Solid-Phase Synthesis of Highly Diverse Purine-Hydroxyquinolinone Bisheterocycles

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Solid-phase synthesis of bisheterocyclic compounds that contain purine and the 3-hydroxyquinolin-4(1*H*)-one skeleton connected with an aliphatic spacer of a different length/structure is described. The reaction sequence started from the primary amines immobilized on aminomethylated polystyrene resin equipped with an acid-labile linker (4-(4-formyl-3-methoxyphenoxy)butyric acid). After the arylation of amines with 2,6-dichloropurine via its C^6, purine N^9 was alkylated and subsequently the chlorine at purine C^2 was substituted with aliphatic diamines. The resulting terminal amino group was used as the starting point for the synthesis of 3-hydroxyquinolin-4(1*H*)-one precursors based on the acylation with 3-amino-4-(methoxycarbonyl)benzoic acid followed by the saponification of the methyl ester and esterification of the resulting carboxylic acid with various haloketones. The intermediates were cleaved from the resin, and their cyclization to the target purine-hydroxyquinolinone bisheterocycles was accomplished by heating in acetic or trifluoroacetic acid.

Introduction

Synthetic derivatives of 2-substituted-3-hydroxyquinolin-4(1*H*)-ones (termed "hydroxyquinolinones" hereafter) represent quite a new class of biologically promising substances.[1] Among their biological effects which have been observed belong, for instance anticancer[2−4] or immunosuppressive activity.[3] In the literature, different routes for the preparation of hydroxyquinolinones from various starting materials have been described;[5−8] however, an acidic thermal cylization of anthranilates is considered as the most advantageous method.[9−11] Although hydroxyquinolinones have been subjected to relatively intense biological research in a past decade, only little attention has been paid to their combination with other condensed heterocyclic systems. The only paper focusing on the preparation and study of these compounds described hydroxyquinolinone-oxazole derivatives such as inosine 5′-monophosphate dehydrogenase inhibitors.[12] In one of our previous articles we introduced a general methodology for the preparation of bisheterocyclic compounds using solid-phase synthesis.[13] Our most recent goal was to apply the described strategy for the synthesis of bisheterocyclic derivatives in which the hydroxyquinolinone skeleton is accompanied by another heterocyclic moiety. The second heterocycle was selected with respect to a known biological potential of single heterocycles and their derivatives. Concerning biological properties, nucleic bases (i.e., purines and pyrimidines) are in a privileged position among the nitrogenous heterocycles as they are being included in large number of active compounds.[14] Purine derivatives are well-known for their ability to interact with both nucleic acids and enzymes acting in their biological transformations, quinolinones are widely used as inhibitors of topoisomerase, enzyme responsible for a cleavage and recombination of DNA. Hydroxyquinolinones were also described as reverse transcriptase inhibitors,[15] providing further evidence of their ability to interact with nucleic acids. In this article our intention was to develop a methodology for the construction of a chemical library consisting of purine-hydroxyquinolinone bisheterocycles with five diversity positions: Two positions at purine (C^6 and N^9 substitution), two positions at hydroxyquinolinone (C^2 and the carboxamide C^7 *N*-substitution), and the fifth diversity position resulting from the presence of a spacer between both heterocyclic systems (Figure 1).

Although a number of potential combinations in terms of the spacer location on each heterocycle exist, this initial study is focused on the connection of both heterocycles via hydroxyquinolinone C^7 and purine C^2 position. The synthetic route partially takes advantage of the previously described solid-phase synthesis of hydroxyquinolinone-7-carboxamides.[10] According to our latest experiments, selected hydroxy-

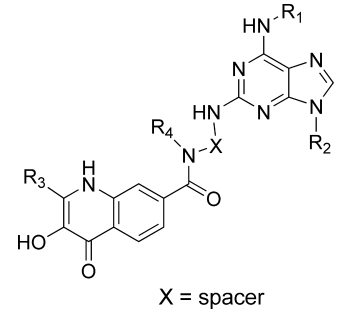

Figure 1. Suggested general structure of the target bisheterocycles.

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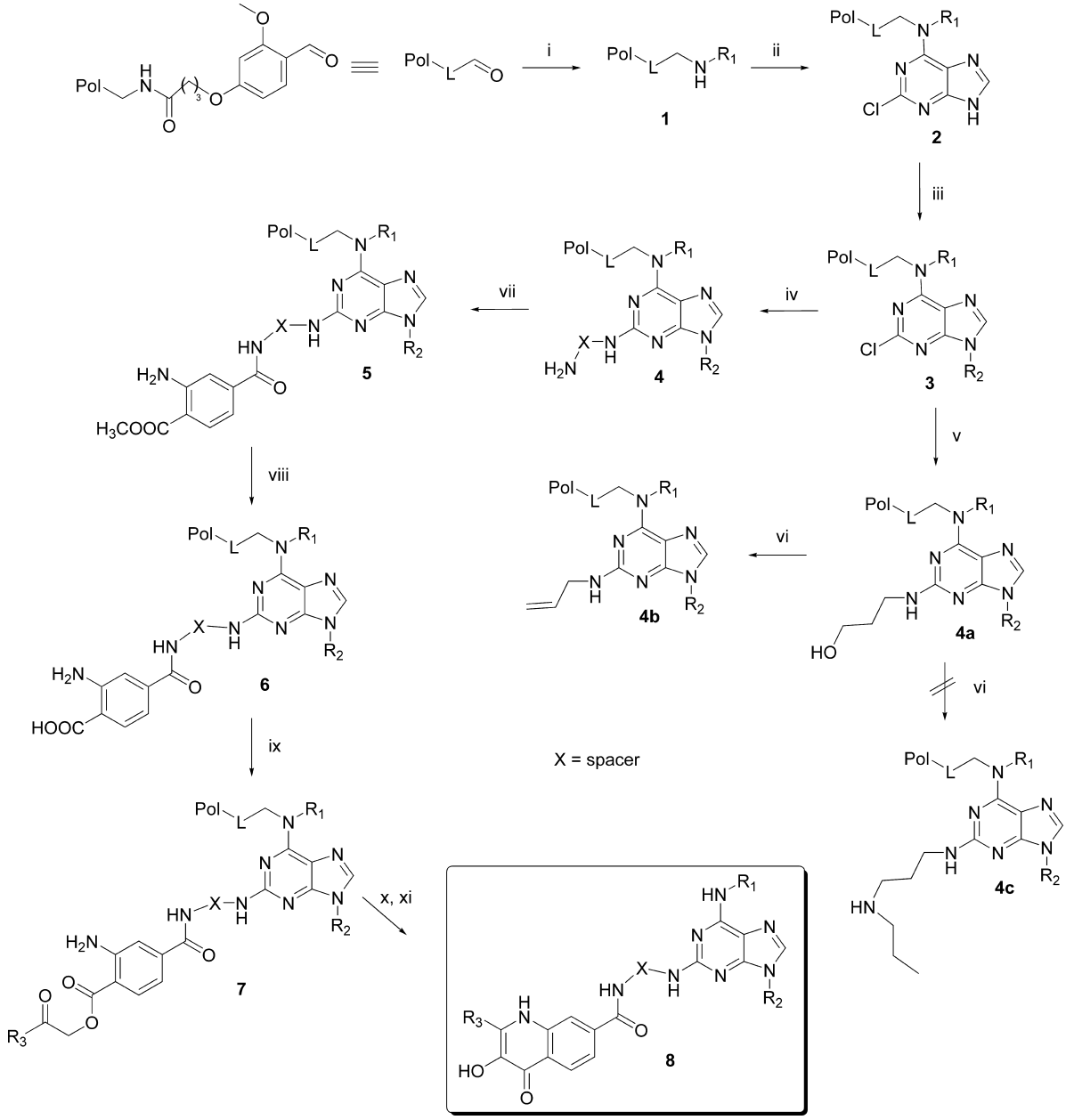
Scheme 1. Preparation of Purine-hydroxyquinolinone Bisheterocycles (**8**)[a]



[a] Reagents: (i) amine, 10% AcOH/DMF, overnight, $NaBH(OAc)_3$, 4 h; (ii) 2,6-dichloropurine, DIEA, THF, 50 °C, overnight; (iii) alkyl iodide, DBU, DMSO, 50 °C, overnight, (repeated once in THF); (iv) diamine, diethylene glycol diethyl ether, 150 °C, 24 h; (v) aminopropanol, diethylene glycol diethyl ether, 150 °C, 24 h; (vi) mesylchloride, pyridine, room temp., 2 h, then propylamine, DMSO, room temp., overnight; (vii) 3-amino-4-(methoxycarbonyl)benzoic acid, DIC, BtOH, DCM, DMF, room temp., overnight; (viii) TMSOK, THF, room temp., 24 h; (ix) haloketones, DIEA, DMF, room temp., 2 h; (x) TFA, DCM, room temp., 30 min.; (xi) AcOH, reflux, 3 h or TFA, reflux, 2 h.

quinolinone-7-carboxamides exhibit strong anticancer activity in vitro (IC_{50} < 500 nmol/L) which strongly predisposes such compounds for further structure modification and biological research.

Results and Discussion

The synthesis was carried out on aminomethylated polystyrene resin equipped with an acid-labile linker (4-(4-formyl-3-methoxyphenoxy)butyric acid) according to Scheme 1. After the immobilization of the primary amines using reductive amination[16] the resulting secondary amines (**1**) were regioselectively arylated with 2,6-dichloropurine to give the intermediates (**2**). In a next step, the purine N^9 had to be alkylated. Alkylation of purine derivatives using solution-phase synthesis is usually performed with alkyliodides in the presence of a suitable base whereas the N^9 modification of solid-supported purine derivatives takes advantage of the Mitsunobu protocol which allows alkylation with various alcohols under mild conditions.[17] In our case, when Mitsunobu alkylation of the intermediates (**2**) with ethanol or isopropanol was tested, reaction yields were unsatisfactory although various conditions and reagents were tested. As a result we used triphenylphosphine or tributylphosphine in various concentrations, different solvents (dry THF or NMP), and varied reaction times as well as repeating the reaction step, however, without success in achieving quantitative

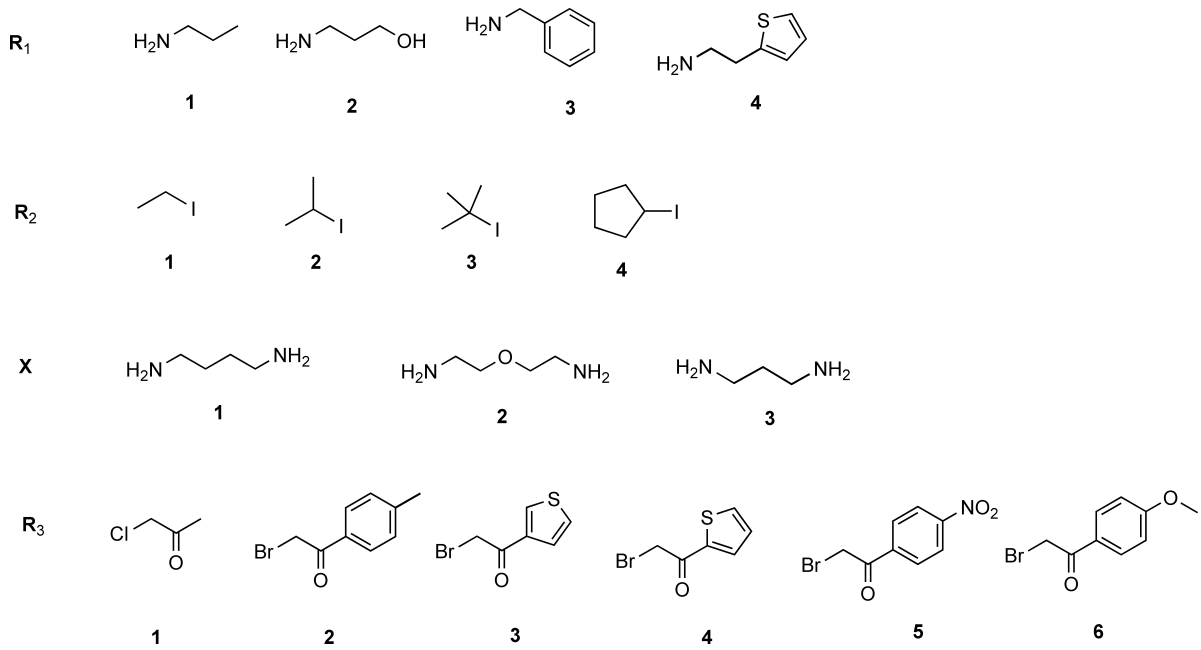
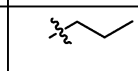
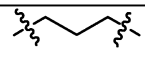
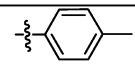
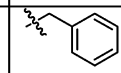
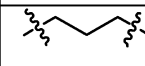
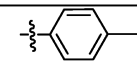
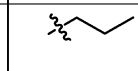
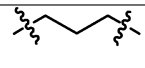
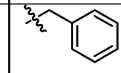
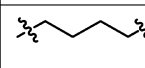
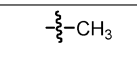
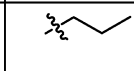
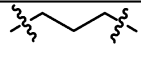
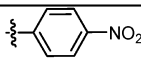
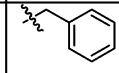
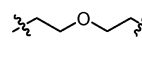
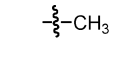
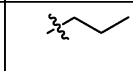
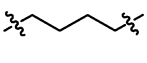
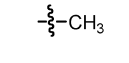
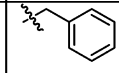
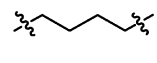
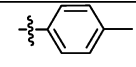
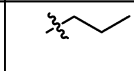
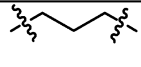
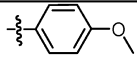
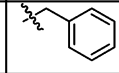
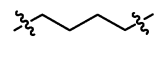
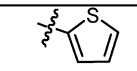


Figure 2. Building blocks successfully used for the evaluation of the synthetic route.

conversion. Additionally, the purity of the products was diminished by the appearance of number of unknown impurities when the alkylation step was repeated 2 or 3 times. Discouraged by such problems we turned our attention to the solution-phase method of alkylation with alkyl iodides, and were finally successful in transferring this strategy to the solid-phase. The reaction was performed in dimethylsulfoxide (DMSO) at elevated temperature (50 °C). Various bases were tested (such as DIEA, NaH, Na_2CO_3, Cs_2CO_3) but the best results (70−80% of conversion, HPLC-UV traces) were obtained with diazabicyclo[5.4.0]undec-7-ene (DBU). For the quantitative yield, the reaction was repeated in tetrahydrofuran (THF) giving intermediates (**3**) of high purity. If the reaction was repeated in DMSO instead of in THF, the yield did not exceed 90%. The developed alkylation method was shown to be limited by the character of the alkyl iodide used. It did not work for methyl iodide and cyclohexyl iodide but it was successfully tested for ethyl iodide, isopropyl iodide, *t*-butyl iodide, and cyclopentyl iodide. Among all used amines and alkyl iodides (see Figure 2) only one exception was detected: The alkylation of the 6-*N*-benzylamine intermediate (**2**) with *t*-butyl iodide furnished only 60% of conversion.

The subsequent substitution of the chlorine atom at purine C^2 required a high temperature (150 °C) to proceed; it was tested for various aliphatic diamines. First, the reaction was performed in solvents typically used for a nucleophilic substitution such as DMSO, DMF or NMP but the intermediates (**4**) were obtained in limited purity. In each case, formation of unknown side-products (20−30%, HPLC-UV traces) was observed which was probably caused by the reaction of intermediates (**3**) with the solvent under the harsh conditions required. The selection of the suitable solvent was crucial at this step - when the reaction was carried out in inert diethylene glycol diethyl ether, the intermediates (**4a**) were obtained in excellent purity. Surprisingly, the method was successfully tested for propylenediamine, butane-1,4-diamine and 3-oxapentane-1,5-diamine but the same reaction with ethylenediamine afforded only a mixture of unknown compounds. In this step we also attempted to increase the diversity of the target bisheterocycles. Instead of diamines, the intermediates (**3**) were reacted with an aminoalcohol (aminopropanol in our case) and the derivatives (**4a**) were obtained. Our intention was to modify the terminal hydroxyl group using mesylation and subsequent reaction with primary amines (propylamine in our case) resulting in possible formation of the secondary aminoderivatives (**4c**). Unfortunately, this modification was unsuccessful and afforded only mixtures of compounds whose major products corresponded to the formation of alkene derivatives (**4b**). Compounds **4** were acylated with 3-amino-4-(methoxycarbonyl)benzoic acid to give intermediates (**5**). If aminopropanol was used for the reductive amination (step i), its hydroxy group was also partially acylated (20%, HPLC-UV traces); however, this side product was removed in the next step, when saponification of the intermediates (**5**) was performed with potassium trimethylsilanolate leading to the carboxylic acids (**6**). Surprisingly, the saponification took place very slowly, and a longer reaction time (at least 24 h) was necessary for the quantitative hydrolysis. After the esterification of the carboxylic acids (**6**) with haloketones of aliphatic, aromatic, or heterocyclic character, the precursors (**7**) were obtained in excellent purity above 90% (HPLC-UV traces). The final cyclization to bisheterocycles (**8**) was performed after the acid-mediated cleavage of the precursors (**7**) from the resin. Two cyclization methods were tested. When trifluoroacetic acid (method A) was used as the cyclizing agent, the target products were usually obtained in excellent purity; in several cases, however, the partial acidic hydrolysis of the esters (**8**) resulting in a contamination of the products with derivatives (**6**) was observed. Thus, we also tested the cyclization in acetic acid (method B) which did not cause the competitive hydrolysis and afforded the target products in very good purity, typically over 90% (HPLC-UV traces). Nevertheless, when the method B was used for the cyclization of the hydroxygroup-containing intermediates (obtained

Table 1. Summary of the Prepared Products

Comp.	R_1	R_2	X	R_3	Comp.	R_1	R_2	X	R_3
8{1,1,3,2}					8{3,1,3,2}				
8{1,2,3,3}					8{3,1,1,1}				
8{1,2,3,5}					8{3,2,2,1}				
8{1,2,1,1}					8{3,2,1,2}				
8{1,3,3,6}					8{3,2,1,4}				
8{1,4,1,1}					8{3,2,3,2}				
8{2,2,3,6}					8{4,1,1,2}				
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8{3,1,3,3}					8{4,2,1,4}				
8{4,3,1,5}					8{4,3,3,6}				

by the reductive amination with aminopropanol, step i), the final compounds were not obtained as the hydroxyl derivatives but the corresponding *O*-acetyl derivatives were isolated. Generally, TFA cyclization can be considered as the more versatile method; however, the cyclization of derivatives (**7**) bearing a nitro group needed to be performed using method B, otherwise the cyclization was not observed.

The purification of the crude products was accomplished by simple sonification in diethylether and subsequent filtration of the precipitated material.

The building blocks used for the synthesis were selected with respect to the highest skeletal diversity of the target substances as well as the commercial availability of the synthones. As can be seen in Figure 2, ligands with aliphatic, aromatic, and heterocyclic character were introduced for R_1 and R_2, also substituted phenyls for R_3 were successfully tested (with both electron-donating and electron-withdrawing functional groups). Three different aliphatic spacers of various length/constitution were selected. Other spacers such as aromatic skeleton containing ligands have been not tested but could be of interest in the future.

In conclusion, we have developed a method of solid-phase synthesis of novel purine-hydroxyquinolinone bisheterocycles with four diversity positions. The versatility of the method was demonstrated by the preparation, isolation and full characterization of 20 compounds (see Table 1). After careful optimization of the reaction sequence the target compounds could be obtained in very good crude purity (typically over 90%) and good yield (about 60% on average) (Table 2). Although the time needed for the entire reaction sequence is approximately 10 days, the methodology is robust, variable, and applicable for the preparation of a large chemical library from commercially available synthones. The target molecules consist of two heterocyclic systems of high biological potential. Hence they represent excellent substrates for biological research.

Table 2. Purity and Yield of the Crude Products[a]

compound	purity (%)	yield(%)	compound	purity (%)	yield (%)
8(1,1,3,2)	90	80	8(3,1,1,1)	88	89
8(1,2,3,3)	93	80	8(2,2,2,1)	90	12
8(1,2,3,5)	98	43	8(3,2,1,2)	93	45
8(1,2,1,1)	85	82	8(3,2,1,4)	98	42
8(1,3,3,6)	90	79	8(3,2,3,2)	95	97
8(1,4,1,1)	82	65	8(4,1,1,2)	95	98
8(2,2,3,6)	95	67	8(4,1,3,2)	99	95
8(2,2,2,2)	98	14	8(4,2,1,4)	95	25
8(3,1,3,3)	86	52	8(4,3,3,6)	65	98
8(3,1,3,2)	80	81	8(4,3,1,5)	86	95

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

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

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