NUCLEOSIDE PRODRUGS OF A_3 ADENOSINE RECEPTOR AGONISTS AND ANTAGONISTS

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This paper is dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his outstanding contributions to the area of nucleoside chemistry.

9-(β-D-Ribosfuranosyluronamide)adenine derivatives that are selective agonists and antagonists of the A_3 adenosine receptor (AR) have been derivatized as prodrugs for *in vivo* delivery. The free hydroxy groups at the 2′ and 3′ positions of the agonists 2-chloro-N^6-(3-iodobenzyl)-9-(*N*-methyl-(β-D-ribosfuranosyluronamide)adenine **2b**, the corresponding 4′-thio nucleoside **2c**, and antagonists **4a** and **4b** (5′-*N*,*N*-dimethylamides related to **2b** and **2c**, respectively) were derivatized through simple acylation reactions. The prodrug derivatives were tested in radioligand binding assays at ARs and in a functional assay of adenylate cyclase at the A_3AR and found to be considerably less active than the parent drugs. The hydrolysis of nucleoside 2′,3′-diesters to regenerate the parent compound in the presence of human blood was demonstrated. 2′,3′-Dipropionate esters of **2b** and **4a** were readily cleaved in a two-step reaction to regenerate the parent drug, on a time scale of two hours. The cleavage of a 2′,3′-dihexanoate ester occurred at a slower rate. This indicates that the prodrugs are suitable as masked forms of the biologically active A_3AR agonists and antagonists for future evaluation *in vivo*.

Keywords: Purines; Nucleosides; G protein-coupled receptors; Adenosine receptors; Receptor binding assays; Adenylate cyclase; Prodrugs.

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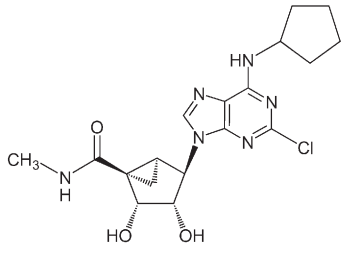
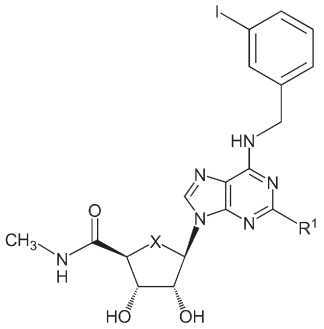
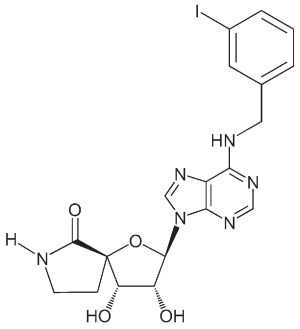
The A_3 adenosine receptor (AR) is associated with effects of adenosine in the cardiovascular, immune, visual, and central nervous systems[1]. Numerous A_3AR-selective agonists (e.g. **1**, **2**) and antagonists have been developed[2–5], and both categories of ligands have displayed cerebroprotective properties in models of ischemia and treatment regimens[6,7]. Selective agonists are also under development as cardioprotective agents. In cardiac myocytes, activation of both A_1 and A_3ARs contribute to the phenomenon of ischemic preconditioning[8,9]. Recently, dual-acting A_1 and A_3AR agonists, such as MRS3630 (**1**), were shown to be highly efficacious in the Langendorf ischemic mouse heart model[10]. Moreover, a selective A_3AR agonist, IB-MECA (CF101) (**2a**), is currently in Phase II clinical trials for colon cancer/rheumatoid arthritis[11–13]. Selective antagonists of the A_3AR have also been proposed for anti-inflammatory applications and for the treatment of glaucoma[14–16].



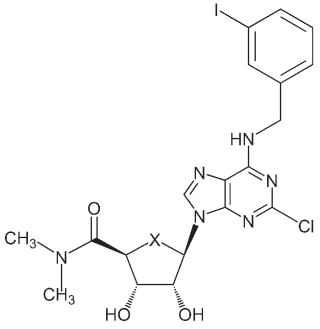
1



2a X = O, R^1 = H
2b X = O, R^1 = Cl
2c X = S, R^1 = Cl



3



4a X = O
4b X = S

The initial approach to the development of A_3AR antagonists was the screening of chemically diverse libraries[2]. A variety of heterocyclic classes were identified and optimized as A_3AR antagonists of high affinity and selectivity[4,5]. Unfortunately, while most of these antagonists showed promising results at the human A_3AR, the murine receptors were less responsive[16]. A more productive approach to the design of selective A_3AR antagonists that act across species arose from the observation that subtle changes of the nucleoside structure of selective A_3AR agonists could diminish the efficacy to the null point, i.e. agonists transformed into antagonists. Examples of such antagonists are MRS1292 (**3**)[16], MRS3771 (**4a**), and MRS3642 (LJ1256) (**4b**)[17].

As has been the case with other attempted therapeutics based on ARs, synthetic adenosine agonists under clinical development for the A_3AR may cause unwanted side effects because of the widespread distribution of ARs [1,12]. Consequently, we sought to design prodrug derivatives, such as esters, which would be relatively inactive in the masked form, but would gradually gain potency upon cleavage *in vivo*. This may be a means to prolong the half-life or to use in lipophilic delivery or a nanocarrier scheme[21]. Furthermore, the development of these nucleoside ester prodrugs could facilitate the penetration through the blood-brain barrier because of increased lipophilicity of these molecules[18,19]. Towards these goals, we have made acyl derivatives of the nucleoside-based A_3AR agonists and antagonists through reaction at the 2′ and 3′ hydroxy groups. This work follows from previously reported ester prodrugs of nucleoside derivatives that act as selective A_1AR agonists[19–21].

EXPERIMENTAL

Chemical Synthesis

Cl-IB-MECA (**2b**) was purchased from Tocris Bioscience (Ellisville, MO). Compound **4a** was synthesized in our laboratory as described[17]. Compounds **2c** and **4b** were synthesized as reported[17,22]. Other reagents and solvents were purchased from Sigma–Aldrich (St. Louis (MO), U.S.A.).

^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer using $CDCl_3$ as a solvent. The chemical shifts are expressed as ppm downfield from tetramethylsilane, coupling constants (*J*) are given in Hz.

Purity of compounds was checked using a Agilent 1100 Series HPLC equipped with a Zorbax Eclipse 5 μm XDB-C18 analytical column (250 × 4.6 mm; Agilent Technologies Inc, Palo Alto (CA), U.S.A.). System A: linear gradient solvent system: H_2O–CH_3CN from 50:50 to 0:100 in 20 min, then isocratic for 10 min; the flow rate was 1 ml/min. Peaks were detected

by UV absorption with a diode array detector. All derivatives tested for biological activity showed >98% purity in the HPLC systems.

TLC analysis was carried out on aluminum sheets precoated with silica gel F_{254} (0.2 mm) from Aldrich. High-resolution mass spectra (HRMS) measurements were performed on a Micromass/Waters LCT Premier Electrospray Time of Flight (TOF) mass spectometer coupled with a Waters HPLC system.

General Procedure for the Preparation of 2′- and/or 3′-*O*-Esters of 9-(β-D-Ribosfuranosyluronamide)adenine Derivatives

Procedure A. To a solution of the corresponding nucleoside (0.005 mmol) and DMAP (5 mg, 0.02 mmol) in pyridine (1 ml) was added dropwise the appropriate acid anhydride (0.02 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and then at room temperature for 3–18 h. The solvent was removed in vacuo, and the residue was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 97:3) to afford the desired compounds.

Procedure B. To a solution of the corresponding nucleoside (0.005 mmol) and DMAP (13 mg, 0.11 mmol) in CH_2Cl_2 (1 ml) was added dropwise the appropriate acid chloride (0.11 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and then at room temperature for 2–24 h. The solvent was removed in vacuo, and the residue was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 97:3) to afford the desired compounds.

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(methylcarbamoyl)-tetrahydrofuran-3,4-diyl Dipropionate (**5**)

Procedure A. Compound **5** (8 mg, 33%) was obtained as a white solid from **2b** (20 mg, 0.04 mmol) and propionic anhydride (6 µl, 0.05 mmol). The reaction mixture was stirred at r.t. for 16 h. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 95:5). ^{1}H NMR ($CDCl_3$): 8.49 (br s, 1 H); 7.77 (s, 1 H); 7.74 (s, 1 H); 7.65 (d, *J* = 7.5, 1 H); 7.36 (d, *J* = 7.8, 1 H); 7.10 (t, *J* = 7.8, 1 H); 6.46 (br s, 1 H); 5.98 (d, *J* = 8.0, 1 H); 5.85 (dd, *J* = 8.0 and 5.0, 1 H); 5.72 (dd, *J* = 5.0 and 1.6, 1 H); 4.78 (m, 2 H); 4.72 (d, *J* = 1.6, 1 H); 3.04 (d, *J* = 4.5, 3 H); 2.46 (m, 2 H); 2.23 (q, *J* = 7.5, 2 H); 1.20 (t, *J* = 7.7, 3 H); 1.05 (t, *J* = 7.5, 3 H). ^{13}C NMR ($CDCl_3$): 172.8, 172.6, 168.4, 155.3, 155.0, 149.4, 140.0 (2 C); 137.1 (2 C); 130.7, 127.5, 120.2, 94.8, 87.3, 82.7, 73.0, 71.1, 44.1, 27.5, 27.2, 26.7, 9.2, 8.8. HRMS, *m/z*: found 657.0716 $(M + H)^+$. $C_{24}H_{27}ClIN_6O_6$ requires 657.0725. HPLC (system A) 12.7 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(methylcarbamoyl)-tetrahydrofuran-3,4-diyl Dihexanoate (**6**)

Procedure B. Compound **6** (1.8 mg, 26%) was obtained as a white solid from **2b** (5 mg, 0.01 mmol), DMAP (11 mg, 0.09 mmol) and hexanoyl chloride (33 µl, 0.23 mmol). The reaction mixture was stirred at r.t. for 24 h. ^{1}H NMR ($CDCl_3$): 8.49 (br s, 1 H); 7.77 (s, 1 H); 7.74 (s, 1 H); 7.65 (d, *J* = 7.8, 1 H); 7.35 (d, *J* = 7.5, 1 H); 7.10 (t, *J* = 7.7, 1 H); 6.27 (br s, 1 H); 5.97 (d, *J* = 7.8, 1 H); 5.83 (m, 1 H); 5.71 (dd, *J* = 4.8 and 1.5, 1 H); 4.77 (m, 2 H); 4.71 (d, *J* = 1.8, 1 H); 3.04 (d, *J* = 4.8, 3 H); 2.42 (m, 2 H); 2.20 (t, *J* = 7.7, 2 H); 1.68 (m, 2 H); 1.53 (m, 2 H); 1.36 (m, 4 H); 1.23 (m, 4 H); 0.93 (m, 3 H); 0.85 (t, *J* = 6.9, 3 H). ^{13}C NMR ($CDCl_3$): 172.0, 171.9, 168.5, 155.4, 155.2, 149.3, 140.3, 139.6, 137.4, 136.9, 130.5, 127.7, 119.4, 94.7, 87.5, 82.8, 72.9, 71.2, 44.0, 34.1, 33.7, 31.5, 31.2, 26.7, 24.7, 24.3, 22.5, 22.4, 14.1, 14.0. HRMS,

m/z: found 741.1680 $(M + H)^+$. $C_{30}H_{39}ClIN_6O_6$ requires 741.1664. HPLC (system A) 18.7 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(methylcarbamoyl)-tetrahydrofuran-3,4-diyl Dioctanoate (**7**)

Procedure B. Compound **7** (3 mg, 40%) was obtained as a white solid from **2b** (5 mg, 0.01 mmol), DMAP (11 mg, 0.09 mmol) and octanoyl chloride (40 µl, 0.23 mmol). The reaction mixture was stirred at r.t. for 24 h. ^{1}H NMR ($CDCl_3$): 8.50 (br s, 1 H); 7.77 (s, 1 H); 7.74 (s, 1 H); 7.65 (d, *J* = 7.8, 1 H); 7.36 (d, *J* = 7.8, 1 H); 7.10 (t, *J* = 7.8, 1 H); 6.31 (br s, 1 H); 5.97 (d, *J* = 7.5, 1 H); 5.83 (m, 1 H); 5.71 (dd, *J* = 5.0 and 1.6, 1 H); 4.77 (m, 2 H); 4.71 (d, *J* = 1.6, 1 H); 3.04 (d, *J* = 4.8, 3 H); 2.42 (m, 2 H); 2.20 (t, *J* = 7.5, 2 H); 1.64 (m, 2 H); 1.52 (m, 2 H); 1.29 (m, 16 H); 0.88 (m, 6 H). HRMS, *m/z*: found 797.2317 $(M + H)^+$. $C_{34}H_{47}ClIN_6O_6$ requires 797.2290. HPLC (system A) 24.3 min (99%).

(2*S*,3*S*,4*R*,5*R*)-5-{2-Chloro-6-[(3-iodobenzyl)amino)]-9*H*-purin-9-yl}-4-hydroxy-2-(methylcarbamoyl)-tetrahydrofuran-3-yl Propionate (**8a**) and
(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino)]9*H*-purin-9-yl}-4-hydroxy-5-(methylcarbamoyl)-tetrahydrofuran-3-yl Propionate (**8b**)

Procedure A. Compounds **8a**, **8b** (10 mg, 45%) were obtained as a white solid from **2b** (20 mg, 0.04 mmol) and propionic anhydride (6 µl, 0.05 mmol). The reaction mixture was stirred at r.t. for 16 h. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 95:5). ^{1}H NMR ($CDCl_3$): 8.49 (br s, 2 H); 7.82 (s, 1 H); 7.76 (s, 1 H); 7.75 (s, 2 H); 7.66 (d, *J* = 7.8, 2 H); 7.36 (d, *J* = 6.9, 2 H); 7.10 (t, *J* = 7.8, 2 H); 6.38 (br s, 2 H); 6.07 (d, *J* = 7.8, 1 H); 5.79 (d, *J* = 8.1, 1 H); 5.55 (d, *J* = 5.7, 1 H); 5.50 (m, 1 H); 5.03 (m, 1 H); 4.80 (m, 5 H); 4.73 (m, 1 H); 4.64 (m, 1 H); 3.04 (m, 6 H); 2.50 (q, *J* = 7.5, 2 H); 2.38 (m, 2 H); 1.22 (t, *J* = 7.5, 3 H); 1.11 (t, *J* = 7.5, 3 H). HRMS, *m/z*: found 601.0461 $(M + H)^+$. $C_{21}H_{23}ClIN_6O_5$ requires 601.0463. HPLC (system A) 7.5 min (99%).

(2*S*,3*S*,4*R*,5*R*)-5-{2-Chloro-6-[(3-iodobenzyl)amino)]-9*H*-purin-9-yl}-4-hydroxy-2-(methylcarbamoyl)tetrahydrofuran-3-yl Hexanoate (**9a**) and
(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino)]-*H*-purin-9-yl}-4-hydroxy-5-(methylcarbamoyl)tetrahydrofuran-3-yl Hexanoate (**9b**)

Procedure B. Compounds **9a**, **9b** (2.6 mg, 44%) were obtained as a white solid from **2b** (5 mg, 0.01 mmol), DMAP (11 mg, 0.09 mmol), and hexanoyl chloride (33 µl, 0.23 mmol). The reaction mixture was stirred at r.t. for 24 h. ^{1}H NMR ($CDCl_3$): 8.60 (br s, 1 H); 8.50 (br s, 1 H); 7.80 (s, 1 H); 7.74 (m, 3 H); 7.63 (d, *J* = 7.8, 2 H); 7.35 (d, *J* = 7.8, 2 H); 7.08 (t, *J* = 7.8, 2 H); 6.78 (br s, 2 H); 6.05 (d, *J* = 8.1, 1 H); 5.76 (d, *J* = 8.1, 1 H); 5.52 (d, *J* = 4.5, 1 H); 5.47 (m, 1 H); 4.99 (m, 1 H); 4.77 (m, 5 H); 4.71 (m, 1 H); 4.65 (m, 1 H); 3.01 (d, *J* = 4.8, 6 H); 2.49–2.27 (m, 4 H); 1.72–1.17 (m, 12 H); 0.94–0.81 (m, 6 H). HRMS, *m/z*: found 643.0924 $(M + H)^+$. $C_{24}H_{29}ClIN_6O_5$ requires 643.0933. HPLC (system A) 11.0 and 11.4 min (99%).

(2*S*,3*S*,4*R*,5*R*)-5-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-4-hydroxy-2-(methylcarbamoyl)tetrahydrofuran-3-yl Octanoate (**10a**) and (2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-4-hydroxy-5-(methylcarbamoyl)tetrahydrofuran-3-yl Octanoate (**10b**)

Procedure B. Compounds **10a**, **10b** (3.1 mg, 50%) were obtained as a white solid from **2b** (5 mg, 0.01 mmol), DMAP (11 mg, 0.09 mmol), and octanoyl chloride (40 µl, 0.23 mmol). The reaction mixture was stirred at r.t. for 24 h. ^{1}H NMR ($CDCl_3$): 8.59 (br s, 1 H); 8.51 (br s, 1 H); 7.81 (s, 1 H); 7.76 (m, 3 H); 7.65 (d, *J* = 7.8, 2 H); 7.36 (d, *J* = 7.2, 2 H); 7.09 (t, *J* = 7.8, 2 H); 6.69 (br s, 2 H); 6.06 (d, *J* = 7.8, 1 H); 5.77 (d, *J* = 8.4, 1 H); 5.53 (d, *J* = 4.8, 1 H); 5.47 (m, 1 H); 5.01 (m, 1 H); 4.79 (m, 5 H); 4.72 (m, 1 H); 4.66 (m, 1 H); 3.03 (d, *J* = 4.8, 6 H); 2.50–2.30 (m, 4 H); 1.74–1.16 (m, 20 H); 0.92–0.81 (m, 6 H). HRMS, *m/z*: found 671.1227 $(M + H)^+$. $C_{26}H_{33}ClIN_6O_5$ requires 671.1246. HPLC (system A) 13.5 and 13.6 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(methylcarbamoyl)-tetrahydrothiophene-3,4-diyl Dipropionate (**11**)

Procedure A. Compound **11** (3.5 mg, 97%) was obtained as a white solid from **2c** (3 mg, 0.005 mmol), DMAP (6 mg, 0.05 mmol), and propionic anhydride (7 µl, 0.05 mmol). The reaction mixture was stirred at r.t. for 16 h. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 95:5). ^{1}H NMR ($CDCl_3$): 8.03 (br s, 1 H); 7.91 (s, 1 H); 7.75 (s, 1 H); 7.65 (d, *J* = 7.8, 1 H); 7.37 (d, *J* = 7.2, 1 H); 7.10 (t, *J* = 7.7, 1 H); 6.31 (br s, 1 H); 6.04 (m, 2 H); 5.93 (m, 1 H); 4.78 (m, 2 H); 4.08 (d, *J* = 2.4, 1 H); 3.07 (d, *J* = 4.8, 3 H); 2.47 (q, *J* = 7.6, 2 H); 2.25 (q, *J* = 7.6, 2 H); 1.21 (t, *J* = 7.6, 3 H); 1.05 (t, *J* = 7.6, 3 H). HRMS, *m/z*: found 673.0472 $(M + H)^+$. $C_{24}H_{27}ClIN_6O_5S$ requires 673.0497. HPLC (system A) 12.7 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Diacetate (**12**)

Procedure A. Compound **12** (2.8 mg, 81%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and acetic anhydride. The reaction mixture was stirred at r.t. for 16 h. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 95:5). ^{1}H NMR ($CDCl_3$): 8.57 (s, 1 H); 7.74 (s, 1 H); 7.63 (d, *J* = 7.5, 1 H); 7.35 (d, *J* = 7.5, 1 H); 7.08 (t, *J* = 8.0, 1 H); 6.51 (d, *J* = 7.5, 1 H); 6.34 (br s, 1 H); 5.89 (dd, *J* = 7.5 and 4.8, 1 H); 5.61 (d, *J* = 4.8, 1 H); 5.01 (s, 1 H); 4.77 (br s, 2 H); 3.11 (s, 3 H); 3.03 (s, 3 H); 2.21 (s, 3 H); 2.04 (s, 3 H). HRMS, *m/z*: found 643.0559 $(M + H)^+$. $C_{23}H_{25}ClIN_6O_6$ requires 643.0569. HPLC (system A) 11.0 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Dipropionate (**13**)

Procedure A. Compound **13** (3.1 mg, 86%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and propionic anhydride. The reaction mixture was stirred at r.t. for 3 h. ^{1}H NMR ($CDCl_3$): 8.59 (s, 1 H); 7.74 (s, 1 H); 7.63 (d, *J* = 7.8, 1 H); 7.35 (d, *J* = 7.8, 1 H); 7.08 (t, *J* = 7.8, 1 H); 6.50 (d, *J* = 7.7, 1 H); 6.35 (br s, 1 H); 5.89 (dd, *J* = 7.7 and 4.6, 1 H); 5.63 (d, *J* = 4.6, 1 H); 5.01 (s, 1 H); 4.77 (br s, 2 H); 3.12 (s, 3 H); 3.03 (s, 3 H); 2.47 (q, *J* = 7.6, 2 H); 2.31 (q, *J* = 7.6, 2 H); 1.21 (t, *J* = 7.6, 3 H); 1.08 (t, *J* = 7.6, 3 H). ^{13}C NMR ($CDCl_3$): 173.7,

173.0, 167.9, 155.1, 154.9, 151.0, 140.4, 139.8, 137.1, 137.0, 130.6, 127.4, 118.7, 94.8, 84.8, 79.8, 74.5, 73.3, 44.1, 37.3, 36.4, 27.6, 27.3, 9.2, 9.0. HRMS, *m/z*: found 671.0876 $(M + H)^+$. $C_{25}H_{29}ClIN_6O_6$ requires 671.0882. HPLC (system A) 14.0 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Dipentanoate (**14**)

Procedure A. Compound **14** (3.2 mg, 83%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and pentanoic anhydride. The reaction mixture was stirred at r.t. for 6 h. ^{1}H NMR ($CDCl_3$): 8.61 (s, 1 H); 7.73 (s, 1 H); 7.63 (d, *J* = 7.8, 1 H); 7.35 (d, *J* = 7.5, 1 H); 7.08 (t, *J* = 7.7, 1 H); 6.51 (d, *J* = 7.5, 1 H); 6.35 (br s, 1 H); 5.87 (dd, *J* = 7.5 and 4.8, 1 H); 5.61 (d, *J* = 4.8, 1 H); 5.00 (s, 1 H); 4.77 (br s, 2 H); 3.11 (s, 3 H); 3.03 (s, 3 H); 2.45 (t, *J* = 7.7, 2 H); 2.28 (t, *J* = 7.8, 2 H); 1.73–1.17 (m, 8 H); 0.96 (t, *J* = 7.4, 3 H); 0.85 (t, *J* = 7.4, 3 H). HRMS, *m/z*: found 727.1526 $(M + H)^+$. $C_{29}H_{37}ClIN_6O_6$ requires 727.1508. HPLC (system A) 19.0 min (98 %).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Dihexanoate (**15**)

Procedure B. Compound **15** (1.6 mg, 39%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol), DMAP (26 mg, 0.21 mmol), and hexanoyl chloride (30 µl, 0.21 mmol). The reaction mixture was stirred at r.t. for 24 h. ^{1}H NMR ($CDCl_3$): 8.64 (s, 1 H); 7.74 (s, 1 H); 7.62 (d, *J* = 8.4, 1 H); 7.36 (d, *J* = 7.5, 1 H); 7.08 (t, *J* = 7.8, 1 H); 6.72 (br s, 1 H); 6.51 (d, *J* = 7.8, 1 H); 5.86 (dd, *J* = 7.8 and 4.7, 1 H); 5.60 (d, *J* = 4.7, 1 H); 5.00 (s, 1 H); 4.76 (br s, 2 H); 3.12 (s, 3 H); 3.03 (s, 3 H); 2.44 (t, *J* = 7.5, 2 H); 2.27 (t, *J* = 7.4, 2 H); 1.76–1.14 (m, 12 H); 0.92 (m, 3 H); 0.83 (m, 3 H). HRMS, *m/z*: found 755.1780 $(M + H)^+$. $C_{31}H_{41}ClIN_6O_6$ requires 755.1821. HPLC (system A) 22.2 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Dioctanoate (**16**)

Procedure B. Compound **16** (1.8 mg, 41%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol), DMAP (26 mg, 0.21 mmol), and hexanoyl chloride (37 µl, 0.21 mmol). The reaction mixture was stirred at r.t. for 24 h. ^{1}H NMR ($CDCl_3$): 8.68 (s, 1 H); 7.76 (s, 1 H); 7.61 (d, *J* = 7.8, 1 H); 7.39 (br s, 1 H); 7.37 (d, *J* = 8.1, 1 H); 7.07 (t, *J* = 8.0, 1 H); 6.51 (d, *J* = 7.6, 1 H); 5.85 (dd, *J* = 7.6 and 4.5, 1 H); 5.59 (d, *J* = 4.2, 1 H); 5.00 (s, 1 H); 4.75 (br s, 2 H); 3.12 (s, 3 H); 3.03 (s, 3 H); 2.44 (m, 2 H); 2.27 (m, 2 H); 1.72–1.15 (m, 20 H); 0.88 (m, 6 H). HRMS, *m/z*: found 811.2435 $(M + H)^+$. $C_{35}H_{49}ClIN_6O_6$ requires 811.2447. HPLC (system A) 27.3 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Bis(3-methylbutanoate) (**17**)

Procedure B. Compound **17** (2.8 mg, 72%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and 3-methylbutanoyl chloride. The reaction mixture was stirred at r.t. for 5 h. ^{1}H NMR ($CDCl_3$): 8.63 (s, 1 H); 7.73 (s, 1 H); 7.63 (d, *J* = 7.5, 1 H); 7.35 (d, *J* = 7.8, 1 H); 7.08 (t, *J* = 7.7, 1 H); 6.51 (d, *J* = 7.6, 1 H); 6.33 (br s, 1 H); 5.87 (dd, *J* = 7.6 and 4.9, 1 H); 5.60 (d, *J* = 4.9, 1 H); 4.99 (s, 1 H); 4.76 (br s, 2 H); 3.12 (s, 3 H); 3.03 (s, 3 H); 2.33 (d, *J* = 6.9, 2 H); 2.16 (m, 3 H); 1.99 (m, 1 H); 1.02 (d, *J* = 6.6, 6 H); 0.85 (d, *J* = 6.6, 3 H); 0.84 (d, *J* =

6.6, 3 H). HRMS, *m/z*: found 727.1494 $(M + H)^+$. $C_{29}H_{37}ClIN_6O_6$ requires 727.1508. HPLC (system A) 18.1 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Dibenzoate (**18**)

Procedure A. Compound **18** (3.3 mg, 80%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and benzoic anhydride. The reaction mixture was stirred at r.t. for 6 h. ^{1}H NMR ($CDCl_3$): 8.63 (s, 1 H); 8.08 (d, *J* = 8.1, 1 H); 8.07 (d, *J* = 8.4, 1 H); 7.97 (d, *J* = 8.4, 1 H); 7.96 (d, *J* = 8.1, 1 H); 7.73 (s, 1 H); 7.63 (m, 2 H); 7.50 (m, 3 H); 7.37 (m, 3 H); 7.07 (t, *J* = 8.0, 1 H); 6.81 (d, *J* = 7.4, 1 H); 6.35 (br s, 1 H); 6.17 (dd, *J* = 7.4 and 4.7, 1 H); 5.98 (d, *J* = 4.7, 1 H); 5.26 (s, 1 H); 4.76 (br s, 2 H); 3.21 (s, 3 H); 3.09 (s, 3 H). HRMS, *m/z*: found 767.0892 $(M + H)^+$. $C_{33}H_{29}ClIN_6O_6$ requires 767.0882. HPLC (system A) 17.1 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Bis(phenylacetate) (**19**)

Procedure B. Compound **19** (3.2 mg, 74%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and phenylacetyl chloride. The reaction mixture was stirred at r.t. for 2 h. ^{1}H NMR ($CDCl_3$): 8.55 (s, 1 H); 7.75 (s, 1 H); 7.63 (d, *J* = 8.1, 1 H); 7.41–7.19 (m, 9 H); 7.10 (m, 3 H); 6.50 (d, *J* = 7.5, 1 H); 6.28 (br s, 1 H); 5.82 (dd, *J* = 7.5 and 4.8, 1 H); 5.59 (d, *J* = 4.8, 1 H); 4.96 (s, 1 H); 4.77 (br s, 2 H); 3.58 (s, 2 H); 3.42 (s, 2 H); 3.04 (s, 3 H); 2.99 (s, 3 H). HRMS, *m/z*: found 795.1167 $(M + H)^+$. $C_{35}H_{33}ClIN_6O_6$ requires 795.1195. HPLC (system A) 17.4 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Bis(3-phenylpropanoate) (**20**)

Procedure B. Compound **20** (3.6 mg, 82%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol), DMAP (6 mg, 0.05 mmol), 3-phenylpropenoyl chloride (8 µl, 0.05 mmol). The reaction mixture was stirred at r.t. for 2 h. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 98:2). ^{1}H NMR ($CDCl_3$): 8.57 (s, 1 H); 7.75 (s, 1 H); 7.63 (d, *J* = 7.8, 1 H); 7.39–7.14 (m, 9 H); 7.09 (m, 3 H); 6.61 (br s, 1 H); 6.44 (d, *J* = 7.6, 1 H); 5.83 (dd, *J* = 7.6 and 4.8, 1 H); 5.56 (d, *J* = 4.8, 1 H); 4.81 (s, 1 H); 4.77 (br s, 2 H); 3.01 (s, 3 H); 3.00 (s, 3 H); 2.98 (m, 2 H); 2.82 (t, *J* = 4.8, 2 H); 2.72 (m, 2 H); 2.53 (m, 2 H). HRMS, *m/z*: found 823.1501 $(M + H)^+$. $C_{37}H_{37}ClIN_6O_6$ requires 823.1508. HPLC (system A) 17.5 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Bis(methyl carbonate) (**21**)

Procedure B. Compound **21** (0.8 mg, 22%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and methyl chloroformate. The reaction mixture was stirred at r.t. for 16 h. ^{1}H NMR ($CDCl_3$): 8.43 (s, 1 H); 7.73 (s, 1 H); 7.63 (d, *J* = 7.5, 1 H); 7.35 (d, *J* = 7.8, 1 H); 7.08 (t, *J* = 8.0, 1 H); 6.50 (d, *J* = 6.8, 1 H); 6.31 (br s, 1 H); 5.85 (dd, *J* = 6.8 and 4.7, 1 H); 5.72 (dd, *J* = 4.7 and 1.9, 1 H); 5.09 (d, *J* = 1.9, 1 H); 4.76 (br s, 2 H); 3.86 (s, 3 H); 3.77 (s, 3 H); 3.12 (s, 3 H); 3.03 (s, 3 H). HRMS, *m/z*: found 675.0477 $(M + H)^+$. $C_{23}H_{25}ClIN_6O_8$ requires 675.0467. HPLC (system A) 11.1 min (99%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Bis(benzyl carbonate) (**22**)

Procedure B. Compound **22** (1.9 mg, 43%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and using benzyl chloroformate as esterification agent. The reaction mixture was stirred at r.t. for 2 h. ^{1}H NMR ($CDCl_3$): 8.41 (s, 1 H); 7.73 (s, 1 H); 7.63 (d, *J* = 8.1, 1 H); 7.39–7.21 (m, 11 H); 7.08 (t, *J* = 7.8, 1 H); 6.49 (d, *J* = 6.7, 1 H); 6.26 (br s, 1 H); 5.86 (dd, *J* = 6.7 and 4.8, 1 H); 5.64 (dd, *J* = 4.8 and 2.0, 1 H); 5.16 (s, 2 H); 5.09 (s, 1 H); 5.08 (s, 1 H); 5.07 (d, *J* = 2.0, 1 H); 4.77 (br s, 2 H); 3.09 (s, 3 H); 3.01 (s, 3 H). HRMS, *m/z*: found 827.1088 $(M + H)^+$. $C_{35}H_{33}ClIN_6O_8$ requires 827.1093. HPLC (system A) 15.9 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrofuran-3,4-diyl Dimesylate (**23**)

Procedure B. Compound **23** (3.6 mg, 95%) was obtained as a white solid from **4a** (3 mg, 0.005 mmol) and mesyl chloride. The reaction mixture was stirred at r.t. for 5 h. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 98:2). ^{1}H NMR ($CDCl_3$): 8.10 (s, 1 H); 7.73 (s, 1 H); 7.64 (d, *J* = 7.5, 1 H); 7.35 (d, *J* = 7.8, 1 H); 7.09 (t, *J* = 7.7, 1 H); 6.36 (d, *J* = 4.2, 1 H); 6.32 (br s, 1 H); 6.02 (t, *J* = 4.8, 1 H); 5.80 (t, *J* = 4.7, 1 H); 5.09 (d, *J* = 4.8, 1 H); 4.76 (br s, 2 H); 3.32 (s, 3 H); 3.14 (s, 3 H); 3.12 (s, 3 H); 3.00 (s, 3 H). HRMS, *m/z*: found 714.9917 $(M + H)^+$. $C_{21}H_{25}ClIN_6O_8S_2$ requires 714.9909. HPLC (system A) 9.8 min (98%).

(2*R*,3*R*,4*S*,5*S*)-2-{2-Chloro-6-[(3-iodobenzyl)amino]-9*H*-purin-9-yl}-5-(dimethylcarbamoyl)-tetrahydrothiophene-3,4-diyl Diacetate (**24**)

Procedure A. Compound **24** (1.3 mg, 99%) was obtained as a white solid from **4b** (1.1 mg, 0.002 mmol) and acetic anhydride. The reaction mixture was stirred at r.t. for 16 h. ^{1}H NMR ($CDCl_3$): 8.42 (s, 1 H); 7.74 (s, 1 H); 7.63 (d, *J* = 7.8, 1 H); 7.35 (d, *J* = 8.1, 1 H); 7.08 (t, *J* = 7.8, 1 H); 6.39 (d, *J* = 7.0, 1 H); 6.34 (br s, 1 H); 6.25 (dd, *J* = 7.0 and 3.7, 1 H); 5.77 (dd, *J* = 3.7 and 2.6, 1 H); 4.76 (br s, 2 H); 4.28 (d, *J* = 2.6, 1 H); 3.06 (s, 3 H); 3.05 (s, 3 H); 2.19 (s, 3 H); 2.00 (s, 3 H). HRMS, *m/z*: found 659.0364 $(M + H)^+$. $C_{23}H_{25}ClIN_6O_5S$ requires 659.0340. HPLC (system A) 10.8 min (99%).

Biological Assays

Methods used for the binding assays using agonist radioligands at the recombinant human A_1, A_{2A}, and A_3 ARs expressed in mammalian cells and a functional assay of adenylate cyclase inhibition were as reported[10,17,22,23].

Kinetic Experiments in Human Whole Blood

The regeneration of the parent drugs **2b** and **4a** from the ester prodrugs was followed by methods similar to those previously described[20,24]. Blood samples were obtained from the NIH Clinical Center Dept. of Transfusion Medicine. They were obtained from healthy human volunteers and directly transferred to heparinized glass tubes. Three ml of blood was used for each experiment. The blood was spiked with a prodrug solution (12 μl of a 5 mM solution in DMSO) to achieve a final concentration of 20 μM and a maximal DMSO content not to exceed 0.5%. During the cleavage experiment, the blood mixture was maintained in

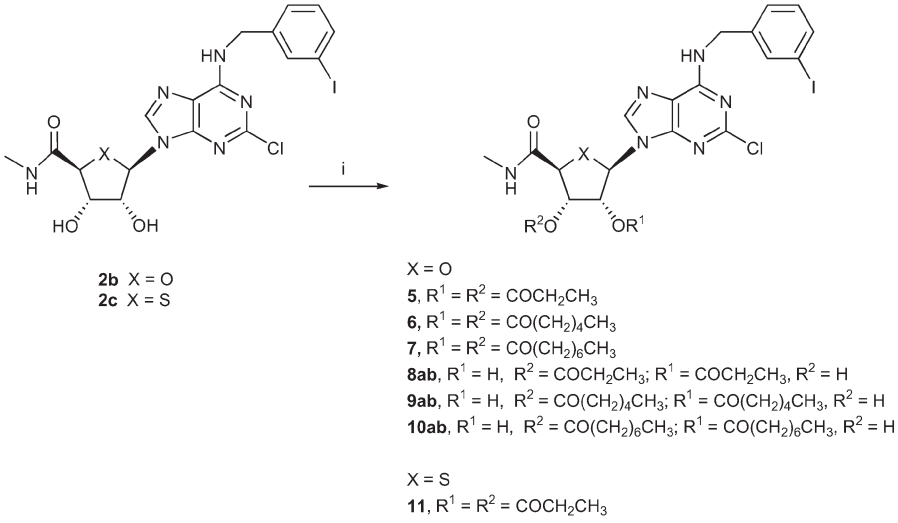
an incubator at 37 °C with gentle shaking. At regular time intervals of 5 min, and 0.5, 1, 2, and 4 h, 200 µl samples of the blood mixture were taken, immediately hemolyzed in Eppendorf tubes prefilled with 1.0 ml of ice-chilled water, and stored at –20 °C until analysis. An internal standard consisting of 100 µl of a 40 µM DMSO solution of the N^6-(fluorenylmethyl) derivative of adenosine[23] and 100 µl of a 10% solution of sulfosalicylic acid were added to each sample. After gently vortexing for 5 min, the samples were extracted three times, successively, with 0.5 ml of water-saturated ethyl acetate. Each extraction consisted of the addition of ethyl acetate, vortexing for 5 min, centrifugation for 5 min at 2000 *g*, and manual separation of supernatant with an automatic pipette. The extract fractions were combined and evaporated to dryness under a stream of nitrogen gas.

The residue was then reconstituted in 50 µl of the HPLC mobile phase system A (above). Using an HPLC system (Hewlett–Packard 1100), 40 µl of this solution was injected for each chromatographic run to provide a kinetic profile for conversion of the prodrug into its parent nucleoside. The chromatography was performed at room temperature using a reversed-phase column (Zorbax Eclipse 5 µm XDB-C18 analytical column, 250 × 4.6 mm) equipped with a guard column packed with C-18 material. The mobile phase consisted of system A (as above). The flow rate was 1.0 ml/min, and the detection wavelength of 280 nm was used. The retention times of the parent drugs **2b** and **4a** were 5.1 and 5.2 min, respectively. The retention time of the internal standard was 4.1 min. The within-day coefficients of variation were generally less than 20%. The detection limit for nucleoside was estimated to be approximately 0.1 nmol. In parallel, the appearance of the parent drug and intermediate monoesters was followed to account for the consumed prodrug. Values for three separate determinations were averaged. The time course for relative concentration of each adenosine derivative was calculated based on the fractional percentage of total nucleoside detected and was plotted using Excel software (Microsoft, Redmond (WA), U.S.A.).

RESULTS AND DISCUSSION

To generate prodrugs of potent A_3AR agonists **2b** and **2c**, straight-chain fatty acids were selected as ideal acyl groups. The diester forms **5**–**11** of the adenosine and thioadenosine 5′-uronamide derivatives **2b** and **2c** were prepared as shown in Scheme 1. The N^6-(3-iodobenzyl) and 2-chloro groups serve to enhance potency and selectivity at the A_3AR, and the 5′-*N*-methyluronamide group was associated with receptor agonism[2]. Both the diester derivatives **5**–**7** and **11** and monoester mixtures **8**–**10** were prepared using the appropriate anhydride or chloride. By controlling the molar equivalents of the esterification agent it was possible to isolate from the same reaction the fully acylated diester and the corresponding monoester mixture. While it was not feasible to separate the monoester species, the isomeric mixtures were isolated and characterized by NMR to establish the isomeric ratio, which was 1:1 for of all the compounds **8**–**10**. Attempts to synthesize pure positional isomers of the monoesters, for example using *O*-silyl protection at either the 3′ or 4′ position, was not productive due to the tendency of

the final *O*-acyl group to migrate between the hydroxy groups after deprotection.

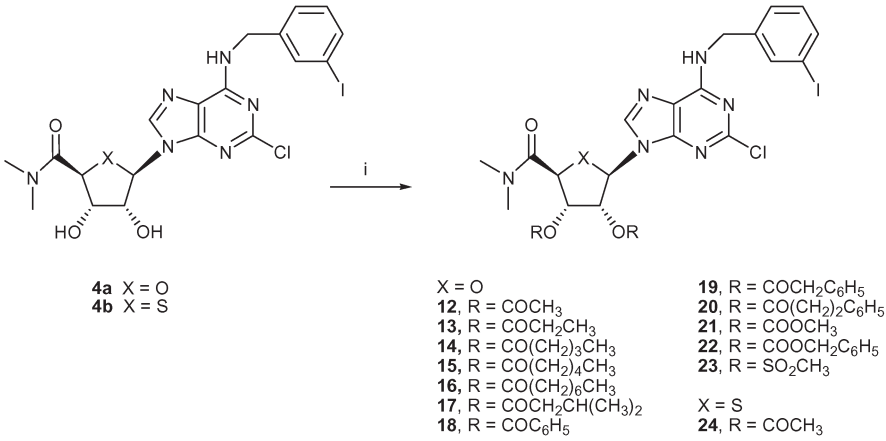


SCHEME 1
Synthesis of prodrugs of A_3AR agonist. Reagents and conditions: (i) Procedure *A*. ROR, DMAP, Py, r.t.; Procedure *B*. RCl, DMAP, CH_2Cl_2, r.t.

Similar ester derivatives, although more structurally diverse, were prepared from the antagonists **4a** and **4b**, as shown in Scheme 2. The 5′-*N*,*N*-dimethyluronamide moiety was associated with receptor antagonism[17], and the other substitution of the adenine moiety was identical to that of agonists **2b** and **2c**. Several aryl-containing esters **18**–**20**, carbonate derivatives **21** and **22**, and a sulfonate ester **23** are also included.

Selected prodrug derivatives were examined in standard binding assays at the A_1, A_{2A}, and A_3ARs (Table I). These three recombinant human ARs were stably expressed in mammalian cell lines (either CHO for A_1 and A_3, or HEK-293 for A_{2A}). The diesters of **2b** and **2c** displayed greatly reduced affinity at all subtypes. For example, the measured affinity of the dipropionate **5** at the A_3AR was 130-fold less than that of the parent agonist **2b**. The agonist-derived monopropionate mixture **8a**, **8b** retained considerable affinity at the A_3AR. Similarly for the diesters of **4a** and **4b**, there was reduced affinity at the ARs. The antagonist-derived diester **24** was 60-fold less potent at the A_3AR than the parent antagonist **4b**, while the diester **13** displayed no measurable affinity at any of the ARs. To avoid side effects it would be desirable for the prodrug preparations to lack activity at the A_3AR, so that the 3′,4′

diesters are more suitable as potential therapeutic drugs. Therefore, **13** displays desirable properties as an antagonist prodrug.



4a X = O
4b X = S

X = O
12, R = $COCH_3$
13, R = $COCH_2CH_3$
14, R = $CO(CH_2)_3CH_3$
15, R = $CO(CH_2)_4CH_3$
16, R = $CO(CH_2)_6CH_3$
17, R = $COCH_2CH(CH_3)_2$
18, R = COC_6H_5

19, R = $COCH_2C_6H_5$
20, R = $CO(CH_2)_2C_6H_5$
21, R = $COOCH_3$
22, R = $COOCH_2C_6H_5$
23, R = SO_2CH_3

X = S
24, R = $COCH_3$

SCHEME 2
Synthesis of prodrugs of A_3AR antagonist. Reagents and conditions: (i) Procedure *A*. ROR, DMAP, Py, r.t.; Procedure *B*. RCl, DMAP, CH_2Cl_2, r.t.

It is unknown whether the binding activity observed for various ester derivatives resides with the ester derivative itself, or due to a trace impurity or product of ester cleavage during the binding assay. An earlier publication described residual binding activity of 5′-esters of the A_1AR agonist N^6-cyclopentyladenosine to cleavage under the conditions of the binding assay, with the assumption that the prodrug derivative would not bind to the receptor[20].

A functional assay at the A_3AR based on measurement of adenosine cyclic 3′,5′-monophosphate (cAMP) was performed. Both **5** and **8a**, **8b** were partially efficacious at the maximum measured concentration of 10 μM.

We examined the stability of adenosine ester prodrug derivatives and their conversion to the parent, active drug upon *in vitro* incubation in human whole blood. Each compound was incubated at 37 °C in fresh whole blood obtained from healthy human volunteers, aliquots of the incubation mixture were hemolyzed at regular time intervals, and extracts were analyzed by HPLC. Results from the various acylated derivatives were compared. The dipropionate ester of Cl-IB-MECA **5** upon incubation in whole blood, was found to effectively regenerate the parent Cl-IB-MECA over a 4 h period (Fig. 1a). By the end of two hours, 70–80% of the expected Cl-IB-MECA was detected. The intermediate mixture of monoesters **8a**, **8b**

TABLE I
Affinity at three human ARs for the adenosine agonists **2b**, **2c** and antagonists **4a**, **4b** and their prodrugs, and the efficacy at the A_3AR[a]

Compd	K_i at A_1[b], nM (or % inhibition)	K_i at A_{2A}[b], nM (or % inhibition)	K_i at A_3[b], nM (or % inhibition)	Efficacy[c,d] at A_3, %
2b[e]	222 ± 22	5360 ± 2470	1.4 ± 0.3	100
2c[f]	193 ± 46	223 ± 36	0.38 ± 0.07	114 ± 9
4a[e,g]	5870 ± 930	(21 %)	29.0 ± 4.9	0
4b[e,g]	6220 ± 640	(28 %)	15.3 ± 3.1	0
5[h]	(55 ± 7 %)	(28 ± 3 %)	179 ± 59	83 ± 6
6[h]	(9 ± 3 %)	(4 ± 2 %)	1070 ± 160	ND
7[h]	(–1 ± 1 %)	(13 ± 5 %)	(30 ± 9 %)	ND
8a,b[h]	(79 ± 1 %)	(49 ± 9 %)	8.0 ± 1.0	62 ± 5
10a,b[h]	1190 ± 310	(36 ± 3 %)	60 ± 18	ND
11[h]	(15 ± 3 %)	(32 ± 3 %)	1550 ± 360	ND
12[i]	(12 ± 4 %)	(15 ± 2 %)	3740 ± 630	ND
13[i]	(10 ± 4 %)	(11 ± 6 %)	>10000	ND
16[i]	(5 ± 3 %)	(3 ± 0 %)	(25 ± 9 %)	ND
24[i]	(16 ± 1 %)	(18 ± 10 %)	910 ± 330	0

Pharmacological methods used were as reported[10,17,22,23].
ND, not determined.
[a] All experiments were done on CHO or HEK-293 cells stably expressing one of three subtypes of human ARs. The binding affinity for A_1, A_{2A} and A_3ARs was expressed as K_i values and was determined by using agonist radioligands (1 nM [^{3}H]2-chloro-N^6-cyclopentyladenosine, 10 nM [^{3}H]CGS21680 2-(2-(4-(2-carboxyethyl)phenyl)ethylamino)-9-(*N*-ethyl-(β-D-ribosfuranosyluronamide)adenine), and 0.5 nM [^{125}I]I-AB-MECA (N^6-(4-amino-3-iodobenzyl)-9-(*N*-methyl-(β-D-ribosfuranosyluronamide)adenine, respectively). Data are expressed as mean ± standard error of mean for 3–4 experiments. [b] K_i in binding, unless noted. [c] At a concentration of 10 μM, in comparison to the maximal effect of a full agonist NECA at 10 μM. [d] cAMP assay. The efficacy at A_3ARs was determined by inhibition of forskolin-stimulated cAMP production in AR-transfected CHO cells, as described in ref.[23] [e] Values from ref.[17]. [f] K_i values from ref.[22]. [g] **4a**, MRS3771; **4b**, LJ-1256. [h] Agonist derivatives. [i] Antagonist derivatives.

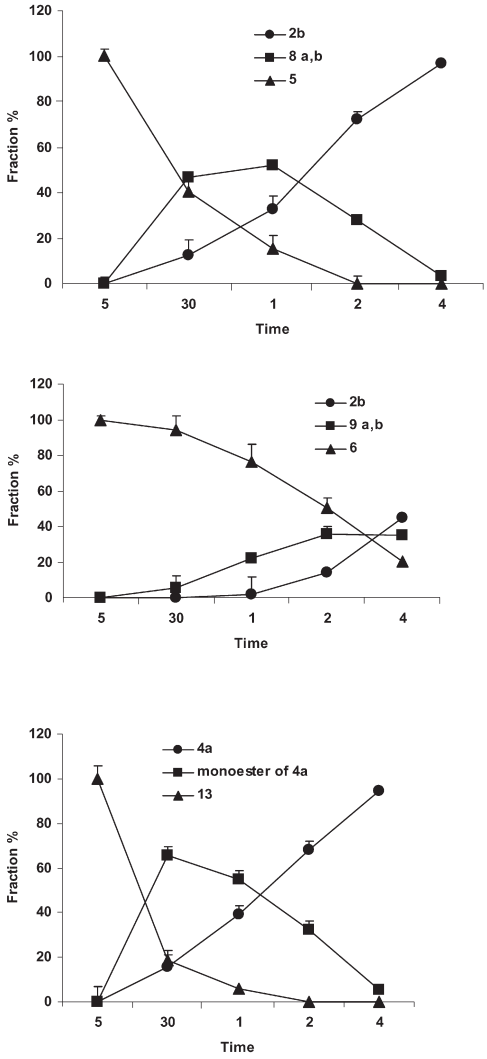


FIG. 1

Time course of degradation of prodrug diesters (▲), compounds **5** (a), **6** (b), and **13** (c) and the appearance of the parent 2′,3′-dihydroxy nucleosides (●), i.e. **2b** for (a) and (b) and **4a** for (c), in whole human blood at 37 °C. In each case, the appearance of the isomeric mixture of 2′ and 3′ monoesters (■), an intermediate stage in the regeneration of the parent compounds, was also followed. Analysis was performed following cell lysis and ethyl acetate extraction, as described in Experimental. The *y*-axis corresponds to fraction of each nucleoside derivative (the prodrug diester, the parent free nucleoside, or monoesterified nucleoside) expressed as % of the initial diesterified prodrug concentration. HPLC determination used UV detection at 280 nm. Results are the mean ± s.e.m (std. error of mean) from three separate experiments

appeared as a single HPLC peak, rose to roughly 50% of the nucleoside mixture at 30–60 min., and dropped to 28% at the end of the 2 h period and 3% after 4 h. The area under the peak corresponding to the internal standard was constant for at least 2 h. Thus, the dipropionate **5** displayed kinetics of cleavage on a timescale compatible with *in vivo* biological applications. The dihexanoate of Cl-IB-MECA **6** was cleaved on a considerably slower time scale, such that there was only partial regeneration (approximately 40%) of Cl-IB-MECA by the end of a 2 h period (Fig. 1b). Cleavage of the antagonist prodrug **13** proceeded at approximately the same rate as cleavage of **5** (Fig. 1c). Thus, the presence of a second *N*-methyl group at the 5′-position in **13** did not impede the hydrolysis at the 3′ and 4′ positions. It is expected that a diacetate, such as **12**, would tend to be cleaved more rapidly (not tested).

For the previously reported 5′-ester prodrugs of N^6-cyclopentyladenosine, optimal kinetics of one-step cleavage occurred with the 5′-*O*-octanoyl derivative[20]. A roughly comparable rate of two-step cleavage in the present set of ester prodrugs occurred with the 3′,4′-dipropionates. Evidently, the hydrolysis at the more sterically hindered secondary alcohols at the 3′,4′ positions occurs at a generally slower rate than at the primary alcohol of the 5′ position. Other 2′,3′-esters of nucleosides, specifically adenosine derivatives, have been described and hydrolysis experiments were performed[25].

The formation of ester prodrugs increases the hydrophobicity of the nucleosides, which may be expected to enhance their bioavailability[26]. The calculated log *P* values for the parent drugs **2b** and **4a** are 1.20 and 1.69, respectively[17], and the values for the prodrugs **5**, **6,** and **13** are 3.96, 7.1, and 4.45, respectively. It is to be noted that other prodrug schemes[27,28] for delivering nucleosides and nucleotides *in vivo* are designed for passage through the plasma membrane. In the case of adenosine agonists and antagonists, the site of action of these substances is on the extracellular surface of the plasma membrane, so the prodrug delivery is desired for passage across other biological barriers. It would likely be disadvantageous if administration of these prodrugs would lead to increased accumulation of the drug molecules intracellularly, where it might have undesired actions.

Adenosine derivatives containing a free 5′-OH, such as the A_1AR-selective agonist N^6-cyclopentyladenosine, are subject to metabolism in erythrocytes, mainly through the action of adenosine 5′-kinase (AK)[29]. The agonists and antagonists of the A_3AR used in this study are not subject to metabolism by AK. This is consistent with a relatively long half-life observed for the agonist **2a** in human trials[12].

In conclusion, acylation of the nucleoside derivatives greatly reduced their biological activity in a bioreversible fashion. The nucleoside 2′,3′-diesters were subject to enzymatic hydrolysis in the presence of human blood to regenerate the parent compound. 2′,3′-Dipropionates of **2b** and **4a** were readily cleaved in a two-step reaction to regenerate the parent drug, in two hours, while the cleavage of a 2′,3′-dihexanoate occurred at a slower rate. These results indicate that the prodrugs are suitable as masked forms of the biologically active A_3AR agonists and antagonists for future evaluation *in vivo*.

Abbreviations: AK, adenosine 5′-kinase; AR, adenosine receptor; cAMP, 3′,5′-cyclic adenosine monophosphate; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMSO, dimethyl sulfoxide; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)-9-(*N*-methyl-(β-D-ribosfuranosyluronamide)adenine; IB-MECA, N^6-(3-iodobenzyl)-9-(*N*-methyl-(β-D-ribosfuranosyluronamide)adenine; MRS3771, 2-chloro-N^6-(3-iodobenzyl)-9-(*N*,*N*-dimethyl-(β-D-ribosfuranosyluronamide) adenine; LJ-568, 5-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methylamide; MRS3630, 4-(2-chloro-6-(cyclopentylamino)purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylic acid methylamide; MRS1292, 2-(N^6-3-iodobenzyl)adenos-9-yl)-7-aza-1-oxa-6-oxospiro[4.4]-nonan-4,5-diol; MRS3642, 5-(2-chloro-6-(3-iodobenzylamino)-purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid dimethylamide; HPLC, high pressure liquid chromatography; HRMS, high resolution mass spectrum; CHO, Chinese hamster ovary; HEK, human embryonic kidney.

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REFERENCES

1. Jacobson K. A., Gao Z. G.: *Nat. Rev. Drug Discovery* **2006**, *5*, 247.
2. Joshi B. V., Jacobson K. A.: *Curr. Top. Med. Chem.* **2005**, *5*, 1275.
3. Müller C.: *Curr. Top. Med. Chem.* **2003**, *3*, 445.
4. Baraldi P. G., Fruttarolo F., Tabrizi M. A., Romagnoli R., Preti D., Carrion M. D., Iaconinoto A., Borea P. A.: *Expert Opin. Ther. Patents* **2005**, *15*, 1507.
5. Moro S., Gao Z. G., Jacobson K. A., Spalluto G.: *Med. Res. Rev.* **2006**, *26*, 131.
6. von Lubitz D. K. J. E., Lin R. C.-S., Popik P., Carter M. F., Jacobson K. A.: *Eur. J. Pharmacol.* **1994**, *263*, 59.

7. Pugliese A. M., Coppi E., Spalluto G., Corradetti R., Pedata F.: *Br. J. Pharmacol.* **2006**, *147*, 524.
8. Liang B. T., Jacobson K. A.: *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6995.
9. Headrick J. P., Peart J.: *Vascul. Pharmacol.* **2005**, *42*, 271.
10. Jacobson K. A., Gao Z. G., Tchilibon S., Duong H. T., Joshi B. V., Sonin D., Liang B. T.: *J. Med. Chem.* **2005**, *48*, 8103.
11. Bar-Yehuda S, Madi L., Silberman D., Gery S., Shkapenuk M., Fishman P.: *Neoplasia* **2005**, *7*, 85.
12. van Troostenburg A. R., Clark E. V., Carey W. D., Warrington S. J., Kerns W. D., Cohn I., Silverman M. H., Bar-Yehuda S., Fong K. L., Fishman P.: *Int. J. Clin. Pharmacol. Ther.* **2004**, *42*, 534.
13. Baharav E., Bar-Yehuda S., Madi L., Silberman D., Rath-Wolfson L., Halpren M., Ochaion A., Weinberger A., Fishman P.: *J. Rheumatol.* **2005**, *32*, 469.
14. Avila M. Y., Stone R. A., Civan M. M.: *Br. J. Pharmacol.* **2001**, *134*, 241.
15. Okamura T., Kurogi Y., Nishikawa H., Hashimoto K., Fujiwara H., Nagao Y.: *J. Med. Chem.* **2002**, *45*, 3703.
16. Yang H., Avila M. Y., Peterson-Yantorno K., Coca-Prados M., Stone R. A., Jacobson K. A., Civan M. M.: *Curr. Eye Res.* **2005**, *30*, 747.
17. Gao Z. G., Joshi B. V., Klutz A., Kim S. K., Lee H. W., Kim H. O., Jeong L. S., Jacobson K. A.: *Bioorg. Med. Chem. Lett.* **2006**, *16*, 596.
18. Jong A., Huang S. H.: *Curr. Drug Targets – Infect. Disorders* **2005**, *5*, 65.
19. Maillard M., Nikodijevic O., LaNoue K. F., Berkich D. A., Ji X.-D., Bartus R., Jacobson K. A.: *J. Pharm. Sci.* **1994**, *83*, 46.
20. Dalpiaz A., Scatturin A., Menegatti E., Bortolotti F., Pavan B., Biondi C., Durini E., Manfredini S.: *Pharm. Res.* **2001**, *18*, 531.
21. Leo E., Contado C., Bortolotti F., Pavan B., Scatturin A., Tosi G., Manfredini S., Angusti A., Dalpiaz A.: *Int. J. Pharm.* **2006**, *307*, 103.
22. Jeong L. S., Lee H. W., Jacobson K. A., Kim H. O., Shin D. H., Lee J. A., Gao Z. G., Lu C., Duong H. T., Gunaga P., Lee S. K., Jin D. Z., Chun M. W., Moon H. R.: *J. Med. Chem.* **2006**, *49*, 273.
23. Tchilibon S., Kim S. K., Gao Z. G., Harris B. A., Blaustein J., Gross A. S., Melman N., Jacobson K. A.: *Biooorg. Med. Chem.* **2004**, *12*, 2021.
24. Mathot R. A. A., Appel S., van Schaick E. A., Soudijn W., IJzerman A. P., Danhof M.: *J. Chromatogr.* **1993**, *620*, 113.
25. Kehraus S., Gorzalka S., Hallmen C., Iqbal J., Müller C. E., Wright A. D., Wiese M., Konig G. M.: *J. Med. Chem.* **2004**, *47*, 2243.
26. Bamat M. K., von Borstel R. W.: U.S. 5470838, 1995.
27. De Clercq E., Field H. J.: *Br. J. Pharmacol.* **2006**, *147*, 1.
28. Zidek Z., Franková D., Holý A., Boubelík M., Draber P.: *Biochem. Pharmacol.* **1997**, *54*, 855.
29. Pavan B., IJzerman A.: *Biochem. Pharmacol.* **1998**, *56*, 1625.