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Combinatorial Libraries of Bis-Heterocyclic Compounds with Skeletal Diversity

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Abstract

Combinatorial solid-phase synthesis of bis-heterocyclic compounds, characterized by the presence of two heterocyclic cores connected by a spacer of variable length/structure, provided structurally heterogeneous libraries with skeletal diversity. Both heterocyclic rings were assembled on resin in a combinatorial fashion.

Keywords

Heterocycles; Solid-phase synthesis; highly efficient solid-phase organic synthesis; skeletal diversity

Introduction

The exclusive role of heterocyclic compounds for drug discovery is best documented by the occurrence of a heterocyclic moiety in current drugs; the majority of drugs are heterocyclic compounds. Since there is no doubt that heterocyclic compounds are relevant targets for drug discovery, a substantial effort has been dedicated to the development of chemistries, both solid- and solution-phase, for combinatorial syntheses of heterocyclic libraries.[1-4] Traditionally, generic combinatorial libraries contained one heterocyclic scaffold and the library diversity was accomplished through derivatization of the common heterocyclic core by diverse R groups, portrayed in Figure 1 for a three combinatorial step library (R^1, R^2 and R^3 in the structure in Figure 1). Albeit building blocks for a generic library were selected from among the most diverse ones, the library diversity is limited by the presence of one identical core structure (Het Core). Not surprisingly, building blocks (BB) for the introduction of R groups were also selected among heterocyclic compounds (Het BB). In such cases, compounds containing two heterocyclic moieties connected by a spacer, bis-heterocyclic compounds, were synthesized.

Recent reports described libraries of derivatized heterocycles where the most active library compound was a bis-heterocycle.[5,6] As an example, Pfizer scientists discovered a number of potent inhibitors of cyclin-dependent kinase 5/p25 in a library of 2-aminothiazoles.[7] The most active compound (IC_{50} = 5 nM) contained isoquinoline in the position of R^3.

The relevance of compounds composed from two or more heterocyclic rings for drug discovery, irrespective of the target, can be best documented by the frequency with which bis-heterocyclic compounds were identified as the most potent ones. An average six publications in every issue

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Supporting Information Available Supporting information contains the details of experimental procedures and spectroscopic data for synthesized compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

of the Journal of Medicinal Chemistry reported bis-heterocycles as being the most potent ones in 2008.

Increased structural complexity of pharmacologically relevant compounds is also apparent in currents drugs. Among the top 50 prescription drugs in 2004, three active substances had a bis-heterocyclic structure.[8] In 2007 the number had increased to 12 (Figure 2);[9] a fourth-fold increase in three years. Among the list of the next 50 top drugs there are 12 bis-heterocycles (Lunesta, Geodon Oral, Cozaar, Atripla/Truvada, Evista, Hyzaar, Cialis, Omnicef, Avelox, Benicar HCT, Avapro) including Imatinib (Gleevec/Glivec™), a potent and selective inhibitor of BCR-ABL and c-kit oncogenic tyrosine kinases that contains three heterocyclic rings (99th in 2007).

Even though medicinal chemistry efforts covered a wide range of structurally and functionally derivatized heterocyclic compounds, there has not been a dedicated effort to explore bis-heterocyclic compounds and libraries of bis-heterocyclic compounds have not been extensively studied so far. The Houghten group described thiohydantoin benzimidazolinethiones and thiohydantoin tetrahydroquinoxalinediones,[10] and Lin and Sun recently reported a library of bis-benzimidazoles using microwave-assisted parallel synthesis[11] (Figure 3).

Our strategy deviates from the traditional approach. Library compounds are composed of two different heterocyclic rings connected by a spacer. This provides access to classes of compounds not systematically studied so far, but with a substantial potential to become drugs. Library compounds are highly diverse because a library can contain a combination of different heterocyclic cores connected at various positions by dissimilar spacers (skeletal diversity), and both heterocyclic rings are assembled in a combinatorial fashion during the synthesis.

Results and discussion

Our objective was to develop efficient chemical routes for solid-phase synthesis of bis-heterocyclic libraries characterized by combinatorial assembly of two heterocyclic systems connected by a spacer of variable length/structure. Combinatorial libraries were synthesized on solid phase (SP). An inherent simplicity of isolation of intermediates and target compounds synthesized on SP combined with a potentially highly efficient sequence of transformations made the SP synthesis the method of choice. In addition, SP synthesis is suited to very efficient split-and-pool (both random and directed) combinatorial synthesis, not to mention that it is amenable to integration and/or automation of the process.

We designed and synthesized three bis-heterocyclic libraries of thiazolo-benzimidazoles that differed by the position of the thiazole on the benzimidazole ring (Figure 4).

Design of libraries

Linking strategy

There are two scenarios for immobilization of bis-heterocyclic compounds to the SP: (i) *via* one of the heterocyclic rings (Figure 5, cartoon A) and (ii) *via* the spacer (Figure 5, cartoon B). Our initial effort was focused on the immobilization of the heterocyclic core. In this favorable scenario, the immobilization strategy should allow synthesis of target compounds in a traceless manner, i.e. target products will not have a residual function group attached to them that originated from a linker used to attach the first synthon to solid support. A typical “trace” of a linker is a carboxylate or a carboxamide used for immobilization of carboxylate-containing building blocks to Wang or Rink linkers, respectively (unless carboxylate is an inherent part of target structures, such as in peptides). One of the strategies for a traceless synthesis of heterocycles is to take advantage of the presence of the heteroatom, particularly nitrogen in N-

heterocycles, and immobilizing the first nitrogen containing synthon to a suitable linker *via* the nitrogen atom. We, and others, have used this strategy on several occasions for the synthesis of single core heterocycles (reviewed in articles[2,3,12]).

The first heterocycle

Numerous heterocyclic skeletons have served as a core structure for drugs and drug-like molecules. The combination of two heterocyclic cores in one molecule increases the number of potential targets by the square power. When selecting the heterocycle for our synthesis of bis-heterocyclic compounds we applied several criteria: (i) drug-likeness, (ii) facile combinatorial SP synthesis, (iii) commercial availability of building blocks, and (iv) versatility of resin-bound intermediates.

All criteria were fulfilled by 1,2-phenylenediamines (among other structures) that serve as very useful intermediates for the transformation to five, six, and seven membered heterocycles (Figure 6). In addition, diverse 1,2-phenylenediamines were conveniently accessed from 1-fluoro-2-nitrobenzenes and amines,[2] providing intermediates with two diversity positions. Moreover, there is straightforward route to resin-bound 1,2-phenylenediamines on an acid-labile electron-rich benzyl-type linker allowing convenient release of target compounds from the resin by acid-containing cocktails or even gas.[13,14]

We used resin-bound *o*-phenylenediamines in traceless syntheses of several heterocycles.[14] For the synthesis of our first biheterocyclic libraries we selected our traceless synthesis of benzimidazoles with three diversity positions (Scheme 1).[15] In order to finish the assembly of the benzimidazole precursor, the polymer-supported 1,2-phenylenediamines were acylated by carboxylic acids. Cyclization to benzimidazoles occurred after cleavage from the resin by heating in acetic acid.

The spacer

The spacer served to connect both heterocycles and it was contained in a building block used to assemble the first heterocyclic core. The building blocks were selected to enable introduction of functionalized spacer in any of three diversity positions of benzimidazoles. For our first bis-heterocyclic library, the benzimidazole core was synthesized with three different points of attachment of the spacer for the subsequent synthesis of the second heterocycle (Figure 7).

The spacer contained a functional group that facilitated assembly of the second heterocycle. A choice can be from suitable functional groups, including amines, alcohols, carboxylates, and alkynes. We chose the amino group which facilitates the attachment of the second heterocycle or it can be an integral part of the second heterocycle. The benzimidazole core was synthesized using the traceless SP synthesis with three combinatorial steps. We prepared three types of resin-bound intermediates, **I-3**, **II-3**, and **III-3**, to introduce the spacer at three different positions. (i) The functionalized spacer in position 1 of the benzimidazole ring was incorporated in the first combinatorial step by reductive amination of the aldehyde linker. Amino alcohols (3-amino-1-propanol to demonstrate the concept) were preferred to diamines that would have to be selectively protected. Polymer-supported alcohols were converted to amines *via* mesylation followed by reaction with amines later in the synthesis. This reaction enabled us to introduce additional diversity at the amine in resin-bound intermediates **I-3**. (ii) For attaching the spacer to the aromatic ring we took advantage of the commercially available 1,2-dichloro-4-fluoro-5-nitrobenzene. After reaction with the polymer-supported secondary amines **1** (Scheme 2), the chlorine was displaced by a diamine (piperazine in intermediate **II-3**) and yielded resin-bound intermediates with an amine-containing spacer attached to the carbocyclic ring. (iii) Acylation of the resin-bound 1,2-phenylenediamines with a protected amino acid (Fmoc-βAla in model compounds) yielded intermediates **III-3** and introduced the spacer in position 2.

The second heterocycle

Using the amino group as an anchor for the next heterocycle, we selected thiazole as the second heterocycle. Thiazoles were prepared by a reaction of resin-bound amines **I-3**, **II-3**, and **III-3** with Fmoc-NCS, followed by Fmoc group cleavage and five membered ring closure using haloketones according to the published protocol.[16] Cleavage from the resin by trifluoroacetic acid (TFA) yielded acyclic precursors that were cyclized by heating in acetic acid. Three libraries of thiazolo-benzimidazole bis-heterocycles were synthesized that differ due to the position of the thiazole on the benzimidazole ring (Figure 8).

Syntheses of libraries

All three thiazolo-benzimidazole bis-heterocyclic libraries shared common intermediates with two diversity positions, the nitroanilines **2** (Scheme 2). Thus, the first two combinatorial steps for all three libraries were identical. However, the selection of individual combination of building blocks (amines and arylfluorides) for each library was different to enable the introduction of a suitable functional group (amine, alcohol) at one of the three diversity positions (Figure 9).

The first combinatorial step - resin-bound amines 1

The immobilization of nitroanilines took advantage of the acid lability of the electron rich benzyl group attached to an aniline. Historically, polymer-supported *N*-alkylated benzylamines were introduced for the synthesis of *N*-alkyl amides.[17-19] *N*-alkylated benzylamines are typically prepared from the aldehyde linker *via* reductive amination. The conditions for reductive amination have been optimized for a large set of diverse amines (more than a hundred). A typical protocol consisted of pre-incubation with an amine, followed by reduction using NaBH(OAc)3 in AcOH/DMF.

A set of seven polymer supported secondary amines **1** was prepared from the BAL resin[19] and primary amines (Figure 9). The reported method[14] for the reductive alkylation was slightly modified for the preparation of the polymer supported 4-methylbenzylamine. The acetate of this amine was precipitated from a DMF solution before reaction, thus the equivalent of TFA was used to form the corresponding soluble trifluoroacetate salt. When the same modification was used for the reductive alkylation with *n*-propylamine, higher resin loading was observed in comparison to the procedure with acetic acid. Three amino alcohols, **A1** though **A3**, were included that allowed transformation of the hydroxy derivatives to amines for the synthesis of the second heterocycle of bis-heterocycles **I**.

To include *N*-unsubstituted derivatives (R^1 = H), resin **1{8}** was prepared from HMPB linker[20] in a two step procedure: the resin-bound alcohol of the HMPB linker was reacted with phthalimide under Mitsunobu conditions and the phthaloyl group cleaved by hydrazine hydrate. The aminomethyl resin **1{8}** can also be alkylated with alcohols *via* 2-nitrobenzenesulfonyl derivative and alkylation using the Fukuyama procedure[21] followed by cleavage of the Nos group.[22]

The second combinatorial step - nitroanilines 2

The second diversity position (derivatization of the benzene ring of benzimidazoles) was introduced using commercially available 1-fluoro-2-nitrobenzenes (Figure 9). The reaction conditions depended on the reactivity of arylfluorides. The arylations with reactive 1-fluoro-2-nitro-4-trifluoromethyl-benzene (**F2**) and 1,2-dichloro-4-fluoro-5-nitrobenzene (**F3**) were quantitative at room temperature after an overnight reaction. Reaction with 1-fluoro-2-nitrobenzene (**F1**) and 4-fluoro-3-nitrobenzoic acid (**F4**) required an elevated temperature (80

°C). Longer reaction time, or higher temperature, caused O-arylation of nitroanilines prepared from amino alcohols in the first combinatorial step.

Two immobilized nitroanilines **2{R^1,3}**‡ and **2{R^1,4}** prepared from 1,2-dichloro-4-fluoro-5-nitrobenzene **F3** and 4-fluoro-3-nitrobenzoic acid **F4**, were further transformed by nucleophilic substitution of chlorine and secondary amide formations, respectively (Scheme 3). This transformation expanded the diversity of substituents at the carbocyclic ring. For the synthesis of the library we used piperidine and 1-aminopropanol.

Bis-heterocycles I

Thiazole ring of bis-heterocycles **I** was attached *via* the substitution at the benzimidazole nitrogen and synthesis was carried out according to Scheme 4. Nitroanilines **I-2** (**I-2** refers to a subset of nitroanilines **2** used for the synthesis of bis-heterocycles **I**) for this library were prepared using amino alcohols **A1** and **A2** and arylfluorides **F1** and **F2** (Figure 9). The resin-bound alcohols **I-2** enabled transformation of the hydroxyl groups to amines **I-3** for the synthesis of thiazoles. Skeletal diversity of the library compounds was enhanced by using four structurally different spacers; two spacers in combination with amino alcohol **A1** and two different spacers for amino alcohol **A2**. To access *N*-unsubstituted derivatives (R^3 = H), the alcohols **I-2{1,R^2}** were reacted with phthalimide under Mitsunobu conditions followed by phthaloyl group cleavage by hydrazine hydrate to yield **I-3{1,R^2,1}**. Acylating half the resin with Fmoc-Pro-OH provided an extended spacer in resins **I-3{1,R^2,2}**. To introduce R^3 substituent at the spacer amino group, alcohols **I-2{2,R^2}** were mesylated and reacted with two different amines, ethanolamine and piperazine, yielding intermediates **I-3{2,R^2,3}** and **I-3{2,R^2,4}**.

Resin-bound thiazoles **I-5** were formed using the published protocol.[16] Resin-bound amines **I-3** were reacted with freshly prepared Fmoc-NCS in dry THF. The Fmoc group was cleaved by piperidine and thioureas **I-4** were reacted with haloketones. We used bromoketone **H1** and dichloroacetone. The chloromethyl derivatives prepared using dichloroacetone were further reacted with piperidine to increase the diversity of compounds.

After the assembly of the thiazoles **I-5**, the nitro group was reduced by tin(II) chloride in the presence of a tertiary amine base (DIEA). The acylation of **I-6** was carried out by acid chlorides or symmetrical anhydrides. In some cases, the reactivity of aniline nitrogen was reduced by the presence of an electron withdrawing group. The reaction conditions for acylation were optimized for individual anilines. Cleavage to linear benzimidazole precursors **I-8** was carried out by TFA in DCM and the subsequent cyclization to **I-9** in AcOH at elevated temperature.

Logistics of library synthesis

The library of bis-heterocycles **I** was synthesized using the split-split approach[23] using polypropylene syringes as reaction vessels on a manually operated Domino Block synthesizer.[24] A full library of 2×2×2×2×2 = 32 compound using amino alcohols **A1** and **A2**, arylfluorides **F1** and **F2**, four spacers, haloketones **H1** and **H9**, and acids **C2** and **C4** was synthesized. To increase the skeletal diversity, two spacers used in combination with amino alcohol **A1** were different from those used with **A2**. All library compounds were purified by semi-preparative HPLC. Selected compounds were characterized by MS and ^{1}H and ^{13}C NMR. All analytical data are summarized in the experimental section.

‡Symbol **2{R^1,3}** denotes a specific subset of compounds: **2{R^1,3}** refers to compounds having any building block in the first position (R^1) and one specific building block in the second position (in this case **F3**)

Bis-heterocycles II

Synthesis of bis-heterocycles **II** with a thiazole ring attached *via* the carbocyclic ring of benzimidazoles was carried out according to Scheme 5. Nitroanilines **II-2** were prepared using five amines (**A2** to **A6**). The side-chain of building block **A7** was introduced by on-resin modification of **A2** later in the synthesis, **A8** was prepared from the HMPB linker. Resin-bound amines were reacted with 1,2-dichloro-4-fluoro-5-nitrobenzene (**F3**). Chlorine in the *para* position to the nitro group is amenable for replacement by diamines to install the amino-derivatized spacer. In this library we used only one amine, piperazine, to prepare resins **II-3**.

At this stage we used two different routes for the synthesis of thiazolo-phenylenediamines **II-7**. Route A included the formation of thiazole (compounds **II-5**), followed by a reduction of the nitro group. The formation of thiazoles was carried out using three bromoketones **H1** to **H3** and two chloroacetones **H8** and **H9**. The alkylation of **II-4** with all chloro/bromoketones in the presence of proton sponge (1,8-bis(dimethylamino)naphthalene) led to spontaneous thiazole ring formation (**II-5**). The nitro derivatives **II-5** were then reduced with tin(II) chloride dihydrate in the presence of a tertiary amine base (DIEA) and yielded corresponding resin-bound anilines **II-7**.

The alternative route B had those two steps interchanged. The nitro group was reduced at the stage of thiourea and the resins **II-6** were reacted with haloketones. Under the conditions used in route A, the LCMS analysis of the intermediates using bromoketones **H1** to **H3** revealed the presence of double alkylated (and in few instances a small amount of three times alkylated) compounds in the case of intermediates prepared from amino alcohol **A3**. The alkylation reaction was optimized to prevent double alkylation by using phenacylbromoketones without a base in DCM for 3 h for amines **A3**, **A2**, and **A7**. Under these new conditions the double alkylation of **II-6** for amine **A3** was still occurring but it was suppressed by the replacement of DCM by toluene.

The practical advantage of route B is the fact that the nitro group reduction was performed on limited number of resin-bound intermediates **II-4**. This happened before the number of compounds was expanded by the combinatorial step with haloketones.

The intermediates prepared using dichloroacetone **H9** yielded chloromethyl derivatives **II-7 {R^1,9}** and enabled additional diversity expansion by reaction with amines. We used piperidine and the conversion in DMSO at ambient temperature was complete after an overnight reaction.

Resin-bound anilines **II-7** were acylated with carboxylic acids **C1** to **C5**, activated as symmetrical anhydrides or chlorides. In order to prepare the trifluoroacetyl derivatives, the anilines were not acylated on the resin but rather the intermediates were cleaved by TFA and trifluoroacetylated and cyclized at the same time (neat TFA, 30°C, overnight).

To further increase the diversity of the library compounds and to demonstrate versatility of the synthetic route, we included a few examples of transformations of final resin-bound intermediates **II-8**. Resins **II-8{2,R^2,R^3}**, prepared using aminopropanol **A2**, were converted to derivatives **II-8{7,R^2,R^3}** *via* mesylation and subsequent substitution with amines (pyrrolidine in Scheme 6). Derivatives **II-8{R^1,R^2,3}**, prepared using 4-chloro-pyridine-2-carboxylic acid, were also treated with pyrrolidine to introduce diversity at the pyridine ring. Intermediates **II-8{R^1,R^2,5}**, prepared using Fmoc-Ala, were mesylated (after Fmoc group cleavage) on the amino group to yield derivatives **II-8{R^1,R^2,11}**.

After the cyclization, hydroxy groups of bis-heterocycles prepared using amino alcohols in the first combinatorial step were partially acetylated. The acetyl side-products were saponified with methanolic sodium hydroxide at 40°C overnight. In general, the cyclization (and possibly

saponification) afforded crude products of good to excellent purity (ranged from 70-90%, HPLC traces).

An interesting side-reaction was observed with compounds **II-9{R^1,R^2,4}**, prepared using TFA as the last building block. Analysis of the crude cyclized product revealed the presence of a side-product (10 to 50%). The MS spectrum of the side-product indicated missing chlorine (Scheme 7). The side-products **II-10{R^1,R^2,4,2}** were isolated (semi-prep HPLC) and MS and NMR spectra confirmed their structures, presumably formed by substitution of the chlorine by hydrogen in TFA. The attempt to drive the substitution reaction to completion by extended exposure to TFA (3 days) was unsuccessful. The ratio between two bis-heterocycles did not change, indicating that the substitution probably occurred in a stage of the linear precursor before the cyclization took place.

Logistics of library synthesis

The library synthesis was split into two parts to compare different synthetic approaches. One part was synthesized following route A (Scheme 5) using amines **A2** to **A6**, carboxylic acids **C1** and **C2** and 4-methylphenacylbromide **H1** (compounds II-9{2,1,1}, II-9{2,1,2}, II-9 {3,1,1}, II-9{3,1,2}, II-9{4,1,1}, II-9{4,1,2}, II-9{5,1,1}, II-9{5,1,2}, II-9{6,1, 1}, and **9 {6,1,2}**). The second part was synthesized with amines **A2**, **A3**, **A7**, **A8**, haloketone **H1** to **H3**, **H8** and **H9**, and carboxylic acids **C1** to **C5** according to route B. Route A represents a more general procedure and can be used for any building block combination without the need to fine tune the reaction conditions. The advantage of route B is the limited the number of resin-bound intermediates that undergo nitro group reduction when using the split-split methodology. However, the conditions for alkylation required optimization of reaction conditions for each particular haloketone **H**.

Bis-heterocycles III

Bis-heterocycles **III** with the thiazole attached to the C2 carbon of the benzimidazole were synthesized according to Scheme 8. Nitro anilines **III-2** used for the synthesis of bis-heterocycles **III** were prepared from four amines **A2** to **A5** and four arylfluorides **F1** to **F4** (Figure 9). The R^2 diversity was further expanded by on-resin transformation of nitroanilines **III-2**. Nitroanilines **III-2{R^1,3}** and **III-2{R^1,4}** prepared from 1,2-dichloro-4-fluoro-5-nitrobenzene **F3** and 4-fluoro-3-nitrobenzoic acid **F4**, were further transformed by nucleophilic substitution of chlorine by piperidine and secondary amide formation using 1-aminopropanol, respectively (Scheme 3).

The reduction of the nitro group with tin(II) chloride required different reaction times depending on the substitution of the starting material (2 hours or overnight). To incorporate the derivatized spacer for the synthesis of thiazoles, the acylation of the aniline was carried out by Fmoc protected amino acid anhydrides. This introduced the amino group for the subsequent thiazole ring formation. Acylation of diverse polymer-supported anilines **III-3** by Fmoc-amino acid anhydrides required different reaction conditions depending on the electronic effect of the substituent on the benzene ring. Polymer supported anilines **III-3** containing electron-withdrawing substituents required forcing acylation conditions. After careful optimization experiments we arrived at an effective acylating procedure using *in situ* preformed symmetrical anhydrides in dry THF at elevated temperature (50 °C) for six hours. The acylating species were exhausted after this time (treatment of the acylating solution sample with *N*-methylbenzylamine showed no amide formation by LC-MS analysis). When required, the acylation was repeated with freshly prepared symmetrical anhydride. However, for some combinations of the amino acids and the electron-poor anilines the reactions were still not quantitative. We observed decreasing acylating efficiency of the anhydrides in the following order: Fmoc-Pro > Fmoc-Ser(O*t*Bu) > Fmoc-Ala > Fmoc-β-Ala > Fmoc-γ-aminobutyric acid.

The reactivity of the resin-bound anilines **III-2** decreased in the order: **III-3{R^1,5}** > **III-3{R^1,1}** > **III-3{R^1,6}** > **III-3{R^1,3}** ~ **III-3{R^1,2}**. For example, acylations with Fmoc-Pro and Fmoc-Ser(O*t*Bu) were quantitative after the first acylation round for all anilines. However, one-round acylations with Fmoc-β-Ala or Fmoc-γ-aminobutyric acid were complete only for reactive anilines **III-3{R^1,5}** and **III-3{R^1,1}**. The remaining combinations required repeated acylation.

N-Hydroxypropylderivatives **III-3{2,R^2}** and **III-3{3,R^2}** were partially acylated on the hydroxy group (~10%, HPLC traces). This side-product was smoothly saponified with potassium trimethylsilanolate in THF.

Fmoc deprotection with piperidine liberated the amino functionality for subsequent thiazole ring formation. The amines **III-4** were treated with a freshly prepared solution of Fmoc-NCS in dry THF and yielded Fmoc-protected thioureas. Subsequent piperidine Fmoc group cleavage yielded thioureas **III-5**.

Alkylation with haloketones led to spontaneous thiazole ring formation. We used eight haloketones **H1** to **H8** (Figure 9). Aromatic bromoketones provided quantitative conversion after overnight reaction while the reaction with chloroacetone was not complete was repeated with fresh reagent solution.

Benzimidazole precursors **III-6** were cleaved from the resin by TFA in DCM. Cyclization of intermediates **III-7** was carried out in acetic acid at elevated temperature, typically 60 °C. The reaction time depended on the nitrogen substitution of the benzimidazole precursors (i.e. on the first place of diversity). While *N*-propyl derivatives cyclized after two hours of heating, the hydroxy derivatives required heating for five hours and *N*-methylbenzyl derivatives cyclized overnight. During the acidic cleavage and the cyclization, the protective *t*Bu group of the derivatives prepared using Fmoc-(*t*Bu)-serine cleaved only partially. The quantitative *t*Bu deprotection required treatment with neat TFA. Intermediates **III-7**, containing any hydroxyl group in the molecule, were partially O-acetylated during the cyclization reaction. Saponification of the acetyl side-products was carried out using methanolic sodium hydroxide. In general, the cyclization (and saponification if necessary) afforded crude products **III-8** in good to excellent purity ranging from 60-90% (HPLC traces).

We observed that derivatives prepared using Fmoc-Ser(*t*Bu) were unstable under acidic conditions at elevated temperature. The C-N bond was cleaved and the target bis-heterocyclic molecules **III-8{R^1,R^2,7,R^4}** were decomposed (Scheme 9). Both components of the cleavage (aminothiazole and acetylbenzimidazole) were detected using LCMS analysis with corresponding masses. 1-[1-(4-Methyl-benzyl)-1H-benzoimidazol-2-yl]-ethanone **12** was isolated by the semi-preparative reversed phase HPLC and its structure confirmed (^{1}H, ^{13}C, ^{13}C APT and COSY NMR spectra). To minimize this side-reaction, the cyclization was carried out at 30 °C overnight.

Logistics of library synthesis

The library was synthesized in two parts using two different methodologies. The first part comprised of 42 compounds was synthesized on loose resin in polypropylene fritted syringes using the split-split method. The following building blocks were used: amine **A4**, arylfluorides **F1**, **F3** and **F5**, carboxylic acids **C5** to **C9**, haloketones **H1** to **H3** and **H5** to **H8** (compounds **III-8{4,R^2,R^3,R^4}**). The resin-bound intermediates corresponding to all combinations of $\mathbf{R}^1$ with $\mathbf{R}^2$ and $\mathbf{R}^3$ were prepared. Different sets of haloketones were reacted with different intermediates **III-5** to increase the diversity of library compounds (c.f. Supporting Information for entire list of compounds).

The second part (92 compounds) was prepared in resin capsules[25] using the directed split-and-pool method. Building blocks: amines **A2**, **A3**, **A5**, arylfluorides **F1** to **F3**, **F5** and **F6**, carboxylic acids **C5** to **C8**, haloketones **H1** to **H8** (compounds **III-8{2, R^2,R^3,R^4}**, **III-8{3, R^2,R^3,R^4}**, **III-8{5, R^2,R^3,R^4}**. All combinations of polymer supported intermediates were prepared using amines (**A2**, **A3**, and **A5**), arylfluorides (**F1** to **F3** and **F5**) and acids (**C5** to **C8**). Intermediates **III-5** were reacted with selected combinations of **H1** to **H8**. This was to avoid synthesis of compounds that differ by only one building block. No significant differences in overall purity/yield of library compounds were found between the split-split and split-and-pool methodologies.

All three sub-libraries were developed and synthesized on commercially available aminomethyl polystyrene-based resin. We used two different batches of the same resin from one supplier. Several reactions carried out on one resin batch experienced significantly slower transformation rates that subsequently lowered the yield of target compounds. We will address the inequality of “equal” resins and simple procedure for evaluating the suitability of a resin for SPOS in a dedicated communication (manuscript in preparation).

All library compounds were submitted for evaluation of biological activities to High Throughput Screening in the Molecular Libraries Probe Production Centers Network and the results are available in PubChem (http://pubchem.ncbi.nlm.nih.gov/.

Conclusion

We described SP synthesis of bis-heterocyclic libraries characterized by a combinatorial assembly of two different heterocyclic rings connected by a spacer with various length/structure. Due to skeletal diversity and up to five combinatorial steps (two for each heterocycle and one for the spacer) this strategy allows synthesis of sizable diverse libraries to match screening throughput of the state of the art screening facilities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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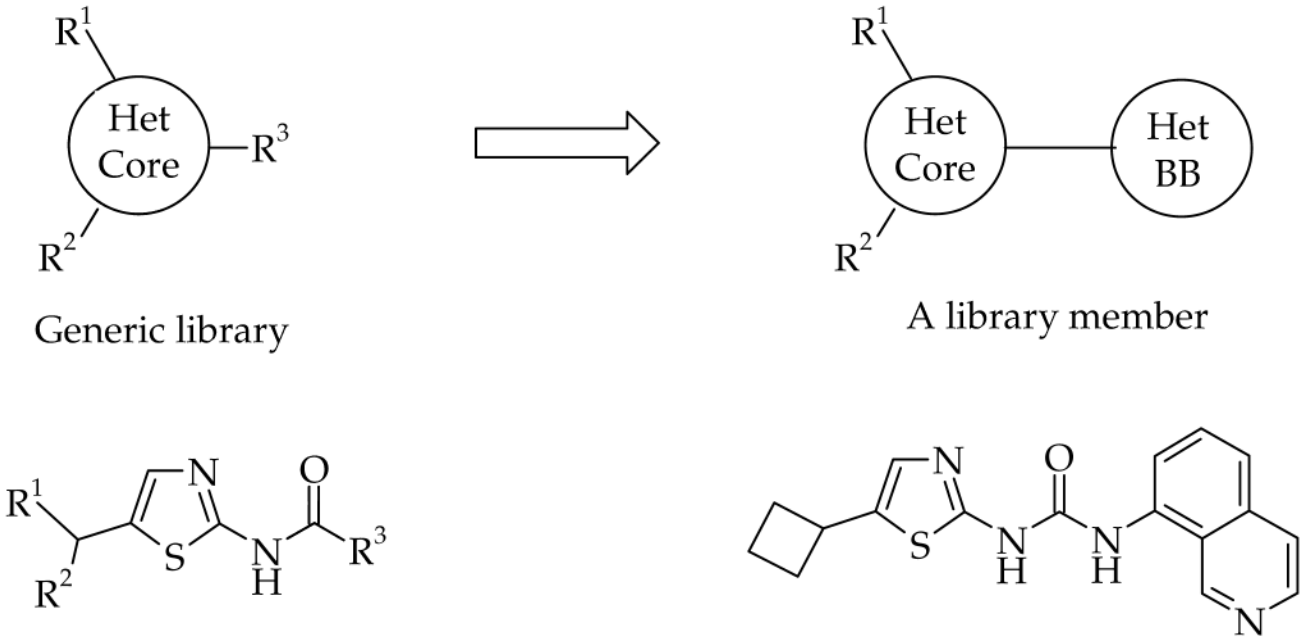


Figure 1.
Bis-heterocycles in combinatorial libraries

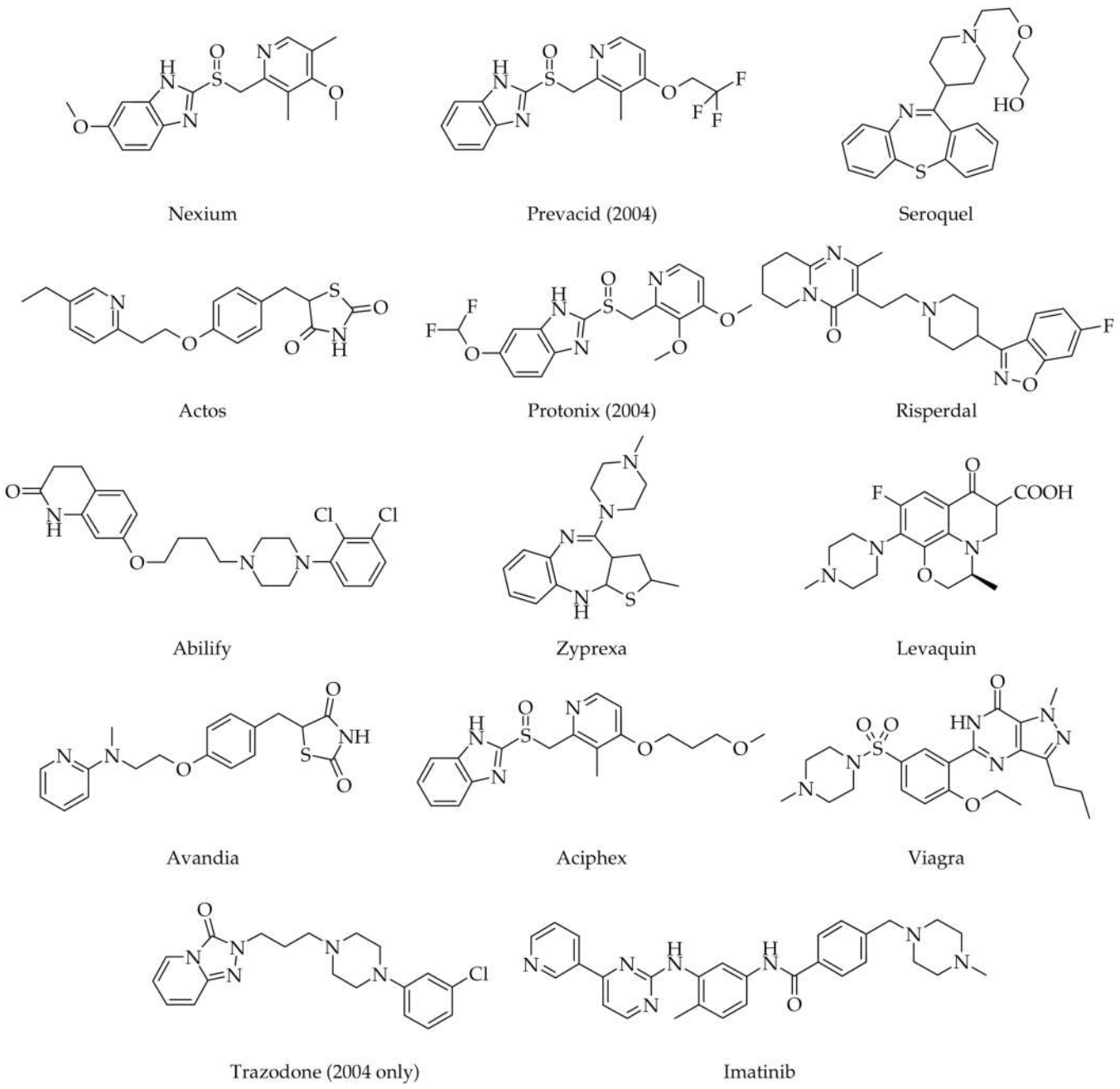


Figure 2.
Top 50 prescription drugs in 2004 and 2007 with a bis-heterocyclic structure

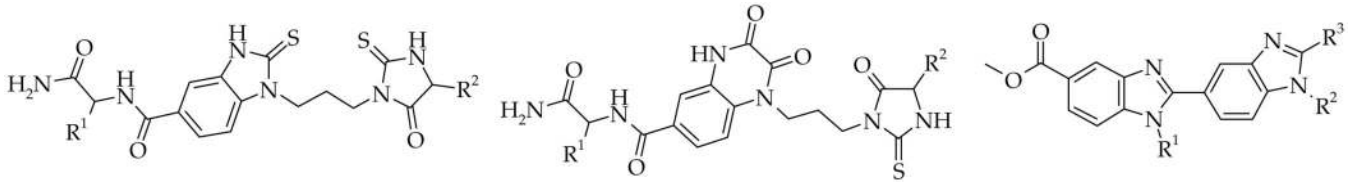


Figure 3.
Bis-heterocyclic libraries

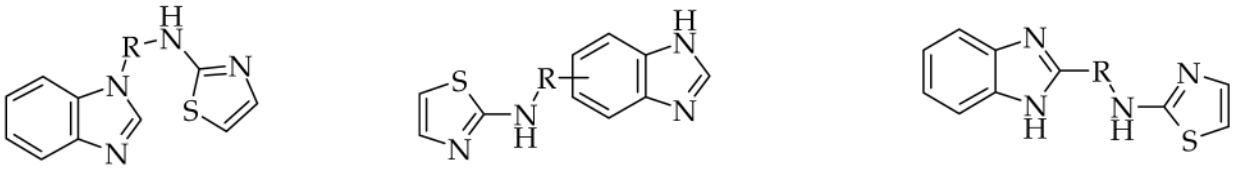


Figure 4.
Generic core structures of three thiazolo-benzimidazole libraries.

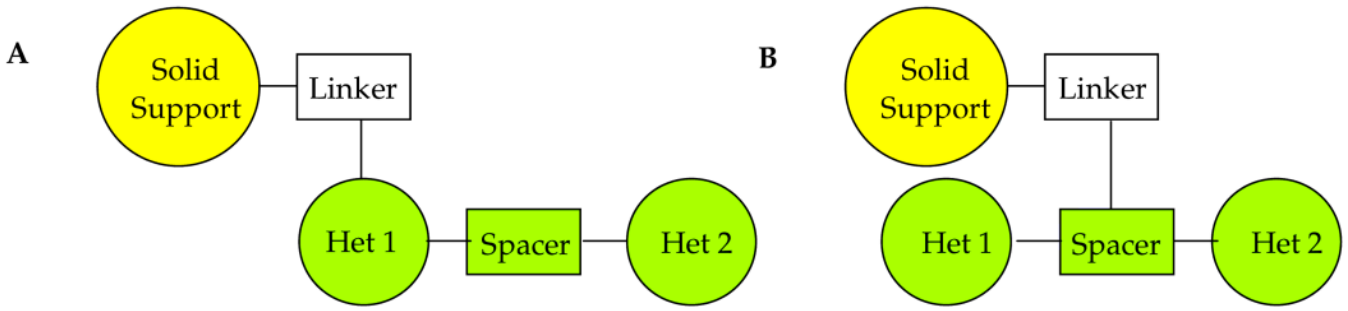


Figure 5.
Two scenarios for immobilization of bis-heterocyclic libraries

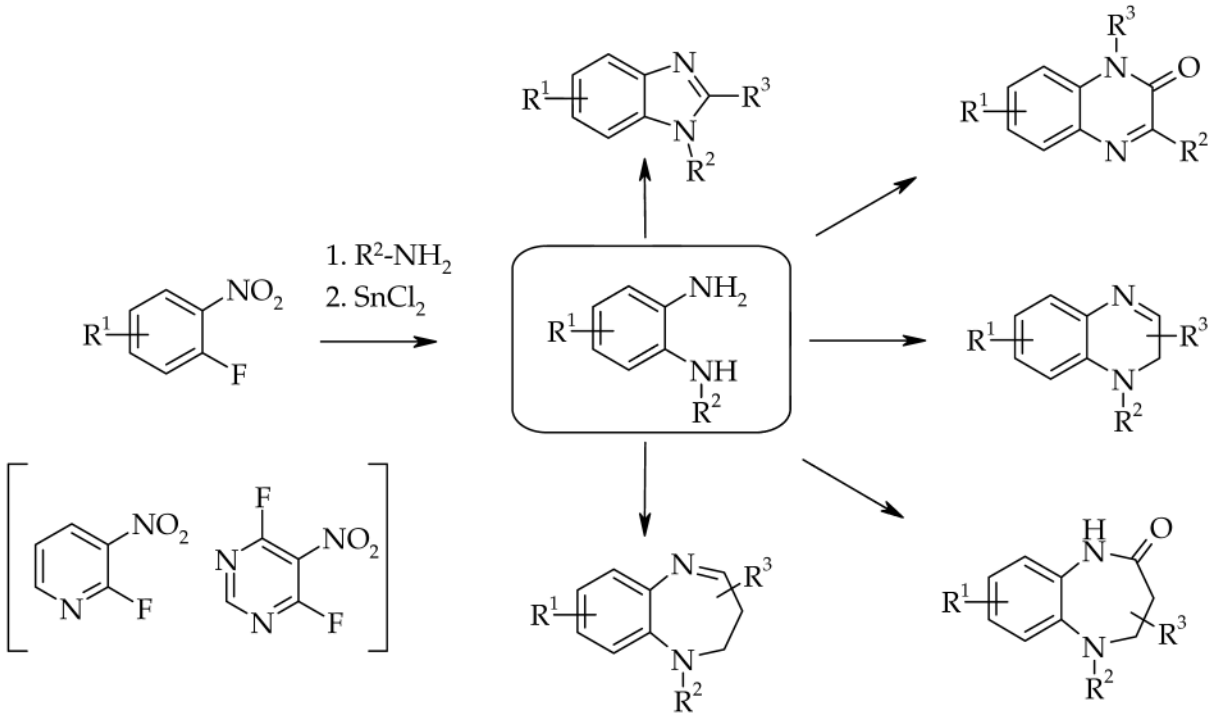
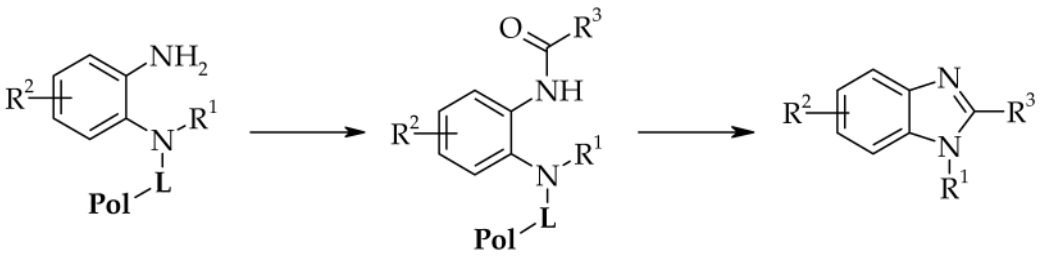


Figure 6.
1,2-Phenylenediamines motif in heterocycle synthesis



Scheme 1.
Traceless SP benzimidazole synthesis

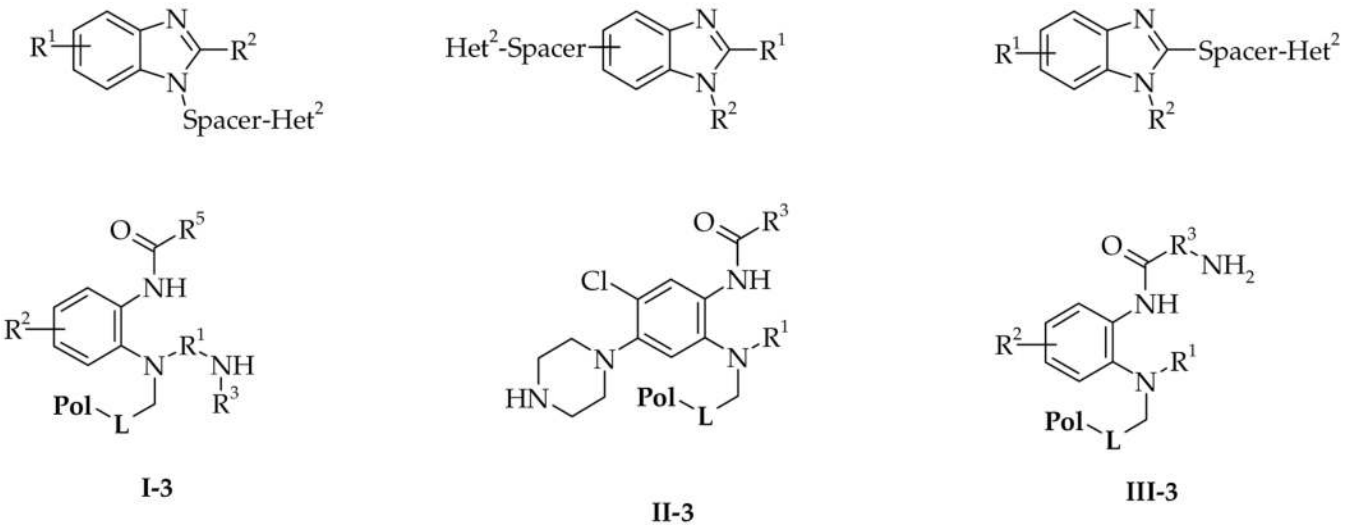


Figure 7.
Three positions of spacers and the respective intermediates

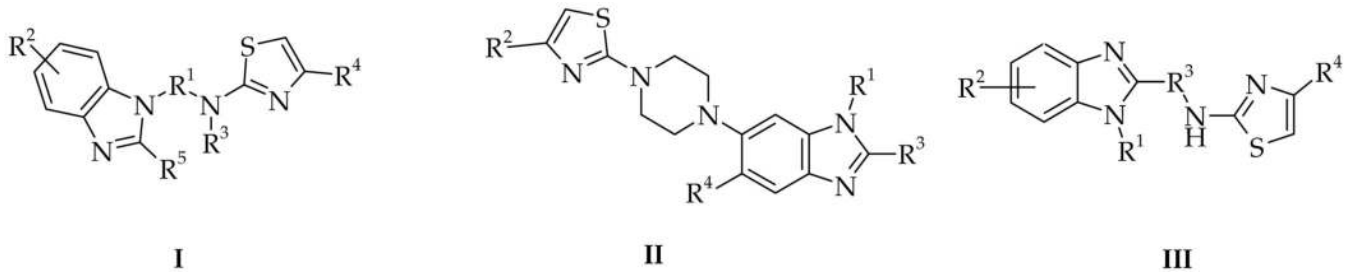
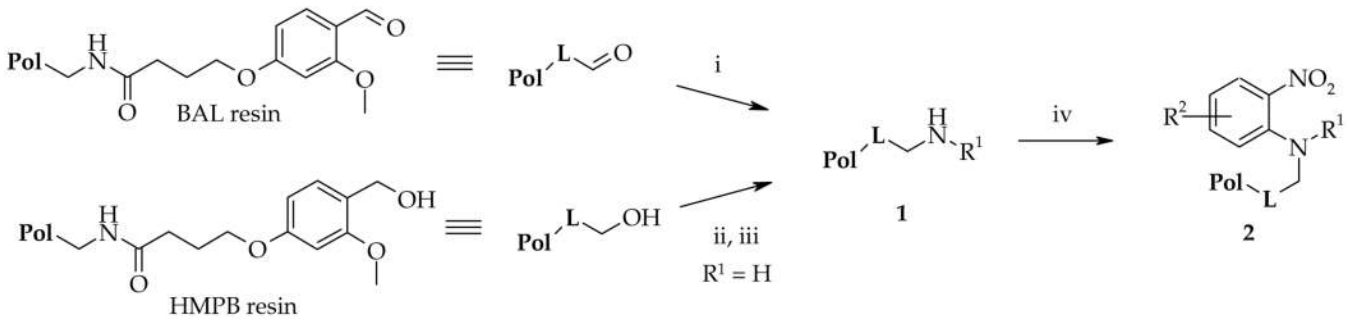


Figure 8.
Three benzimidazole-thiazole bis-heterocycles



Scheme 2.

Synthesis of resin-bound nitroanilines **2**

Reagents and conditions: (i) amine, 10% AcOH/DMF, overnight, then $NaBH(OAc)_3$, 5 h; (ii) phtalimide, PPh_3, NMP, DIAD, overnight; (iii) hydrazine hydrate, MeOH/THF, overnight; (iv) 1-fluoro-2-nitrobenzenes, DIEA, DMSO, for temperature see experimental part, overnight;

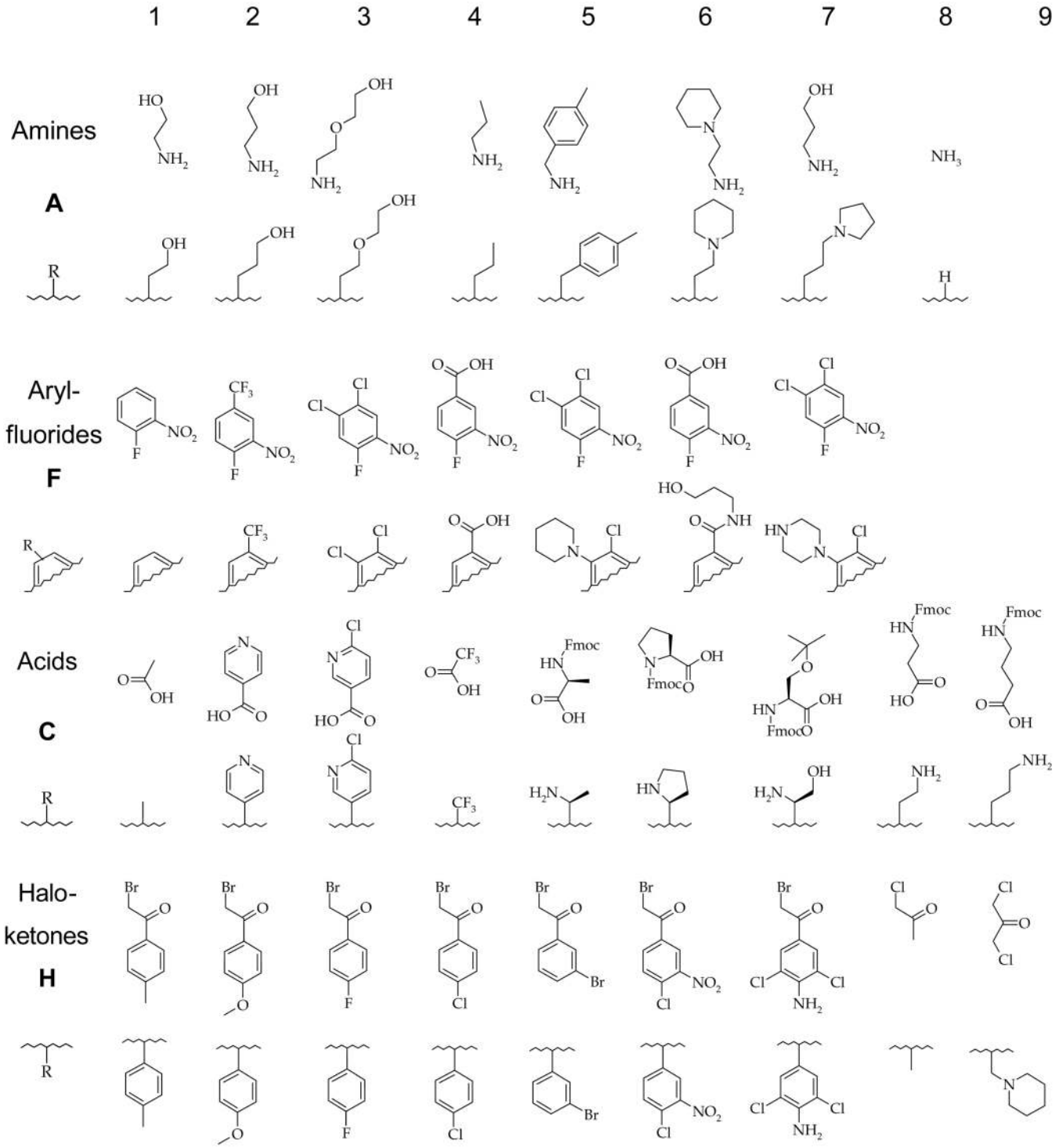
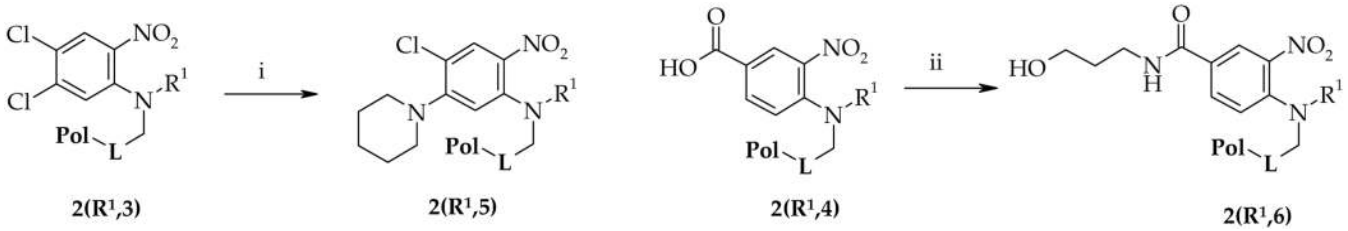


Figure 9.

Building blocks and the corresponding R groups

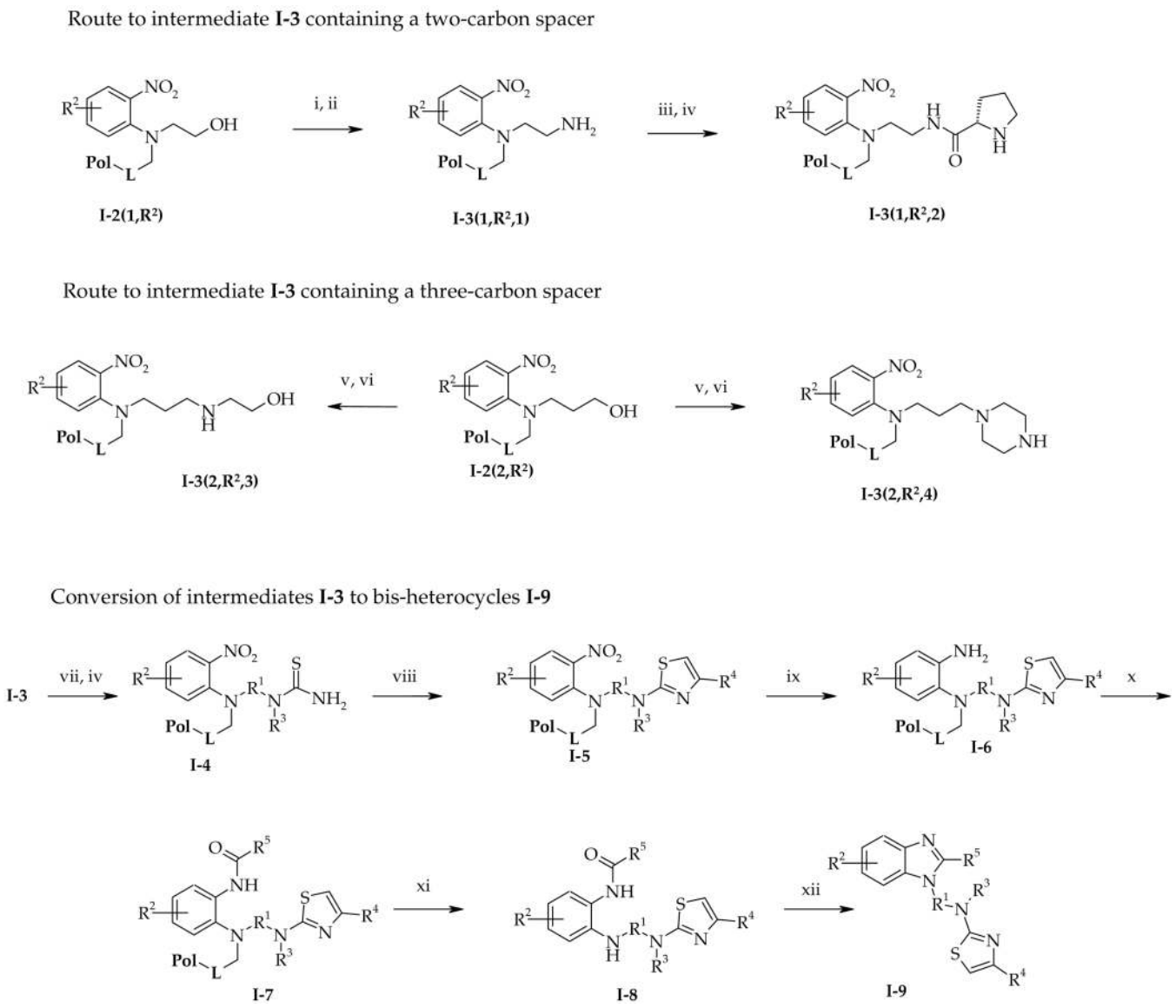
Note: Building block **A8** (R^1 = H) was introduced via the HMPB linker, side chains of building blocks **A7**, **F5** through **F7** and **H9** were installed on the resin (see the text for details)



Scheme 3.

Derivatization of resin-bound nitroanilines

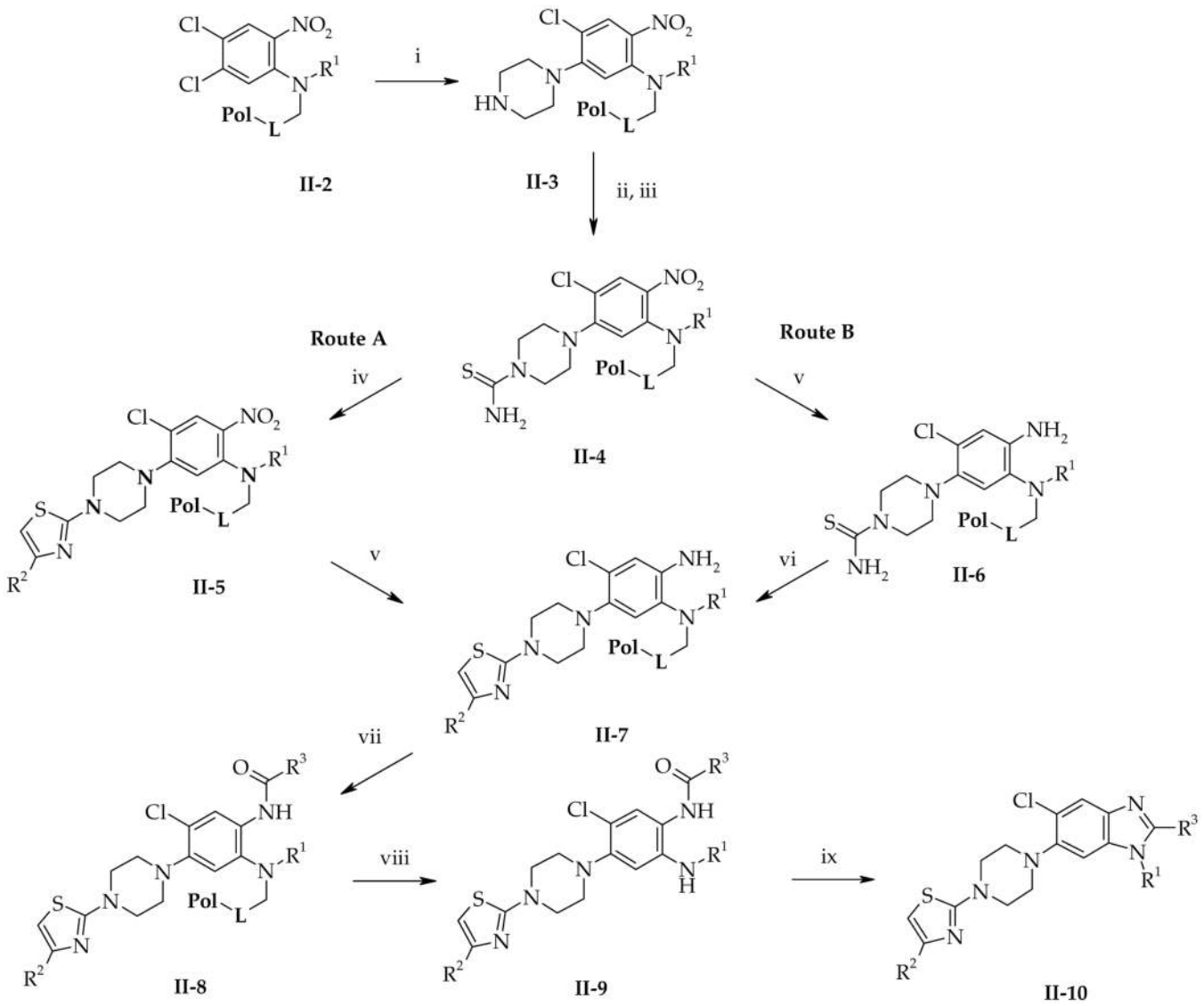
Reagents and conditions: (i) amine, DMSO, for temperature cf. experimental part, overnight; (ii) HOBt, DIC, DMF/DCM, 1h, then amino alcohol, rt, overnight.



Scheme 4.

Synthesis of bis-heterocycles **I**

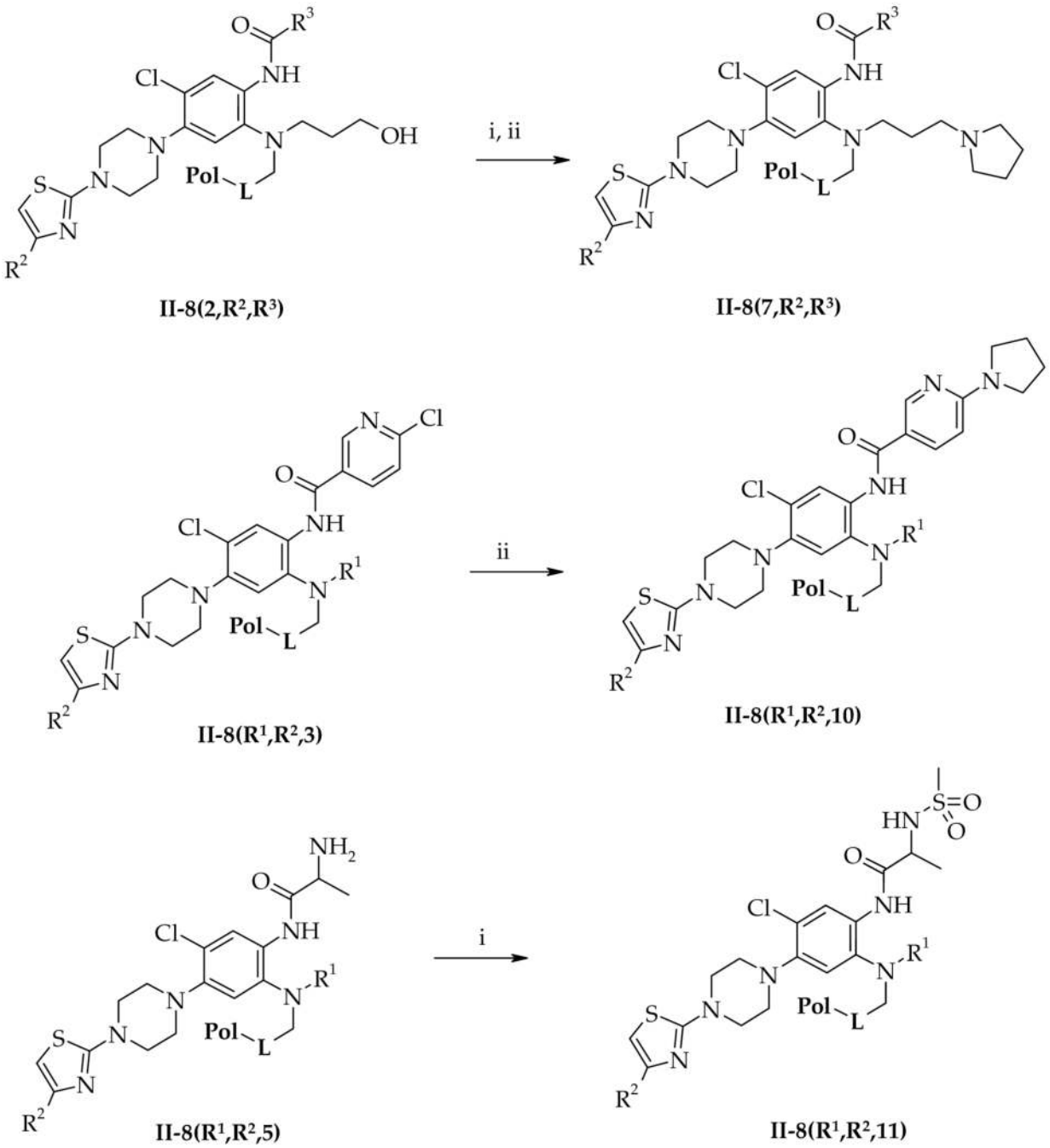
Reagents and conditions: (i) phthalimide, PPh_3, DIAD, THF, 5 h; (ii) hydrazine hydrate, MeOH/THF (1:1), overnight, (iii) Fmoc-Pro-OH, HOBt, DIC, DMF/DCM (1:1), overnight; (iv) 50% piperidine, DMF, rt, 10 min; (v) mesylchloride, pyridine, 1h; (vi) amine, DMSO, for temperature and time cf. experimental part; (vii) Fmoc-NCS, THF, rt, 60 min; (viii) haloketone, 1,8-bis(dimethylamino)naphthalene, DCM, rt, 4h; (ix) $SnCl_2 \cdot 2H_2O$, DIEA, DMF, rt, overnight; (x) for acylating conditions c.f. experimental part; (xi) 50% TFA, DCM, rt, 30 min; (xii) AcOH, 60 °C, 2 - 24 h.



Scheme 5.

Synthesis of bis-heterocycles **II**

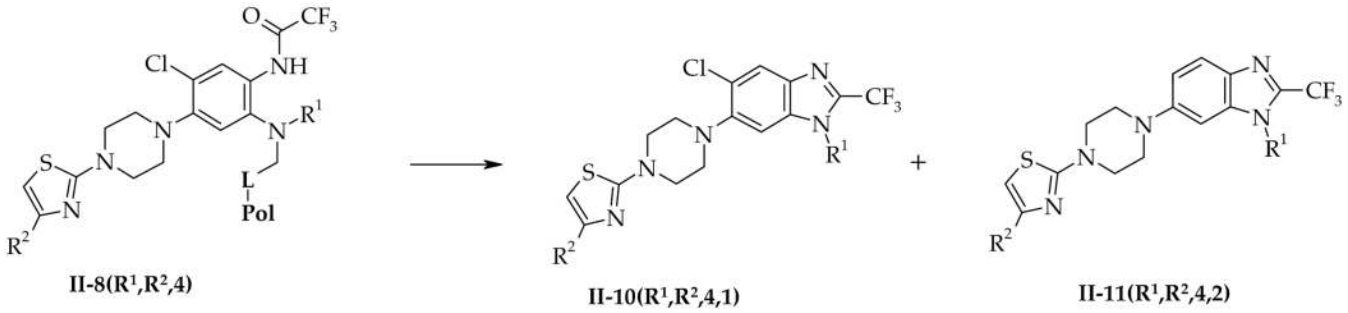
Reagents and conditions: (i) piperazine, DMSO, 60°C, overnight; (ii) Fmoc-NCS, THF, rt, 60 min; (iii) 50% piperidine, DMF, rt, 10 min; (iv) haloketones, 1,8-bis(dimethylamino) naphtalene, DCM, rt, 4h; (v) $SnCl_2 \bullet 2H_2O$, DIEA, DMF, rt, overnight, (vi) for alkylation conditions c.f. experimental part; (vii) for acylating conditions c.f. experimental part; (viii) 50% TFA, DCM, rt, 30 min; (ix) AcOH, 60 °C, 2 - 24 h; then 5% of NaOH in MeOH, overnight (for hydroxyl derivatives)



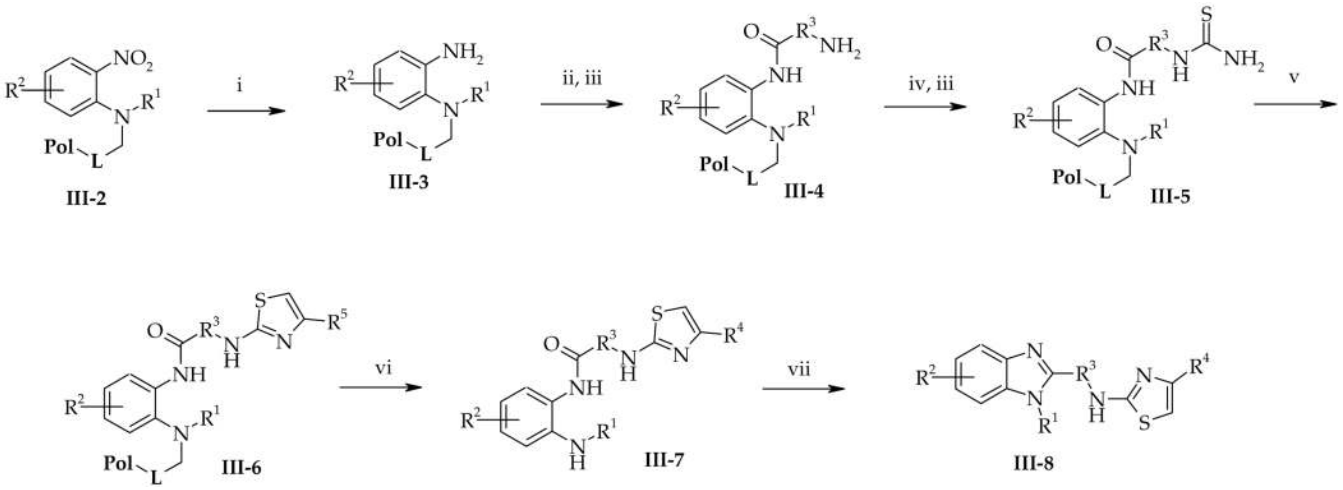
Scheme 6.

On-resin transformations of final intermediates **II-8**

Reagents and conditions: (i) mesyl chloride, pyridine, rt, 1 h; (ii) pyrrolidine, DMSO, rt, overnight. Bis-heterocycle precursors **II-9** were obtained after TFA/DCM cleavage from resins **II-8**. Final cyclization to the corresponding bis-heterocycles **II-10** was carried out in acetic acid at elevated temperature. The reaction time necessary for complete cyclization was dependent on the N-acyl substituent. While the cyclization of compounds **II-9{R^1,R^2,1}** and **II-9{R^1,R^2,2}** was completed after 2 h, **II-9{R^1,R^2,3}** were cyclized after 4 h and the **II-9 {R^1,R^2,5}** and **II-9{R^1,R^2,4}** derivatives required heating overnight.



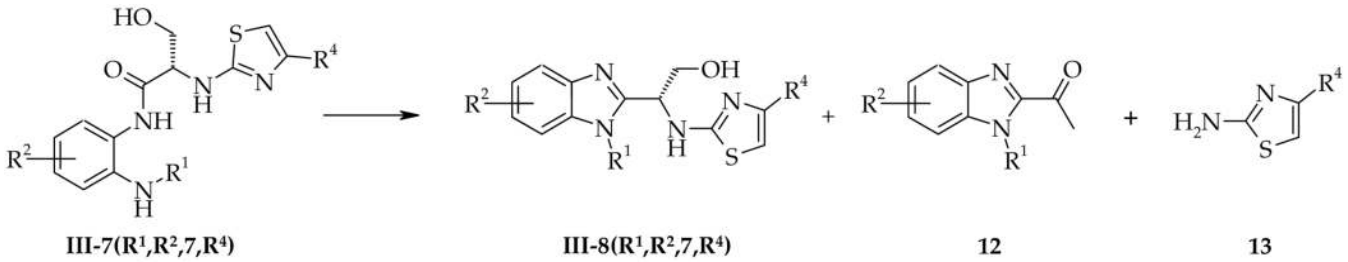
Scheme 7.
De-chlorination of aromatic ring (the last digit "1" refers to chloro derivatives, "2" refers to unsubstituted derivatives)



Scheme 8.

Synthesis of bis-heterocycles **III**

Reagents and conditions: (i) $SnCl_2.H_2O$, DIEA, DMF, rt, 2 h to overnight; (ii) Fmoc-amino acid, DIC, THF, for temperature and time cf. experimental part; (iii) 50% piperidine, DMF, rt, 30 min; (iv) Fmoc-NCS, DCM, rt, 60 min; (v) haloketone, DCM, rt, overnight; (vi) 50% TFA, DCM, rt, 30 min; (vii) AcOH, 60 °C, for reaction time c.f. experimental part, then 5% of NaOH in MeOH, 60 °C, overnight (when amino alcohols were used in the first combinatorial step).



Scheme 9.
The decomposition of hydroxymethylated bis-heterocycles