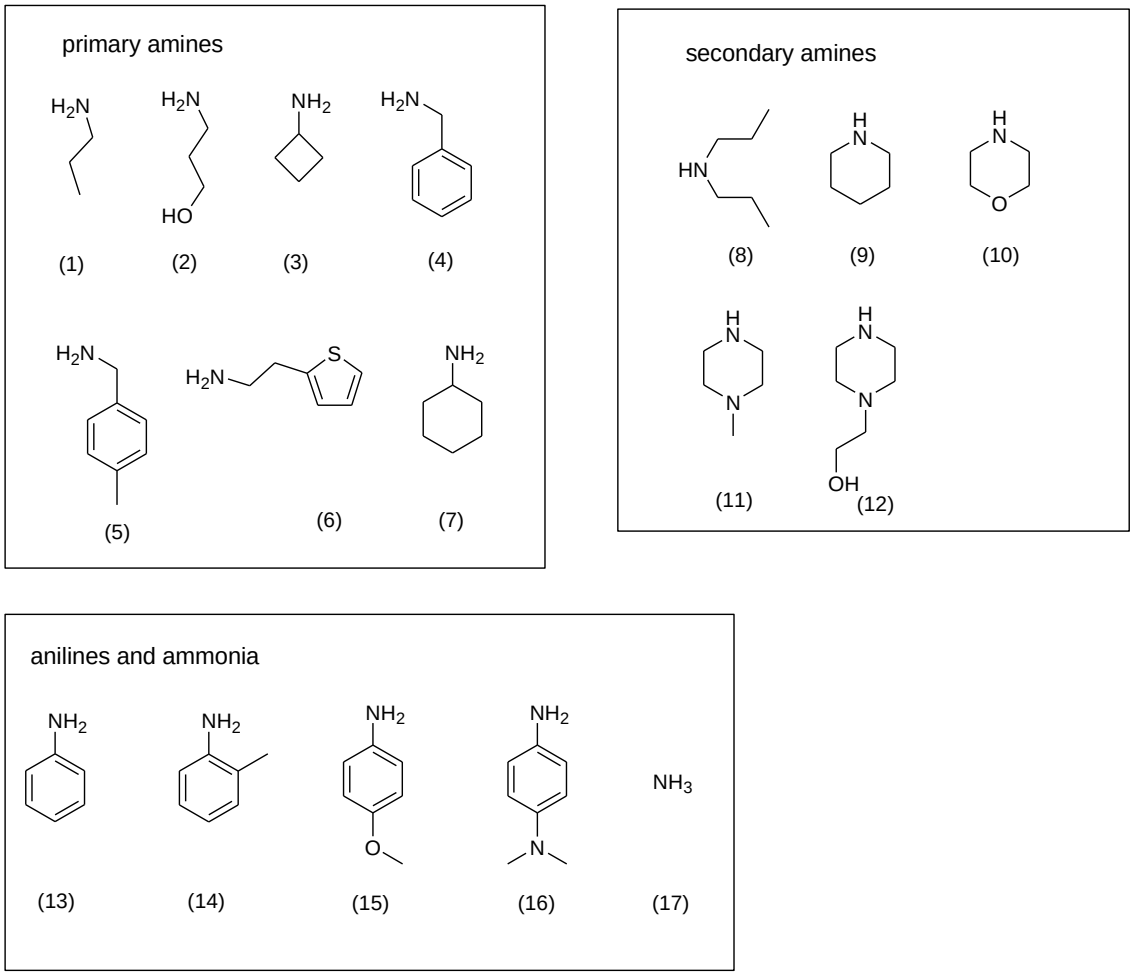
The chloro-nitroanthranilic acid bounded to Rink resin was exposured to a number of nucleophiles including primary and secondary amines (Scheme 24).

Scheme 24: The nucleophilic substitution using various amines[a]

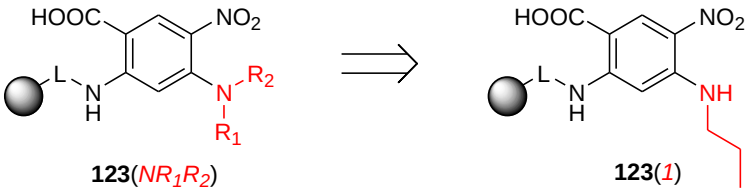


121 **123**

[a]Reagents and conditions: (i) amine, DIEA (for amines 2 and 12), DMSO, 120 °C, 1 day.



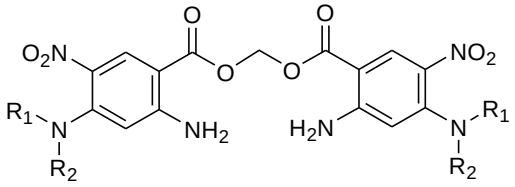
Scheme 25: Encoding of the structures



Although a high temperature and, in the case of amines (2**)** and **(**12), catalysis by DIEA was required for the quantitative reaction, all used amines except ammonia gave the corresponding intermediates **123**. The reaction times were highly dependent on the reactivity of the amines but after 24-hour heating, all tested substitutions were quantitative. Different results were obtained from the reaction with anilines. Only building blocks possessing a group with M+ effect (15) and (16) provided the corresponding nitroanilines **123**, derivatives (13) and (14) were found to be unreactive.

As mentioned in the chapter 4.1.1.1. the residues of DMF or DCM which are ordinarily used for the washing of this resin type entailed a production of side products. Interestingly, we detected the impurity, structure of which was using HPLC-MS and NMR analysis determined as the product of cross-coupling **124**. Molecules of the acid were bounded *via* methylene linker probably originated from DCM.

Figure 14: The structure of beside product

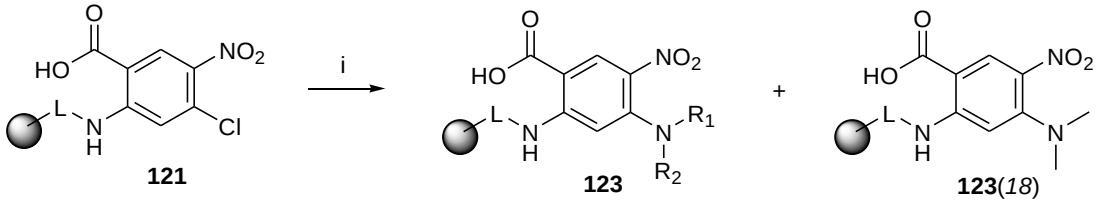


124

Removing of the solvent from the reaction sequence eliminated the formation of the side product. Conversely, we expected that the creation of the side product is accelerated by excess of DCM. Surprisingly, after the reaction performed in DCM the side product was not observed, what could be counted to a higher swelling of the resin resulting in prolonged distances between both immobilized counterparts. Another noteworthy fact was that the side product yielded only reaction with secondary amines, what could be counted to a higher nucleophility of the carboxylate moiety.

Since the replacement of the chlorine atom with amines took place at 120 ^{o}C, the presence of DMF which is unstable under these conditions produced dimethylamino derivative **123***(18)* (the structure was determined by MS analysis) as a side product of the nucleophilic substitution (Scheme 26).

Scheme 26: The formation of dimethylamino derivative **123**(*18*) during the susbtitution of the chlorine atom[a]



121 **123** **123**(*18*)

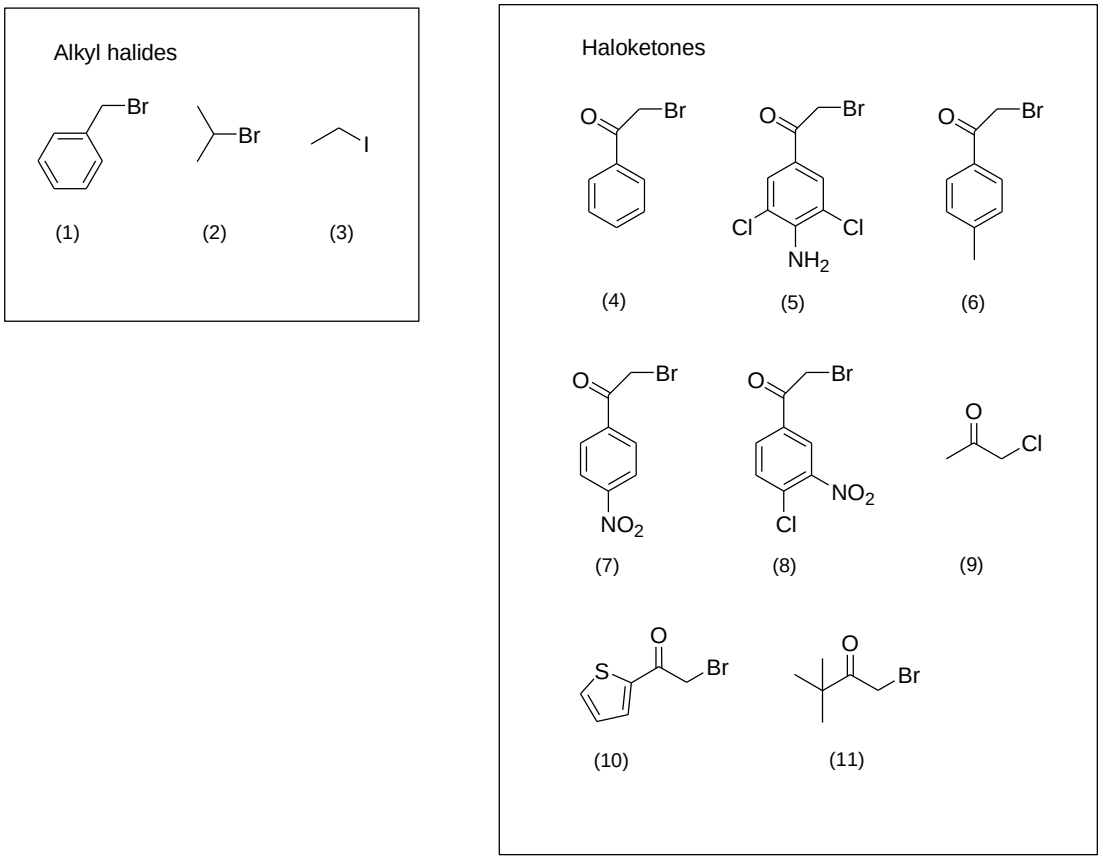
[a]Reagents and conditions: (i) amine, DMSO, residual DMF, (DIEA for amines (2) and (12)), 120 °C, 1 day.

In the case of BAL resin, building blocks (1) and (9) were tested and corresponding nitroanilines were obtained under the same reaction conditions as used for derivatives attached to the Rink resin.

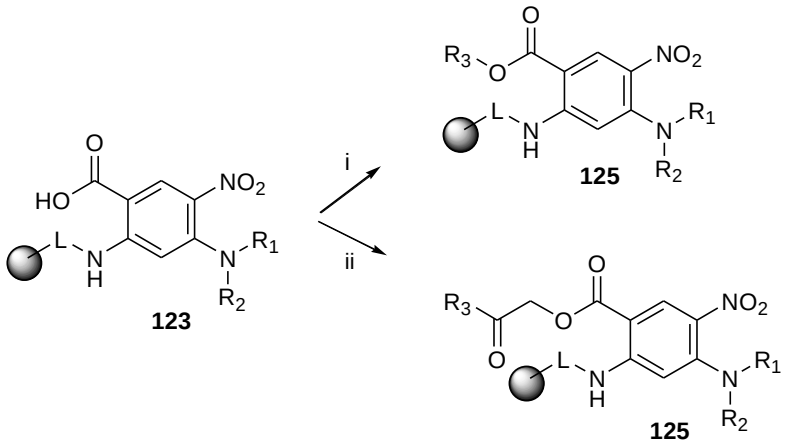
4.1.1.3. Esterification of the carboxylic group

The conversion of the anthranilic acid on Rink resin to anthranilate was performed using various alkyl halides including also haloketones (Scheme 27). Benzyl bromide (1) yielded the appropriate ester **125**(*R_1R_2,1*) after overnight shaking at room temperature, whereas the reaction with aliphatic alkyl halides (2) and (3) required heating at 80 °C for the completion.

Scheme 27: Esterefication of the carboxylic acid using various alkyl halides

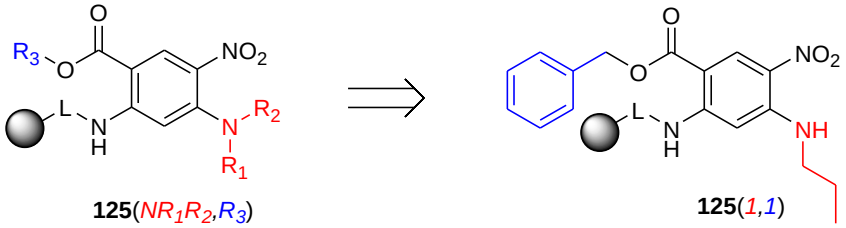


Esterification of the anthranilic acid[a]



[a]Reagents and conditions: (i) alkyl halide, DIEA, DMF, rt (80 °C for (2) and (3)), overnight; (ii) bromoketone, TEA, DMF, rt, 3 – 48h.

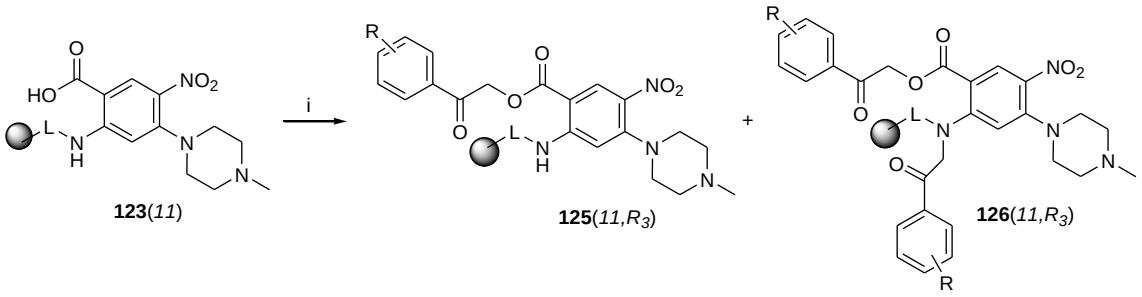
Scheme 28: Encoding of the structures



In the set of haloketones different reaction times depending on the type of haloketones used were observed; for building blocks (4), (5) and (6) only a few hours were sufficient while the reactions with bromoketones (7) and (8) containing strong electron-withdrawing nitro group required 24-48 hours for the quantitative esterification.

In most cases, the reactions afforded the pure corresponding anthranilates **125**. However, during the esterification of the intermediate **123**(*11*), we observed formation of a double alkylated product (Scheme 29). The amount of the side product depended on

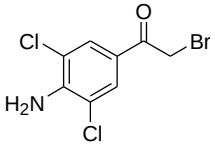
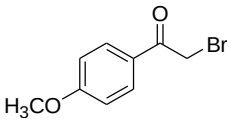
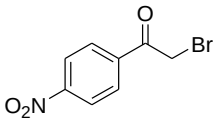
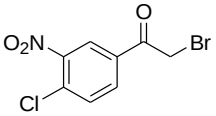
Scheme 29: Formation of a double alkylated product[a]



123(*11*) **125**(*11*,R_3) **126**(*11*,R_3)

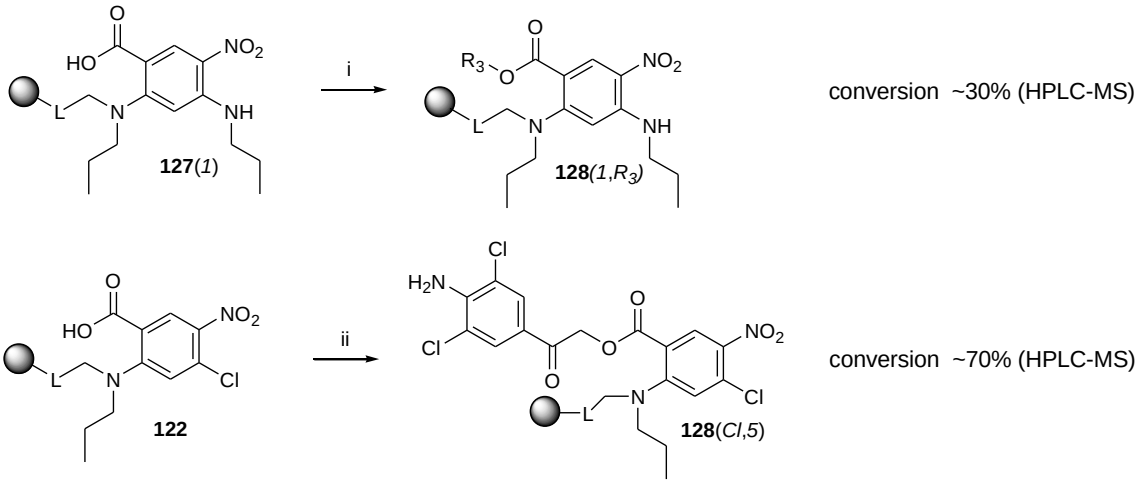
[a]Reagents and conditions: (i) bromoketones, TEA, DMF, rt, overnight for bromoketones (5) and (6) and 2 days for bromoketones (7) and (8).

Table 2: The amount of the side products **126**

bromoketone				
LC-MS (%)	68	25	35	60

each used bromoketone (Table 2). Interestingly, except of the alkylation of intermediate **123**(*11*), the formation of such side products was not detected in any other substrate. These side products were not separated hence their structure was proved only by MS analysis. Regrettably, esterification of the BAL resin-supported acid was less succesful. Reactions performed with the building blocks (1), (4) and (5) proceeded only with a conversion of about 30% (HPLC-MS) which was increased neither by the catalysis with strong base (*t*-BuOK) or by heating. Interestingly, more positive results were obtained when the chloro derivative **121** was esterificated which could be caused by electron properties of the chlorine (Scheme 30).

Scheme 30: Esterification of the amino and chloro derivatives[a]

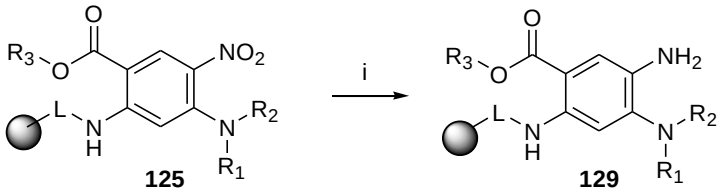


[a]Reagents and conditions: (i) alkyl halide, DIEA, DMF, rt or 80 °C, overnight or alkyl halide, *t*-BuOK, DMF, rt, overnight; (ii) 3,5-dichloro-4-amino-2´-bromacetophenone, TEA, DMF, rt, overnight.

4.1.1.4. Reduction of the nitro group

In general *o*-phenylenediamines can serve as an excellent starting material for a synthesis of nitrogenous heterocycle systems. Thus, the reduction of the nitro group of nitroanilines **125** leading to the diamine derivatives **129** was an object of our interest (Scheme 31).

Scheme 31: Reduction of nitroanilines **125**[a]

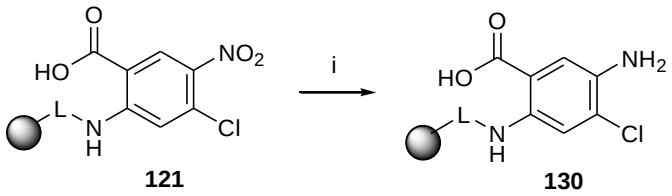


[a]Reagents and conditions: $SnCl_2 \cdot 2H_2O$, DBU, DMF, rt, 1 day.

Our primary attempts to reduce the nitro group were reported as unsuccessful.[80] The nitro group was observed as resistant towards various reducing agents including e.g. tin(II) chloride, diisobutyl aluminum hydride, sodium triacetoxy borohydride, $Na_2S_2O_4$. Since it was found that it was possible to smoothly reduce chloro derivative

121 to aniline **130** (Scheme 32) we explained the resistance of the nitro group toward reduction as an result of double-resonance effect of the electron donating

Scheme 32: Reduction of the chloro derivative **121**[a]



121 **130**

[a]Reagents and conditions: $SnCl_2 \cdot 2H_2O$, DIEA, DMF, rt, overnight.

amino groups in *ortho* and *para* position. However, later optimization gave promising results when concentration of tin(II) chloride was increased from 1.5 to 2.5M solution and the resin was carefully pre-washed several times with degassed DMF. Although the reduction took place under the improved conditions, the resulted *o*-phenylendiamines were accompanied with unknown side product (10-30%, HPLC-MS traces). The side product was detected after the reduction of nitroanilines **125** prepared using primary amines. Further optimization of the tin(II) chloride method consisting in change of the base (1,8-diazabicycloundec-7-ene (DBU) was used instead of *N*,*N*-diisopropylethylamine (DIEA)) eliminated the formation of the side product and resulting *o*-phenylendiamine derivatives **129** were obtained in an excellent purity (over 90%, HPLC-MS traces). Interestingly, excellent results provided also reduction with the suspension of potassium carbonate. However the use of this reagent was excluded due to difficult washing of the resin.

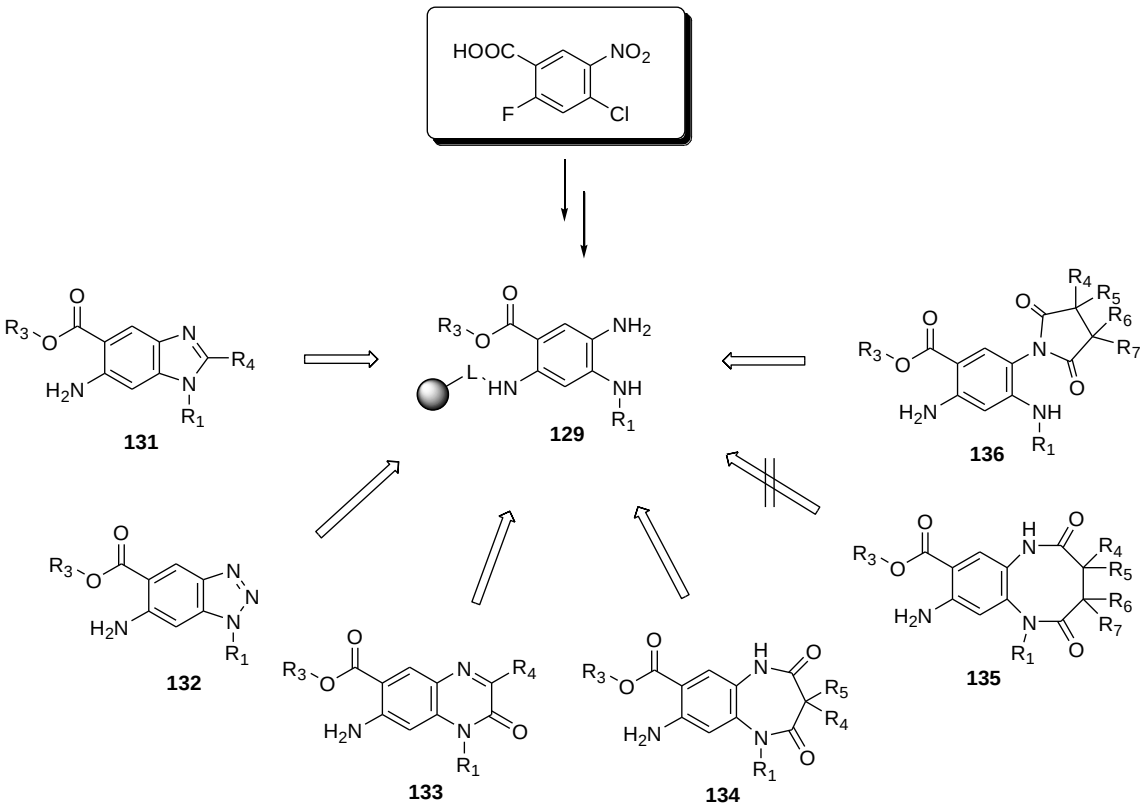
4.1.2. Cyclization methods leading to nitrogenous heterocycles

After preparation of the appropriate intermediates we paid attention to the closing of the various nitrogenous heterocycles. The sets of heterocycles were prepared using DOS strategy where various types of heterocycles were produced as well as TOS strategy focused on the synthesis of variously substituted 3-hydroxy-quinolinone-4(*1H*)-ones.

4.1.2.1. Condensed various-size nitrogenous heterocycles (DOS approach)

In this part we study use of the immobilized anthranilic acid for the diversity oriented solid-phase heterocycles synthesis. After replacement of the chlorine atom with primary amines and reduction of adjacent nitro group we obtained solid-supported derivatives of *o*-phenylenediamine **129**. Their subsequent conversion gave a set of nitrogenous scaffolds varying in size of a cycle (Scheme 33).

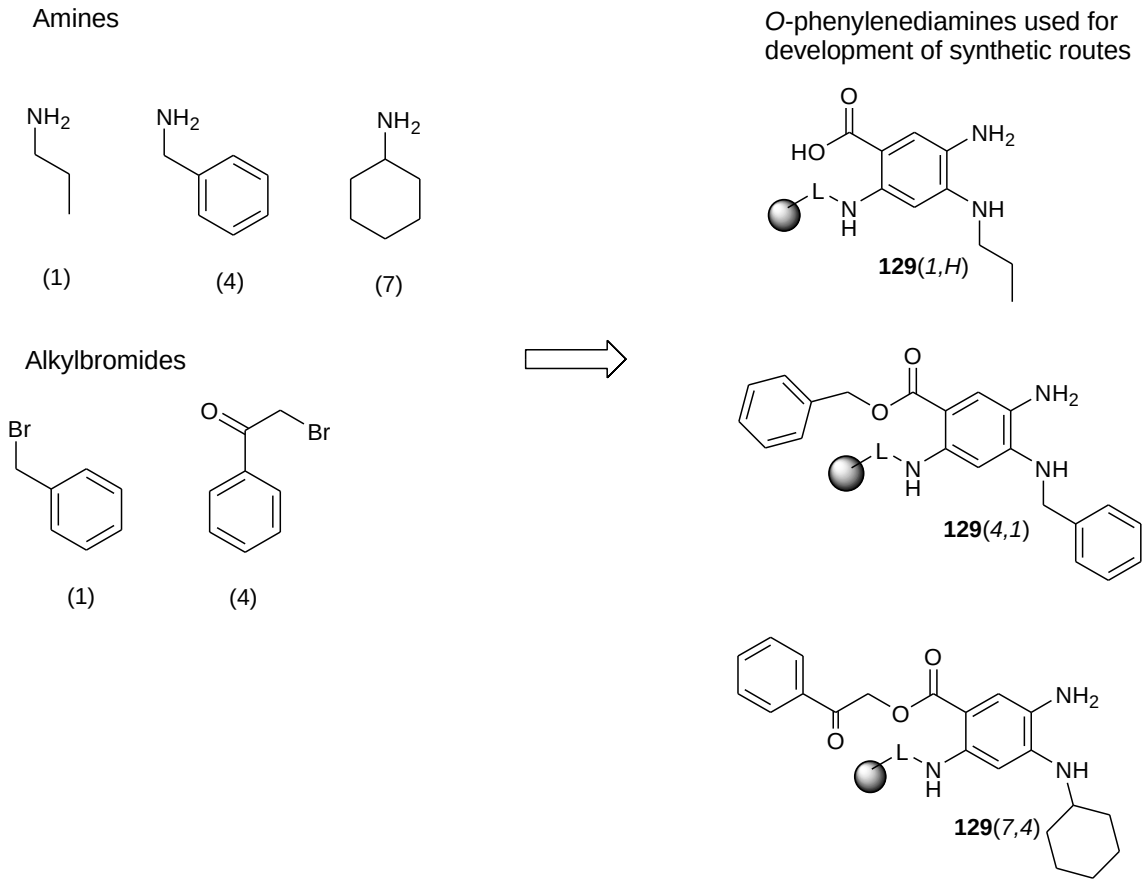
Scheme 33: Use of immobilized *o*-phenylenediamines **129** for the preparation of various nitrogenous heterocycles



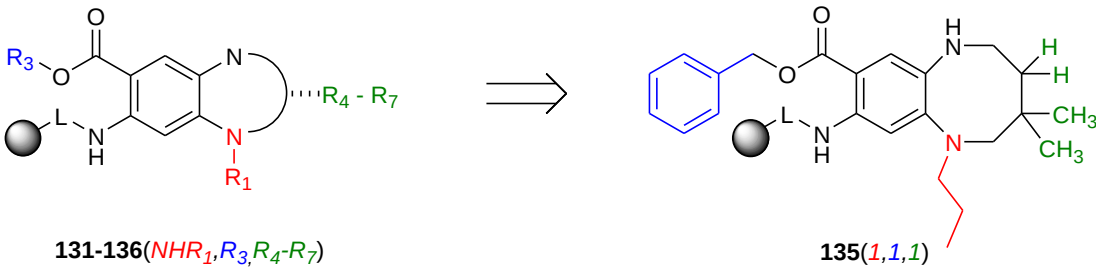
The starting immobilized 4-chloro-5-nitroanthranilic acid **121** was achieved using Rink amide resin. Three primary amines (giving substituents R_1) and two alkylbromides (giving substituents R_3) were used in random combination to verify the synthetic accessibility of the target heterocycles with various substitution of their scaffold. The combination of the building blocks used is depicted in Scheme 34. After reduction of

the nitro group the reactivity of the resulting diamine intermediates **129** was studied to form the target heterocycles.

Scheme 34: Amines and alkylbromides used for the preparation of immobilized *o*-phenylenediamines **129**



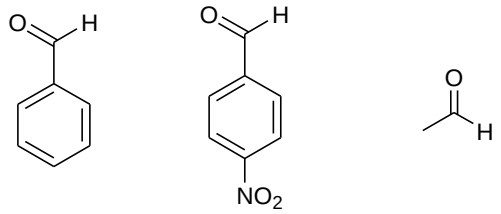
Scheme 35: Encoding of the structures



4.1.2.1.1. Benzimidazoles

To achieved benzimidazole cycles **131** we applied a simple cyclization of immobilized intermediates **129** with aldehydes. Three different aldehydes were tested for this reaction (Table 3) and their different reactivity was observed. Whereas

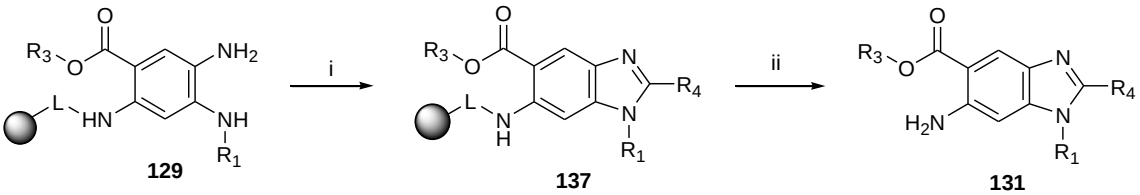
Table 3: Aldehydes used for the cyclization to benzimidazoles **131**



(1) (2) (3)

p-nitrobenzaldehyde and acetaldehyde smoothly yielded benzimidazoles **131**(*1,H,2*) and **131**(*4,1,3*) in DMF after overnight reaction (although acetaldehyde provided the product contaminated with a mixture of small impurities, purity of the product about 60%, HPLC-MS traces) use of unsubstituted benzaldehyde was more complicated. The conversion took place very slowly and afforded the required product in a limited purity (about 50%, HPLC-MS traces). We tried to accelerate the reaction using acidic or basic catalysis or 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) as dehydrating agent, but each attempt resulted in the undefined mixture of products. Another optimization consisted in an alternation of solvent. DMSO, THF and DCM were tested and fortunately the last one significantly accelerated the formation of benzimidazole **131** which was achieved in acceptable purity after overnight reflux (Scheme 36). Apart from

Scheme 36: Preparation of benzimidazoles **131**[a]



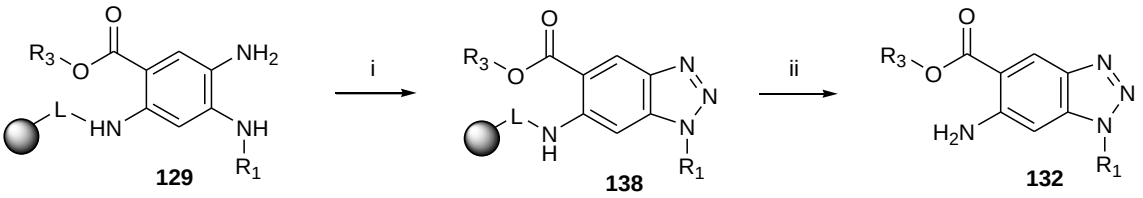
[a]Reagents and conditions: (i) aldehyde, DMF, rt, overnight or DCM, reflux, overnight; (ii) TFA/DCM, rt, 1h.

aldehydes another components such as imidoesters, ester or nitrile of benzoic acid were tested, but none of these reagents yielded required benzimidazoles.

4.1.2.1.2. Benzotriazoles

We tried to introduce the nitrogen atom to the molecule **129** by means of Katritzky method with isoamyl nitrite[95] but the reaction afforded product **132** only as a part of a mixture with another undefined compounds. Optimization of the reaction conditions based on a variation of solvents (DMF and NMP were used), temperature (reaction was carried out at ambient temperature and at 5 °C) or reaction time (0.5 – 12h) did not increase the purity of the products. Subsequently we decided to use sodium nitrite as a source of the nitrosyl cation (Scheme 37). Although in this case a

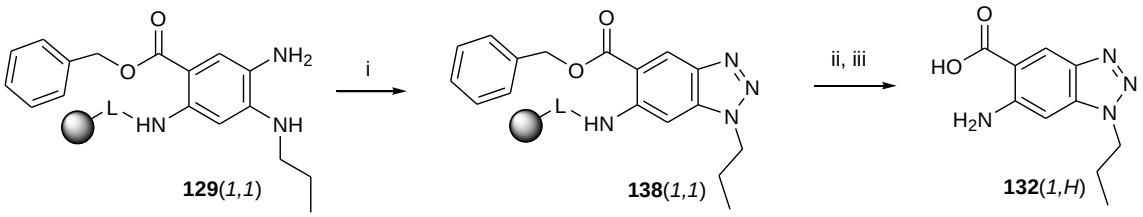
Scheme 37: Preparation of benzotriazoles **132**[a]



[a]Reagents and conditions: (i) $NaNO_2$, AcOH, DMF, rt, 4 days; (ii) TFA/DCM, rt, 1h.

long reaction time was needed (four days), benzotriazoles **132** were obtained in a high purity (about 90%, LC-MS traces). In the case of free carboxylic acid derivative **129**(*1*,*H*) we observed an elimination of amino group (according to HPLC-MS analysis). Thus, the target benzotriazole **132**(*1*,*H*) was achieved using the corresponding benzyl ester which was hydrolyzed after formation of a triazole ring (Scheme 38).

Scheme 38: The preparation of benzotriazole **132**(*1*,*H*)

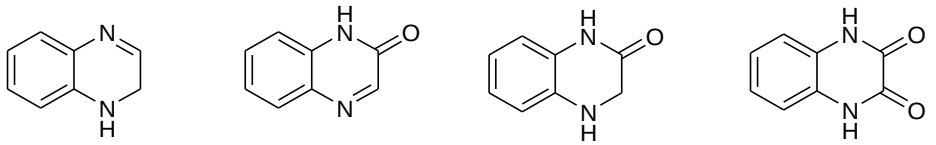


[a]Reagents and conditions: (i) $NaNO_2$, AcOH, DMF, rt, 4 days; (ii) TMSOK, THF, rt, overnight; (iii) TFA/DCM, rt, 1h.

4.1.2.1.3. Quinoxalinones

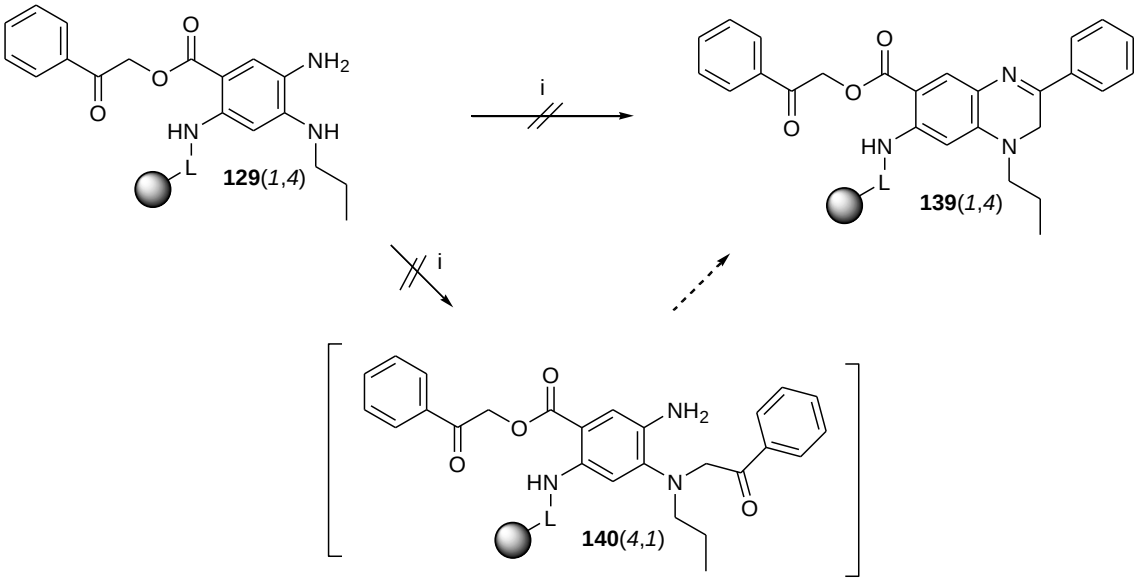
Our primary target was to use above mentioned diamines **129** for the preparation of quinoxalines, quinoxalinones and quinoxaline diones **I-IV** (Figure 15). For the

Figure 15: Structures of the aimed quinoxaline derivatives



preparation of quinoxaline **I** the reaction of diamine precursors **129** with α-bromoacetophenone was chosen. The reaction afforded a mixture of compounds and neither attempt to control the reaction by decreasing of the reaction temperature was not successful (Scheme 39). The similar results were already described by Wu [100] who

Scheme 39: Condensation of the *o*-phenylendiamines **129** with α-bromoacetophenone[a]

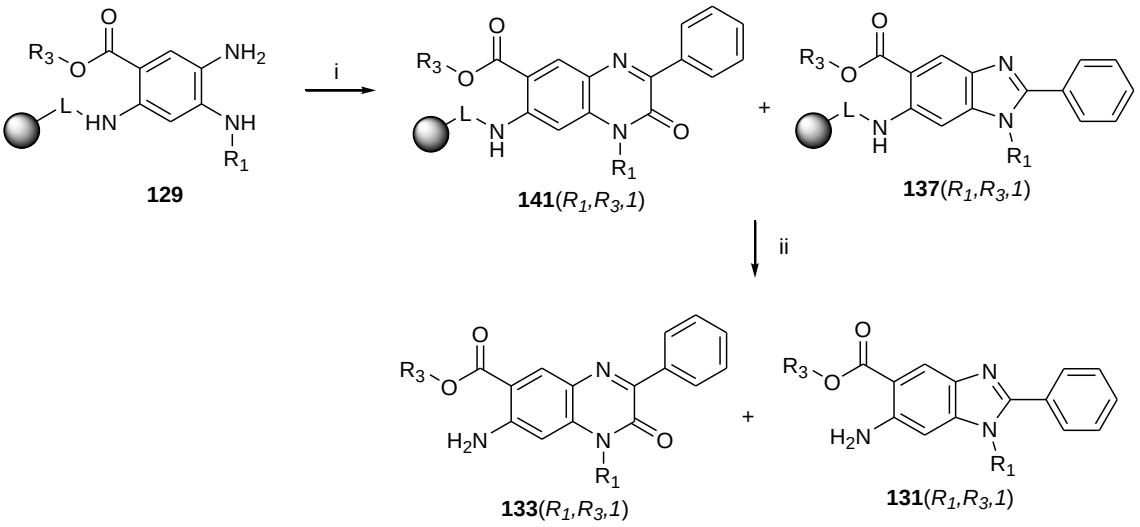


[a]Reagents and conditions: (i) α-bromoacetophenone, DIEA, DMF, rt or 5 °C, overnight.

tried to prepare *N*-substituted 1,2-dihydroquinoxalines by the reaction of *N*-substituted *o*-phenylendiamine with α-bromoacetophenone. For the preparation of quinoxalin-2-one we decided to investigate the alternative application of α-ketocarboxylic acids and for this purpose phenylglyoxylic acid was chosen. First, we tried a direct acylation of immobilized phenylendiamines **129** with phenylglyoxylic acid. Besides expected

quinoxalinone **133** we obtained benzimidazole derivative **131** as a side product (Scheme 40).

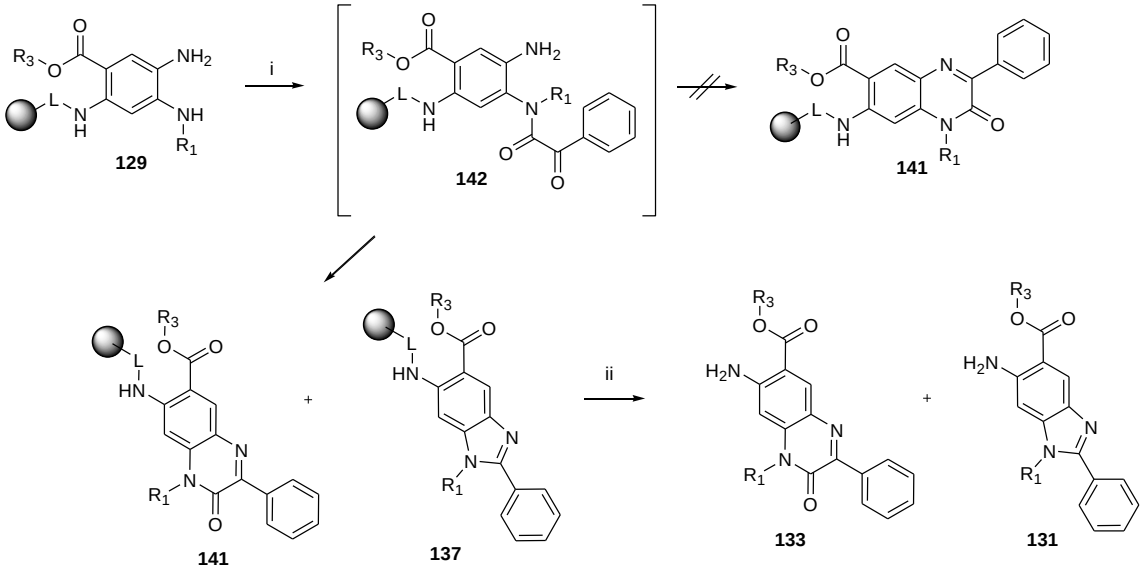
Scheme 40: Unsuccessful attempt to prepare pure quinoxalinones **133**[a]



[a]Reagents and conditions: (i) phenylglyoxylic acid, various solvents (Table 5), rt, overnight or 80 °C (for THF reflux), 2h; (ii) TFA/DCM, rt, 1h.

The formation of benzimidazole derivative during condensation of *o*-phenylenediamine and ketocarboxylic acid was already described. Authors studied reaction of 1,2-substituted *o*-phenylendiamine with 2-oxo-2-(1*H*-pyrrol-2-yl)acetic acid and its usability for synthesis of corresponding benzimidazoles or quinoxalinones in dependence on the reaction conditions and the substitution of a diamine.[140] In order to avoid the undesirable decarboxylation and formation of benzimidazole we attempted to prepare the target molecule *via* convenient hydroxybenzotriazole (HOBt) activated acylation of the one amino group followed by the condensation of the second amino group. In the case of the suitable regioselectivity the quinoxalin-2-one could be prepared in one-pot reaction. Unfortunately, formation of a benzimidazole side product **131** was observed as well (Scheme 41). As another possibility we tested a modification

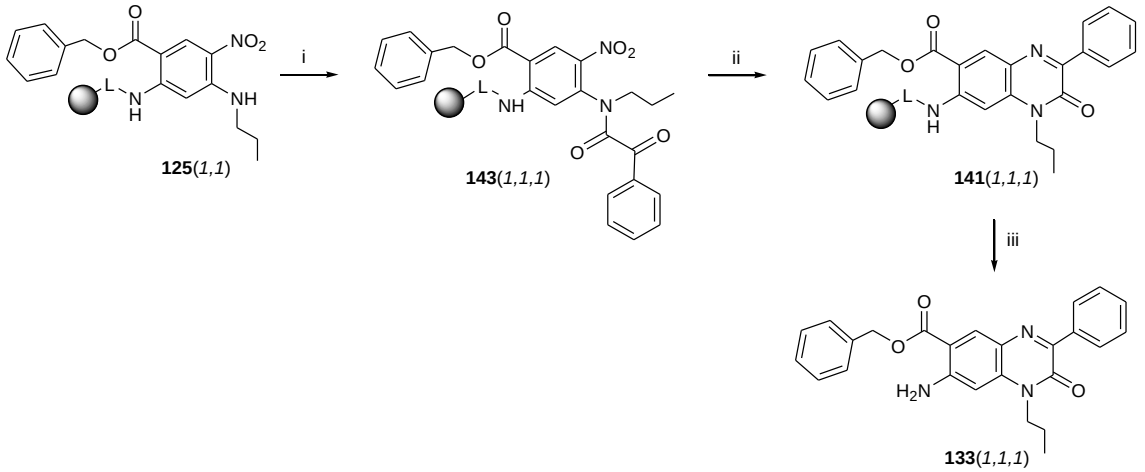
Scheme 41: Synthesis of quinoxalin-2-one **133** *via* acylation of diamine **129**[a]



[a]Reagents and conditions: (i) phenylglyoxylic acid, HOBt, DIC, DMF/DCM, rt or 5 °C, overnight; (ii) TFA/DCM, rt, 1h.

of the reaction sequence based on the acylation of nitroaniline[141] **125** and subsequent nitro group reduction (Scheme 42). Despite strong deactivation of the

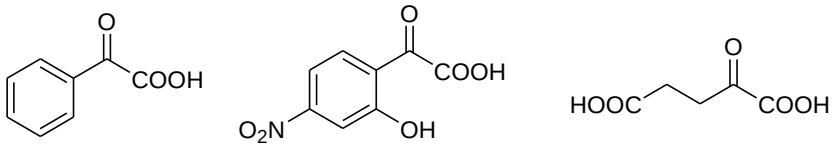
Scheme 42: Formation of quinoxalinone **133** *via* acylation of nitroaniline **125**[a]



[a]Reagents and conditions: (i) chloride of phenylglyoxylic acid, DIEA, dry dichloroethane (DCE), reflux; overnight (ii) $SnCl_2 \cdot 2H_2O$, DBU, DMF, rt, overnight; (iii) TFA/DCM, rt, 1h.

amino group caused by adjacent nitro group we achieved the desired amide intermediate **143**(*1,1,1*) by heating of the resin-bound nitroaniline with phenylglyoxylic chloride. Subsequent reduction with tin(II) chloride resulted in final quinoxaline **133**(*1,1,1*) of a high purity (95%, HPLC-MS traces). Unfortunately, this method is not universal for any type of ketocarboxylic acids. In case of acids (2) and (3) (Table 4) when less reactive

Table 4: α-ketokarboxylic acids used for creation of quinoxalinones

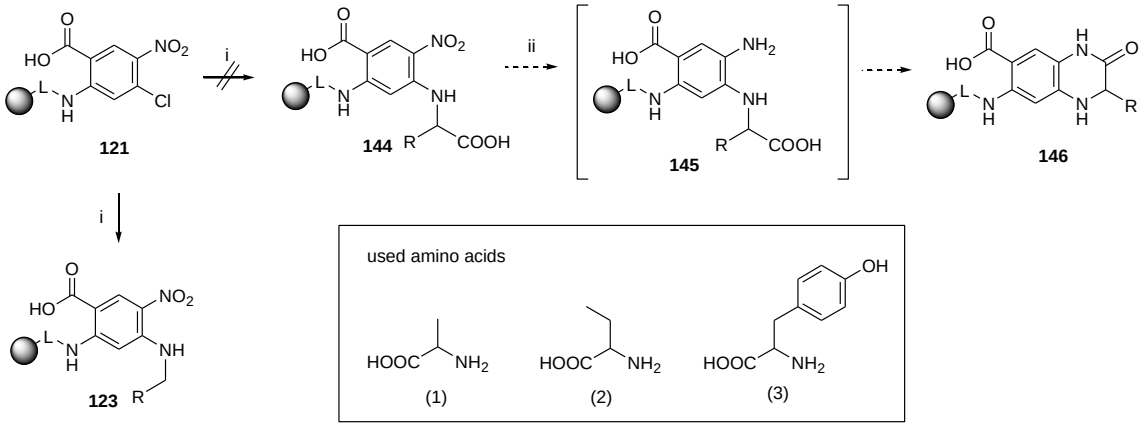


(1) (2) (3)

lactone or anhydride respectively is probably formed initially, the acylation step failed and starting material was recovered.

Amino acids were selected as another candidate possessing suitable functional groups to close quinoxaline cycle **III**. For this purpose, 4-chloro-5-nitroanthranilic acid **121** served as an intermediate which was reacted with amino acids. We supposed the formation of the cycle *via* nucleophilic substitution of the chlorine atom with amino group **144** followed by reduction of the nitro group **145** and subsequent intramolecular acylation of arised diamine (Scheme 43). However, the method failed already in the first

Scheme 43: Use of the amino acids for the preparation of quinoxalin-2-one **146**[a]

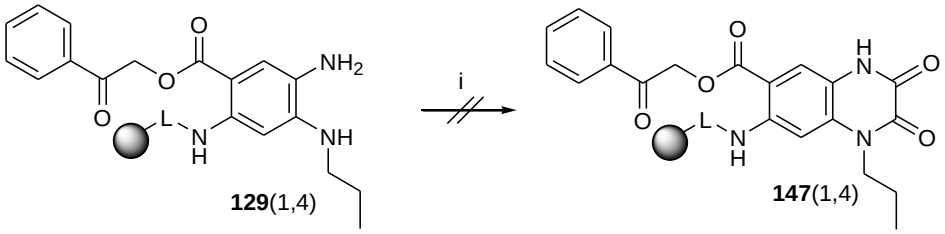


[a]Reagents and conditions: (i) amino acid, DIEA, DMSO, 120 °C, 3h; (ii) $SnCl_2 \cdot 2H_2O$, DBU, DMF, rt, overnight.

step. Since a high temperature (120 °C) was necessary for the substitution and these conditions caused decarboxylation of the amino acids it was not possible to prepare the intermediates **144**.

For the preparation of quinoxaline diones **IV** we tried to use reaction of diamine intermediates **129** with oxalic acid (Scheme 44). However, oxalic acid did not provide

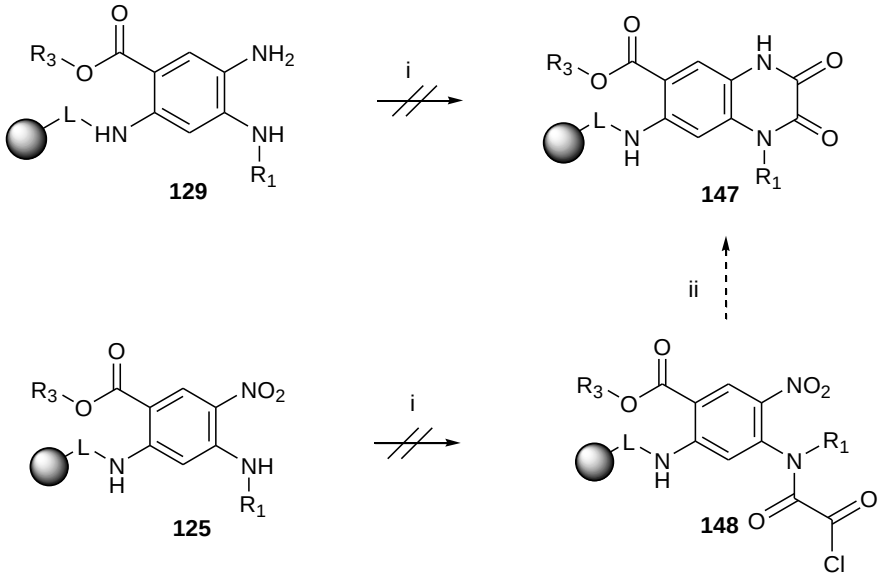
Scheme 44: Use of glyoxylic acid for the preparation of quinoxaline diones **147**[a]



[a]Reagents and conditions: (i) oxalic acid, DMF, (DIEA or AcOH), rt, overnight.

the expected quinoxaline diones **147** and starting diamines were detected even with acidic or basic catalysis. Activation with HOBt supported the acylation but product was obtained in a mixture of compounds. In order to increase purity of the product we tried to acylate nitroaniline intermediates **125**. Unfortunately, we met with resistance of the nitroanilines toward the used reaction conditions (Scheme 45).

Scheme 45: Use of HOBt activated acylation for the preparation of quinoxaline diones **147**[a]



[a]Reagents and conditions: (i) oxalic acid, HOBt, DIC, DMF/DCM, rt, overnight or oxalyl chloride, DIEA, dry DCE, reflux, overnight; (ii) $SnCl_2 \cdot 2H_2O$, DBU, DMF, rt, overnight.

Subsequent attempts to close the quinoxaline diones **147** with diesters of the acid led to negative results as well. Although formation of the target heterocycle was detected, the reaction proceeded very slowly. The reaction was accelerated neither by increasing of temperature nor using various solvents (DMF, NMP, THF, DMSO) and the maximum conversion (about 30%, HPLC-MS traces) was achieved in DCM at room temperature.

After all unsuccessful attempts we returned to already tested coupling of phenylglyoxylic acid (1) with resin-bound diamines **129** (Scheme 40) and decided to pay attention for thorough investigation of this reaction. We observed that the cyclization to quinoxalinone **133** was highly accelerated by acidic catalysis and thus the formation of undesirable benzimidazole **131** was detected only in low yield (about 20%, HPLC-MS traces). The choice of the solvent was another factor influencing the outcome of the reaction. Various solvents and reaction temperatures were combined and tested in order to achieve the target quinoxalinones **133** in as high purity as possible. The best results were obtained by acetic acid catalyzed reaction of diamines **129** with ketocarboxylic acids (1), (2) and (3) at reflux in THF or at 80 °C in toluene (Table 5).

Table 5: Solvents tested for preparation of quinoxalinones **133**

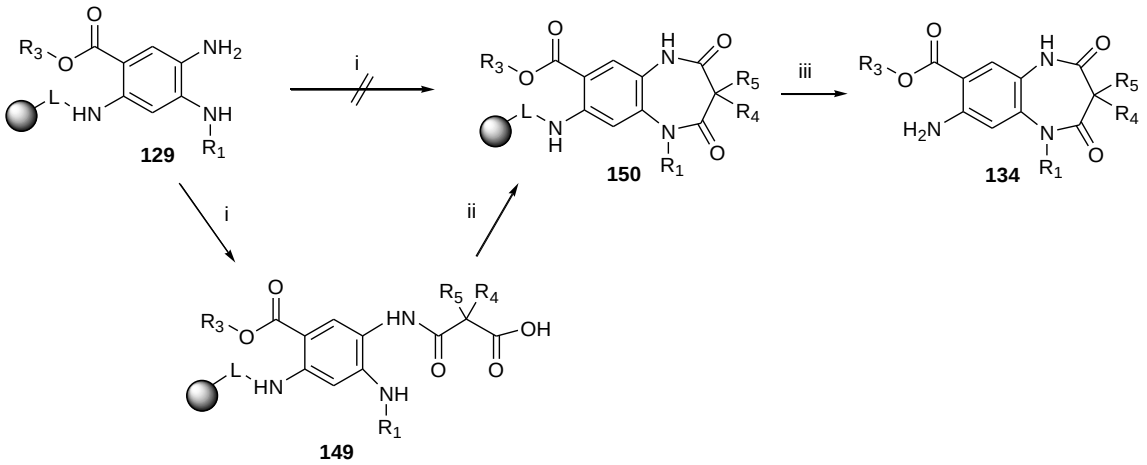
	DMF	AcCN	NMP	dioxane	THF	DCM	toluene
rt	1:1	3:2	n.t.	n.t.	2:3	1:1	1:2
AcOH, 80 °C (reflux for THF)	1:1	2:1	1:4	5:1	10:1	n.t.	10:1

Ratio of products (structure **133**(*4*,*1*,*1*) : structure **131**(*4*,*1*,*1*); n.t. – not tested

4.1.2.1.4. Benzodiazepinediones

In this work malonic acids were applied to close 7-membered ring *via* double acylation of the diamine precursors **129**. We anticipated the simultaneous formation of both amides but reaction with hydroxybenzotriazole (HOBt) activated acids yielded only acyclic amide **149** with free carboxylic group (according to HPLC-MS analysis) and ring closure had to be completed *via* intramolecular cyclization in the additional step (Scheme 46). In the case of symmetrical malonic acid the regioselectivity of the first-step acylation was not determined as both potential structural isomers **149** afford

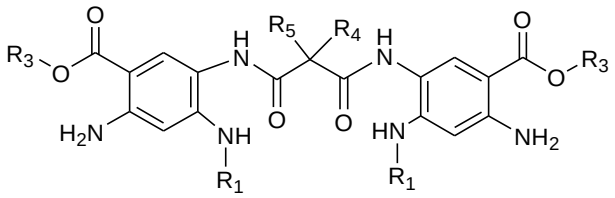
Scheme 46: The two step preparation of benzodiazepinediones **134**[a]



[a]Reagents and conditions: (i) disubstituted malonic acid, HOBt, DIC, DMF/DCM, rt, overnight; (ii) HOBt, DIC, THF, 50 °C, 2 hours; (iii) TFA/DCM, rt, 1h.

the same product **150**. However, we suppose a preferential acylation of the primary amino group which is in accordance with detailed NMR analysis that has been measured for analogical intermediate **153***(2,2,1)* (Scheme 48). HPLC-MS analysis revealed that the intermediates **149** were contaminated with side product which was identified as a result of cross-coupling reaction leading to compound **151**(5-40%, HPLC-MS traces) (Figure 16). We noticed that the outcome of the reaction was significantly influenced by reaction conditions. The yield of the side product **151** varied

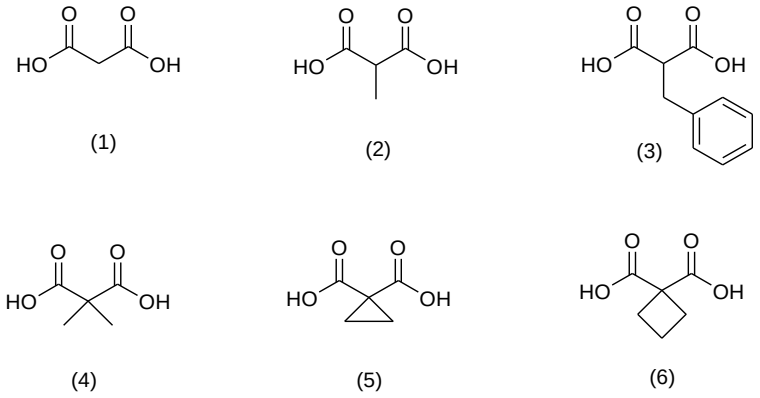
Figure 16: Structure of beside product



151

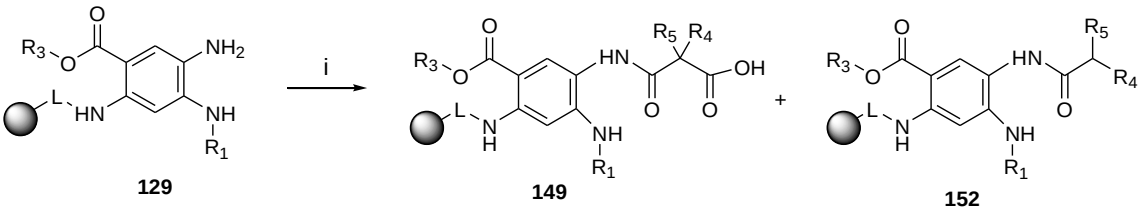
in dependence on a concentration ratio of the acid and HOBt. The use of HOBt excess led to the decrease of the side product. The choice of the solvent affected the reaction as well. Dioxane and DMSO yielded the side product in minimum but the reaction was rapidly decelerated. Another marked effect had NMP which caused decarboxylation of the acid as well as using basic catalysis. Despite the obvious dependence on reaction conditions we did not achieved the complete elimination of the side product. Another noteworthy fact was that the outcome of the first-step acylation reaction was highly dependent on acid used (Table 6). Whereas disubstituted malonic acids (4), (5) and

Table 6: Malonic acids used for preparation of benzodiazepines



(6) provided the corresponding intermediates **149**, preparation of an unsubstituted derivative **149**(*7,4,1*) was unsuccessful due to a decarboxylation of the acid during the reaction. Reaction with monosubstituted malonic acids resulted in a mixture of desired carboxy derivative **149** and product of decarboxylation **152** (Scheme 47). We tried to suppress the decarboxylation by decreasing of reaction temperature or with use of

Scheme 47: Acylation with unsubstituted and monosubstituted malonic acid[a]



[a]Reagents and conditions: (i) malonic acid, HOBt, DIC, DMF/DCM, rt, overnight.

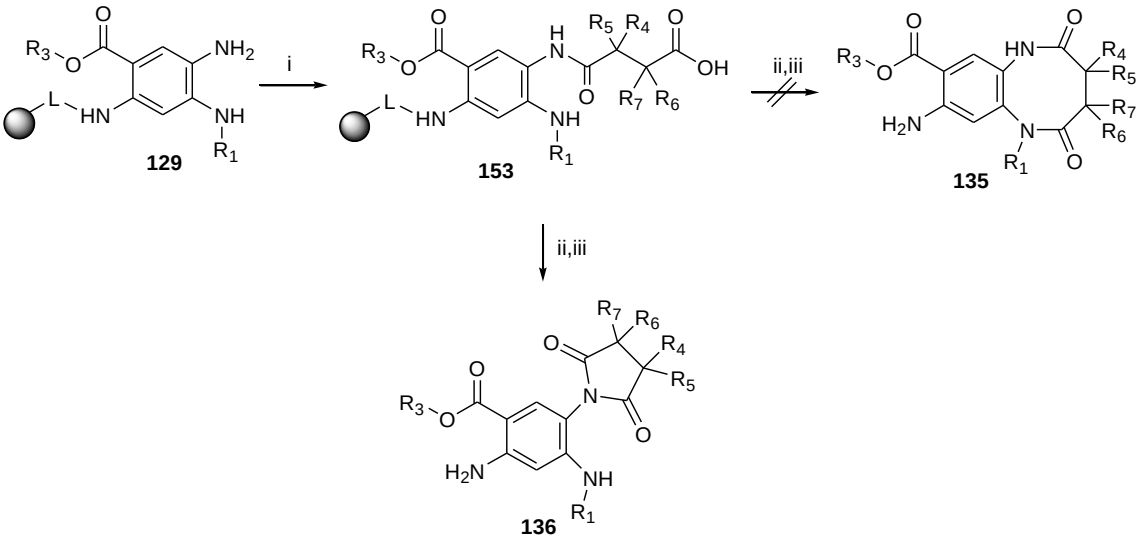
anhydride formed *in situ* with use of *N,N*-diisopropylcarbodiimide (DIC). However, the starting diamine **129** did not react under such reaction conditions. As another alternative the esterification of the malonic acid was done to avoid the undesired elimination of carboxylic group. However, after the reaction with ester of malonic acid only the starting diamines **129** were detected. The harsher reaction conditions achieved by heating in DMSO or DMF yielded the mixture of compounds. Thus, the application of this method was reduced to synthesize only disubstituted derivatives **149**(R_1,R_3,*4*), **149**(R_1,R_3,*5*) and **149**(R_1,R_3,*6*).

Neither cyclization step of succesfully prepared compounds proceeded smoothly. Closing the cycle under mild reaction conditions identical to the first-step acylation provided final heterocycles only with low conversion and significantly contaminated with by-products. Finally, the benzodiazepindiones **134** were obtained *via* HOBt acylation in THF at 50°C. Other methods such as acidic (AcOH) or basic (DIEA) catalysis or the use of alternative activating agents (such as BOP) did not increase the purity.

4.1.2.1.5. Attempt to prepare benzodiazocinediones

After the successful formation of 7-membered ring we tried to extend the methodology for the preparation of 8-membered rings. The double acylation with dicarboxylic acids (Scheme 48) was carried out in two steps as in the case of benzodiazepines **134**.

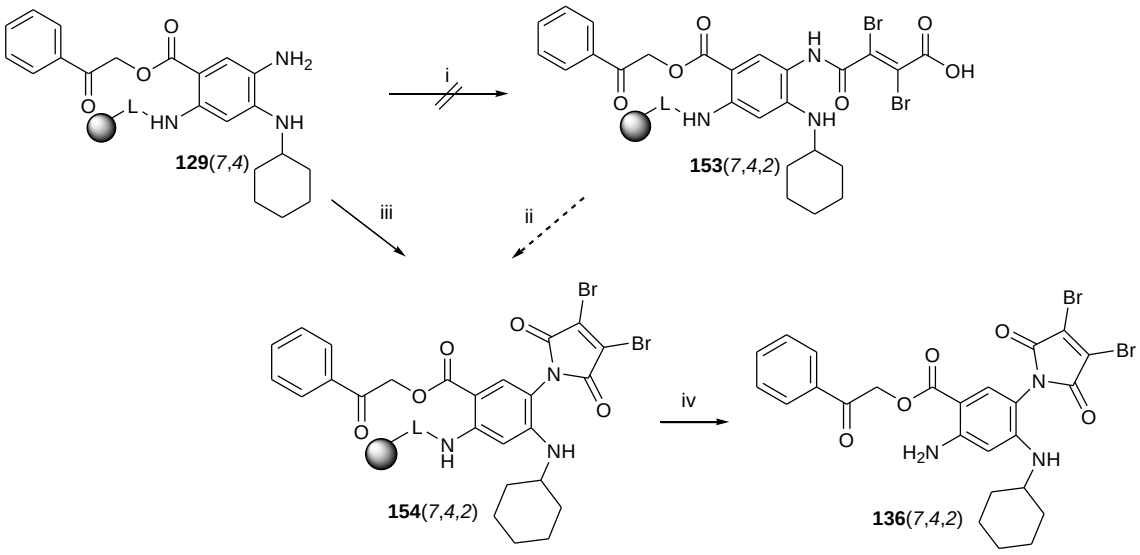
Scheme 48: Attempt to prepare benzodiazocinedione derivatives **135**[a]



[a]Reagents and conditions: (i) dicarboxylic acid, HOBt, DIC, DMF/DCM, rt, overnight; (ii) HOBt, DIC, DMF/DCM, rt, overnight; (iii) TFA/DCM, rt, 1h.

Surprisingly the second-step acylation took place very smoothly (according to HPLC-MS) in comparison to intermediates **149**. However, 2D NMR analysis experiments revealed formation of 5-membered cycle **136** instead of targeted 8-membered cycle **135** (Scheme 48). Similarly, literature described the building of 5-memebered cycle *via* double acylation of diamines with diacids in synthesis of tri- and tetracyclic compounds.[142] Preparation of bromo derivative **153** (*7,4,2*) with use of HOBt activation yielded unidentified mixture of compounds. In this case the cyclization was performed by one step acylation with the acid anhydride formed *in situ* with use of DIC (Scheme 49). Three dicarboxylic acids of aliphatic and aromatic structure were used at

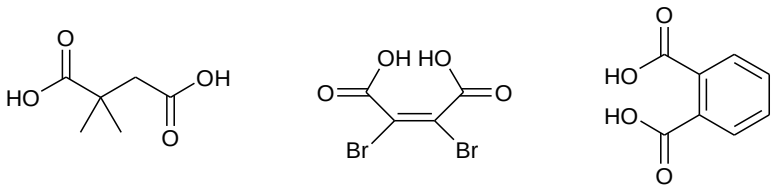
Scheme 49: Preparation of bromo derivative **136**(*7,4,2*)



[a]Reagents and conditions: (i) 2,3-dibromomaleinic acid, HOBt, DIC, DMF/DCM, rt, overnight; (ii) HOBt, DIC, DMF/DCM, rt, overnight; (iv) 2,3-dibromomaleinic acid, DIC, DMF/DCM, rt, overnight; (iii) TFA/DCM, rt, 1h.

this step (Table 7). In each case, the reaction yielded succinimides derivatives **136**(*1,H,3*), **136**(*4,1,1*) and **136**(*7,4,2*).

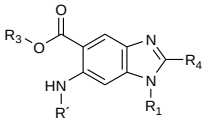
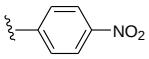
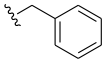
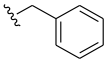
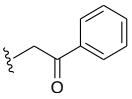
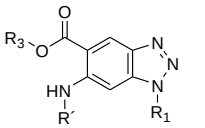
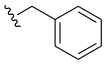
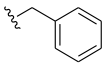
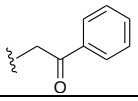
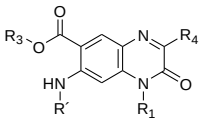
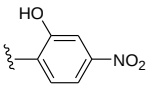
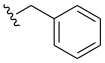
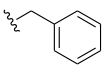
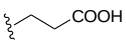
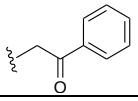
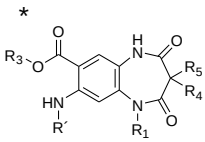
Table 7: Dicarboxylic acids used for preparation of succinimides **136**



(1) (2) (3)

Although considerable limitations and in some cases moderate yields were revealed, the set of novel heterocyclic derivatives excepting benzodiazocines was prepared (Table 8).

Table 8: Summary of the prepared compounds

Scaffold	Product	R_1	R_3	R_4 (R_5, R_6, R_7)	R´	Purity (%)[a]	Yield (%)[b]
	**131**(*1*,*H*,*2*)					75	35
	131(*4*,*1*,*3*)					60	11
	131(*7*,*4*,*1*)					70	37
	**132**(*1*,*H*)			-		85	75
	132(*4*,*1*)			-		95	45
	132(*7*,*4*)			-		90	42
	**133**(*1*,*H*,*2*)					90	20
	133(*4*,*1*,*3*)					96	53
	133(*7*,*4*,*1*)					80	28
* 	**134**(*1*,*H*,*5*)					90	20
	134(*4*,*1*,*6*)					96	53
	134(*7*,*4*,*4*)					80	28
*	**136**(*1*,*H*,*3*)					80	22
	136(*4*,*1*,*1*)					67	24
	136(*7*,*4*,*2*)					77	18

[a]Purity of crude product (LC-MS traces); [b]Yield of product after purification (semipreparative HPLC)

* In the case of derivatives 1**34**(*1*,*H*,*5*) and **136**(*1*,*H*,*3*) acylation of the amino group with TFA was observed during the cleavage from the polymer-support using TFA/DCM.

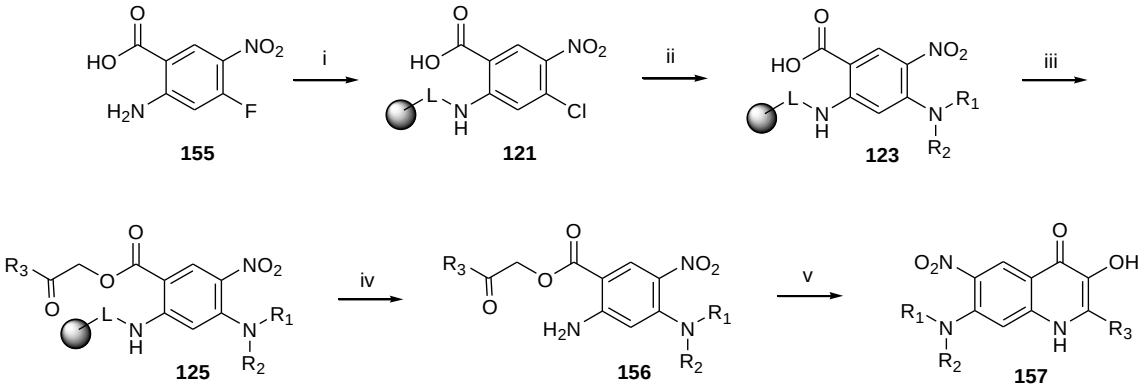
This study was focused on the reactivity of the commercially available 4-chloro-2-fluoro-5-nitrobenzoic acid and its use for the preparation of various condensed nitrogen heterocycles *via* polymer-supported *o*-phenylendiamines. After careful optimization of the reaction conditions synthetic pathways leading to 5, 6 and 7-membered heterocycles were developed. The preparation of 8-membered heterocycles was unsuccessful and 5-membered succinimides were formed instead. The solid-phase synthesis approach applied in this project allows an efficient use of the protocol for a diversity oriented heterocycles synthesis. The methodology can be applied for a chemical library synthesis affording new derivatives for the high throughput screening. Additionaly, substitution of the target compounds allows their use as a starting material for another modification. The prepared derivatives represent heterocyclic analogues of anthranilic acid and can be used for solid phase synthesis of more complex (poly)heterocyclic structures.

4.1.2.2. Synthesis of 3-hydroxy-6-nitroquinolin-4(*1H*)-ones (TOS approach)

Immobilization of anthranilic acid, chlorine atom substitution and subsequent esterification of carboxylic group gave substituted anthranilates which served as precursor for following solution-phase preparation of 3-hydroxyquinolinones.

Theoretically, 4-chloro-2-fluoro-5-nitrobenzoic acid can serve as the starting material for the preparation of *N*-substituted as well as *N*-unsubstituted hydroxyquinolinones depending on the type of immobilized amine used. To evaluate the cycling method we first focused on the preparation of *N*-unsubstituted derivatives using the Rink amide resin (Scheme 50).

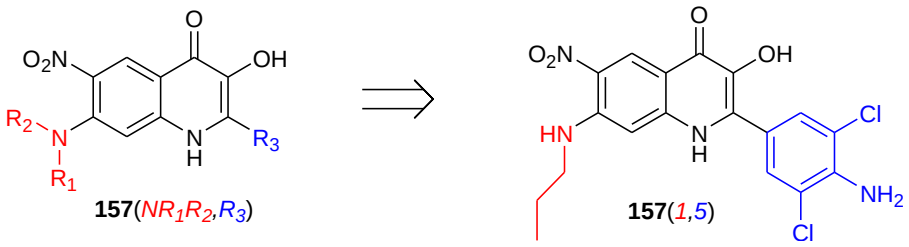
Scheme 50: Synthetic route to 3-hydroxy-6-nitroquinolin-4(1*H*)-ones **157**[a]



[a]Reagents and conditions: (i) Rink amide resin, DMSO, DIEA, 50 °C, overnight; (ii) amine, DIEA for amine (2) and (12), DMSO, 120 °C, overnight; (iii) bromoketone, TEA, DMF, rt, 3 – 48h; (iv) TFA/DCM, rt, 1h; (v) H_2SO_4, 100 °C, 2h or TFA, reflux, 2-8h or acetic acid reflux, 14h.

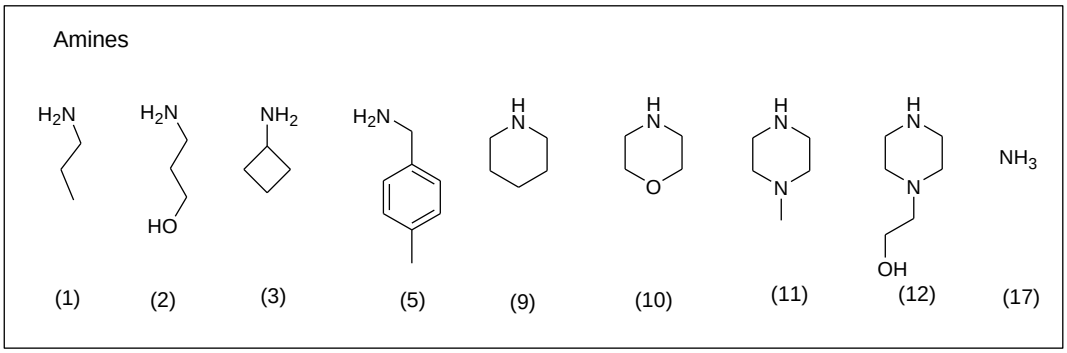
The diversity positions R_1, R_2 and R_3 originated from using various amines and bromoacetophenones in the substitution and esterification steps (Table 9).

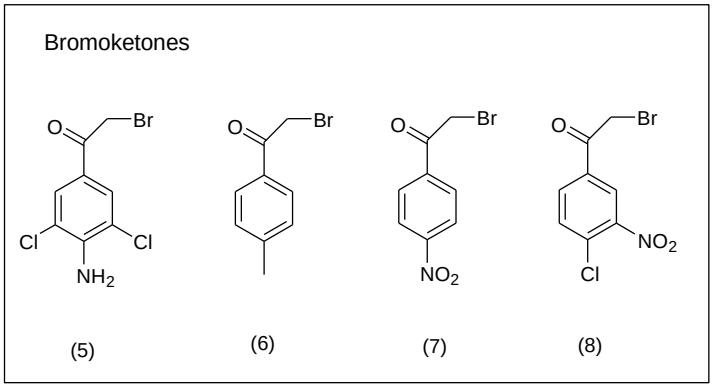
Scheme 51: Encoding of the structures



Intermediates **125** were then cleaved from the resin and their cyclization to the corresponding hydroxyquinolinones **157** was tested by heating in acids. Surprisingly, the previously described protocols with polyphosphoric acid,[113,143-145] trifluoroacetic acid[146] or *N*-methylpyrrolidone[144] were not universally applicable. Polyphosphoric acid and *N*-methylpyrrolidone were unreactive while trifluoroacetic acetic furnished relatively better results: intermediates **156**(*1,5*), (*2,5*), (*11,5*), (*12,5*), (*1,6*), (*2,6*), (*11,6*), and (*12,6*) were successfully cyclized. However, the rest of the intermediates did not afford the corresponding hydroxyquinolinones which demonstrated the structure-reactivity dependence of the TFA cyclization. Hence we tested other potentially suitable cyclizing agents. When heated in acetic acid,

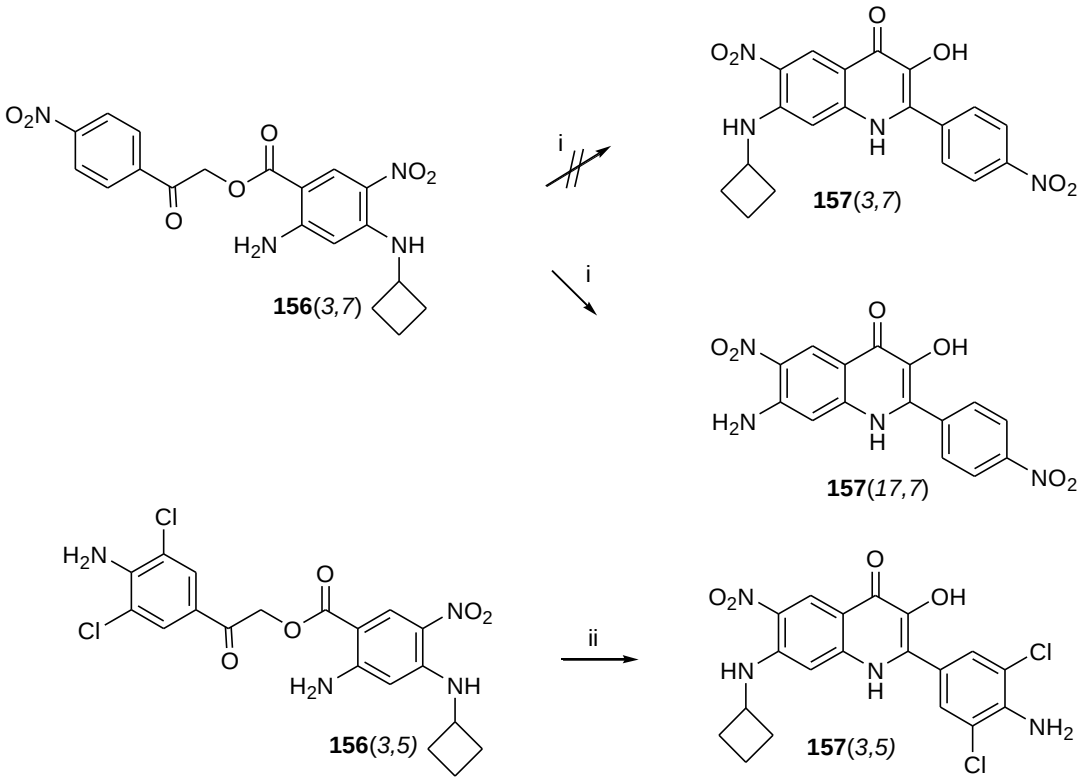
Table 9: The set of amines and bromoacetophenones used for the preparation of anthranilates **125**





only one hydroxyquinolinone **157**(*9,5*) from the whole set resulted. Finally we developed a new, generally applicable procedure using sulfuric acid and tested it successfully for all intermediates **156**. The derivative **157**(*5,7*) was not obtained because of the debenzylation following the cyclization of the intermediate **156**(*5,7*) which produced 7-aminoderivative **157**(*17,7*) (cyclization in sulfuric acid and TFA) (Scheme 52). The dealkylated product **157**(*17,7*) was obtained also when phenacylanthranilate **156**(*3,7*) was cyclized in sulfuric acid (Scheme 52); however, the cyclization of phenacylanthranilate **156**(*3,5*) in TFA afforded required derivate **157**(*3,5*). This indicates that cycloaliphatic amines can be used for the preparation of the corresponding hydroxyquinolinones, but they are not compatible with bromoacetophenones that contain electron-withdrawing nitro group as they do not undergo the cyclization in trifluoroacetic acid. After the cyclization, most of the products were isolated in an excellent crude purity of about 90-95% (HPLC-MS). A small number of hydroxyquinolinones were contaminated with side products (about 10-15%, HPLC-MS traces) which were identified as hydrolysis products **121** and discarded after sonification

Scheme 52: Dealkylation of cycloaliphatic amines in H_2SO_4[a]

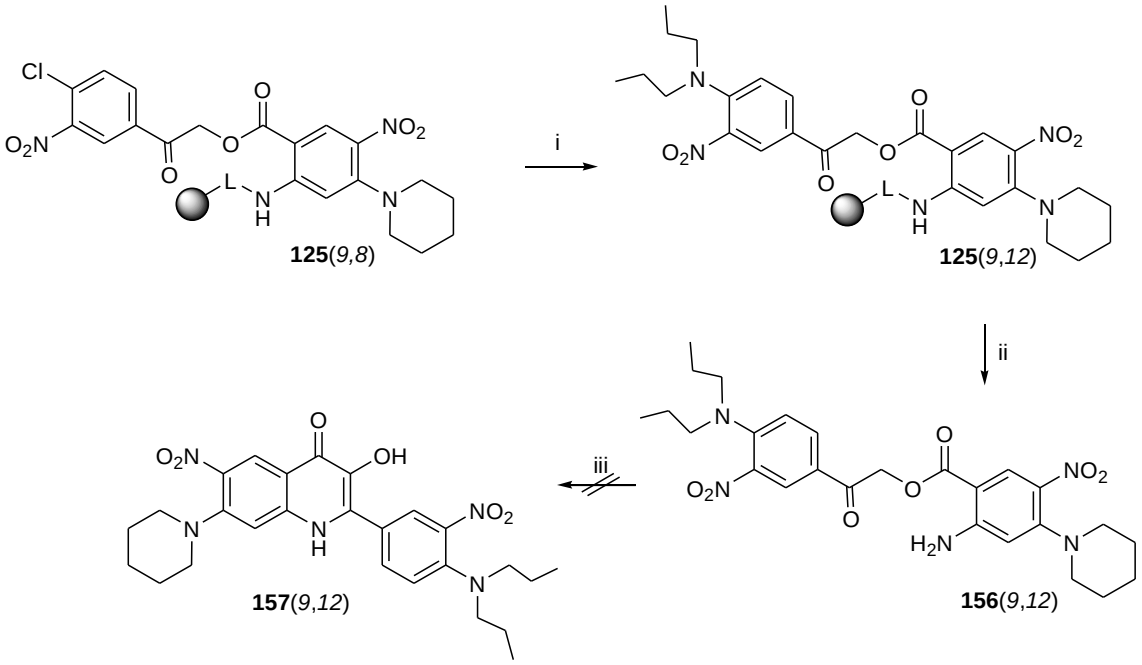


[a]Reagents and conditions: (i) H_2SO_4, 100 °C, 2h; (ii) TFA, reflux, 2h.

of the crude products **157** in potassium carbonate solution or diethyl ether. Products prepared using the amine building block (11) were not isolated because of their contamination after the esterification step (as described above in chapter 4.1.1.3.).

In theory, the diversity of the intermediates **125**(R_1,*8*) can be extended by reaction with nucleophiles, e.g. amino derivatives leading to nitroanilines **125**(R_1,*12*). Some hydroxyquinolinones with this kind of 2-phenylsubstitution have been described earlier as promising cytotoxic and imunosuppresive agents.[114] We tested the possible preparation of the similar hydroxyquinolinones using dipropylamine as the model building block. The nucleophilic substitution of the chlorine atom took place under mild conditions; however, the cyclization of the intermediate **156**(*9*,*12*) was unsuccessful. Heating in various acids such as acetic acid, trifluoroacetic acid or sulfuric acid unfortunately did not give the required hydroxyquinolinone **157**(*9*,*12*) (Scheme 53).

Scheme 53: Unsuccessful preparation of hydroxyquinolinone **157**(*9*,*12*)[a]

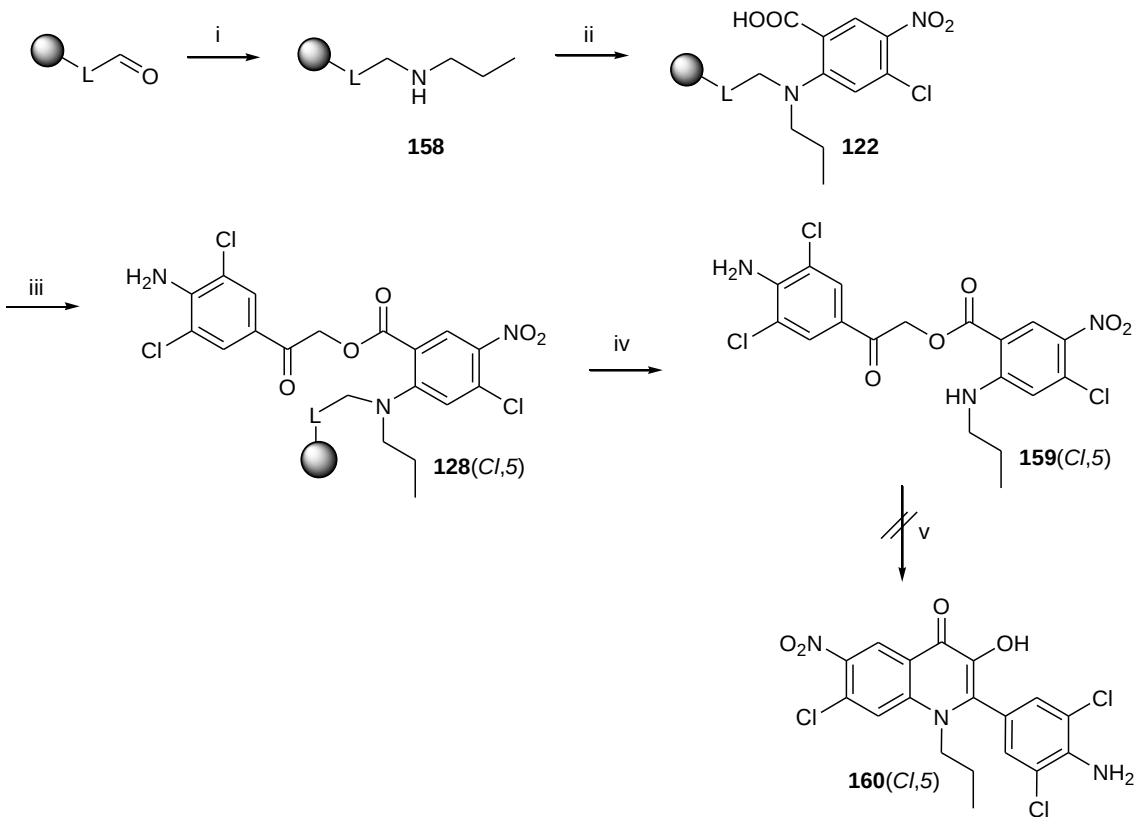


[a]Reagents and conditions: (i) dipropylamine, DMSO, rt, overnight; (ii) TFA/DCM, rt, 1h; (iii) TFA, reflux or AcOH, reflux or H_2SO_4, 100 ^oC.

Finally, we were interested in the utilization of our synthetic route for the preparation of *N*-alkylated hydroxyquinolinones. Concerning the biological effects, *N*-alkylated 2-arylhydroxyquinolinones are totally unexplored group of derivatives hence they still represent focus our interest. For the synthesis of *N*-alkylated hydroxyquinolinones we used aminomethylated polystyrene resin equipped BAL linker and after reductive alkylation with propylamine **158** we immobilized 4-chloro-2-fluoro-5-nitrobenzoic acid as *N*-propyl-4-chloro-5-nitroanthranilic acid **122**. After the esterification with bromoketone (8) we tried to cyclize the intermediate **159**(*Cl*,*5*) to the corresponding *N*-propyl hydroxyquinolinone **160**(*Cl*,*5*). Unfortunately, the required rearrangement did not take place using any above described procedure (Scheme 54).

For comparison, we concurrently tried to cyclize *N*-unsubstituted derivative **159**(*Cl*,*5*) prepared on Rink resin. The corresponding quinolinone product **160**(*Cl*,*5*) was smoothly afforded after reflux in TFA, what confirmed the restriction of the reported method to *N*-unsubstituted hydroxyquinolinones.

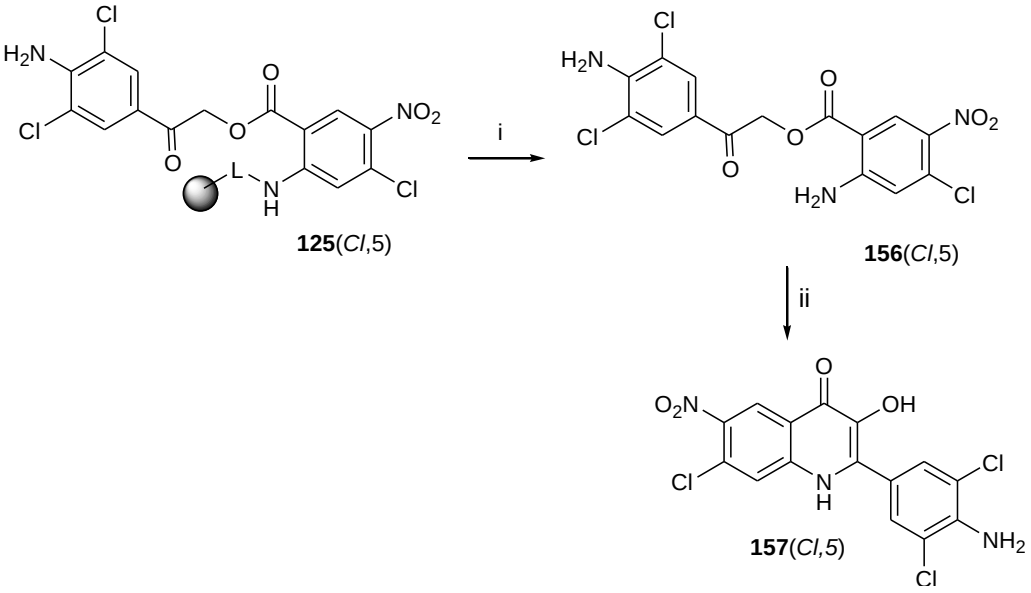
Scheme 54: Attempt to prepare *N*-alkyl hydroxyquinolinones **160**[a]



[a]Reagents and conditions: (i) propylamine, 10%AcOH in DMF, rt, overnight, then $NaBH(OAc)_3$, 5%AcOH in DMF, rt, 3h; (ii) 4-chloro-2-fluoro-5-nitrobenzoic acid, DMSO, DIEA, 50 °C; (iii) 4-amino-3,5-dichloro-2´-bromacetophenone, TEA, DMF, rt, overnight; (iv) TFA/DCM, rt, 1h; (v) TFA, reflux or AcOH, reflux or H_2SO_4, 100 °C or PPA 100 °C.

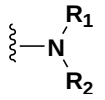
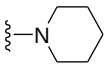
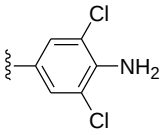
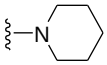
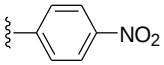
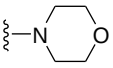
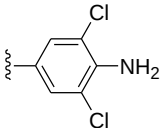
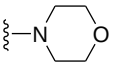
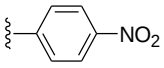
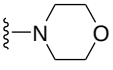
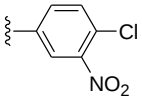
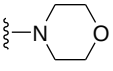
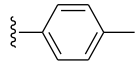
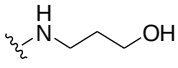
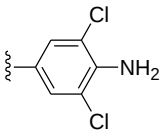
For comparison, we concurrently tried to cyclize *N*-unsubstituted derivative **156**(*Cl*,*5*) prepared on Rink resin (Scheme 55). The corresponding quinolinone product was smoothly afforded after reflux in TFA, what confirmed the restriction of the reported method to *N*-unsubstituted hydroxyquinolinones.

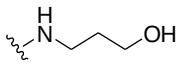
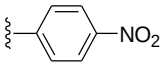
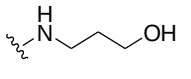
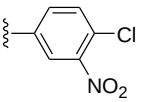
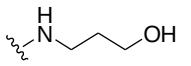
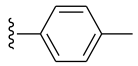
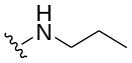
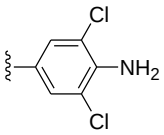
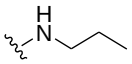
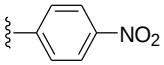
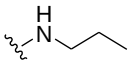
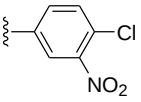
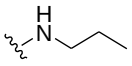
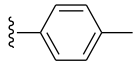
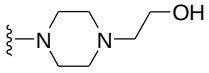
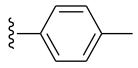
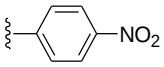
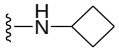
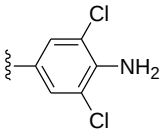
Scheme 55: Preparation of *N*-unsubstituted chloro derivative **157**(*Cl*,*5*)



[a]Reagents and conditions: (i) TFA/DCM, rt, 1h; (ii) TFA, reflux, 2h.

Table 10: The summary of prepared compounds

Product		R_3	Method	Purity (%)[a]	Yield (%)[b]
157(*9*,*5*)			B	85	70
157(*9*,*7*)			C	95	38
157(*10*,*5*)			C	97	43
157(*10*,*7*)			C	97	20
157(*10*,*8*)			C	97	49
157(*10*,*6*)			C	92	23
157(*2*,*5*)			A	96	36

157(*2,7*)			C	75	45
157(*2,8*)			C	92	91
157(*2,6*)			A	98	43
157(*1,5*)			A	90	42
157(*1,7*)			C	70	23
157(*1,8*)			C	72	40
157(*1,6*)			A	91	62
157(*12,6*)			A	87	68
157(*17,7*)			C	88	20
157(*3,5*)			A	90	30

[a]Purity of crude product (HPLC-MS traces); [b]Yield of crude product (compounds were purified only in necessary cases, see text above)

Method A: reflux in TFA; method B: reflux in AcOH; method C: heating in H_2SO_4

In conclusion, we have developed an efficient and simple synthesis of 3-hydroxy-6-nitro-2-phenylsubstituted-quinolin-4(1*H*)-ones with two diversity positions. The cyclization was completed in excellent purity and minor impurities accompanying the products were easily removable by simple purification methods. Reported method is not aplicable for synthesis of *N*-substituted hydroxyquinolinones and a limitation was also found in case of some 2-phenylsubstituted derivatives. Despite the limitation the described procedure offers a powerful tool for the library synthesis of diverse target molecules from the large number of commercially available building blocks such as amines and bromoketones.

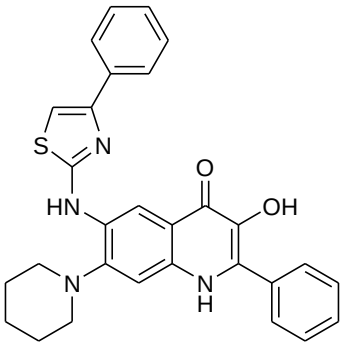
4.1.2.3. Synthesis of 3-hydroxy-6-(thiazol-2-ylamino)-4(*1H*)-quinolinones

Following study was focused on the synthesis of bisheterocycles, group of compounds possessing in general significant biological activity and having an important role in drug research.[111] The primary aminogroup resulting from the reduction of the nitro group of above described 3-hydroxyquinolinones ideally allows smooth introduction of another heterocycle into quinolinone molecule and promises potentially active compounds. This supposition directed our current investigation to the synthesis of 3-hydroxy-2,7-disubstituted-6-(4-substitutedthiazol-2-ylamino)-4(*1H*)-quinolinones - the quinolone-thiazol bisheterocyclic system with three diverse positions.

After achievement of nitro anilines **125** with two diverse positions the reaction sequence continued by reduction of the nitro group and subsequent building of thiazole ring using reported procedure.[147] Due to use of various bromoketones for formation of thiazole the third diverse position was achieved. The cleavage of the intermediates **163** from the resin yielded esters of anthranilic acid **164** ready for exposure to cyclization conditions (Scheme 56).

A simple model compound possessing unsubstituted phenyl moiety in position 2 and in position 4 of thiazole ring and secondary amine in position 7 (Figure 17) was chosen to evaluate the synthetic route. After initial steps (i, ii, iii, iv) the thiourea

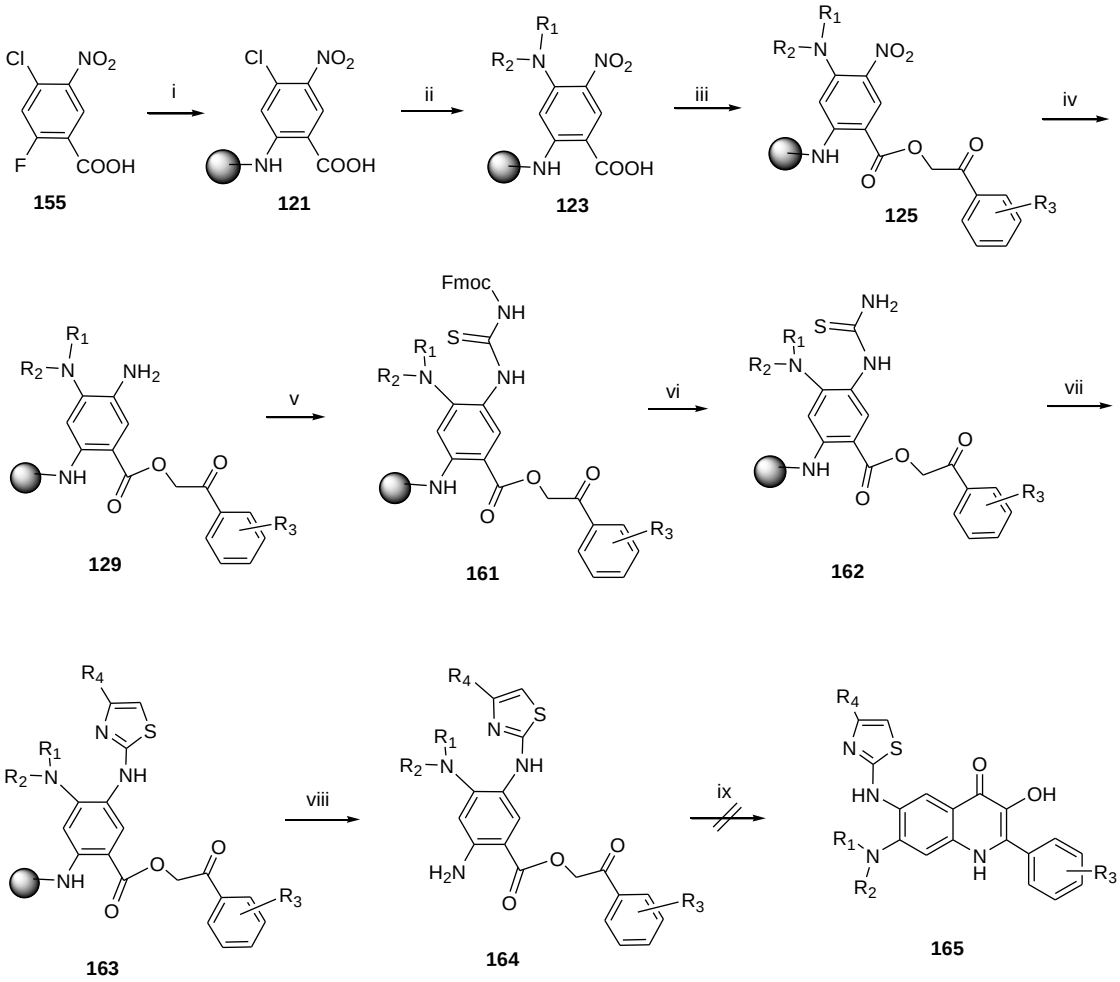
Figure 17: Model structure of the targets molecules



165(*9,4,4*)

derivative **162**(*9,4*) was obtained by reaction of the amino group of compound **129**(*9,4*) with Fmoc-NCS and subsequent piperidine-mediated cleavage of the Fmoc group. In subsequent step, a closure of the thiazole ring using α-bromoacetophenone, we observed formation of a side product. The LC-MS analysis indicated that in addition to the

Scheme 56: Synthetic pathway of 3-hydroxy-2,7-disubstituted-6-(thiazol-2-ylamino)-4(*1H*)-quinolinones **165**[a]



[a]Reagents and conditions : (i) Rink Amide resin, DMSO, DIEA, 50 °C, overnight; (ii) amine, DMSO, 120 °C, overnight; (iii) bromoketone, TEA, DMF, rt, overnight; (iv) $SnCl_2 \cdot 2H_2O$, DBU, DMF, rt, overnight; (v) Fmoc-NCS, THF, rt, 1h; (vi) piperidine, DMF, rt, 15 min.; (vii) bromoketone, TEA, DMF, rt, 2h; (viii) TFA/DCM, rt, 1h; (ix) various method.

thiourea moiety bromoketone alkylated another nucleophilic centre in the molecule. The side product was eliminated by using bromoketone solution of a lower concentration (0.1M instead of 0.25M). Concurrently, the reaction with chloroacetone was performed. The comparison of the results showed an obvious dependence on used haloketones. Whereas reaction with α-bromoacetophenone was completed in 0.1M solution in half an hour, in the case of chloroacetone several hours were needed. In contrast to α-bromoacetophenone, chloroacetone could be used in 0.25M concentration even in the

presence of base (proton sponge) without a detection of undesirable alkylation. After the closure of the thiazole ring, the anthranilate **163**(*9,4,4*) was cleaved from the resin. For the final cyclization step we tested formerly reported trifluoracetic acid[146] and sulfuric acid.[80] Unfortunately, none of them offered required hydroxyquinolinone **165**(*9,4,4*). Therefore several model hydroxyquinolinone precursors **164** were designed and prepare for a thorough survey of the cyclization method. The set of model compounds included derivatives with various combinations of substituents in position 5 and aryl moiety of the ester group (Table 12).

Scheme 57: Encoding of the structures

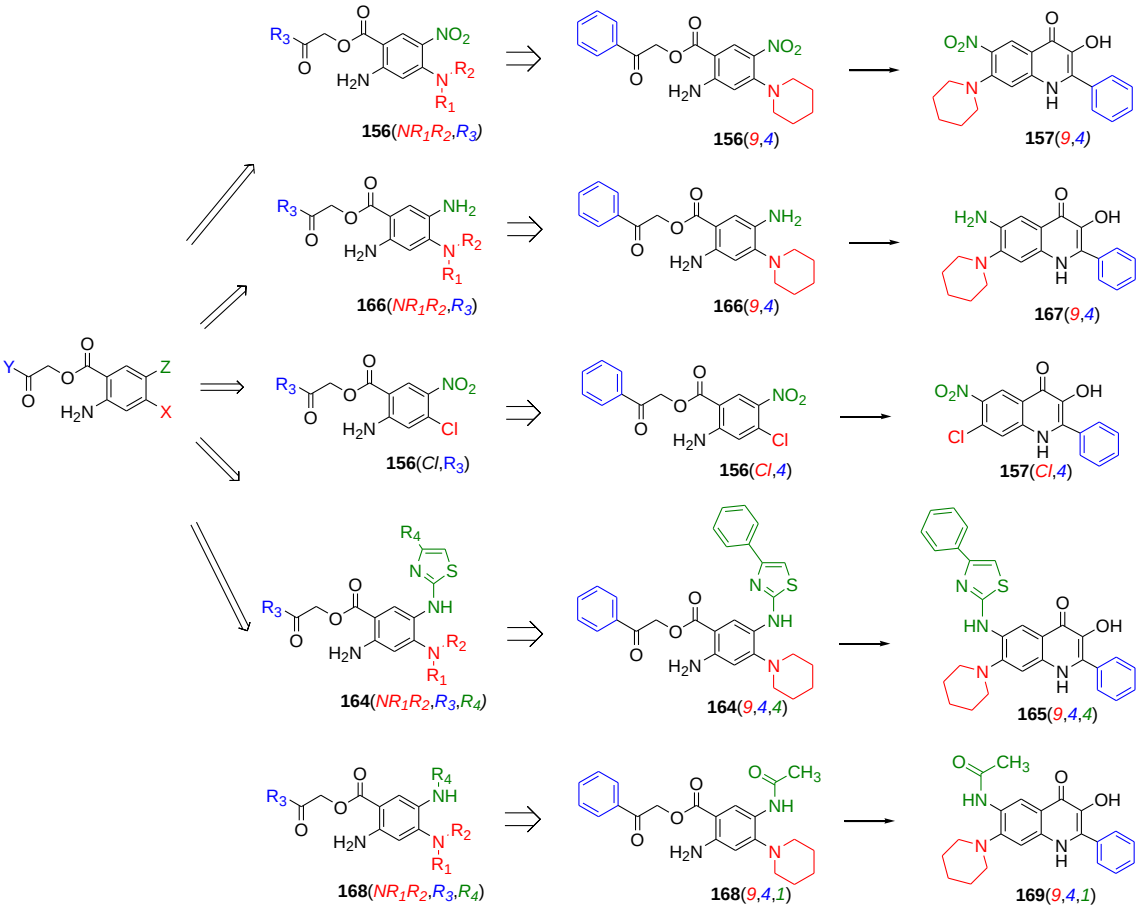


Table 11 : The set of used building blocks

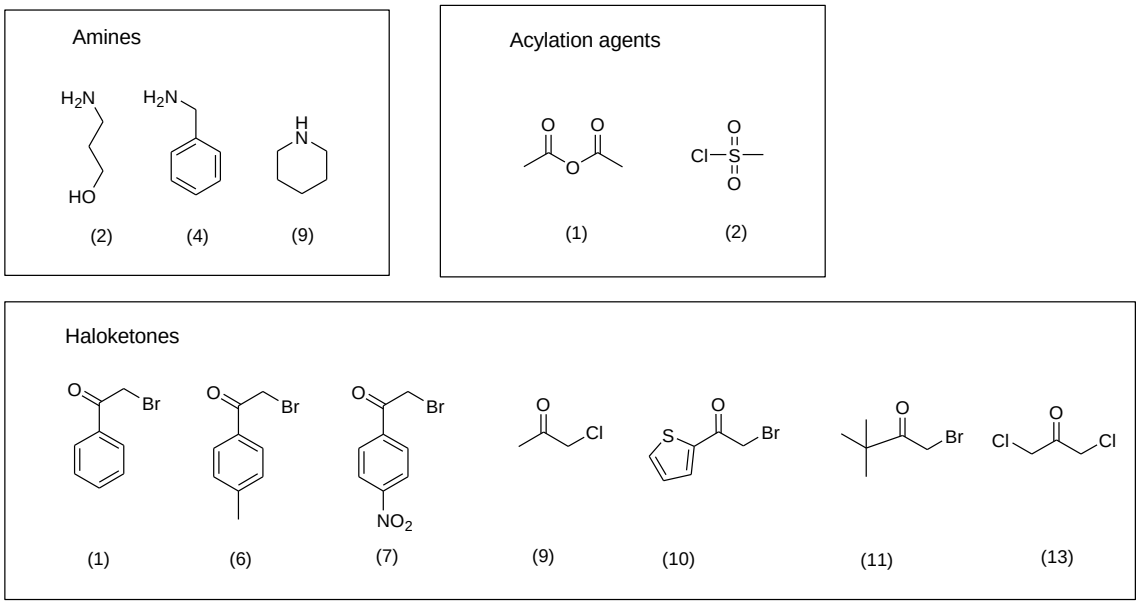
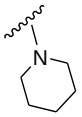
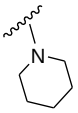
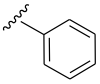
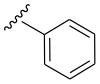
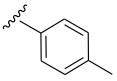
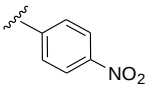
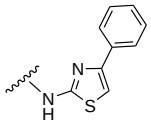
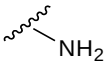
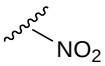
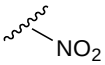
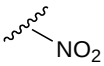
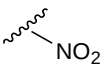


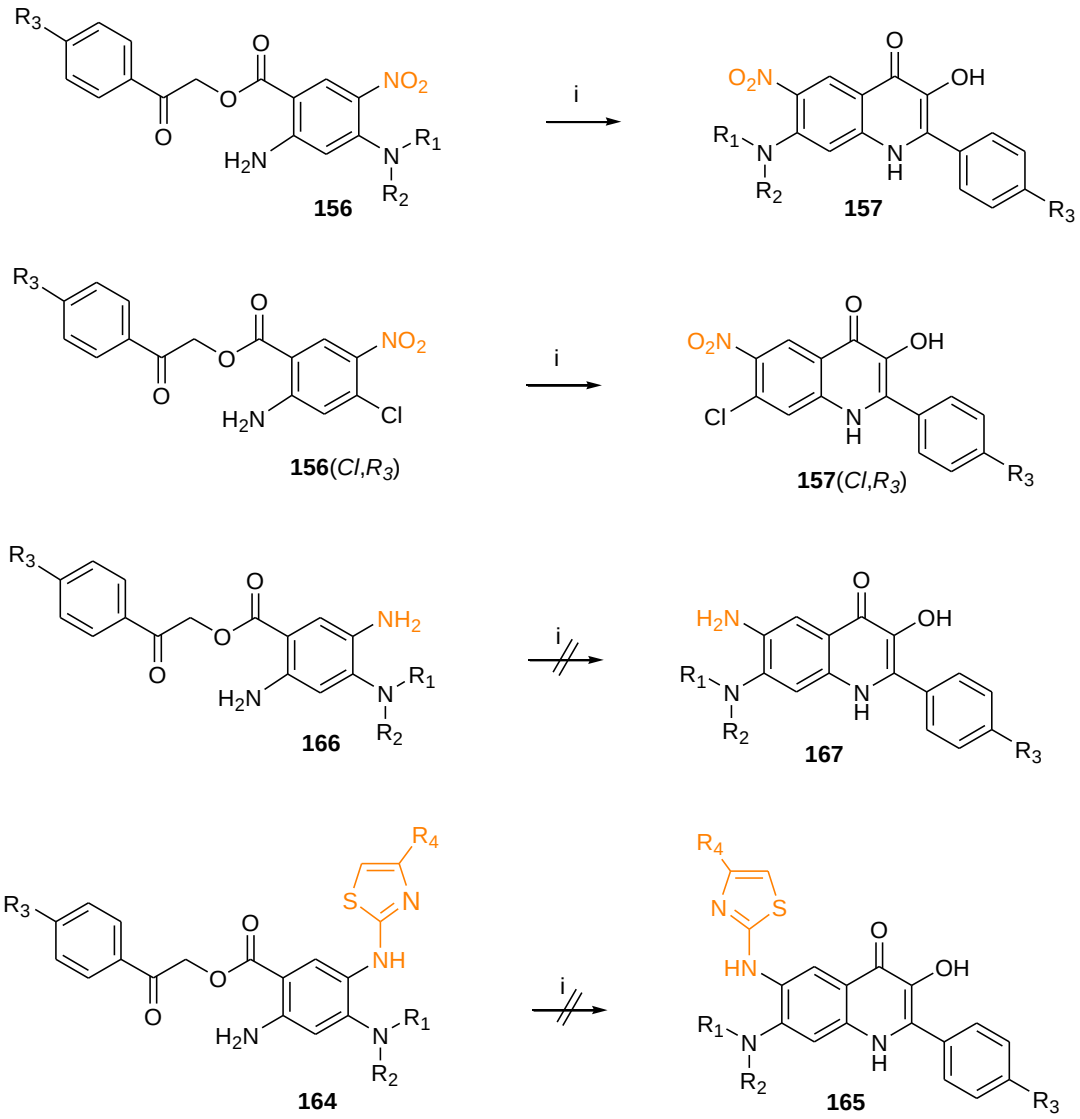
Table 12: The set of hydroxyquinolinone precursors tested for the cyclization

n	**164**(*9,4,4*)	**166**(*9,4*)	**156**(*Cl,4*)	**156**(*9,4*)	**156**(*9,6*)	**156**(*9,7*)
X						
Y						
Z						

A number of cyclization agents comprising TFA, AcOH, trifluoromethanesulfonic acid, H_2SO_4, polyphosphoric acid, HF or BF_3 were tested to achieve acid catalyzed cyclization or DBU to perform basic catalyzed cyclization. Besides conventional heating we tried to accelerate the reaction by using a microwave reactor. Unfortunately, we observed a considerable resistance of the anthranilate intermediates toward the tested conditions as the intermediates were obtained after majority reactions; in some

cases (triflic acid or DBU, 70°C) the reaction conditions caused the hydrolysis of the ester bond. Nevertheless, we found three methods offering target hydroxyquinolinones. Two of these methods using trifluoracetic acid catalyzed with triflic acid and sulfuric acid were applicable only in case of the hydroxyquinolinones possessing nitro group in position 5 (Scheme 58). This finding indicated that reduction of the nitro group

Scheme 58: Different reactivity of nitro and amino derivatives[a]

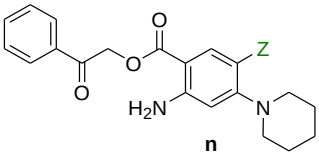
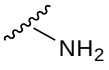
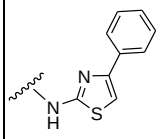
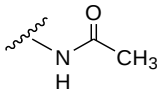
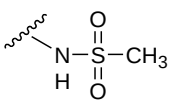


[a]Reagents and conditions: (i) TFA, triflic acid (cat.), 70 °C, 2h or H_2SO_4, 100 °C, 2h.

considerably inhibited the cyclization to the corresponding hydroxyquinolinones. Fortunately, the last method using polyphosphoric acid[143-145,148] provided cyclic target

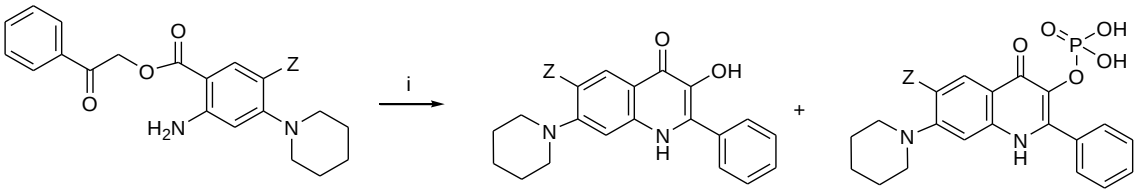
compounds even for models with reduced nitro group. Subsequently, the polyphosphoric acid-mediated cyclization was studied on several precursors (Table 13).

Table 13: Anthranilate precursors tested for polyphosphoric acid-mediated cyclization

 n	**166**(*9,4*)	**164***(9,4,4)*	**168**(*9,4,1*)	**168**(*9,4,2*)
Z				

All derivatives yielded expected heterocycles, but these were accompanied with corresponding phosphate (according to HPLC-MS, Scheme 59).

Scheme 59: The cyclization of anthranilates using polyphosphoric acid[a]

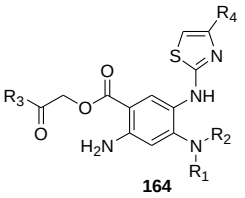
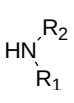
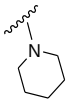
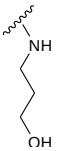
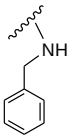
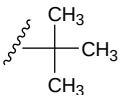
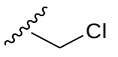


164, 166, 168 **165, 167, 169** **170, 171, 172**

[a]Reagents and conditions: (i) PPA, 100 °C, 2h.

Apart from the acid-catalyzed cyclization, a method using P_2O_5 appeared to be applicable for the closure of the quinolinone cycle. This method when the acyclic precursor is refluxed in n-butanol in the presence of P_2O_5 was applied for the synthesis of model derivative **165**(*9,4,4*) which was obtained in sufficient purity (85%, HPLC-MS traces). Encouraged by this success, we prepared several precursor derivatives (Table 14) to verify the method. Unfortunately, after the cyclization we had to contend with a substantial insolubility avoiding the purification and structure confirmation of the target heterocycle. Regrettably, in our effort to repeat the primary successful cyclization of the derivative **165**(*9,4,4*) in preparative amount we met with the alike problem connected with the separation on RP-HPLC. Neither NMR analysis of the crude product provided

Table 14: Anthranilate precursors tested for the P_2O_5-mediated cyclization

 **164**	**164**(*9,11,4*)	**164**(*2,4,9*)	**164**(*4,10,13*)
			
R_3			
R_4			

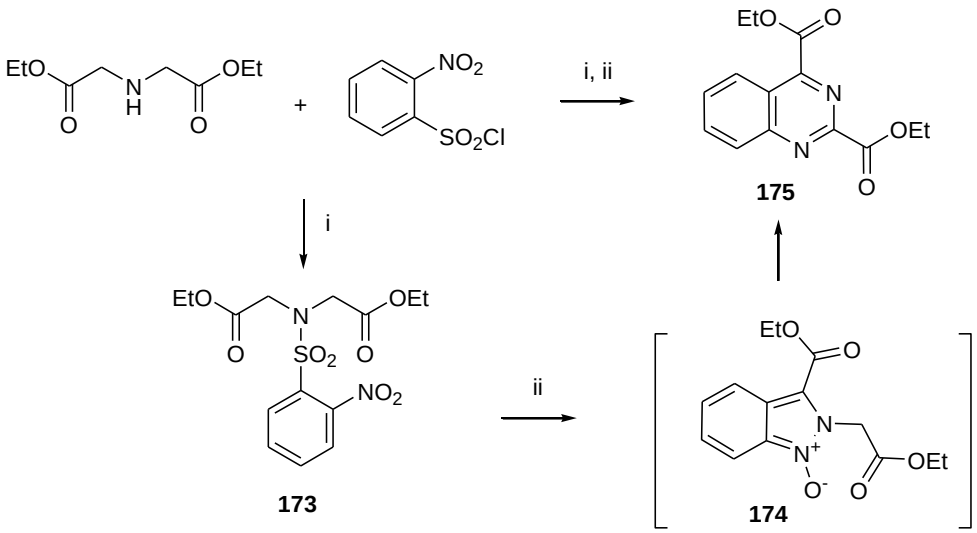
an applicable spectrum what might be caused by insufficient concentration of the sample. Despite all these negative results, there were some points which could support the probability of successful preparation of hydroxyquinolones: a fluorescence of the prepared products which is a characteristic property of hydroxyquinolinones; the fact, that HPLC-MS analysis did not revealed neither the starting compound or other products and difficulties with the separation of hydroxyquinolinone derivatives were noted in some previous works as well.[146] However, in this moment it is not possible to characterize and confirm the product structure.

4.2. SYNTHESIS OF QUINAZOLINES *VIA* 2*H*-INDAZOLE 1-OXIDES

This part of the work deals with a synthesis of quinazolines, a group of pharmaceutically very important compounds. In development of the synthetic method leading to the target heterocycles we utilized base-catalyzed rearrangement of 2*H*-indazole 1-oxides prepared by tandem carbon-carbon followed by nitrogen-nitrogen bond formation from easily accessible *N*-alkyl-2-nitro-*N*-(2-oxo-2-aryl-ethyl)-benzenesulfonamides.

Reaction of diethyl iminodiacetate with 2-nitrobenzenesulfonylchloride in the presence of a mild base, lutidine, yielded the expected sulfonamide **173**. An exposure of crude reaction mixture to a strong base, DBU, in DMF triggered the subsequent transformation to quinazoline **175** (Scheme 60).

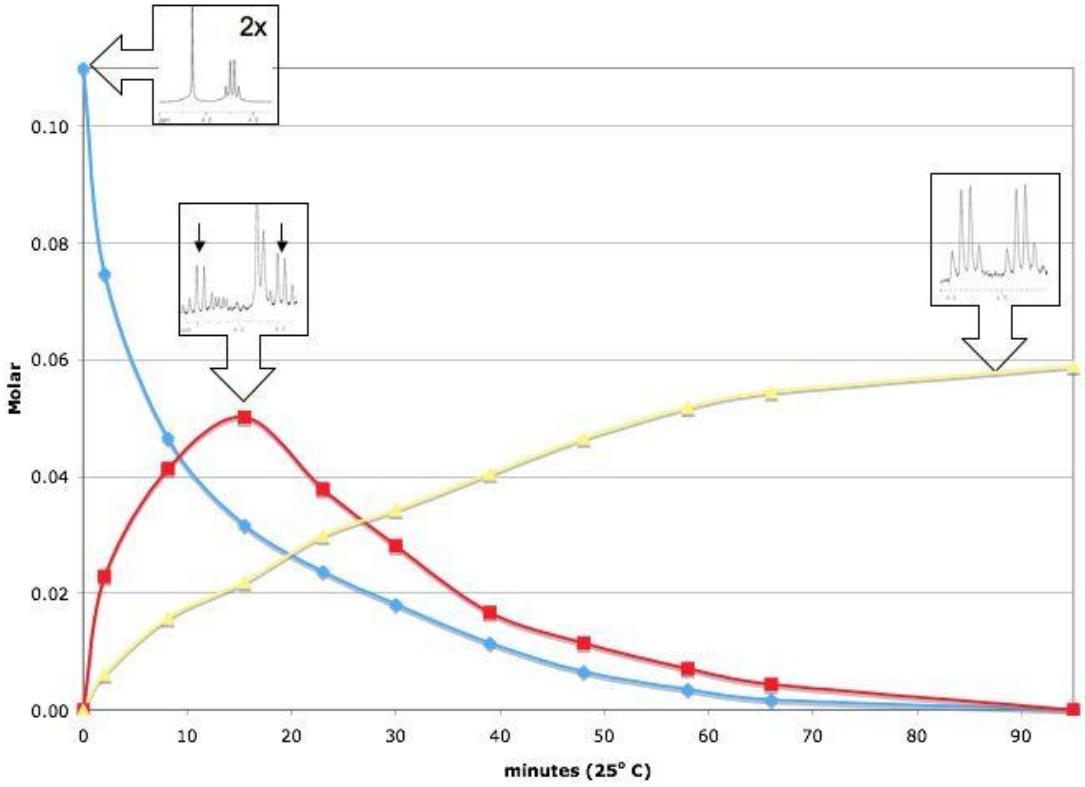
Scheme 60: Synthesis of quinazolines from iminodiacetate and 2-nitrobenzenesulfonylchloride[a]



[a]Reagents and conditions: (i) lutidine, DCM, rt, overnight; (ii) DBU, DMF, rt, overnight.

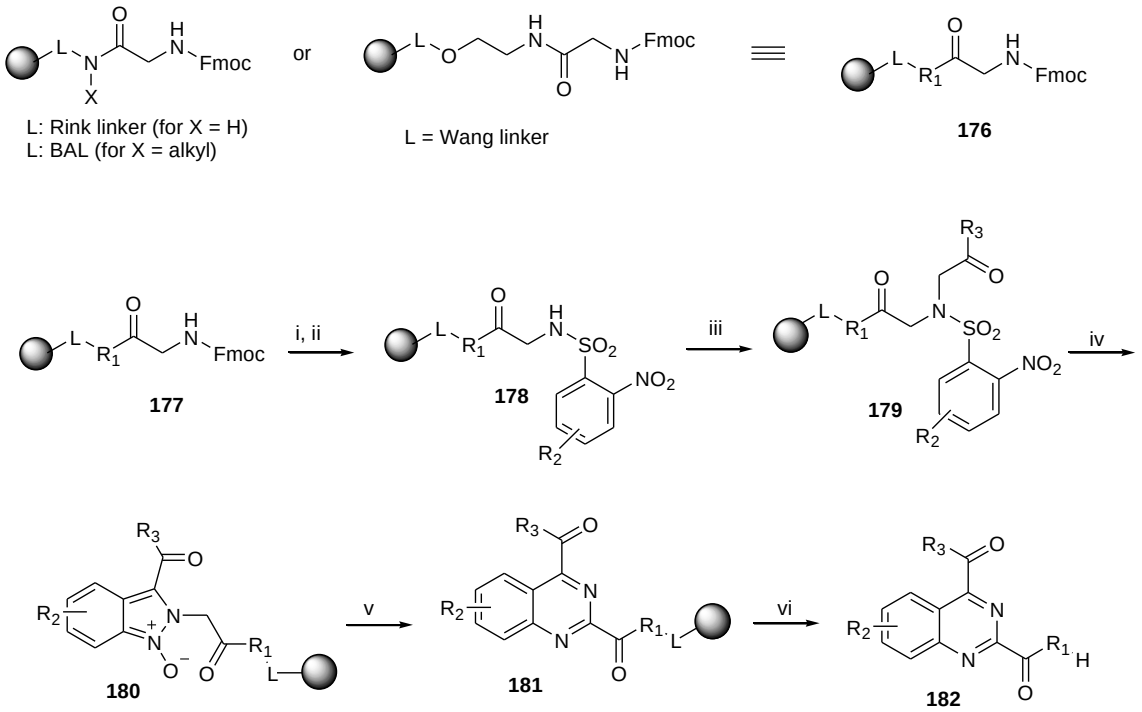
Qualitative details associated with the sequential cyclization of **173** to **175** came from a time-study ^{1}H NMR experiment. When 0.11M solution of **173** in DMSO-d_6 was treated with 3 eq. of DBU, an immediate color change ensued and proton signals (δ 4.16 (s), 4.09 (q)) for sulfonamide **173** rapidly decreased. An intermediate set of signals (δ 4.39 (q), 4.19 (q)) increased in intensity, and then these signals declined with production of quinazoline **175** (δ 4.56 (q), 4.45 (q)) (see Figure 18). Attempts to extract quantitative information for this reaction were complicated by ester hydrolysis and the presence of water in the initial reaction mixture. Ester hydrolysis occurred in all three materials involved in this reaction and accounted for the low overall yield of **175** (54%). The data plotted in Figure 18 resulted only after thorough drying of solvent and DBU.[149,150] In the presence of water, the consumption of **173** occurred at a rate similar to that shown in Figure 18, while cyclization to **175** can take up to 18 h to achieve 50% conversion.

Figure 18: Time-study of the sequential cyclization sulfonamide **173** to quinazoline **175** in the presence of DBU



To increase the diversity of compounds accessible by this ring expansion transformation, we synthesized combinatorial ensembles of linear precursors with three diversity positions on solid phase support. *N*-Alkyl-2-nitro-*N*-(2-oxo-2-aryl-ethyl)-benzenesulfonamides **179** were prepared by using glycine, 2-nitrobenzenesulfonyl chlorides and bromoketones following a route developed for the synthesis of indazole oxides.[151] When the indazole-oxides **180** were exposed to basic condition, we observed rearrangement to a 6-membered ring **181** (Scheme 61). First, we evaluated the scope and limitation of this transformation. Model compounds were synthesized on Rink amide resin acylated with Fmoc-Gly-OH. Later we also used alkylated BAL resin and ethanolamine-derivatized Wang resin.

Scheme 61: Synthesis of quinazolines on solid phase[a]

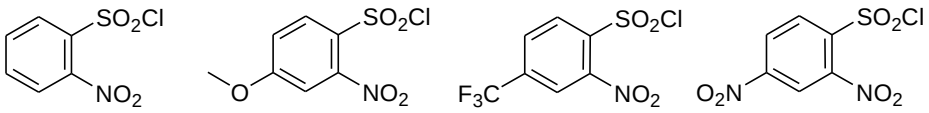


[a]Reagents and conditions: (i) piperidine, DMF, rt, 15 min; (ii) 2-nitrobenzenesulfonyl chloride, lutidine, DCM, rt, overnight; (iii) bromoketone, DMF, rt, 0.5-7 hours (see experimental part); (iv) DBU, DMF, rt, 30 min; (v) DBU, DMF, rt, 10 min to overnight (see experimental part); (vi) TFA/DCM, rt, 1h.

To optimize reaction conditions and to evaluate the effect of diverse substitution pattern on 2-nitrobenzenesulfonyl chlorides and bromoketones on the yield and purity of products, we prepared a set of model compounds on Rink Amide resin acylated with Fmoc-Gly-OH with use of various sulfonyl chlorides and bromoketones (Table 15).
Both 2-Nos chlorides and bromoketones were selected to bear electron-donating as well as electron-withdrawing groups. Synthesis of acyclic precursors followed from a published protocol.[151]

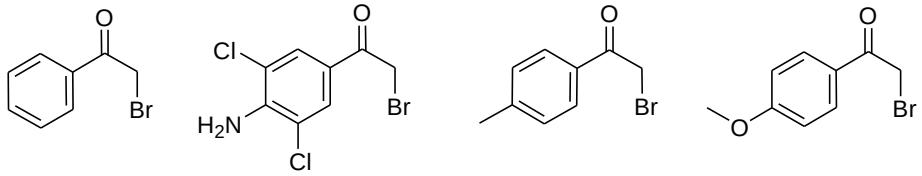
Table 15: Diversity reagents

2-Nitrobenzenesulfonyl chlorides

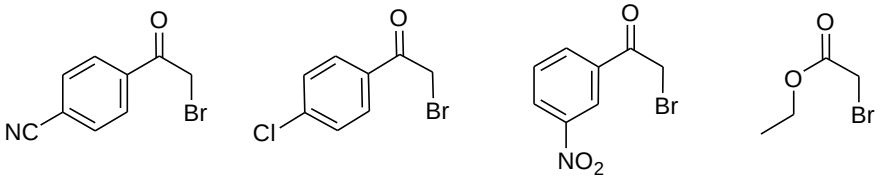


(1) (2) (3) (4)

Bromoketones and esters

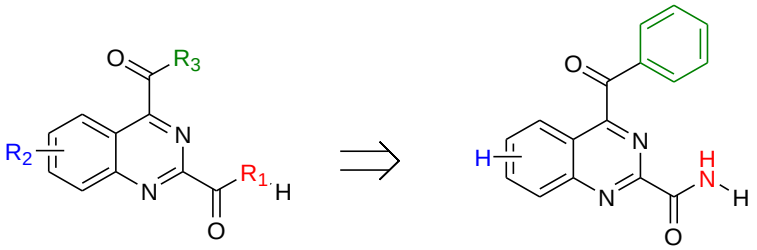


(4) (5) (6) (14)



(15) (16) (17) (18)

Scheme 62: Encoding of the structures



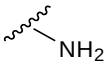
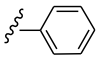
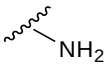
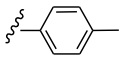
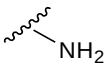
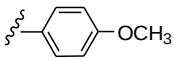
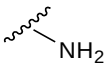
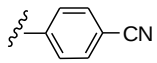
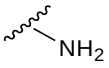
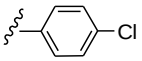
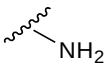
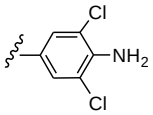
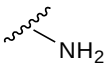
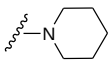
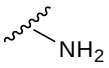
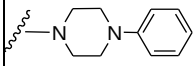
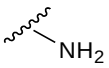
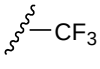
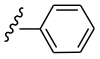
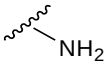
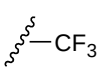
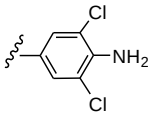
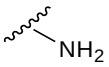
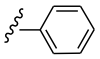
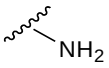
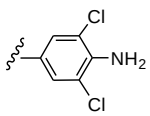
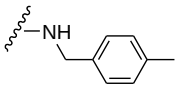
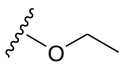
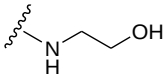
182(*R*$_1$,*R*$_2$,*R*$_3$) **182**(*1*,*1*,*4*)

The rate of *N*-alkylation affording product **179** was dependent upon substitution pattern of both building blocks (bromoketone and benzenesulfonyl chloride). The presence of nitro group on either bromoketone or 2-Nos chloride markedly accelerated the reaction; the alkylation was complete in thirty minutes. By contrast, when electron-donating substituents were present on either the ketone or benzenesulfonyl groups the nitrogen alkylation reaction required several hours for completion. HPLC-MS analysis of linear precursors prepared by alkylation of electron deficient model compounds already revealed the presence of indazole-oxides **180**, an indication that these activated systems cyclized even in the presence of DIEA used in alkylation step.

Exposure of intermediate **179** to DBU caused further transformation of indazole-oxides to quinazolines **181**. The outcome of cyclization was highly dependent upon substitution pattern. The effect was evaluated on a set of model compounds with particular attention given to the Nos substitution pattern, **179**(*1,R_1,1*). Electron withdrawing nitro group accelerated the rearrangement up to quinazolines **181**, so the compounds **179**(*1,4,4*) was transformed by 10 minutes with use of 0.1M DBU. Prolonged reaction time for reactions involving **179**(*1,4,4*) led to product decomposition. In comparison, non-substituted derivative **179**(*1,1,4*) (R_2, R_3 = H) required 0.2 M DBU overnight to achieve maximum yield of **181**(*1,1,4*). The electron-donating methoxy group in model compound **179**(*1,2,4*) cyclized only to indazole-oxides **180**(*1,2,4*). Attempts to facilitate conversion of the indazole-oxide to quinazoline **181**(*1,2,4*) by increasing of the temperature led to decomposition, as demonstrated by NMR analysis of the cleaved resin product.

The structure of bromoketones influenced the conversion to quinazolines as well. The presence of nitro group considerably accelerated the formation of indazole, however 6-nitroquinazoline **181**(*1,1,16*) was obtained only in low yield in a complex mixture of unidentified compounds. Because we observed partial cyclization during alkylation in the presence of DIEA, we attempted to replace the DBU by DIEA. In this case indazole oxide was stable in the presence of DIEA at room temperature. Increased temperature facilitated the rearrangement to desired quinazoline **181**(*1,1,16*), but the purity of crude product was low due to contamination by unidentified components. Derivative possessing combination of electron-donating and electron-withdrawing substituent **179**(*1,2,16*) provided similar results as derivative **179**(*1,1,16*) what indicated a predominant influence of bromoketone on cyclization. A summary of quinazoline formation is presented in Table 16.

Table 16: Summary of the prepared derivatives

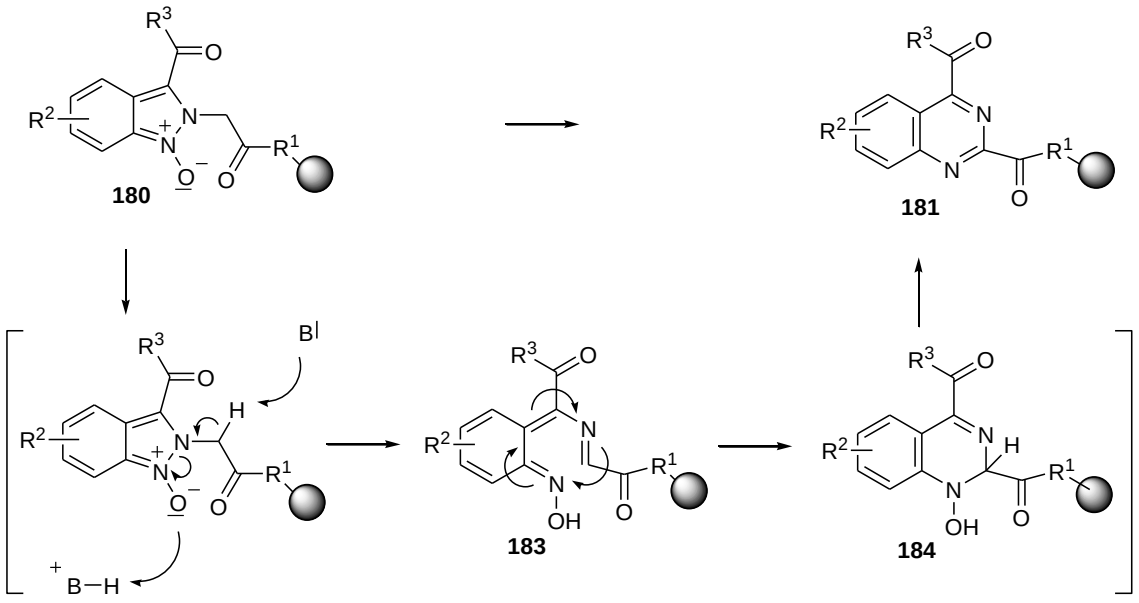
Compound	R_1	R_2	R_3	Method	Purity (%)[a]	Yield (%)[b]
182(*1,1,4*)				A	88	23
182(*1,1,6*)				A	95	12
182(*1,1,14*)				A	90	10
182(*1,1,15*)				B	25	15
182(*1,1,16*)				A	98	11
182(*1,1,5*)				A	90	20
182(*1,1,19*)				A	76	30
182(*1,1,20*)				A	90	41
182(*1,2,4*)				A	91	7
182(*1,2,5*)				A	98	23
182(*1,4,4*)				C	85	33
182(*1,4,5*)				C	92	14
182(*2,1,18*)				A	45	18
182(*3,1,4*)				A	88	48
182(*3,1,6*)				A	79	33
182(*3,1,16*)				A	84	36

[a]Purity of crude product before purification; [b]yield after purification; method A: 0.2M DBU in DMF, rt, overnight; method B: 0.1M DBU in DMF, rt, 1.5 h; method C: 0.1M DBU in DMF, rt, 10 min.

Compounds **182**(*1,1,18*), **182**(*1,1,19*), **182**(*2,1,17*), **182**(*3,1,4*), **182**(*3,1,6*) and **182**(*3,1,15*) were prepared by co-authors.

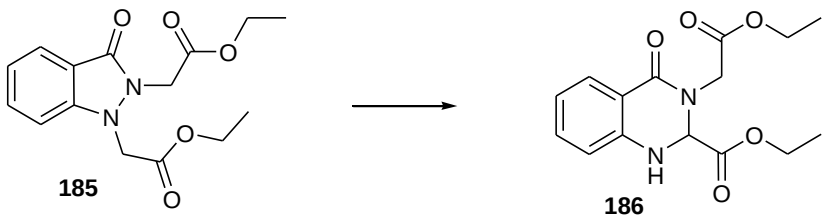
Proposed mechanism of rearrangement. This base-catalyzed rearrangement started with scission of the nitrogen-nitrogen bond caused by the presence of the *N*-oxide which was responsible for electronic activation of the electron-deficient nitrogen and assisted by the DBU-mediated proton subtraction (Scheme 63). Formation of *N*-hydroxy derivative **183** was important for subsequent water elimination facilitated by the carbon-nitrogen bond formation.

Scheme 63: Proposed mechanism of quinazoline formation



No precedent for the transformation of indazole oxides to quinazolines was found. A distant analogy to the ring expansion of indazoles can be found in the rearrangement of diethyl 2,2'-(3-oxo-1*H*-indazole-1,2(3*H*)-diyl)diacetate **185** to ethyl 3-(2-ethoxy-2-oxoethyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-2-carboxylate **186** (Scheme 64).[152,153]

Scheme 64: Rearrangement of 2,2'-(3-oxo-1*H*-indazole-1,2(3*H*)-diyl)diacetate

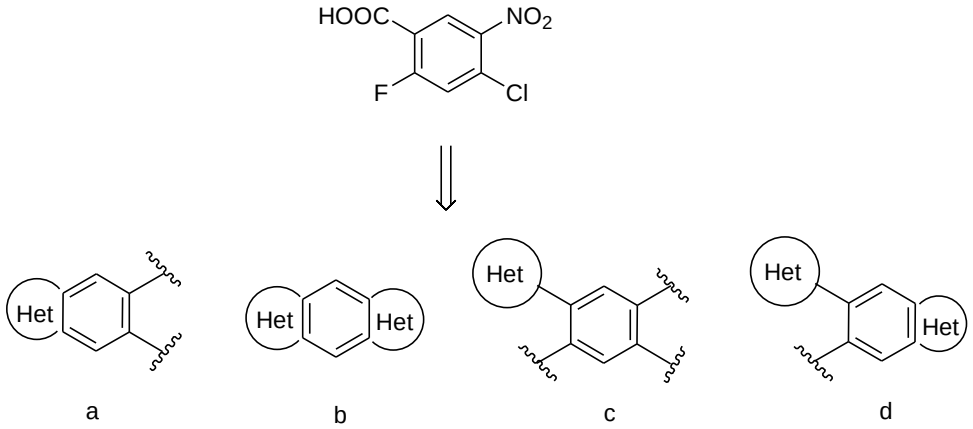


In conlusion, we have developed an efficient synthetic method of quinazolines which utilized the ring expansion of 2*H*-indazoles 1-oxides. The reaction was significantly influenced by a substitution pattern of used building blocks and required an optimization of the reaction conditions according to the type of substitution.

5. Conclusion

In this work we studied the reactivity of the commercially available 4-chloro-2-fluoro-5-nitrobenzoic acid and took advantage of its convenient arrangement to build a heterocycle fused to one (chloro-nitro) or to the other side (carboxy-fluoro) of the benzene ring or attached *via* nitrogenous linker (Figure 19). Despite substantial limitations which appeared in course of the study and required careful optimization of

Figure 19: The utility of 4-chloro-2-fluoro-5-nitrobenzoic acid to the synthesis of various heterocycles



the reaction conditions we developed synthetic methods leading to various heterocyclic scaffolds. The set of prepared heterocycles included benzoimidazoles, benzotriazoles, quinoxalinones and benzodiazepindiones achieved *via o*-phenylendiamines originating from chloro and nitro substitution (a). Reduction of the nitro group allowed introduction of a non-condensed heterocycle to the molecule as well, what was utilized to formation of thiazole derivatives (c). The last representatives of the reported group of heterocycles were 3-hydroxyquinolinones synthesized from anthranilic acid esters built on carboxy-fluoro side (a). Substitution of the target compounds offers further possible modification of the molecule. For this purpose, combination of here described methods can be in future used to achieve novel heterocyclic compounds (b) and (d) (Figure 19). Already tested synthesis of quinolone-thiazol bisheterocycles (d) involving the formation of thiazol heterocycle and hydroxyquinolinone heterocycle was unfortunately unsuccessful and will require further attention.

In the second part of the work we described a convenient preparation of quinazolines *via* based-catalyzed rearrangement of 2*H*-indazoles 1-oxides. The heterocycle intermediates were obtained by tandem carbon–carbon followed by nitrogen–nitrogen bond formations from *N*-alkyl-2-nitro-*N*-(2-oxo-2-aryl-ethyl)-benzenesulfonamides prepared using commercially available building blocks. The transformation tolerated a range of substituents; however it was sensitive to their electronic properties.

6. Experimental part

6.1. Material and methods

Solvents and chemicals were purchased from Aldrich (Milwaukee, IL, www.sigmaaldrich.com), Acros (Geel, Belgium, www.acros.cz) and Fisher (Pittsburgh, PA, www.fishersci.com). The Rink Amide Resin (100-200 mesh, 1% DVB, 1.2 mmol/g) was obtained from Advanced ChemTech (Louisville, KY, www.advancedchemtech.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°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 chromatogram 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°C.

Purification was carried out on C18 column 20 x 100 mm, 5 μm particles, 12 nm poruses, gradient was formed from 10 mM aqueous ammonium acetate and acetonitrile, flow rate 15 ml/min.

NMR $^{1}H/^{13}C$ spectra were obtained on a Bruker Avance (300 MHz) or Varian (400 MHz) instrument. NMR spectra were recorded at ambient temperature (21°C) in DMSO-d_6, pyridine-*d5* and $CDCl_3$ solutions and referenced to the resonance signal of solvent. Chemical shifts δ are reported in ppm and coupling constants *J* in Hz.

6.2. Synthetic procedures

6.2.1. Synthesis of immobilized anthranilic acid intermediates

Immobilized 4-chloro-5-nitroanthranilic acid (121)

A polypropylene fritted syringe was charged with Rink Amide resin (~2 g; substitution 1.1 mmol/g) and 50% piperidine in DMF (20 ml) was added. The resin was shaken for 30 minutes at room temperature, washed 5 times with DMF, 3 times with DCM and dried. 4-Chloro-2-fluoro-5-nitrobenzoic acid (4.4 mmol) was dissolved in DMSO (20 ml) and DIEA (8.8 mmol) was added. The resin was mixed with the solution and shaken at 50°C overnight. The resin was washed 3 times with DMF, 3 times with DCM, 3 times with MeOH and dried.

The yield of the reaction was evaluated after acylation of the resin sample with Fmoc-OSu and DIEA in DCM. No Fmoc-NH_2 was detected indicating the arylation was quantitative.

Immobilized 4-chloro-5-nitro-2-(propylamino)benzoic acid (122)

4-Chloro-2-fluoro-5-nitrobenzoic acid (0.2 mmol) was dissolved in DMSO (5 ml) and DIEA (0.4 mmol) was added. Propylamine attached to BAL resin **158** (~0,5 g) was mixed with the solution and shaken at 50°C overnight. The resin was washed 3 times with DMF, 3 times with DCM, 3 times with MeOH and dried.

Substitution of the chlorine atom of 4-chloro-5-nitroanthranilic acid (123)

10% solution of amine in DMSO was prepared and to the solution of amine (2) and (12) an equivalent of DIEA was added. The resin **121** (~200 mg) was mixed with the solution (5 ml) and shaken at 120°C overnight. The resin was washed 3 times with DMF, 3 times with DCM, 3 times with MeOH and dried.

{[2-Amino-5-nitro-4-(piperidin-1-yl)phenyl]carbonyloxy}methyl 2-amino-5-nitro-4-(piperidin-1-yl)benzoate (124(*9*))

A polypropylene fritted syringe was charged with Rink Amide Resin (~1 g; substitution 1.1 mmol/g) and 50% piperidine in DMF (10 ml) was added. The resin was shaken for 30 minutes at room temperature, washed 5 times with DMF, 3 times with DCM and dried. 4-Chloro-2-fluoro-5-nitrobenzoic acid (2.2 mmol) was dissolved in DMSO (10 ml) and DIEA (4.4 mmol) was added. The resin was mixed with the solution and shaken at 50°C overnight. The resin was washed 3 times with DMF and 3 times with DCM. 5% solution of piperidine in DMSO (10 ml) was added to the resin and shaken at 120°C for 5 hours. The resin was washed 3 times with DMF, 3 times with DCM and isolated.

{[2-Amino-5-nitro-4-(dipropylamino)phenyl]carbonyloxy}methyl 2-amino-5-nitro-4-(dipropylamino)benzoate (124(*8*))

A polypropylene fritted syringe was charged with Rink Amide Resin (~1 g; substitution 1.1 mmol/g) and 50% piperidine in DMF (10 ml) was added. The resin was shaken for 30 minutes at room temperature, washed 5 times with DMF, 3 times with DCM and dried. 4-Chloro-2-fluoro-5-nitrobenzoic acid (2.2 mmol) was dissolved in DMSO (10 ml) and DIEA (4.4 mmol) was added. The resin was mixed with the solution and shaken at 50°C overnight. The resin was washed 3 times with DMF and 3 times with DCM. 5% solution of dipropylamine in DMSO (10 ml) was added to the resin and shaken at 120°C for 1 day. The resin was washed 3 times with DMF, 3 times with DCM and isolated.

Esterification of substituted 5-nitroanthranilic acid (125)

With haloketones

A polypropylene fritted syringe was charged with the resin **123** (~200 mg). The solution of bromoacetophenone (0.4 mmol) and TEA (0.4 mmol) in DMF (2 ml) was added and the syringe was shaken at room temperature overnight (in the case of bromoacethophenone (7) and (8) for 2 days). The resin was washed 3 times with DMF and 3 times with DCM.

With alkylhalides

The polypropylene fritted syringe was charged with the resin **123** (~400 mg) and shaken for 10 minutes in a solution of DIEA (0.8 mmol) in DMF (4 ml). The solution of DIEA was removed and replaced with a solution of alkyl halide (0.8 mmol) in DMF (4 ml). The resin was shaken at room temperature (in the case of alkylhalide (2) and (3) at 80 °C) overnight, washed 3 times with DMF and 3 times with DCM.

4-Chloro-5-nitro-2-propylamino-benzoic acid 2-(4-amino-3,5-dichloro-phenyl)-2-oxo-ethyl ester (128(*Cl*,*5*)**)**

A polypropylene fritted syringe was charged with the resin **122** (*5*) (~50 mg), the solution of 3,5-dichloro-4-amino-2´-bromacetophenone (0.2 mmol) and TEA (0.2 mmol) in DMF (1 ml) was added and the syringe was shaken at room temperature overnight. The resin was washed 3 times with DMF and 3 times with DCM. Product was cleaved and identified by HPLC-MS analysis.
Purity of the cleaved product was 68%, APCI-MS m/z = 460, $[M-H]^-$

Reduction of nitroaniline (129)

The polypropylene fritted syringe was charged with the resin **125** (in the case of free carboxylic acid derivative the resin **123** was used) (~1 g), the resin was washed with degassed DMF. The solution of $SnCl_2 \cdot 2H_2O$ (20 mmol) and DBU (20 mmol) in degassed DMF (10 ml) was added to the resin and shaken at room temperature for 1 day. The resin was washed 5 times with DMF and 3 times with DCM.

2,5-Diamino-4-chlorobenzoic acid (130)

A polypropylene fritted syringe was charged with the resin **121**(~50 mg) and the solution of $SnCl_2 \cdot 2H_2O$ (1 mmol) and DIEA (1 mmol) in degassed DMF (1 ml) was added. The resin was shaken at room temperature overnight, washed 3 times with DMF, 3 times with DCM. Product was cleaved and identified by HPLC-MS analysis.
Purity of the cleaved product 95 %, APCI-MS m/z = 187, $[M+H]^+$

6.2.2. Cyclization methods leading to nitrogenous heterocycles

- with utility of 4-chloro-2-fluoro-5nitrobenzoic acid

- synthesis of quinazolines via 2H-indazole-1-oxides

- with utility of 4-chloro-2-fluoro-5nitrobenzoic acid

Benzimidazoles (131)

The polypropylene fritted syringe was charged with the resin **129** (~400 mg). The solution of aldehyde (0.8 mmol) in DCM (4 ml) was added to the resin and shaken at reflux overnight. The resin was washed 3 times with DMF, 3 times with DCM and the product was isolated.

Benzotriazoles (132)

The polypropylene fritted syringe was charged with the resin **129** (~400 mg). Sodium nitrite (1.2 mmol) was dissolved in the mixture of DMF (3.2 ml) and water (0.8 ml) and acetic acid was added (100 μl). The resin was mixed with the solution and shaken at room temperature for 4 days. The resin was washed 3 times with DMF, 3 times with DCM and the product was isolated.

Benzotriazole (**132**(*1*,*H*))

The polypropylene fritted syringe was charged with the resin **123**(*1*) (~400 mg) and the esterification with benzyl bromide, reduction of the nitro group and synthesis of a benzotriazole scaffold were carried out according to above described procedures.

Hydrolysis of benzylester (**132**(*1,H*))

After closure of triazole ring, the resin was shaken in the solution of potassium trimethylsilanolate (0.8 mmol) in THF (4 ml) at room temperature overnight. The resin was washed 3 times with THF, 3 times with DCM and the product was isolated.

Quinoxalinones (133)

The polypropylene fritted syringe was charged with the resin **129** (~400 mg). The solution of α-ketokarboxylic acid (0.8 mmol) and acetic acid (7 mmol) in THF (4 ml) was added and shaken at reflux for 2 hours. The resin was washed 3 times with THF, 3 times with DCM and the product was isolated.

6-Amino-1-benzyl-2-phenyl-1*H*-benzoimidazole-5-carboxylic acid benzyl ester (131(*4,1,1*))

(formation of the benzimidazole side product)

The polypropylene fritted syringe was charged with the resin **129***(4,1)* (~1 g). The solution of phenylglyoxylic acid (2 mmol) in DMF (10 ml) was added and shaken at room temperature overnight. The resin was washed 3 times with DMF, 3 times with DCM and the product was isolated.

2-Amino-5-nitro-4-[(2-oxo-2-phenyl-acetyl)-propyl-amino]-benzoic acid benzyl ester (**143(*1,1,1*))**

Phenylglyoxalic acid (0.7 mmol) was dissolved in dry DCE (2 ml), thionyl chloride (1.5 mmol) and DMF (cat.) was added and the reaction mixture was refluxed for 2 hours. After cooling to the room temperature, DIEA was added until slightly basic reaction. The solution was combined with resin **125**(*1,1*) (~50 mg) and shaken at reflux overnight. The resin was washed 3 times with DMF, 3 times with DCM.

7-Amino-2-oxo-3-phenyl-1-propyl-1,2-dihydro-quinoxaline-6-carboxylic acid benzyl ester (133(*1,1,1*))

The polypropylene fritted syringe was charged with the resin **143**(*1,1,1*) and resin was washed with degassed DMF. The solution of $SnCl_2 \cdot 2H_2O$ (2 mmol) and DBU (2 mmol) in degassed DMF (1 ml) was added to the resin and shaken at room temperature overnight. The resin was washed 5 times with DMF, 3 times with DCM. Product was cleaved and identified by HPLC-MS analysis.
Purity of the cleaved product 95%, APCI-MS m/z = 414, $[M+H]^+$

Benzodiazepinediones (134)

Step 1: Intermediates (**149**)

The polypropylene syringe was charged with the resin **129** (~400 mg). Dicarboxylic acid (0.8 mmol), HOBt (0.8 mmol) and DIC (0.8 mmol) were dissolved in DMF (2 ml) and DCM (2 ml) was added. After 15 minutes, the solution was added to the resin and shaken at room temperature overnight. The resin was washed 3 times with DMF and 3 times with DCM.

Step 2: Intramolecular acylation (ring closure)

The solution of HOBt (0.8 mmol) and DIC (0.8 mmol) in THF (4 ml) was added to the resin and shaken at 50 °C for 2 hours. The resin was washed 3 times with THF, 3 times with DCM and the product was isolated.

2-Oxo-2-phenylethyl-2-amino-5-(2-{[4-amino-2-(cyclohexylamino)-5-[(2-oxo-2-phenylethoxy)carbonyl]phenyl]carbamoyl}-2,2-dimethylacetamido)-4-(cyclohexylamino)benzoate (151(*7,4,4*))

(formation of the side product)

The polypropylene syringe was charged with the resin **129**(*7,4*) (~1 g). Dicarboxylic acid (2 mmol), HOBt (2 mmol) and DIC (2 mmol) were dissolved in DMF (5 ml) and DCM (5 ml) was added. After 15 minutes, the solution was added to the resin and shaken at room temperature overnight. The resin was washed 3 times with DMF and 3 times with DCM. The resin was combined with the solution of HOBt (2

mmol) and DIC (2 mmol) in mixture of DMF (5ml) and DCM (5 ml) and shaken at room temperature overnight. The resin was washed 3 times with DMF, 3 times with DCM and the product was isolated.

Dioxopyrroles and Dioxoisoindole (136)

Step 1: Intermediates (**153**)

The polypropylene syringe was charged with the resin **129** (~400 mg). Dicarboxylic acid (0.8 mmol), HOBt (0.8 mmol) and DIC (0.8 mmol) were dissolved in DMF (2 ml) and DCM (2 ml) was added. After 15 minutes, the solution was added to the resin and shaken at room temperature overnight. The resin was washed 3 times with DMF and 3 times with DCM.

Step 2: Intramolecular acylation (ring closure)

The solution of HOBt (0.8 mmol) and DIC (0.8 mmol) in DMF (2 ml) and DCM (2 ml) was added to the resin and shaken at room temperature overnight. The resin was washed 3 times with DMF, 3 times with DCM and the product was isolated.

Dibromo derivative (**136**(*7,4,2*))

The polypropylene syringe was charged with the resin **129**(*7,4*) (~400 mg). Dicarboxylic acid (0.8 mmol) and DIC (0.8 mmol) were dissolved in DMF (2 ml) and DCM (2 ml) was added. After 15 minutes, the solution was added to the resin and shaken at room temperature overnight. The resin was washed 3 times with DMF and 3 times with DCM. After this, the process was repeated. After washing the resin, the product was isolated.

Cyclization to hydroxyquinolinones (157)

Cleavage cocktail (50% TFA in DCM) was added to the syringe with resin **125** (~200 mg) and shaken for 30 minutes at room temperature. The cleavage cocktail was collected and the content of the syringe was washed 2 times with fresh 50% TFA in DCM. The washes were collected, the cleavage cocktail was evaporated by a stream of nitrogen and the resulting material was cyclized using following methods:

Method A (using trifluoroacetic acid)

The anthranilate **156**(*1,5*), **156**(*1,6*), **156**(*12,5*), **156**(*3,5*) was dissolved in neat TFA (2 ml) and the solution was refluxed (for the reaction time see the table below). Trifluoracetic acid was evaporated by a stream of nitrogen and the residual oil was sonified in diethyl ether (5 ml) for 5 minutes. The precipitated material was filtered off, washed with diethyl ether and dried.

The anthranilate **156**(*2,5*), **156**(*2,6*) was dissolved in neat TFA (2 ml) and the solution was refluxed (for the reaction time see the table below). TFA was evaporated by a stream of nitrogen, ethanol (5 ml) was added and the solution was stirred at room temperature overnight. The precipitated material was filtered off, washed with ethanol and dried.

anthranilate	**156**(*2,5*), **156**(*3,5*)	**156**(*1,5*)	**156**(*1,6*), **156**(*2,6*)	**156**(*12,6*)
reaction. time	2 hours	6 hours	1 hour	8 hours

Method B (using acetic acid)

The cleaved anthranilate **156**(*9,5*) was dissolved in acetic acid (2 ml) and the solution was refluxed for 14 hours. Acetic acid was evaporated and the residual oil was sonified in diethyl ether (5 ml) for 5 minutes. The precipitate material was filtered off, washed with diethyl ether and dried.

Method C (using sulfuric acid)

The cleaved anthranilate **156**(*9,7*), **156**(*10,5*), **156**(*10,7*), **156**(*10,8*), **156**(*10,6*), **156**(*2,7*), **156**(*2,8*), **156**(*1,7*), **156**(*1,8*), **156**(*5,7*), **156**(*3,7*) was dissolved in sulfuric acid (2 ml), the solution was heated at 100 °C for 2 hours and then poured to an ice. After melting of the ice the precipitated material was centrifuged. The material was separated, stirred up with water and centrifuged again. This procedure was repeated until the neutral pH was detected. The resulting product was collected by suction and vacuum dried.

Summary of the cyclization methods study:

Method	**157**(*9,5*)	**157**(*9,7*)	**157**(*10,5*)	**157**(*10,7*)	**157**(*10,8*)	**157**(*10,6*)	**157**(*2,5*)	**157**(*2,7*)
A	-	-	-	-	-	-	+	-
B	+	-	-	-	-	-	-	-
C	+	+	+	+	+	+	+	+
Method for preparation/ crude purity	B/85%	C/95%	C/97%	C/97%	C/97%	C/92%	A/96%	C/75%

Methods of purification of crude hydroxyquinolinones (only in neccessary cases):

- The crude product **157**(*1,8*) was sonified in diethyl ether (5 ml) for 5 minutes, filtered off, washed with diethyl ether and dried.
- The crude product **157**(*2,7*), **157**(*1,7*) was sonified in 10% solution of potassium carbonate in water, filtered off, washed with water and dried.

2-Amino-5-nitro-4-piperidin-1-yl-benzoic acid 2-(4-dipropylamino-3-nitro-phenyl)-2-oxo-ethyl ester (125(*9,12*))

A polypropylene fritted syringe was charged with the resin **125**(*9,8*) (~50mg) and the solution of dipropylamine (0.2 mmol) in DMSO (1 ml) was added. The resin was shaken at room temperature overnight, washed 3 times with DMF and 3 times with DCM. Product was cleaved and identified by HPLC-MS analysis.
Purity of the cleaved product 90%, APCI-MS m/z = 528, $[M+H]^+$

2-(4-Amino-3,5-dichloro-phenyl)-7-chloro-3-hydroxy-6-nitroquinolin-4(1*H*)-one (157(*Cl,5*))

The resin **121** (50 mg) was esterificated with 3,5-dichloro-4-amino-2´-bromacetophenone acccording to above described protocol. The intermediate **125**(*Cl,5*) was cleaved from the resin and resulting anthranilate **156**(*Cl,5*) was dissolved in neat TFA (1 ml). The solution was refluxed for 2 hours. Trifluoracetic acid was evaporated by a stream of nitrogen and product was identified by HPLC-MS analysis.
Purity of the cleaved product 75%, APCI-MS m/z = 400, $[M+H]^+$

Formation of thiourea derivatives (161)

A polypropylene fritted syringe was charged with the resin **129** (~200 mg) and the resin was shaken in 0.15M solution of Fmoc-NCS in THF (2 ml) for 1 hour at room temperature. The resin was washed 5 times with THF and 3 times with DCM.

Closure of thiazole ring (163)

A polypropylene fritted syringe was charged with the resin **161** (~200 mg) and the resin was shaken in 50% piperidine in DMF (2 ml) for 15 minutes at room temperature, washed 3 times with DMF, 3 times with DCM. The resin was shaken in solution of haloketone (0.2 mmol) in DCM (2 ml) for 5 hours at room temperature, washed 3 times with DMF and 3 times with DCM.

Acylation of amino group (168(*9,4,1*))

A polypropylene fritted syringe was charged with the resin **129**(*9,4*) (~100 mg) and the solution of acetic anhydride (1 mmol) in mixture of DCM and pyridine (1:1) was added. The resin was shaken for 6 hours at room temperature, washed 5 times with DCM and cleaved.

Mesylation of amino group (168(*9,4,2*))

A polypropylene fritted syringe was charged with the resin **129**(*9,4*) (~100 mg) and the solution of methane sulfonyl chloride (0.3 mmol) and lutidine (0.33 mmol) in DCM (1 ml) was added. The resin was shaken at room temperature for 8 hours, washed 5 times with DCM and cleaved.

The set of hydroxyquinolinone precursors tested for cyclization

Compound	Purity of cleaved product(LC-MS)	ms $[M+H]^+$
164(*9,4,4*)	98%	513
166(*9,4*)	98%	354
156(*Cl,4*)	97%	335
156(*9,4*)	95%	384
156(*9,6*)	92%	398
156(*9,7*)	95%	429

Anthranilate precursors tested for polyphosphoric acid-mediated cyclization

Compound	Purity of cleaved product(LC-MS)	ms $[M+H]^+$
168(*9,4,1*)	98%	396
168(*9,4,2*)	98%	432

Anthranilate precursors tested for P_2O_5-mediated cyclization

Compound	Purity of cleaved product(LC-MS)	ms $[M+H]^+$
164(*9,11,4*)	98%	493
164(*2,4,9*)	98%	441
164(*4,10,12*)	97%	513

- synthesis of quinazolines via 2H-indazole-1-oxides

Quinazoline-2,4-dicarboxylic acid diethyl ester (175)

Solution of diethyl iminodiacetate (1 mmol) in DCM (2 ml) and solution of 2-nitrobenzenesulfonyl chloride (1 mmol) in DCM (1 ml) were combined. Lutidine (1 mmol) was added, and the reaction mixture was kept at ambient temperature overnight. The DCM was evaporated by a stream of nitrogen, and the residual material was dissolved in anhydrous DMF and DBU (4 mmol) and added. The reaction mixture

remained at ambient temperature overnight. The product was isolated after purification by semi-preparative HPLC.

Synthesis of the resins **178** was carried out according to published protocol.[151]

Alkylation with bromoketones (179)

The polypropylene fritted syringe was charged with the resin **178** (~250 mg), the resin was washed 3 times with DCM and 3 times with DMF. A solution of bromoketone (1.5 mmol) and DIEA (3 mmol) in DMF (3 ml) was added and shaken at room temperature for 7 hours. Alkylation of resins **178** prepared from 4-CF_3-2-Nos-Cl and 4-NO_2-2-Nos-Cl and alkylation with 4-CN and 3-NO_2 bromoketones was complete in 30 min. The resin was washed 3 times with DMF and 3 times with DCM.

Quinazolines (182)

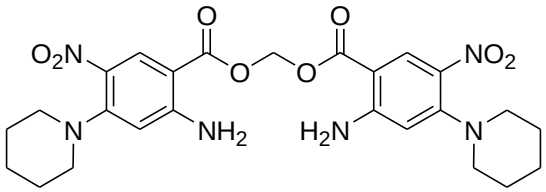
The polypropylene fritted syringe was charged with the resin **179** (~250 mg), the resin was washed 3 times with DCM and 3 times with anhydrous DMF. Cyclization was carried out at ambient temperature in DBU solution in DMF (5 ml): Method A: 0.2M DBU, overnight; Method B: 0.1M DBU, 1.5h; Method C: 0.1M DBU, 10 min. The resin was washed 3 times with DMF, 3 times with DCM, 2 times with 5% AcOH in DCM, 5 times with DCM, 3 times with MeOH and the product was isolated.

Isolation

The resin was treated with 50% TFA in DCM and shaken at room temperature for 1 hour. The cleavage cocktail was collected and the content of the syringe was washed 2 times with 50% TFA in DCM. The washes were collected and evaporated by a stream of nitrogen. Residual material was dissolved in AcCN and purified by semi-preparative HPLC.

6.3. Analytical data

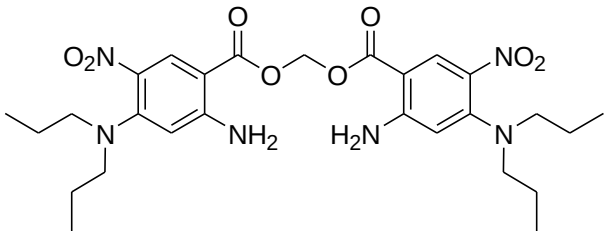
{[2-Amino-5-nitro-4-(piperidin-1-yl)phenyl]carbonyloxy}methyl 2-amino-5-nitro-4-(piperidin-1-yl)benzoate (124(*9*))



Yield of the purified product 2%, purity of the crude product 20%, purity of the purified product 95%

^{1}H NMR (300 MHz ,DMSO-d_6) δ = 8.43 (s, 2 H), 7.46 (s, 4 H), 6.35 (s, 2 H), 6.08 (s, 2 H), 2.95 (br. s., 8 H), 1.58 (br. s., 12 H).
APCI-MS m/z = 543, $[M+H]^+$

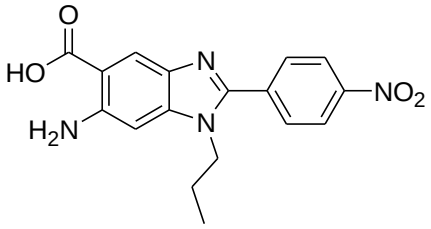
{[2-Amino-5-nitro-4-(dipropylamino)phenyl]carbonyloxy}methyl 2-amino-5-nitro-4-(dipropylamino)benzoate (124(*8*))



Yield of the crude product 7%, purity of the crude product 52%, purity of the purified product 96%

^{1}H NMR (300 MHz ,DMSO-d_6) δ = 8.34 - 8.29 (m, 2 H), 6.34 (s, 2 H), 6.05 (s, 2 H), 3.03 (t, *J* = 7.0 Hz, 8 H), 1.59 - 1.43 (m, 8 H), 0.79 (t, *J* = 7.3 Hz, 12 H).
APCI-MS m/z = 575, $[M+H]^+$

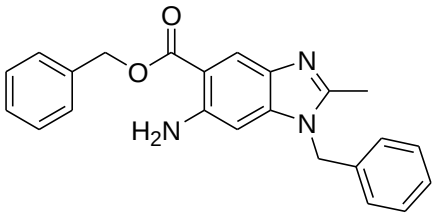
6-Amino-2-(4-nitro-phenyl)-1-propyl-1*H*-benzoimidazole-5-carboxylic acid (131(*1,H,2*))



Yield of the purified product 35%, purity of the crude product 75%, purity of the purified product 98%

^{1}H NMR (300 MHz, Pyr-d_5) δ = 9.14 (s, 1 H), 8.36 (d, *J* = 8.8 Hz, 2 H), 8.07 (d, *J* = 8.8 Hz, 2 H), 7.06 (s, 1 H), 4.04 (t, *J* = 7.7 Hz, 2 H), 1.75 - 1.60 (m, 2 H), 0.70 (t, *J* = 7.4 Hz, 3 H). ^{13}C NMR (75 MHz, Pyr-d_5) δ = 173.3, 171.9, 151.4, 149.8, 148.2, 141.9, 137.3, 130.1, 124.6, 124.1, 94.9, 46.4, 22.8, 21.2, 10.9.
APCI-MS m/z = 341, $[M+H]^+$

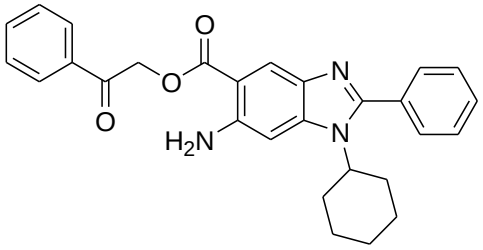
6-Amino-1-benzyl-2-methyl-1*H*-benzoimidazole-5-carboxylic acid benzyl ester (131(*4,1,3*))



Yield of the purified product 11%, purity of the crude product 60%, purity of the purified product 94%

^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.97 (s, 1 H), 7.53 - 7.30 (m, 8 H), 7.09 (s, 1 H), 7.07 (s, 1 H), 6.60 (s, 1 H), 6.40 (br. s., 2 H), 5.29 (s, 2 H), 5.28 (s, 2 H), 2.41 (s, 3 H). ^{13}C NMR (101 MHz , DMSO-d_6) δ = 168.1, 148.5, 137.3, 137.2, 129.4, 129.1, 128.5, 128.3, 128.3, 128.1, 127.6, 127.0, 121.0, 106.2, 94.9, 65.7, 49.1, 46.7, 14.1.
APCI-MS m/z = 372, $[M+H]^+$

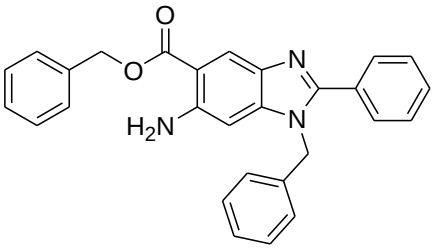
6-Amino-1-cyclohexyl-2-phenyl-1*H*-benzoimidazole-5-carboxylic acid 2-oxo-2-phenyl-ethyl ester (131(*7,4,1*))



Yield of the purified product 37%, purity of the crude product 70%, purity of the purified product 97%

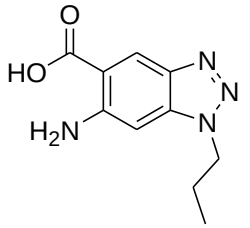
^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.18 (s, 1 H), 8.03 (d, *J* = 7.1 Hz, 2 H), 7.76 - 7.53 (m, 8 H), 7.16 (s, 1 H), 6.44 (br. s., 2 H), 5.71 (s, 2 H), 4.22 - 4.10 (m, 1 H), 2.30 - 2.14 (m, 2 H), 1.94 - 1.77 (m, 5 H), 1.33 - 1.18 (m, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 193.9, 167.5, 154.6, 148.1, 140.0, 135.6, 134.6, 134.5, 131.1, 130.2, 129.7, 129.5, 129.3, 128.3, 122.5, 106.5, 97.6, 67.1, 56.7, 30.5, 26.0, 25.2.
APCI-MS m/z = 454, $[M+H]^+$

6-Amino-1-benzyl-2-phenyl-1*H*-benzoimidazole-5-carboxylic acid benzyl ester (131(*4,1,1*))



Yield of the purified product 4%, purity of the crude product 30%, purity of the purified product 96%
^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.17 (s, 1 H), 7.68 - 7.59 (m, 2 H), 7.51 - 7.45 (m, 5 H), 7.40 (t, *J* = 7.2 Hz, 2 H), 7.37 - 7.21 (m, 4 H), 7.01 (d, *J* = 7.4 Hz, 2 H), 6.59 (s, 1 H), 6.52 (s, 2 H), 5.38 (s, 2 H), 5.32 (s, 2 H). ^{13}C NMR (101 MHz , DMSO-d_6) δ = 167.9, 154.4, 148.8, 142.1, 137.1, 137.0, 134.7, 130.3, 130.2, 129.4, 129.2, 129.1, 129.0, 128.4, 128.2, 127.9, 126.3, 122.2, 107.2, 95.2, 65.8, 47.9.
APCI-MS m/z = 434, $[M+H]^+$

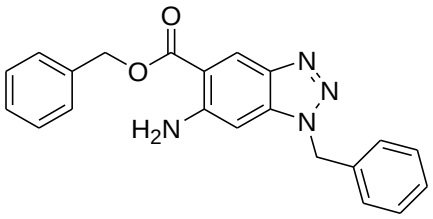
6-Amino-1-propyl-1*H*-benzotriazole-5-carboxylic acid (132(*1,H*))



Yield of the purified product 75%, purity of the crude product 85%, purity of the purified product 98%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.43 (s, 1 H), 6.76 (s, 1 H), 4.44 (t, *J* = 6.9 Hz, 2 H), 1.95 - 1.79 (m, 2 H), 0.85 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 169.8, 150.6, 138.6, 137.1, 124.2, 112.1, 90.9, 49.0, 22.7, 11.6.
APCI-MS m/z = 221, $[M+H]^+$

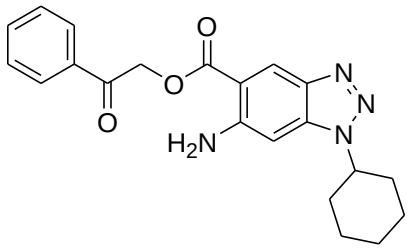
6-Amino-1-benzyl-1H-benzotriazole-5-carboxylic acid benzyl ester (132(*4,1*))



Yield of the purified product 45%, purity of the crude product 95%, purity of the purified product 98%

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.50 (s, 1 H), 7.52 - 7.46 (m, *J* = 7.0 Hz, 2 H), 7.42 - 7.26 (m, 6 H), 7.25 - 7.20 (m, *J* = 7.0 Hz, 2 H), 6.78 (br. s., 2 H), 6.72 (s, 1 H), 5.75 (s, 2 H), 5.33 (s, 2 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 167.2, 150.5, 138.7, 137.0, 136.6, 136.2, 129.3, 129.0, 128.5, 128.4, 128.4, 127.8, 124.0, 111.1, 91.3, 66.4, 50.9.
APCI-MS m/z = 359, $[M+H]^+$

6-Amino-1-cyclohexyl-1H-benzotriazole-5-carboxylic acid 2-oxo-2-phenyl-ethyl ester (132(*7,4*))

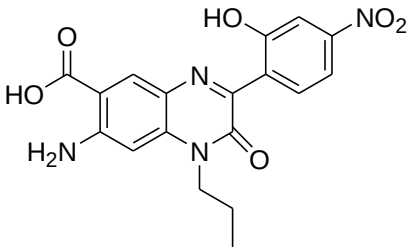


Yield of the purified product 42%, purity of the crude product 90%, purity of the purified product 95%

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.56 (s, 1 H), 8.01 (d, *J* = 7.4 Hz, 2 H), 7.70 (t, *J* = 7.4 Hz, 1 H), 7.57 (t, *J* = 7.6 Hz, 2 H), 6.90 (s, 1 H), 6.69 (br. s., 2 H), 5.74 (s, 2 H), 4.57 (t, *J* = 11.3 Hz, 1 H), 2.11 - 2.00 (m, 2 H), 2.00 - 1.81 (m, 4 H), 1.70 (s, 1 H), 1.48 (q, *J* = 12.5 Hz, 2 H), 1.35 - 1.18 (m, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 193.4, 166.9, 150.1, 138.7, 136.4, 134.5, 134.3, 129.4, 128.3, 124.1, 110.7, 91.6, 67.4, 57.8, 32.2, 25.3.

APCI-MS m/z = 379, $[M+H]^+$

7-Amino-3-(2-hydroxy-4-nitro-phenyl)-2-oxo-1-propyl-1,2-dihydro-quinoxaline-6-carboxylic acid (132(*1,H,2*))

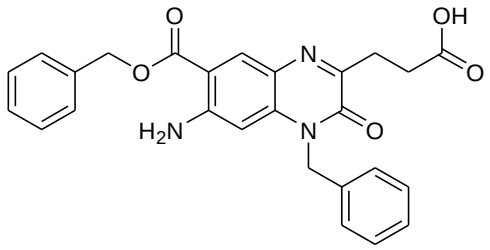


Yield of the purified product 20%, purity of the crude product 90%, purity of the purified product 97%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 13.38 (b.s. 1 H), 9.32 (d, *J* = 2.9 Hz, 1 H), 8.13 (s, 2 H), 7.07 (s, 1 H), 6.75 (s, 1 H), 4.04 (t, *J = 7.3* Hz, 2 H), 1.75 – 1.63 (m, 2 H), 0.99 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 169.1, 164.9, 154.6, 153.8, 145.7, 139.4, 137.7, 133.7, 127.2, 126.9, 122.1, 120.8, 118.1, 97.9, 44.3, 21.6, 20.1, 11.8.

APCI-MS m/z = 385, $[M+H]^+$

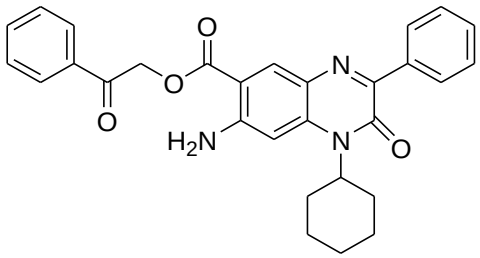
7-Amino-1-benzyl-3-(2-carboxy-ethyl)-2-oxo-1,2-dihydro-quinoxaline-6-carboxylic acid benzyl ester (132(4,*1*,*3*))



Yield of the purified product 53%, purity of the crude product 95%, purity of the purified product 95%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.07 (s, 1 H), 7.51 - 7.21 (m, 10 H), 7.08 (s, 2 H), 6.59 (s, 1 H), 5.37 - 5.24 (m, 4 H), 2.99 (t, *J* = 6.9 Hz, 2 H), 2.66 (t, *J* = 6.9 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 166.8, 155.2, 153.8, 152.3, 138.0, 136.8, 135.9, 133.0, 129.2, 129.1, 128.7, 128.6, 127.9, 127.4, 123.9, 106.7, 99.1, 66.3, 45.5, 41.0, 30.4, 28.4.
APCI-MS m/z = 458, $[M+H]^+$

7-Amino-1-cyclohexyl-2-oxo-3-phenyl-1,2-dihydro-quinoxaline-6-carboxylic acid 2-oxo-2-phenyl-ethyl ester (132(*7*,*4*,*1*))



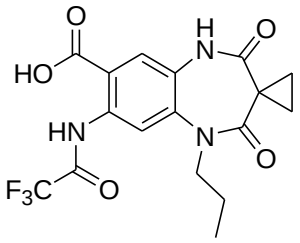
Yield of the purified product 28%, purity of the crude product 80%, purity of the purified product 96%

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.62 (s, 1 H), 8.22 - 8.13 (m, 2 H), 7.99 (d, *J* = 7.3 Hz, 2 H), 7.69 - 7.61 (m, 1 H), 7.57 - 7.41 (m, 6 H), 6.68 (br. s., 1 H), 5.58 (s, 2 H), 1.96 (s, 2 H), 1.89 - 1.71 (m, 4 H), 1.56 - 1.25 (m, 4 H). ^{13}C NMR (75 MHz , $CDCl_3$) δ = 192.4,

166.4, 155.6, 151.3, 136.1, 135.3, 134.1, 134.0, 129.5, 129.2, 128.9, 127.9, 127.9, 126.0, 107.3, 66.3, 40.8, 28.2, 26.4, 25.3.

APCI-MS m/z = 482, $[M+H]^+$

2,4-Dioxo-1-propyl-8-(2,2,2-trifluoro-acetylamino)-1,2,4,5-tetrahydro-spiro[benzo[b][1,4]diazepine-3,1'-cyclopropane]-7-carboxylic acid (134(*1*,*H*,*5*))

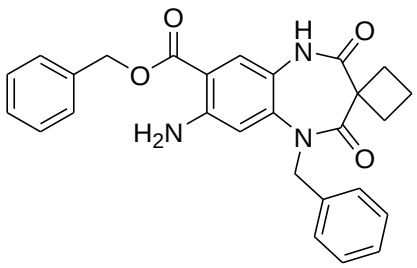


Yield of the purified product 19%, purity of the crude product 90%, purity of the purified product 94%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.49 (s, 1 H), 7.83 (s, 1 H), 1.63 - 1.42 (m, 3 H), 1.31 - 1.06 (m, 1 H), 1.06 - 0.83 (m, 4 H), 0.75 (t, *J* = 7.1 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.0, 168.7, 167.5, 154.8, 154.4, 137.2, 135.2, 128.2, 125.0, 123.4, 113.6, 50.1, 31.8, 21.2, 11.4.

APCI-MS m/z = 398, $[M-H]^-$

2,4-Dioxo-1-benzyl-8-amino-1,2,4,5-tetrahydro-spiro[benzo[b][1,4]diazepine-3,1'-cyclobutane]-7-carboxylic acid benzyl ester (134(*4*,*1*,*6*))



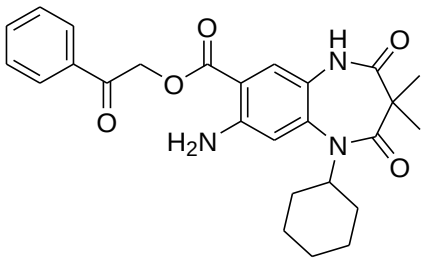
Yield of the purified product 20%, purity of the crude product 78%, purity of the purified product 95%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.11 (s, 1 H), 7.58 (s, 1 H), 7.51 - 7.17 (m, 9 H), 7.10 (d, *J* = 7.1 Hz, 2 H), 6.83 (s, 1 H), 6.57 (br. s., 2 H), 5.31 - 5.21 (m, 3 H), 4.93 (d, *J*

= 16.1 Hz, 1 H), 2.74 - 2.54 (m, 2 H), 2.03 - 1.90 (m, 1 H), 1.90 - 1.76 (m, 1 H), 1.72 - 1.56 (m, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.0, 169.6, 166.6, 149.3, 139.5, 137.8, 136.7, 129.1, 128.7, 128.6, 127.5, 126.8, 124.6, 121.3, 108.7, 107.5, 66.2, 53.7, 51.7, 29.9, 24.6, 15.4.

APCI-MS m/z = 456, $[M+H]^+$

8-Amino-1-cyclohexyl-3,3-dimethyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[b][1,4]diazepine-7-carboxylic acid 2-oxo-2-phenyl-ethyl ester (134(*7,4,4*))

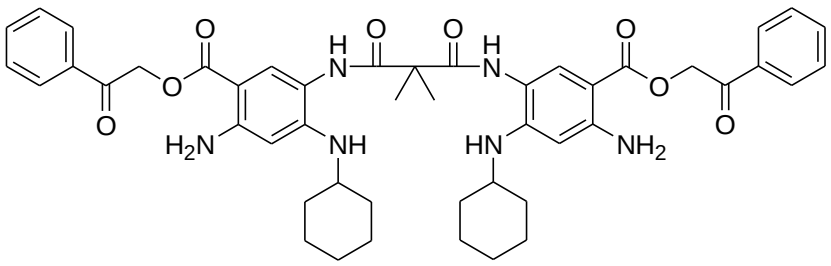


Yield of the purified product 17%, purity of the crude product 50%, purity of the purified product 92%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.05 - 7.96 (m, 3 H), 7.74 - 7.67 (m, 1 H), 7.63 - 7.52 (m, 3 H), 6.04 (s, 1 H), 5.58 (s, 2 H), 1.99 - 1.80 (m, 2 H), 1.80 - 1.63 (m, 2 H), 1.63 - 1.49 (m, 2 H), 1.50 - 1.37 (m, 7 H), 1.36 - 1.10 (m, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 194.1, 175.4, 166.3, 153.9, 147.5, 134.6, 134.4, 130.6, 129.5, 128.3, 128.3, 107.3, 97.6, 95.9, 66.5, 59.8, 51.3, 32.3, 25.9, 24.9, 18.0.

APCI-MS m/z = 464, $[M+H]^+$

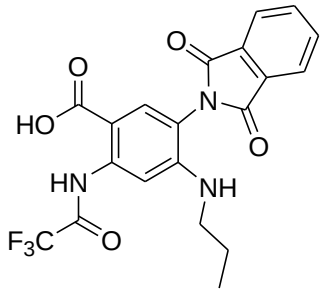
2-Oxo-2-phenylethyl-2-amino-5-(2-{[4-amino-2-(cyclohexylamino)-5-[(2-oxo-2-phenylethoxy)carbonyl]phenyl]carbamoyl}-2,2-dimethylacetamido)-4-(cyclohexylamino)benzoate (151(*7,4,4*))



Yield of the purified product 2%, purity of the crude product 25%, purity of the purified product 93%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.92 (s, 2 H), 8.00 (d, *J* = 7.3 Hz, 4 H), 7.70 (t, *J* = 7.4 Hz, 2 H), 7.57 (t, *J* = 7.6 Hz, 4 H), 7.43 (s, 2 H), 5.95 (s, 2 H), 5.59 - 5.53 (m, 4 H), 3.16 (br. s., 2 H), 2.01 - 1.85 (m, 4 H), 1.79 - 1.64 (m, 4 H), 1.56 (s, 6 H), 1.50 - 1.08 (m, 12 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 194.2, 174.6, 166.6, 153.0, 149.3, 134.7, 134.4, 131.2, 129.4, 128.3, 113.4, 97.0, 95.1, 66.2, 51.3, 51.1, 32.7, 25.9, 25.0, 23.9.
APCI-MS m/z = 831, $[M+H]^+$

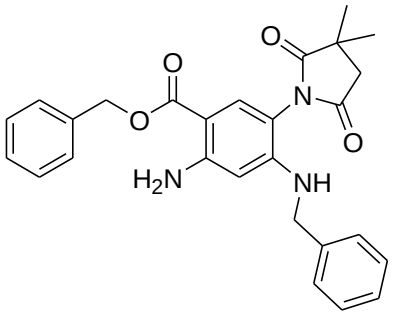
5-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-propylamino-2-(2,2,2-trifluoro-acetylamino)- benzoic acid (136(*1,H,3*))



Yield of the purified product 22%, purity of the crude product 80%, purity of the purified product 96%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 13.22 (br. s., 1 H), 8.00 - 7.78 (m, 6 H), 7.00 (t, *J* = 5.4 Hz, 1 H), 3.07 (q, *J* = 6.2 Hz, 2 H), 1.54 (qd, *J* = 7.1, 14.2 Hz, 2 H), 0.86 (t, *J* = 7.3 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.3, 168.1, 151.1, 141.5, 134.6, 134.4, 133.3, 123.6, 114.2, 113.1, 103.9, 101.1, 44.4, 21.6, 11.8.
APCI-MS m/z = 434, $[M-H]^-$

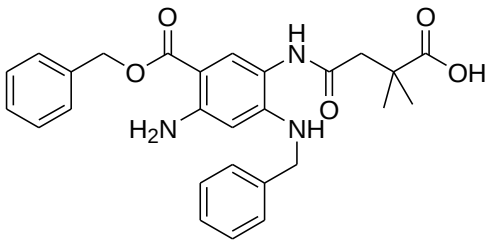
2-Amino-4-benzylamino-5-(3,3-dimethyl-2,5-dioxo-pyrrolidin-1-yl)-benzoic acid benzyl ester (136(*4,1,1*))



Yield of the purified product 24%, purity of the crude product 67%, purity of the purified product 93%

^{1}H NMR (400MHz, DMSO-d_6) δ = 7.35 (d, *J* = 4.4 Hz, 4 H), 7.33 - 7.16 (m, 7 H), 6.61 (s, 2 H), 6.47 (t, *J* = 6.2 Hz, 1 H), 5.73 (s, 1 H), 5.24 (d, *J* = 12.8 Hz, 1 H), 5.16 (d, *J* = 12.8 Hz, 1 H), 4.29 (d, *J* = 6.2 Hz, 2 H), 2.76 (d, *J* = 17.6 Hz, 1 H), 2.61 (d, *J* = 17.6 Hz, 1 H), 1.35 (s, 3 H), 1.28 (s, 3 H). ^{13}C NMR (101 MHz , DMSO-d_6) δ = 183.8, 176.5, 166.9, 153.9, 149.4, 139.6, 137.5, 132.3, 128.9, 128.7, 128.3, 128.2, 127.0, 126.8, 108.5, 98.1, 95.2, 64.9, 45.4, 43.4, 26.5, 24.8.
APCI-MS m/z = 458, $[M+H]^+$

2-Amino-4-benzylamino-5-(3-carboxy-3-methyl-butyrylamino)-benzoic acid benzyl ester (153(*4,1,1*))



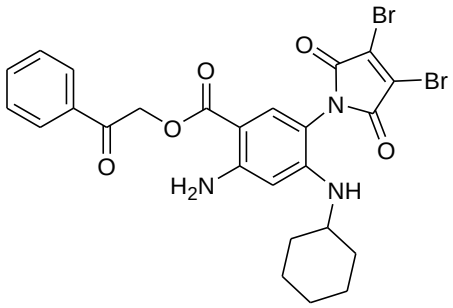
Yield of the purified product 18%, purity of the crude product 65%, purity of the purified product 95%

^{1}H NMR (300MHz, DMSO-d_6) δ = 8.98 (s, 1 H), 7.41 - 7.28 (m, 11 H), 6.46 (br. s., 2 H), 6.06 (t, *J* = 5.8 Hz, 1 H), 5.76 (s, 1 H), 5.20 (s, 2 H), 4.31 (d, *J* = 5.9 Hz, 2 H), 2.53 (s, 2 H), 1.19 (s, 6 H). ^{13}C NMR (75MHz ,DMSO-d_6) δ = 179.0, 170.9, 167.2, 152.4,

149.2, 139.7, 137.7, 129.7, 129.0, 128.8, 128.3, 128.1, 127.4, 127.3, 113.8, 97.6, 95.5, 64.9, 46.2, 45.5, 40.8, 26.0.

APCI-MS m/z = 476, $[M+H]^+$

2-Amino-4-cyclohexylamino-5-(3,4-dibromo-2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-benzoic acid 2-oxo-2-phenyl-ethyl ester (136(*7,4,2*))

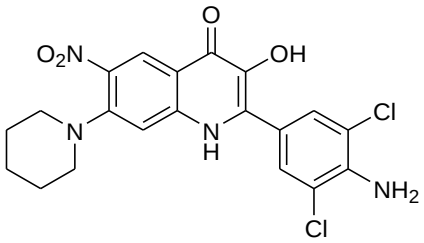


Yield of the purified product 18%, purity of the crude product 77%, purity of the purified product 95%

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.02 (d, *J* = 7.1 Hz, 2 H), 7.72 (t, *J* = 7.5 Hz, 1 H), 7.63 (s, 1 H), 7.59 (t, *J* = 7.5 Hz, 2 H), 6.65 (br. s., 2 H), 6.00 (s, 1 H), 5.91 (d, *J* = 7.5 Hz, 1 H), 5.56 (s, 2 H), 3.21 - 3.12 (m, 1 H), 1.94 - 1.86 (m, 2 H), 1.80 - 1.75 (m, 1 H), 1.69 - 1.59 (m, 1 H), 1.38 - 1.08 (m, 6 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 194.0, 166.3, 164.8, 154.3, 149.8, 134.5, 134.3, 134.0, 130.3, 129.4, 128.2, 106.6, 97.4, 94.7, 66.2, 51.6, 32.7, 25.9, 25.4.

APCI-MS m/z = 606, $[M+H]^+$

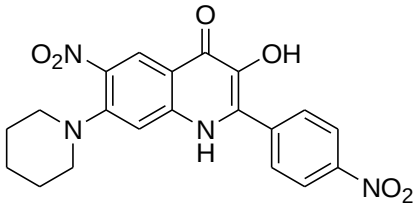
2-(4-Amino-3,5-dichlorophenyl)-3-hydroxy-6-nitro-7-(piperidin-1-yl)quinolin-4(1*H*)-one (157(*9,5*))



Yield of the purified product 70%, purity of the purified product 93%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.35 (s, 1 H), 8.50 (s, 1 H), 7.78 (s, 2 H), 7.27 (s, 1 H), 6.07 (s, 2 H), 3.06 - 2.93 (m, 4 H), 1.72 - 1.52 (m, 6 H). ^{13}C NMR could not be collected due to low solubility of the compound in DMSO-d_6.
APCI-MS m/z = 449, $[M+H]^+$

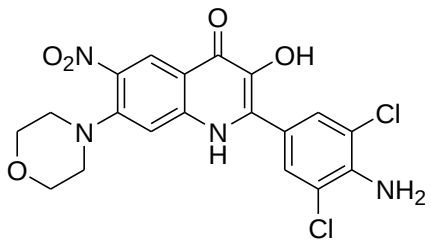
3-Hydroxy-6-nitro-2-(4-nitrophenyl)-7-(piperidin-1-yl)quinolin-4(1*H*)-one (157(*9*,*7*))



Yield of the crude product 38%, purity of the crude product 95%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.69 (s, 1 H), 8.54 (s, 1 H), 8.41 (d, *J*=8.97 Hz, 2 H), 8.08 (d, *J*=8.97 Hz, 2 H), 7.25 (s, 1 H), 3.05 - 2.95 (m, 4 H), 1.72 - 1.52 (m, 6 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.18, 147.27, 140.99, 138.55, 138.37, 138.20, 130.37, 129.04, 124.45, 123.22, 114.66, 106.28, 51.93, 25.13, 23.30.
APCI-MS m/z = 411, $[M+H]^+$

2-(4-Amino-3,5-dichlorophenyl)-3-hydroxy-7-(morpholin-4-yl)-6-nitroquinolin-4(1*H*)-one (157(*10*,*5*))

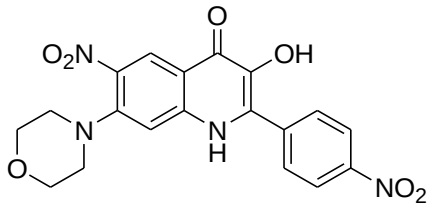


Yield of the crude product 43%, purity of the crude product 97%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.44 (s, 1 H), 8.56 (s, 1 H), 7.78 (s, 2 H), 7.30 (s, 1 H), 3.78 - 3.71 (m, 4 H), 3.04 - 2.98 (m, 4 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.08, 146.33, 142.09, 138.06, 137.68, 129.55, 128.38, 124.52, 119.54, 117.32, 115.10, 106.88, 65.79, 51.25.

APCI-MS m/z = 451, $[M+H]^+$

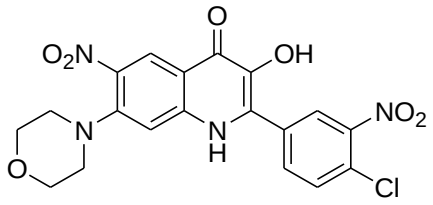
3-Hydroxy-7-(morpholin-4-yl)-6-nitro-2-(4-nitrophenyl)quinolin-4(1*H*)-one (157(*10,7*))



Yield of the crude product 20%, purity of the crude product 97%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.76 (br. s., 1 H), 8.59 (s, 1 H), 8.40 (d, *J*=8.97 Hz, 2 H), 8.08 (d, *J*=8.78 Hz, 2 H), 7.27 (s, 1 H), 3.81 - 3.69 (m, 4 H), 3.08 - 2.93 (m, 4 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.19, 147.35, 146.56, 140.94, 138.75, 138.25, 130.40, 129.21, 124.70, 123.23, 115.23, 106.76, 65.76, 51.15.
APCI-MS m/z = 413, $[M+H]^+$

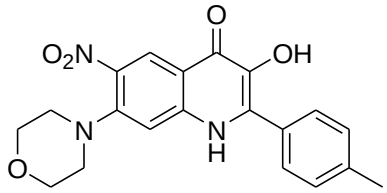
2-(4-Chloro-3-nitrophenyl)-3-hydroxy-7-(morpholin-4-yl)-6-nitroquinolin-4(1*H*)-one (157(*10,8*))



Yield of the crude product 49%, purity of the crude product 95%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.76 (s, 1 H), 8.58 (s, 1 H), 8.49 (d, *J*=2.01 Hz, 1 H), 8.13 (dd, *J*=8.42, 2.01 Hz, 1 H), 8.00 (d, *J*=8.60 Hz, 1 H), 7.23 (s, 1 H), 3.81 - 3.69 (m, 4 H), 3.06 - 2.98 (m, 4 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.23, 146.59, 140.86, 138.62, 134.24, 131.54, 130.64, 128.08, 127.71, 125.84, 124.73, 115.31, 106.70, 65.76, 51.49.
APCI-MS m/z = 447, $[M+H]^+$

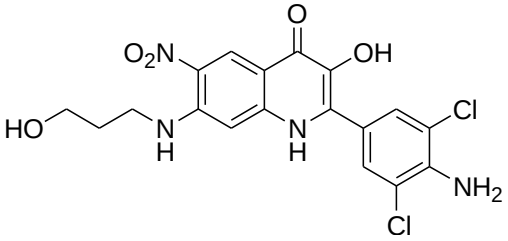
3-Hydroxy-2-(4-methylphenyl)-7-(morpholin-4-yl)-6-nitroquinolin-4(1*H*)-one (157(*10,6*))



Yield of the crude product 23%, purity of the crude product 92%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.59 (s, 1 H), 8.59 (s, 1 H), 7.71 (d, *J*=8.05 Hz, 2 H), 7.38 (d, *J*=8.05 Hz, 2 H), 7.33 (s, 1 H), 3.79 - 3.68 (m, 4 H), 2.95 - 3.05 (m, 4 H), 2.40 (s, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 169.58, 146.30, 140.64, 139.18, 137.80, 132.23, 128.78, 124.51, 115.13, 113.66, 106.99, 65.77, 51.22, 20.88.
APCI-MS m/z = 382, $[M+H]^+$

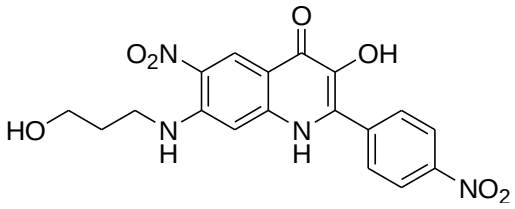
2-(4-Amino-3,5-dichlorophenyl)-3-hydroxy-7-[(3-hydroxypropyl)amino]-6-nitroquinolin-4(1*H*)-one (157(*2,5*))



Yield of the crude product 36%, purity of the crude product 96%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.02 (s, 1 H), 8.85 (s, 1 H), 8.63 (br. s., 1 H), 7.96 (t, *J*=4.67 Hz, 1 H), 7.77 (s, 2 H), 6.88 (s, 1 H), 6.05 (s, 2 H), 4.73 (br. s., 1 H), 3.58 (t, *J*=5.31 Hz, 2 H), 1.85 (quin, *J*=6.04 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.47, 144.38, 142.77, 141.97, 136.13, 130.27, 129.34, 128.27, 125.81, 119.76, 117.30, 112.31, 96.64, 58.74, 30.55.
APCI-MS m/z = 439, $[M+H]^+$

3-Hydroxy-7-[(3-hydroxypropyl)amino]-6-nitro-2-(4-nitrophenyl)quinolin-4(1*H*)-one (157(*2,7*))

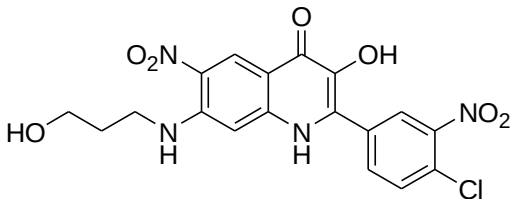


Yield of the purified product 45%, purity of the purified product 79%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.89 (s, 1 H), 8.39 (d, *J*=7.87 Hz, 2 H), 8.09 (dd, *J*=8.60, 3.66 Hz, 2 H), 6.90 (d, *J*=10.25 Hz, 1 H), 3.61 - 3.52 (m, 2 H), 1.90 - 1.77 (m, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.57, 147.22, 144.51, 142.71, 138.14, 137.11, 130.30, 125.92, 123.13, 112.26, 96.64, 63.53, 58.78, 30.45, 27.72.

APCI-MS m/z = 401, $[M+H]^+$

2-(4-Chloro-3-nitrophenyl)-3-hydroxy-7-[(3-hydroxypropyl)amino]-6-nitroquinolin-4(1*H*)-one (157(*2,8*))

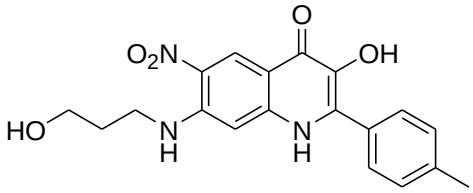


Yield of the crude product 91%, purity of the crude product 92%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.89 (s, 1 H), 8.51 (s, 1 H), 8.14 (d, *J*=9.70 Hz, 1 H), 7.98 (d, *J*=8.23 Hz, 1 H), 7.87 (t, *J*=5.03 Hz, 1 H), 6.87 (s, 1 H), 3.87 (t, *J*=5.95 Hz, 2 H), 2.01 -1.87 (m, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 171.01, 147.27, 144.48, 142.88, 137.17, 134.10, 132.19, 131.46, 130.61, 127.94, 126.11, 125.78, 125.30, 112.57, 96.76, 63.39, 27.76.

APCI-MS m/z = 435, $[M+H]^+$

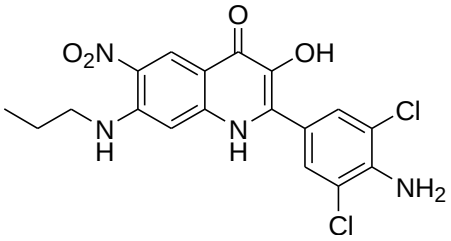
3-Hydroxy-7-[(3-hydroxypropyl)amino]-2-(4-methylphenyl)-6-nitroquinolin-4(1*H*)-one (157(*2*,*6*))



Yield of the crude product 43%, purity of the crude product 98%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.15 (s, 1 H), 8.88 (s, 1 H), 7.96 (t, *J*=4.76 Hz, 1 H), 7.70 (d, *J*=1.00 Hz, 2 H), 7.36 (d, *J*=1.00 Hz, 2 H), 6.91 (s, 1 H), 3.58 (t, *J*=5.95 Hz, 2 H), 2.39 (s, 3 H), 1.84 (quin, *J*=6.22 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.78, 144.35, 142.82, 138.94, 136.35, 131.44, 130.33, 129.07, 128.73, 125.93, 112.45, 96.69, 58.76, 30.53, 20.88.
APCI-MS m/z = 370, $[M+H]^+$

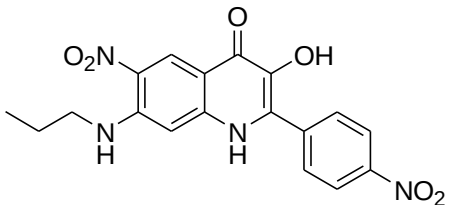
2-(4-Amino-3,5-dichlorophenyl)-3-hydroxy-6-nitro-7-(propylamino)quinolin-4(1*H*)-one (157(*1*,*5*))



Yield of the purified product 42%, purity of the purified product 99%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.01 (s, 1 H), 8.85 (s, 1 H), 8.64 (br. s., 1 H), 7.82 (t, *J*=4.67 Hz, 1 H), 7.76 (s, 2 H), 6.88 (s, 1 H), 6.07 (br. s., 2 H), 3.30 - 3.20 (m, 2 H), 1.75 - 1.64 (sxt, *J*=7.06 Hz, 2 H), 0.99 (t, *J*=7.32 Hz, 3 H). ^{13}C NMR could not be collected due to low solubility of the compound in DMSO-d_6.
APCI-MS m/z = 423, $[M+H]^+$

3-Hydroxy-6-nitro-2-(4-nitrophenyl)-7-(propylamino)quinolin-4(1*H*)-one (157(*1,7*))

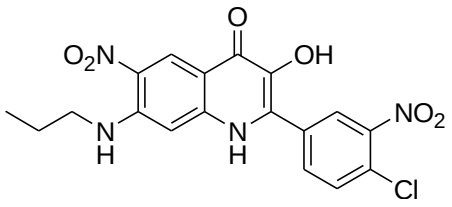


Yield of the purified product 23%, purity of the purified product 93 %

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.33 (s, 1 H), 9.05 (s, 1 H), 8.90 (s, 1 H), 8.40 (d, *J*=8.97 Hz, 2 H), 8.08 (d, *J*=8.96 Hz, 2 H), 7.87 (t, *J*=5.58 Hz, 1 H), 6.87 (s, 1 H), 3.25 (q, *J*=6.34 Hz, 2 H), 1.76 - 1.65 (m, 2 H), 0.98 (t, *J*=7.32 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 147.21, 144.35, 142.91, 138.34, 137.35, 130.42, 130.24, 128.77, 126.11, 123.16, 112.50, 96.57, 44.37, 20.79, 11.29.

APCI-MS m/z = 385, $[M+H]^+$

2-(4-Chloro-3-nitrophenyl)-3-hydroxy-6-nitro-7-(propylamino)quinolin-4(1*H*)-one (157(*1,8*))

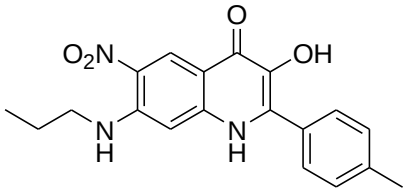


Yield of the purified product 40%, purity of the purified product 97%

^{1}H NMR (300 MHz, DMSO-d_6) 11.30 (s, 1 H), 8.88 (s, 1 H), 8.48 (d, *J*=2.01 Hz, 1 H), 8.12 (dd, *J*=8.60, 2.01 Hz, 1 H), 7.98 (d, *J*=8,7 Hz, 1 H), 7.88 - 7.82 (m, 1H), 6.81 (s, 1 H), 3.19 - 3.30 (m, 2 H), 1.79 - 1.61 (m, 2 H), 0.98 (t, *J*=7.41 Hz, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 171.04, 147.29, 144.52, 142. 90, 137.17, 134.15, 132.16, 130.58, 127.90, 125.74, 125.36, 112.58, 96.57, 44.37, 20.78, 11.29.

APCI-MS m/z = 419, $[M+H]^+$

3-Hydroxy-2-(4-methylphenyl)-6-nitro-7-(propylamino)quinolin-4(1*H*)-one (157(*1,6*))

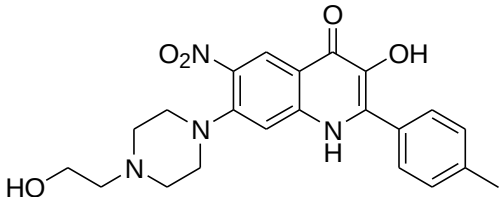


Yield of the purified product 62%, purity of the purified product 96%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.16 (s, 1 H), 8.88 (s, 1 H), 7.69 (d, *J*=7.87 Hz, 2 H), 7.33 (d, J=8.2 Hz, 2 H), 6.91 (s, 1 H), 2.39 (s, 3 H), 1.75 – 1.65 (m, 2 H), 0.98 (t, *J*=7.32 Hz, 3 H). ^{13}C NMR could not be collected due to low solubility of the compound in DMSO-d_6.

APCI-MS m/z = 354, $[M+H]^+$

3-Hydroxy-7-[4-(2-hydroxy-ethyl)-piperazin-1-yl]-6-nitro-2-p-tolylquinolin-4(1*H*)-one (157(*12,6*))

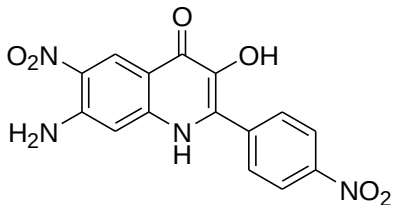


Yield of the purified product 68%, purity of the purified product 90%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.69 (s, 1 H), 8.71 (s, 1 H), 7.71 (d, *J*=7.87 Hz, 2 H), 7.46 - 7.33 (m, 2 H), 5.44 (br. s., 1 H), 3.82 – 3.50 (m, 4 H), 3.32 - 3.14 (m, 8 H), 2.41 (s, 3H). ^{13}C NMR could not be collected due to low solubility of the compound in DMSO-d_6.

APCI-MS m/z = 425, $[M+H]^+$

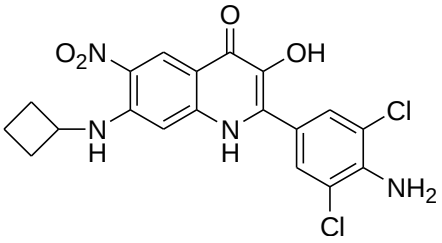
7-Amino-3-hydroxy-6-nitro-2-(4-nitro-phenyl)quinolin(1*H*)-4-one (157(*17,7*))



Yield of the crude product 20%, purity of the crude product 90%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.85 (s, 1 H), 8.38 (d, *J*=8.78 Hz, 2 H), 8.04 (d, *J*=8.78 Hz, 2 H), 6.95 (s, 1 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 171.09, 147.27, 145.83, 142.52, 138.36, 136.80, 130.53, 130.34, 129.49, 125.51, 123.10, 113.36.
APCI-MS m/z = 341, $[M+H]^+$

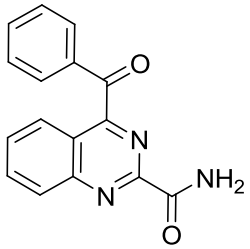
2-(4-Amino-3,5-dichloro-phenyl)-7-(cyclobutylamino)-3-hydroxy-6-nitroquinolin-4(1*H*)-one (157(*3,5*))



Yield of the purified product 30%, purity of the purified product 94%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.05 (s, 1 H), 8.85 (s, 1 H), 7.75 (s, 2 H), 6.79 (s, 1 H), 6.07 (br. s., 2 H), 3.92 - 4.04 (m, 1 H), 1.98 - 2.12 (m, 2 H), 1.78 - 1.91 (m, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 170.40, 142.90, 142.59, 142.01, 136.24, 130.18, 129.40, 128.29, 125.71, 119.73, 117.30, 112.64, 97.54, 47.76, 29.64, 15.03.
APCI-MS m/z = 435, $[M+H]^+$

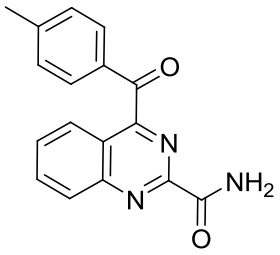
4-Benzoylquinazoline-2-carboxamide (182(*1,1,4*))



Yield of the purified product 23%, purity of the crude product 88%, purity of the purified product 98%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.39 - 8.25 (m, 2 H), 8.19 (t, *J* = 7.5 Hz, 1 H), 8.03 (d, *J* = 8.0 Hz, 1 H), 8.00 - 7.91 (m, 3 H), 7.87 (t, *J* = 8.0 Hz, 1 H), 7.79 (t, *J* = 7.5 Hz, 1 H), 7.60 (t, *J* = 7.7 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO- d_6) δ = 192.8, 165.0, 164.3, 153.7, 150.3, 135.9, 135.1, 134.6, 130.6, 130.5, 129.2, 129.2, 125.6, 121.0.
ESI-MS *m/z* =278[M+H]$^{+}$, HRMS *m/z* calcd for $C_{16}H_{11}N_3O_2$ [M + H]$^{+}$ 278.0924, found 278.0915

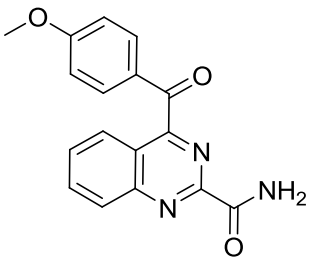
4-(4-Methyl-benzoyl)-quinazoline-2-carboxamide (182(*1,1,6*))



Yield of the purified product 12%, purity of the crude product 95%, purity of the purified product 97%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.36 - 8.23 (m, 2 H), 8.18 (t, *J* = 6.9 Hz, 1 H), 8.03 - 7.93 (m, 2 H), 7.91 - 7.77 (m, 3 H), 7.40 (d, *J* = 8.0 Hz, 2 H), 2.42 (s, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 197.8, 170.7, 169.8, 159.2, 155.7, 151.5, 141.3, 137.7, 136.1, 135.8, 135.2, 134.7, 131.1, 126.4.
ESI-MS *m/z* = 292, [M+H]$^{+}$, HRMS *m/z* calcd for $C_{17}H_{13}N_3O_2$ [M + H]+ 292.1081, found 292.1077

4-(4-Methoxy-benzoyl)-quinazoline-2-carboxamide (182(*1,1,13*))

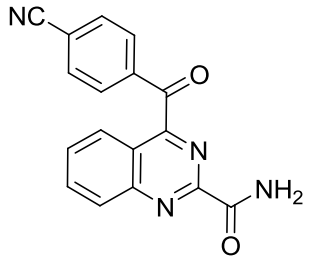


Yield of the purified product 10%, purity of the crude product 90%, purity of the purified product 95%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.33 (br. s., 1 H), 8.26 (d, *J* = 8.4 Hz, 1 H), 8.18 (t, *J* = 8.4 Hz, 1 H), 8.03 - 7.78 (m, 5 H), 7.12 (d, *J* = 8.9 Hz, 2 H), 3.87 (s, 3 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 196.5, 171.0, 170.1, 169.8, 159.2, 155.6, 141.3, 138.5, 135.8, 134.7, 133.0, 131.1, 126.5, 120.0, 61.3.

ESI-MS *m/z* = 308, $[M+H]^+$, HRMS *m/z* calcd for $C_{17}H_{13}N_3O_3$ [M + H]+ 308.1030, found 308.1021

4-(4-Cyano-benzoyl)-quinazoline-2-carboxamide (182(*1,1,14*))

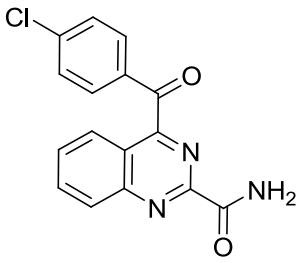


Yield of the purified product 15%, purity of the crude product 25%, purity of the purified product 94%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.36 - 8.12 (m, 6 H), 8.12 - 8.05 (m, 2 H), 7.97 (br. s., 1 H), 7.90 (t, *J* = 7.7 Hz, 1 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 191.9, 164.2, 163.1, 153.5, 150.6, 137.9, 136.0, 132.9, 131.2, 130.6, 129.2, 125.8, 121.1, 118.1, 116.5.

ESI-MS *m/z* = 303, $[M+H]^+$, HRMS (FAB) *m/z* calcd for $C_{17}H_3N_3O_3$ [M + H]+ 303.0877, found 303.0873

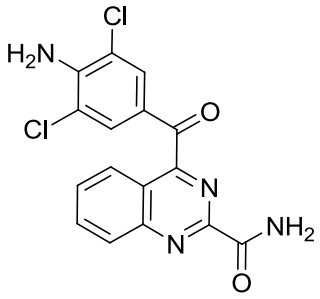
4-(4-Chloro-benzoyl)-quinazoline-2-carboxamide (182(*1,1,15*))



Yield of the purified product 11%, purity of the crude product 98%, purity of the purified product 99%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.39 - 8.26 (m, 2 H), 8.20 (t, *J* = 7.7 Hz, 1 H), 8.08 (d, *J* = 8.3 Hz, 1 H), 8.03 - 7.94 (m, 3 H), 7.88 (dt, *J* = 8.3, 1.1 Hz, 1 H), 7.67 (d, *J* = 8.6 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 191.7, 164.3, 164.1, 153.6, 150.5, 140.1, 135.9, 133.4, 132.5, 130.5, 129.3, 125.7, 121.0.
ESI-MS *m/z* = 312, $[M+H]^+$, HRMS *m/z* calcd for $C_{16}H_{10}ClN_3NaO_2$ $[M+Na]^+$ 334.0354, found 334.0343

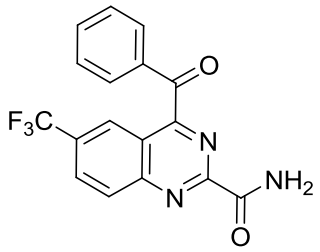
4-(4-Amino-3,5-dichloro-benzoyl)-quinazoline-2-carboxamide (182(*1,1,5*))



Yield of the purified product 20%, purity of the crude product 90%, purity of the purified product 97%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.33 (br. s., 1 H), 8.24 (d, *J* = 8.3 Hz, 1 H), 8.16 (t, *J* = 8.0 Hz, 1 H), 8.01 - 7.95 (m, 2 H), 7.89 - 7.79 (m, 3 H), 6.92 (s, 2 H). ^{13}C NMR (75 MHz, DMSO- *d*6) δ = 188.6, 164.8, 164.4, 153.6, 150.3, 147.2, 135.7, 130.8, 130.1, 129.2, 125.8, 122.9, 121.2, 117.5.
ESI-MS *m/z* = 361, $[M+H]^+$, HRMS *m/z* calcd for $C_{16}H_{10}Cl_2N_4NaO_2$ $[M+Na]^+$ 383.0073, found 383.0070

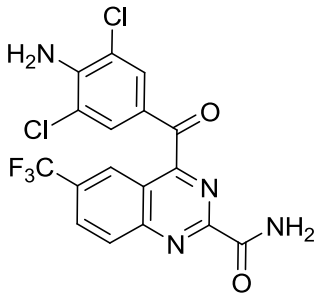
4-Benzoyl-6-trifluormethyl-quinazoline-2-carboxamide (182(*1,2,4*))



Yield of the purified product 7%, purity of the crude product 91%, purity of the purified product 96%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.65 (s, 1 H), 8.40 (br. s., 1 H), 8.31 (d, *J* = 8.6 Hz, 1 H), 8.18 - 8.03 (m, 2 H), 8.00 (d, *J* = 7.2 Hz, 2 H), 7.80 (t, *J* = 7.5 Hz, 1 H), 7.61 (t, *J* = 7.7 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 192.3, 165.4, 163.9, 154.8, 149.9, 135.3, 134.5, 130.8, 129.1, 128.1, 126.9 (q, *J* = 4.18 Hz), 125.5, 122.8.
ESI-MS *m/z* = 346, $[M+H]^+$, HRMS *m/z* calcd for $C_{17}H_{10}F_3N_3NaO_2$ $[M+Na]^+$ 368.0617, found 368.0617

4-(4-Amino-3,5-dichloro-benzoyl)-6-trifluoromethyl-quinazoline-2-carboxamide (182(*1,2,5*))

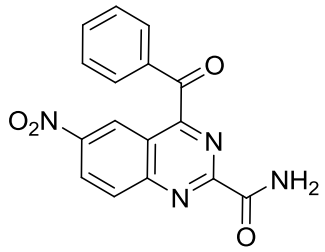


Yield of the purified product 23%, purity of the crude product 98%, purity of the purified product 99%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.60 (s, 1 H), 8.40 (br. s., 1 H), 8.25 (d, *J* = 8.6 Hz, 1 H), 8.16 - 8.00 (m, 2 H), 7.89 (s, 2 H), 6.96 (s, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 188.0, 165.4, 164.0, 154.7, 149.9, 147.4, 134.8, 134.4, 131.0, 128.2, 126.8 (q, *J* = 4.18 Hz), 125.2 (d, *J* = 2.09 Hz), 123.0, 122.7, 121.5, 117.5.

ESI-MS *m/z* = 429, $[M+H]^+$, HRMS *m/z* calcd for $C_{17}H_9Cl_2F_3N_4NaO_2$ $[M+Na]^+$ 450.9947, found 450.9922

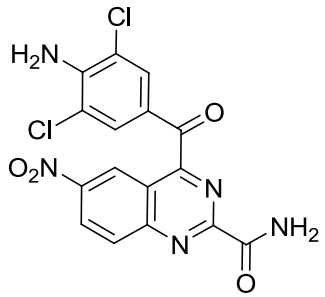
4-Benzoyl-6-nitroquinazoline-2-carboxamide (182(*1,4,4*))



Yield of the purified product 33%, purity of the crude product 85%, purity of the purified product 95%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.99 (d, *J* = 1.9 Hz, 2 H), 8.59 - 8.43 (m, 2 H), 8.34 (d, *J* = 9.1 Hz, 1 H), 8.10 (br. s., 1 H), 8.01 (d, *J* = 7.5 Hz, 2 H), 7.81 (t, *J* = 7.5 Hz, 1 H), 7.60 (t, *J* = 7.7 Hz, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 192.1, 165.5, 163.7, 155.1, 151.4, 150.3, 135.4, 134.4, 130.9, 129.2, 128.5, 124.7, 123.7, 123.5.
ESI-MS *m/z* = 323, $[M+H]^+$, HRMS *m/z* calcd for $C_{16}H_{10}N_4NaO_4$ $[M+Na]^+$ 345.0594, found 345.0582

4-(4-Amino-3,5-dichloro-benzoyl)-6-nitro-quinazoline-2-carboxamide (182(*1,4,5*))



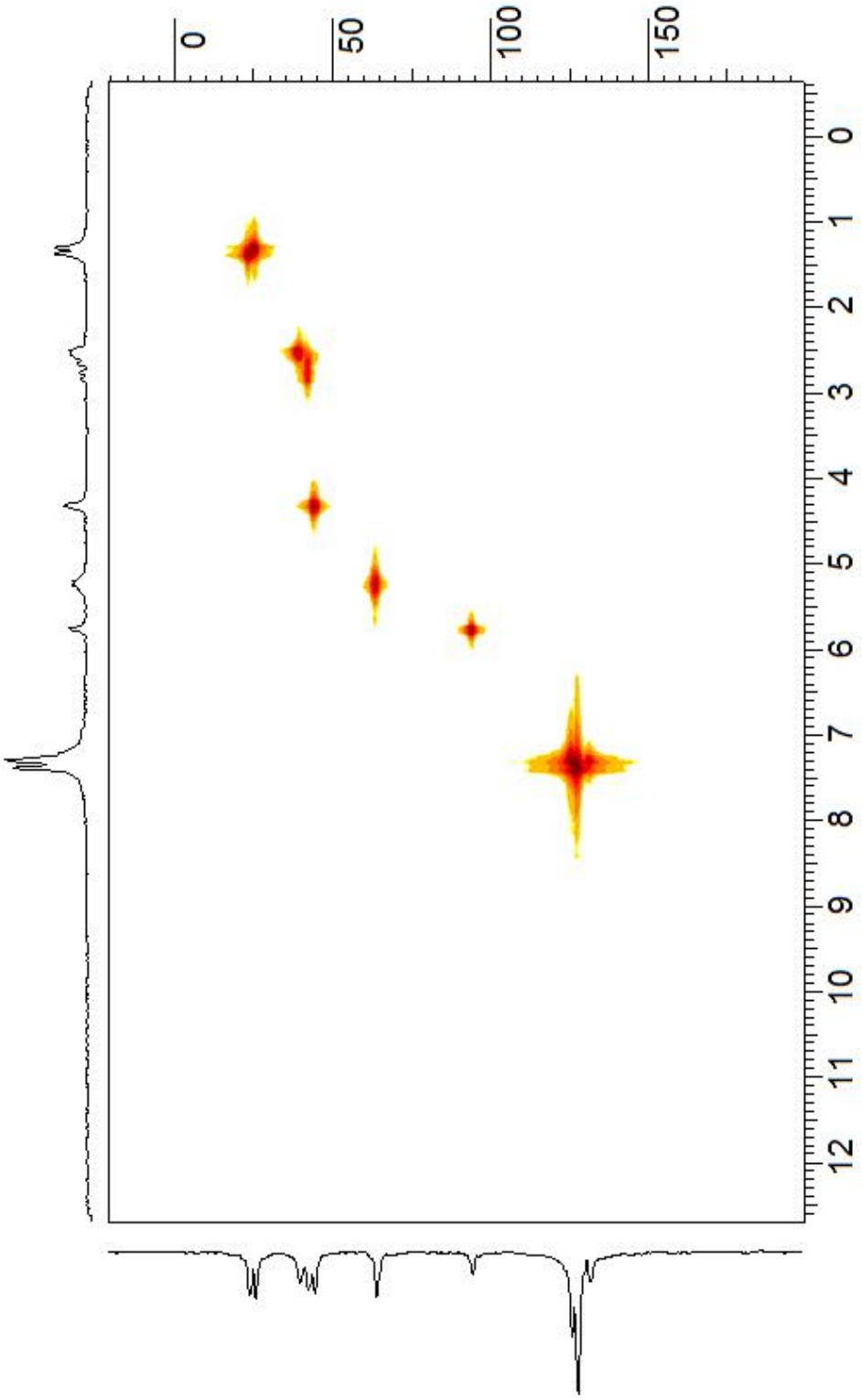
Yield of the purified product 14%, purity of the crude product 92%, purity of the purified product 97%

^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.96 (d, *J* = 1.9 Hz, 1 H), 8.55 - 8.42 (m, 2 H), 8.28 (d, *J* = 9.1 Hz, 1 H), 8.08 (br. s., 1 H), 7.87 (s, 2 H), 6.95 (s, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 187.8, 165.5, 163.8, 155.1, 151.3, 150.3, 147.4, 131.1, 128.6, 124.6, 124.0, 123.3, 122.6, 117.5, 48.6.

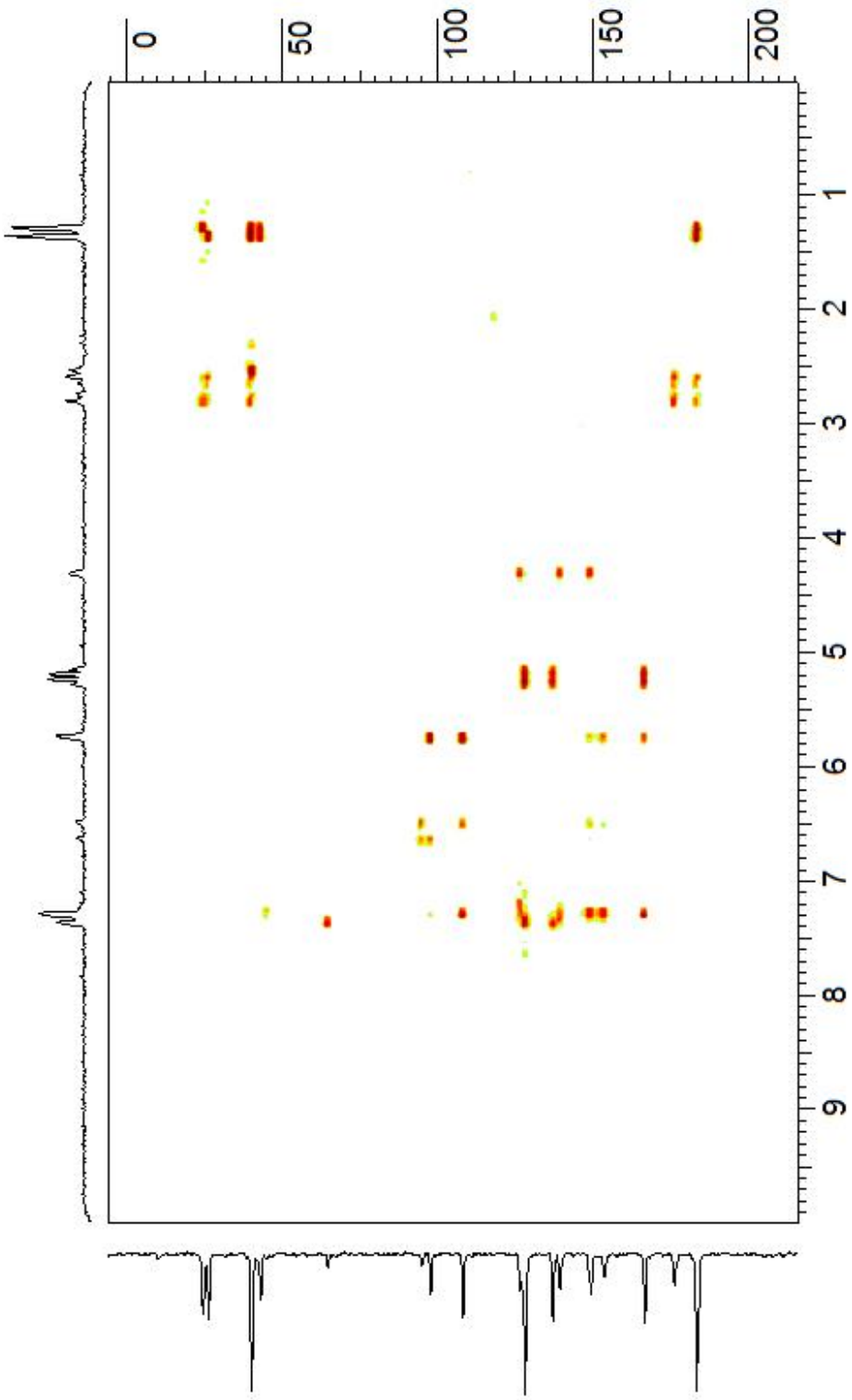
ESI-MS *m/z* = 406, $[M+H]^+$, HRMS *m/z* calcd for $C_{16}H_9Cl_2N_5NaNaO_4$ $[M+Na]^+$ 427.9924, found 427.9906

6.4. Supplement data

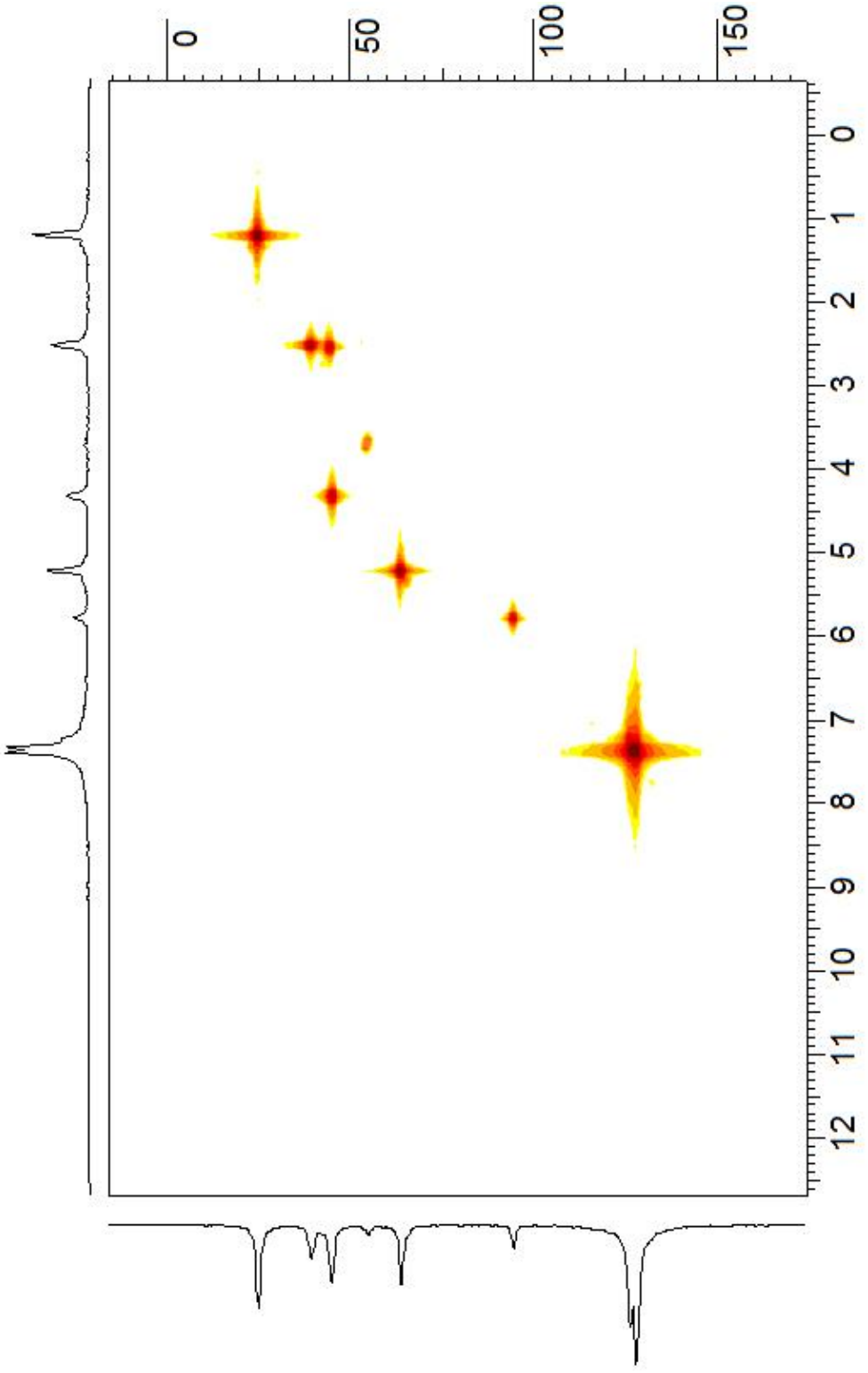
HSQC NMR of 2-amino-4-benzylamino-5-(3,3-dimethyl-2,5-dioxo-pyrrolidin-1-yl)-benzoic acid benzyl ester (136(*4,1,1*))



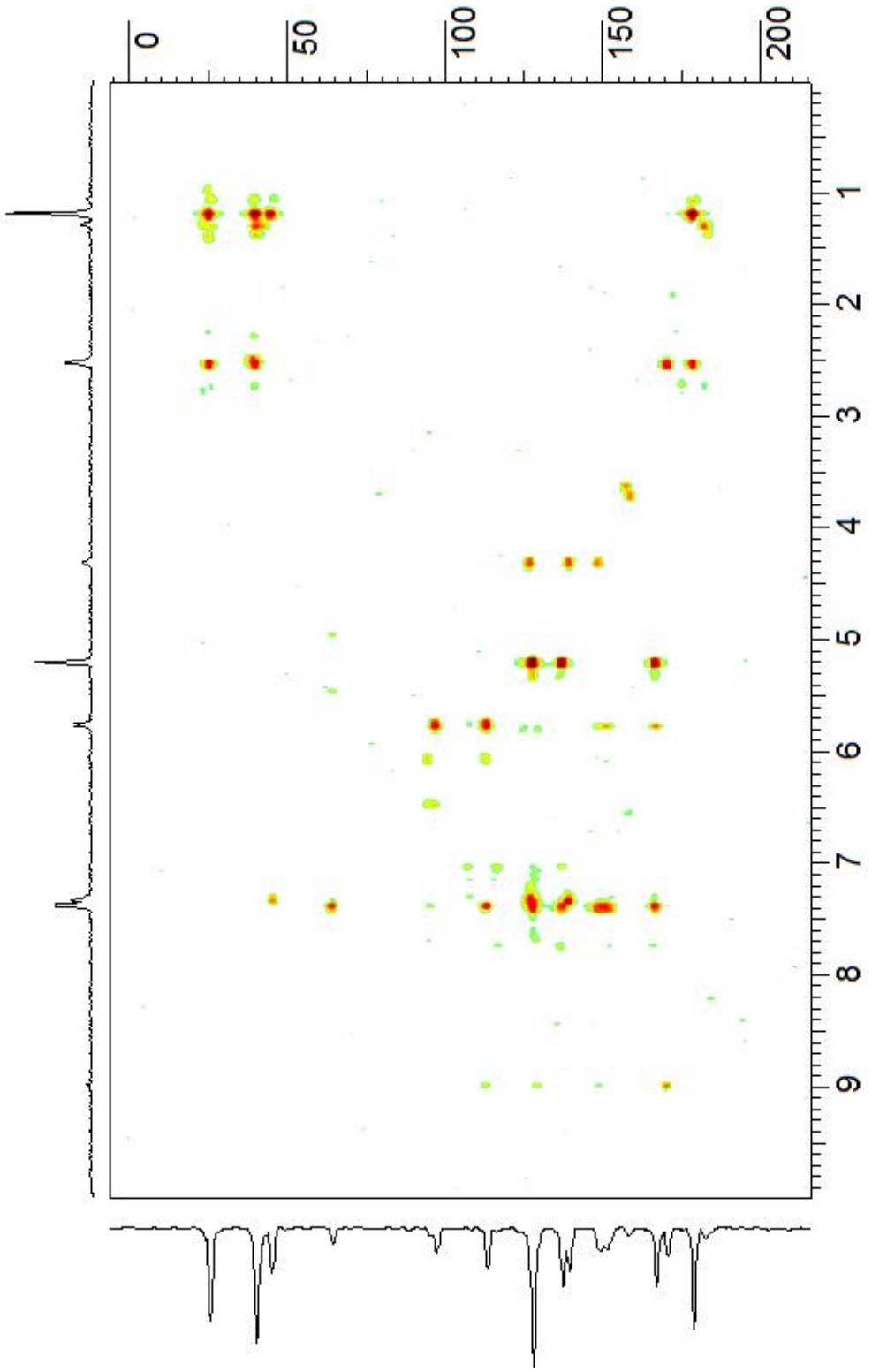
HMBC NMR of 2-amino-4-benzylamino-5-(3,3-dimethyl-2,5-dioxo-pyrrolidin-1-yl)-benzoic acid benzyl ester (136(*4,1,1*))



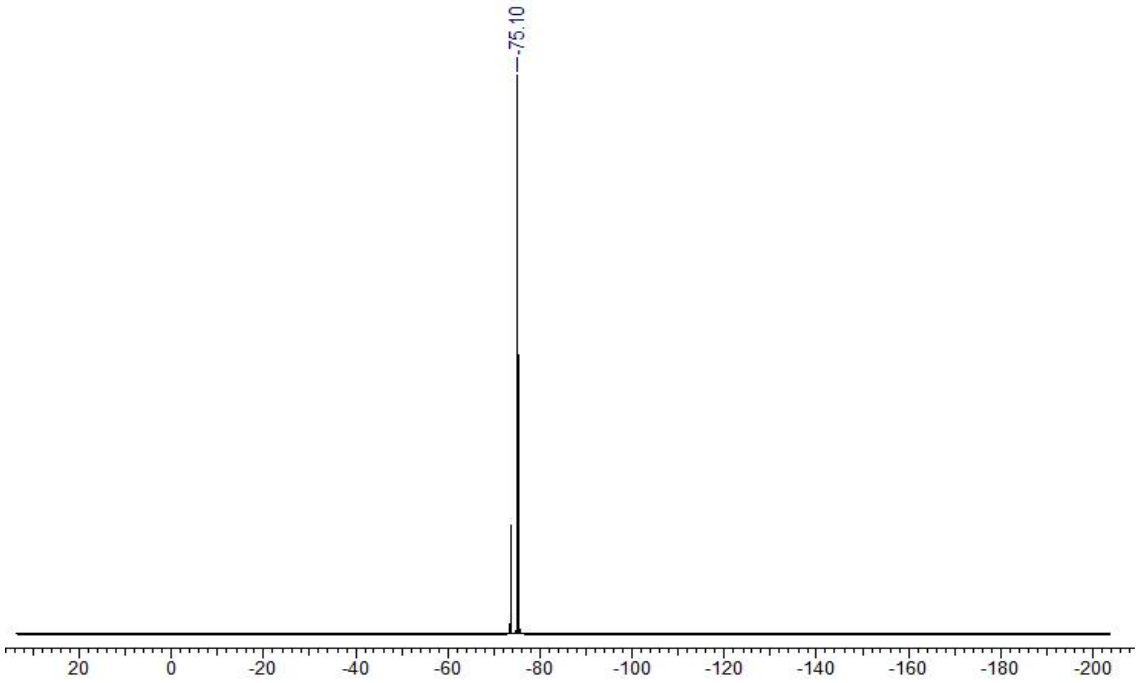
HSQC NMR of 2-amino-4-benzylamino-5-(3-carboxy-3-methyl-butyrylamino)-benzoic acid benzyl ester (153(*4,1,1*))



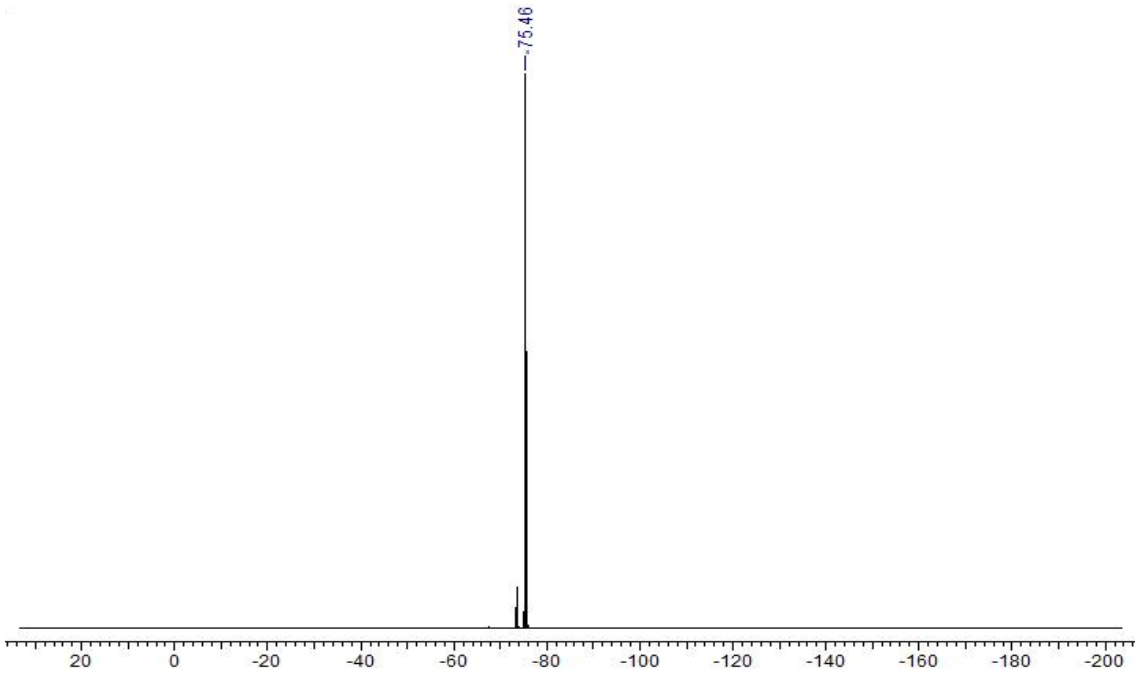
HMBC NMR of 2-amino-4-benzylamino-5-(3-carboxy-3-methyl-butyrylamino)-benzoic acid benzyl ester (153(*4,1,1*))



^{19}F NMR of 2,4-dioxo-1-propyl-8-(2,2,2-trifluoro-acetylamino)-1,2,4,5-tetrahydro-spiro[benzo[b][1,4]diazepine-3,1'-cyclopropane]-7-carboxylic acid (134(*1*,*H*,*5*))



^{19}F NMR of 5-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-propylamino-2-(2,2,2-trifluoro-acetylamino)- benzoic acid (136(*1*,*H*,*5*))



7. List of Abbreviations

A549	tumor cells of lung carcinoma
AcOH	acetic acid
Ac	acyl
Ar	aryl
B/C/P strategy	build/couple/pair strategy
BAL	4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid
BB	building block
BEMP	2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine
Bn	benzyl
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
CEM	subline of acute lymphoblastic leukemia
CEM-DNR-bulk	subline of acute lymphoblastic leukemia resistant on daunorubicin
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichlorethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DIC	*N*,*N'*-diisopropylcarbodiimid
DEAD	diethyl azodicarboxylate
DIEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DOS	diversity-oriented synthesis
Et	ethyl
FDA	Food and Drug Administration
Fmoc	fluorenylmethyloxycarbonyl
Fmoc-Gly-OH	Fmoc-glycine (fluorenylmethyloxycarbonyl glycine)
HATU	O-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyl-uronium-hexafluorophosphate

Het	heterocycle
HIV	human immunodeficiency virus
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
HTOS	high throughput organic synthesis
HTS	high throughput screening
IMPDH	inosin monophosphate dehydrogenase
*i*Pr	isopropyl
K562	subline of chronic myelogeneous leukemia
K562-Tax	subline of chronic myelogeneous leukemia resistant on paclitaxel
HPLC-MS	liquid chromatography – mass spectrometry
Mes	mesyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n.t.	not tested
NBS	*N*-bromosuccinimide
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
Nos	nitro-benzenesulfonyl
OBOC	one bead one compound
OTf	triflate
PEG	poly(etylene glykol)
Ph	phenyl
PPA	poly phosphoric acid
PPTS	pyridinium *p*-toluenesulfonate
PyBroP	bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
Pyr	pyridine
RCM	ring-closing metathesis
Shh	Sonic hedgehog homolog
t-BuOK	potassium *tert*-butoxide
TEA	triethylamine
TFA	trifuoracetic acid
THF	tetrahydrofuran

TMSOK	potassium trimethylsilanolat
TOS	target-oriented synthesis
TPP	triphenylphosphine
Tos	tosyl
RP-HPLC	reversed phase high-performance liquid chromatography
Rt	room temperature
VS	virtual screening

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