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Synthesis of Novel N^9-Substituted Purine Derivatives from Polymer Supported α-Amino Acids

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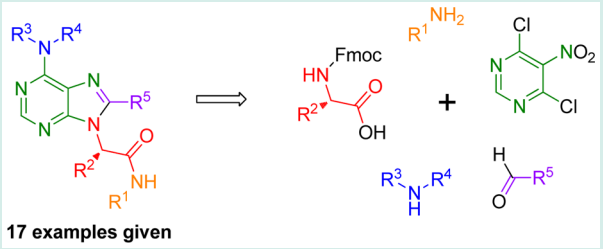
Supporting Information

ABSTRACT: Solid-phase synthesis of purine derivatives bearing an α-amino acid motif in position 9 is described herein. Polymer supported amines were acylated with various Fmoc-α-amino acids and, after cleavage of the protecting group, arylation with 4,6-dichloro-5-nitropyrimidine or 2,4-dichloro-5-nitropyrimidine was performed. The second chlorine atom was replaced with various amines. Subsequent reduction of the nitro group, followed by reaction with aldehydes, afforded the purine scaffold. After cleavage from the polymer support, the target compounds were obtained in very good crude purity, good overall yields, and excellent enantiomeric purity. The anticancer activity of prepared compounds was tested in vitro against human cancer cell lines MCF7 and K562, and they were found to have mild, but clear dose-dependent effects.



KEYWORDS: *α-amino acids, solid-phase synthesis, purine derivatives, anticancer activity*

INTRODUCTION

The most frequently studied derivatives within the group of nitrogenous heterocycles are those which contain a purine skeleton. The purine scaffold is considered very important as it is heavily present in the structure of nucleic acids. Therefore, a tremendous amount of research into the use of purines in the field of drug discovery has been undertaken and various biologically active purine derivatives have been produced.[1] The subsequent structure−activity relationship studies have been focused on different patterns of the purine scaffold in positions C^2, C^6, and C^8. Similarly, the N^9 position of synthetic purines have been substituted with different (non)functionalized ligands, based on aromatic, aliphatic, alicyclic, or heterocyclic moieties (Figure 1) and various biological properties have been reported.[2−7]

The high biological potential of purine derivatives inspired us to develop a synthetic method yielding novel derivatives with specifically functionalized N^9 position. We focused on the incorporation of a ligand based on the structure of natural α-amino acids (Figure 1, R^2 refers to a side chain of the acid). Natural α-amino acids represent excellent building blocks for the preparation of various biologically active compounds due to their large commercial availability, structural diversity and high interaction potential with biomolecules. For this reason, the core structure (Figure 1, R^2−R^5 = H) has been introduced as the key part of well-known peptide nucleic acids (PNAs).[8,9] Such compounds have been typically obtained from adenine-9-yl acetic acid, which was presynthesized and subsequently used for the modification of selected peptides.[10] Our goal was to develop a simple procedure, applicable for the quick preparation of diverse target derivatives, with highly variable types of individual ligands. With respect to the large number of available building blocks (primary amines, secondary amines, Fmoc-amino acids, aldehydes), we suggested the use of a high-throughput synthetic concept based on polymer-supported chemistry. Solid-phase synthesis in connection with combinatorial chemistry has frequently been applied in medicinal chemistry to easily produce various collections of compounds (chemical libraries),[11] for structure−activity relationship studies of pharmacologically relevant substances. Herein, we report its use for the preparation of desired molecules inspired by methodologies for solid-phase synthesis of purines and deazapurines recently reported.[12−16]

RESULTS AND DISCUSSION

The synthetic approach leading to target compounds is depicted in Scheme 2. To test its applicability, four representative building blocks were selected: propylamine,

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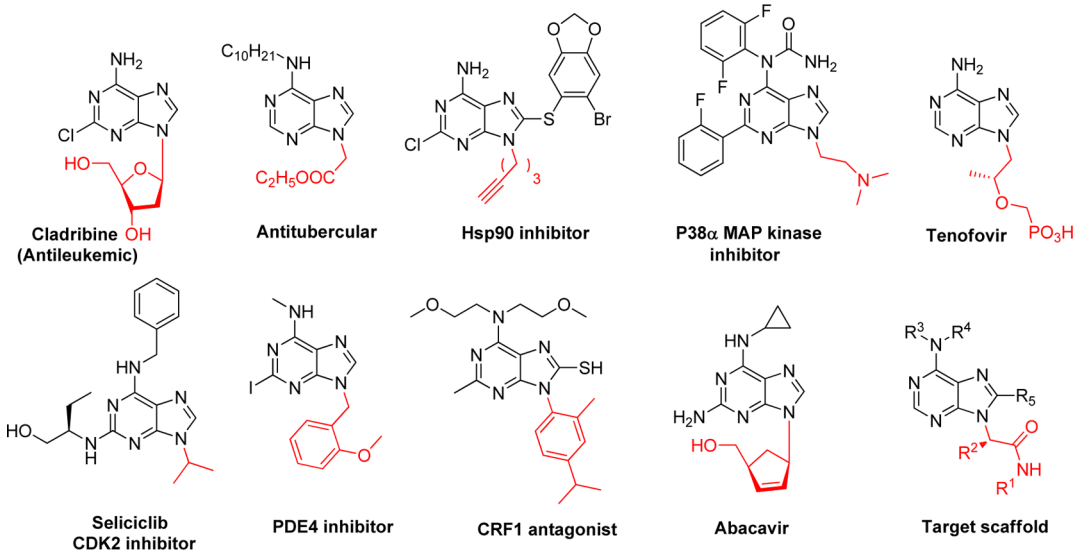
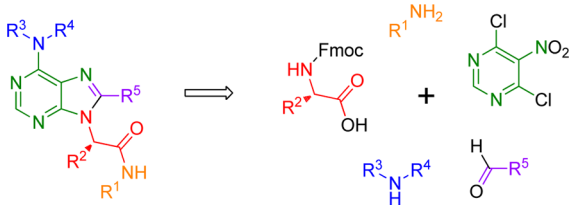


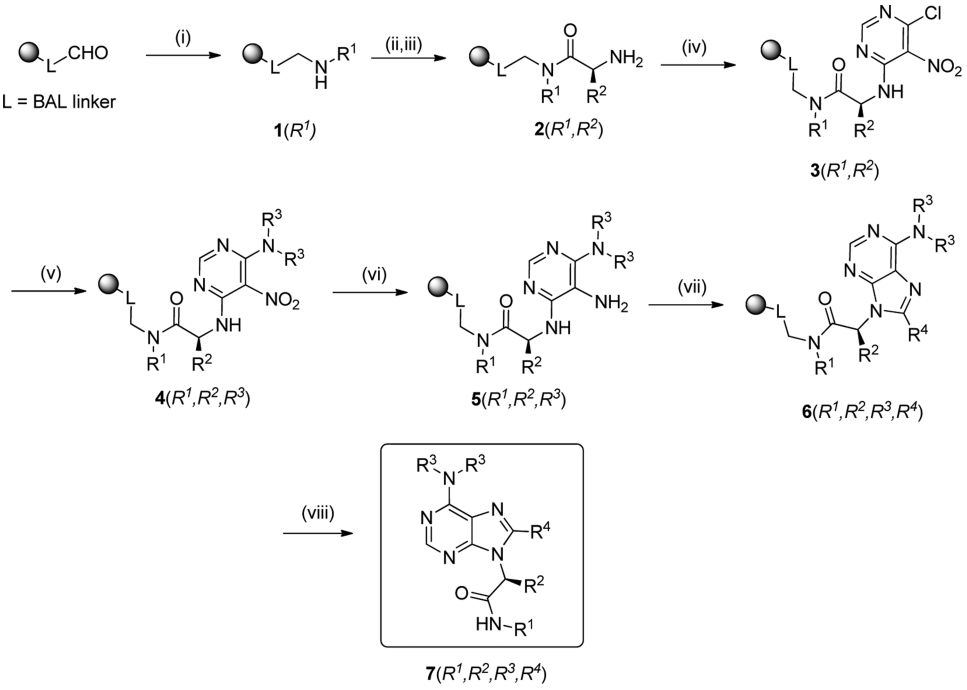
Figure 1. Representative examples of bioactive purines with a different substitution pattern in N^9 position and specification of the target scaffold.

Scheme 1. Building Blocks and Their Positioning in the Target Scaffold



Fmoc-Ala-OH, piperidine, and benzaldehyde. In the first step, aminomethyl polystyrene resin equipped with backbone amide linker (4-(4-formyl-3-methoxyphenoxy)butanoic acid)[17] was reductively aminated with propylamine to give resin **1**(*1*). After acylation with Fmoc-Ala-OH and deprotection with piperidine, polymer supported alanine derivative **2**(*1,1*) was obtained. Arylation with 4,6-dichloro-5-nitropyrimidine, followed by the replacement of the chlorine atom with piperidine, yielded resin **4**(*1,1,1*). It is worth mentioning that cleavage of resin **3**(*1,1*) for analytical purposes always afforded the expected product

Scheme 2. General Synthetic Pathway Leading to Target Compounds 7[a]



[a]Reagents: (i) Amine, 10% AcOH, dry dimethylformamide (DMF), rt, 16 h, then $NaBH(OAc)_3$, rt, 4 h; (ii) Fmoc-amino acid, 1-hydroxybenzotriazole (HOBt), *N,N*-diisopropylcarbodiimide (DIC), DMF, dichloromethane (DCM), rt, 16 h; (iii) piperidine, DMF, rt, 30 min; (iv) 4,6-dichloro-5-nitropyrimidine, *N,N*-diisopropylethylamine (DIPEA), dry DMF, rt, 16 h; (v) 10% secondary amine in dry DMF, 60 °C, 16 h; (vi) $Na_2S_2O_4$, K_2CO_3, tetrabutylammonium hydrogen sulfate (TBAHS), DCM, H_2O, rt, 16 h; (vii) aldehyde, dimethyl sulfoxide (DMSO), 100 °C, conventional heating, 72 h or MW heating, 100 °C, 60 min; (viii) 50% trifluoroacetic acid (TFA) in DCM, rt, 2 h.

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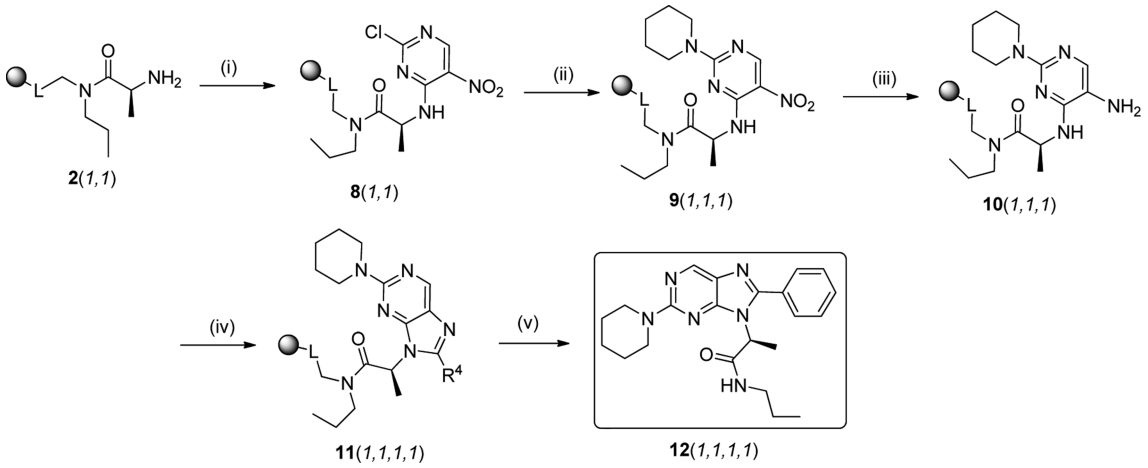
Scheme 3. Synthesis of Target Compound 12(*1,1,1,1*)[a]



[a]Reagents: (i) 2,4-Dichloro-5-nitropyrimidine, DIPEA, dry DMF, rt, 16 h; (ii) 10% piperidine in dry DMF, 60 °C, 16 h; (iii) $Na_2S_2O_4$, K_2CO_3, TBAHS, DCM, H_2O, rt, 16 h; (iv) benzaldehyde, DMSO, 100 °C, 72 h; (v) 50% TFA in DCM, rt, 2 h.

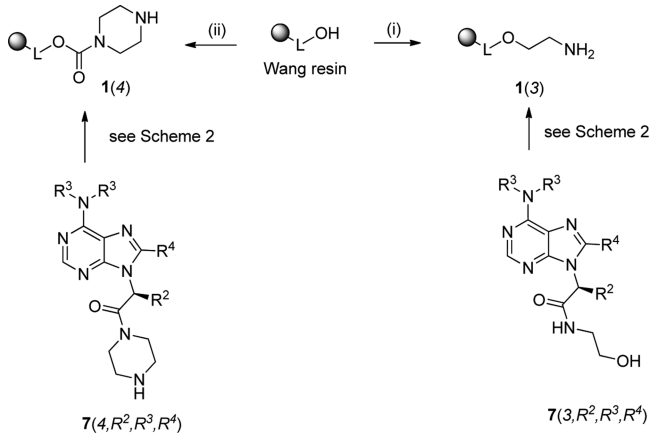
accompanied by the corresponding 4-hydroxy derivative, which was formed during the cleavage step. Subsequently, the nitro group was reduced by sodium dithionite and the polymer supported purine derivative **6**(*1,1,1,1*) was synthesized with use of benzaldehyde thermal cyclization, reported recently for the chemistry of deazapurines.[16] A long reaction time was needed for the completion of the last step (3 days) under conventional heating. For this reason, we tested the microwave heating alternative which yielded the product **6**(*1,1,1,1*) after only 60 min. The final acidic cleavage from the resin gave the target compound **7**(*1,1,1,1*) in a crude purity of 94% (calculated from LC-UV traces) and an overall yield of 38% (after semipreparative HPLC purification).

Encouraged by the result, we tested the possible application of the chemistry for the preparation of analogical purine derivatives, bearing ligand $(R^3)_2N$ in position 2 instead of position 6 (Scheme 3). For this purpose, resin **2**(*1,1*) was arylated with 2,4-dichloro-5-nitropyrimidine to give resin **8**(*1,1*). Subsequent amination with piperidine followed by reduction of the nitro group gave the resin-bound diamine **10**(*1,1,1,1*). Unexpectedly, the cyclization step with benzaldehyde was very difficult to complete. We tested various conditions: different solvents (DMSO, DMF, *N*-methyl-2-pyrrolidon (NMP)), temperatures (100−200 °C) including microwave heating, catalysis with AcOH or *p*-toluensulfonic acid, but the conversion of the starting material was low. Also, the method employing 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) described previously for similar purine derivatives did not help.[18] For this reason, the target compound **12**(*1,1,1,1*) was obtained in limited crude purity 53% and an overall yield of 7% (after semipreparative HPLC purification).

For functionalized amino derivatives (such as diamines, amino alcohols), to give amino- and hydroxy-substituted ligand R^1, a different method of attachment to the resin has been used. Starting amines were attached to Wang resin via a carbamate or ether bond to protect the hydroxy and amino groups from undesired acylation/arylation reactions (Scheme 4). With use of this strategy, two representative compounds have been synthesized using polymer supported aminoethanol and piperazine as model building blocks (Table 1).

To evaluate limitations and scope of the method, we subsequently tested various starting materials for each diversity

Scheme 4. Synthesis of Target Compounds with Use of Wang Resin[a]



[a]Reagents: (i) (a) Trichloroacetonitrile, 1,8-diazabicycloundec-7-ene (DBU), DCM, rt, 1 h, (b) 2-(Fmoc-amino)ethanol, $BF_3{\cdot}Et_2O$, THF, rt, 0.5 h, (c) 20% piperidine in DMF, rt, 20 min; (ii) (a) *N*,*N*-carbonyldiimidazole (CDI), pyridine, DCM, rt, 2 h, (b) piperazine, DCM, rt, 16 h.

position: propylamine, benzylamine, Fmoc-aminoethanol and piperazine for R^1, Fmoc-amino acids with a different substitution on the side chain to give R^2, five different secondary amines to modify position 6 of the resulting purine scaffold and aldehydes of aromatic, aliphatic, and heterocyclic moiety. In each case, the target compounds **7** were obtained in very good crude purity and good overall yields. All tested building blocks are depicted in Figure 2. Synthesized derivatives are summarized in Table 1.

Although we began this synthetic route with enantiomerically pure α-amino acids (L-isomers), we kept in mind a possible racemization within the reaction sequence. In our recent work, it was demonstrated that either full[19] or partial[20] racemization of the α-amino acid stereocenter can occur, even under mild reaction conditions. To evaluate the stereochemical outcome of this synthetic method, compound $\mathbf{7}^{DL}$(*1,3,1,1*) was prepared as the racemic standard. For this purpose, an equimolar mixture of Fmoc-L-Ser-(O*t*Bu)-OH and Fmoc-D-Ser-(O*t*Bu)-OH was used

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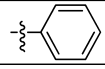
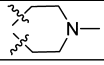
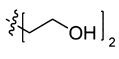
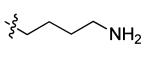
Table 1. Synthesized Derivatives 7 and 12[d]

Compound	R^1	R^2	R^3	R^4	Purity (%)[a]	Enant. purity (%)[b]	Yield (%)[c]
7(*1,1,1,1*)		Me			94	100*	38
7(*1,1,1,2*)		Me			84	NT	35
7(*1,1,1,3*)		Me			80	100*	35
7(*1,1,1,4*)		Me		Me	90	100*	37
7(*1,1,2,1*)		Me			94	100*	37
7(*1,1,3,1*)		Me			76	99.0	47
7(*1,1,4,1*)		Me			66	99.5	29
7(*1,1,5,1*)		Me			91	100*	52
7(*1,2,1,1*)					79	100*	32
7(*1,3,1,1*)					51	98.5	26
7(*1,4,1,1*)					82	NT	33
7(*1,5,1,1*)					78	NT	47
7(*1,6,1,1*)					76	NT	58
7(*2,1,1,1*)		Me			82	95.0	30
7(*3,1,1,1*)		Me			78	99.0	42
7(*4,1,1,1*)		Me			89	99.7	61
12(*1,1,1,1*)		Me			53	100*	7

[a]Crude purity after the reaction sequence according to integrated HPLC-UV chromatograms. [b]Enantiomeric purity according to integrated HPLC-UV chromatograms determined with use of the chiral separation of purified compounds, *Second isomer not detected by the method. [c]Overall yields after all reaction steps and HPLC purification. [d]NT: Not tested.

for the acylation of resin **2**(*1*) and the target derivative was synthesized according to Scheme 1. For the racemic sample, a method for the separation of enantiomers with use of chiral HPLC was developed. Analysis of the corresponding product **7**(*1,3,1,1*) by this method (Figure 3) confirmed that the amino acid stereocenter did not undergo racemization. The method was further applied to evaluate the enantiomeric purity of selected derivatives and similar results have been obtained (Table 1). Only in some cases, the negligible racemization within the reaction sequence was observed. The presence of D-isomers did not originate from the starting amino acids, although their enantiomeric purity was not determined. This fact can be demonstrated by the different enantiometric purity of products **7**(R^1,*1*,R^3,R^4) that have been synthesized from the same batch of Fmoc-Ala-OH.

To demonstrate the biological potential of target compounds, the anticancer activity against breast carcinoma and myelogenous leukemia cell lines MCF7 and K562, respectively, was evaluated using Calcein AM assay. Although clear concentration-dependent activity was observed for most compounds, IC_{50} values were determined for only 5 derivatives because of the solubility limitations (Table 2). The highest activity was detected for compound **7**(*2,1,1,1*) with IC_{50} approximating Seliciclib (roscovitine) against K562 cell line.

For this reason, we turned our attention to the mechanism of antiproliferative activity of the most potent compound. We have treated K562 cells with various doses of **7**(*2,1,1,1*) for 24 h

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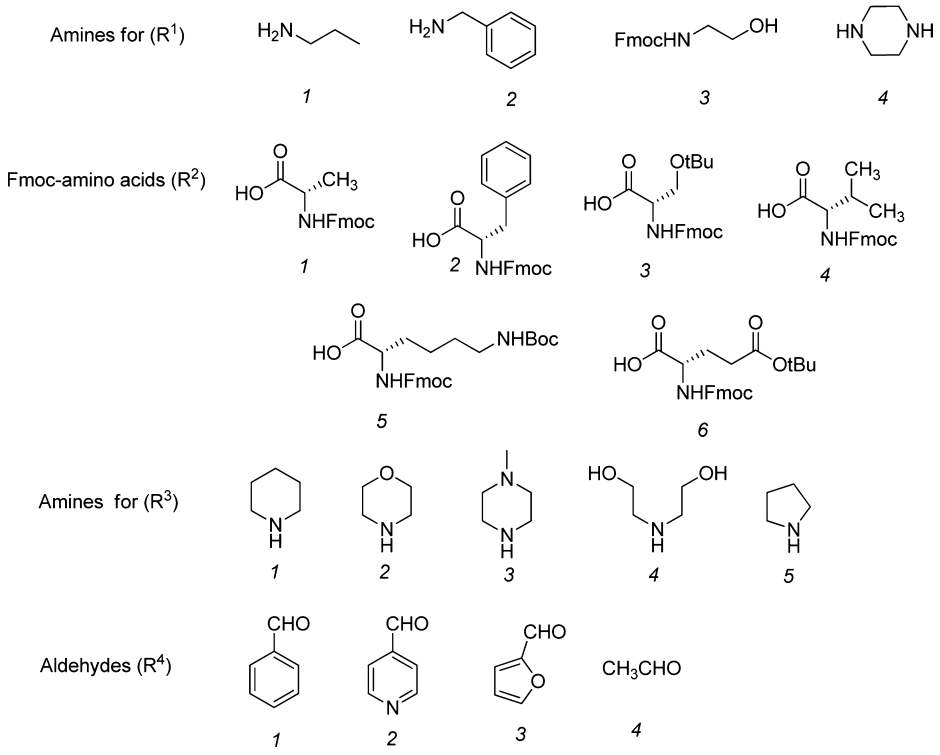

Figure 2. List of successfully tested building blocks.

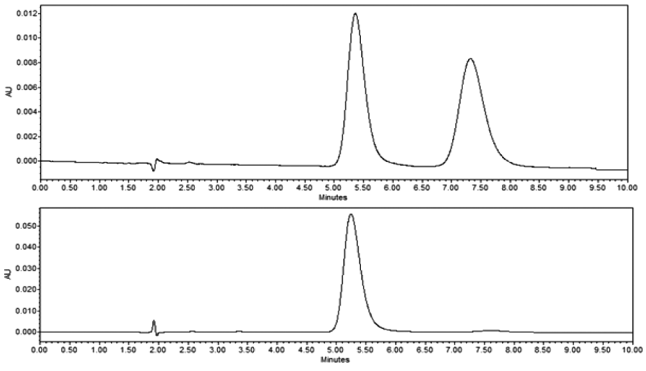

Figure 3. Chiral separation of $7^{DL}(1,3,1,1)$ enantiomers and analysis of the corresponding sample $7(1,3,1,1)$. Conditions: Chiralpak HSA (150 × 4.0 mm i.d.; particle size, 5 μm; Chiral, Illkirch Cedex, France). Mobile phase: Aqueous ammonium acetate buffer 90% (25 mM) and isopropylalcohol 10%, flow, 0.8 mL/min.

and analyzed its effect using flow cytometry. As documented by Figure 4A, the compound had no effect on the distribution of cell cycle phases even in the highest concentration used (80 μM). However, the compound did cause an increase in the sub-G1 population, which is indicative of ongoing apoptotic cell death. The increase of sub-G1 was clearly dose-dependent (Figure 4B). Apoptotic cell death progression is based on cascade activation of specific proteases—caspases. We therefore analyzed caspases 3/7 activity using a fluorimetry-based assay in lysates prepared from treated K562 cells. The assay revealed again a dose-dependent increase of caspase activity, confirming apoptosis as the mechanism of cell death caused by $7(2,1,1,1)$ (Figure 4C).

In conclusion, we have developed a simple method for the solid-phase synthesis of novel, highly diverse purine derivatives that contain natural α-amino acid motif in position N^9. The

Table 2. In Vitro Antiproliferative Activity of Compounds 7

	IC_{50} (μM)	
compound	K562	MCF7
$7(1,1,1,1)$	81	>100
$7(1,1,1,3)$	92	>100
$7(1,1,1,4)$	>100	>100
$7(1,1,1,2)$	>100	>100
$7(1,1,5,1)$	>100	>100
$7(1,1,2,1)$	>100	>100
$7(1,1,3,1)$	>100	>100
$7(1,1,4,1)$	>100	>100
$7(1,3,1,1)$	>100	>100
$7(1,2,1,1)$	75	87
$7(1,4,1,1)$	>100	>100
$7(1,6,1,1)$	>100	>100
$7(1,5,1,1)$	84	>100
$7(2,1,1,1)$	53	88
$7(3,1,1,1)$	>100	>100
$7(4,1,1,1)$	>100	>100
roscovitin	47	14

target compounds have been obtained in very good crude purity, good overall yields, and good retention of the chiral center configuration. Considering the large number of commercially available starting materials (primary and secondary amines, Fmoc-amino acids, and aldehydes), this method can be used for the simple preparation of sizable chemical libraries of diversely substituted purines. Additionally, the strategy eliminates the problem with potential formation of N^7 alkylated isomers as described earlier for N^9 alkylation of the purine scaffold.[21,22] The versatility of this synthetic route was highlighted using a variety of starting materials and demonstrated by the preparation and full characterization of 17 representative compounds. The model derivatives have been

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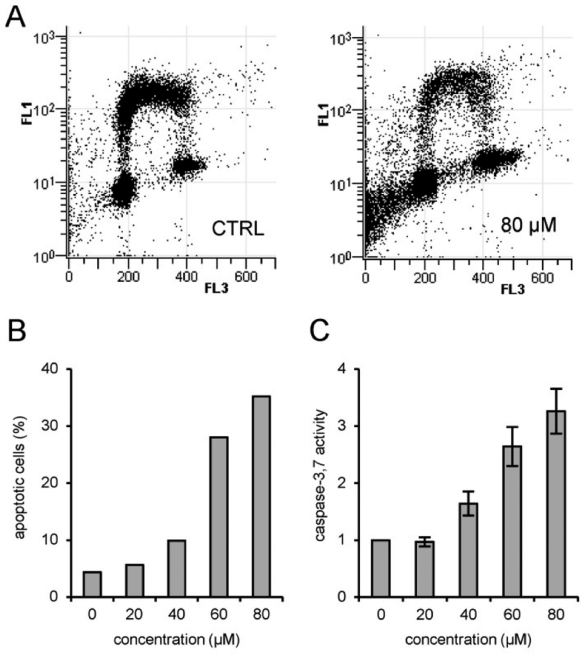

Figure 4. Effect of compound 7(*2,1,1,1*) on K562 cell proliferation and apoptosis after 24 h treatment. Cell cycle was analyzed by flow cytometry after staining of DNA by propidium iodide (FL3 signal) and 5-bromo-2́-deoxyuridine (FL1 signal) (a) and the sub-G1 cell population (b) was quantified by a Multicycle AV software (Phoenix Flow Systems, version 3.00). The activities of caspases 3/7 were measured in lysates using the fluorogenic substrate Ac-DEVD-AMC (c).

screened against two representative cancer cell lines to show their biological potential. One compound exhibited promising activity and has been further studied in terms of its antiproliferative activity mechanism. With respect to the robustness of the developed synthetic method, the desired properties can be further optimized with use of combinatorial solid-phase synthesis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of experimental, synthetic, and analytical procedures, along with spectroscopic data for synthesized compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.5b00071.

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Notes

The authors declare no competing financial interest.

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