



Published in final edited form as:

Bioorg Med Chem Lett. 2001 September 3; 11(17): 2295–2300.

Synthesis and Purine Receptor Affinity of 6-Oxopurine Nucleosides and Nucleotides Containing (N)-Methanocarba-pseudoribose Rings

Gnana Ravi^a, Kyeong Lee^a, Xiao-duo Ji^a, Hak Sung Kim^a, Kelly A. Soltysiak^a, Victor E. Marquez^b, and Kenneth A. Jacobson^{a,*}

^aMolecular Recognition Section, LBC, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA

^bLaboratory of Medicinal Chemistry, National Cancer Institute, Frederick, MD 21702, USA

Abstract

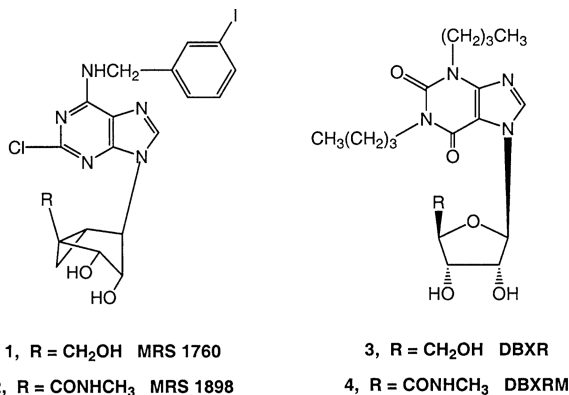
6-Oxopurine derivatives containing a northern (N) methanocarba modification (i.e., fused cyclopropane and cyclopentane rings in place of the ribose) were synthesized and the adenosine receptor affinity measured. Guanine or hypoxanthine was coupled at the 7-position, or 1,3-dibutylxanthine was coupled at the 9-position. The pseudoribose ring was also substituted at the 5'-position with an *N*-methyluronamide or with phosphate groups.

We recently examined conformational requirements of the ribose moiety in adenosine derivatives acting as agonists or partial agonists at P2 receptors and at subtypes of adenosine receptors (A₁, A_{2A}, and A₃ ARs).^{1–3} Using a methanocarba modification originally introduced by Marquez and co-workers,⁴ the ribose-like moiety of these derivatives was constrained to either a northern [(N), 2'-*exo*] or southern [(S), 2'-*endo*] conformation through fused cyclopropane and cyclopentane rings. Such analogues helped to define the role of ribofuranosyl puckering in stabilizing the active AR-bound conformation, and thereby allowed identification of the (N)-methanocarba conformation as the isomer which preserved affinity at the A₃ and to a lesser extent, A₁ AR. Thus, the (N)-methanocarba analogues, **1** and **2**, were highly potent as full agonist (**2**)³ or partial agonist (**1**) at the human A₃ AR.² These analogues contained substituent groups known to enhance A₃ affinity in the ribose series [i.e., *N*⁶-(3-iodobenzyl) and 2-chloro]. The affinity of adenosine methanocarba analogues at the A_{2A} AR was greatly decreased for the (N)-methanocarba isomer and nearly absent for the (S)-methanocarba isomer.

In this study, we combined the (N)-methanocarba modification with known purine receptor ligands containing a 6-oxo-substitution. Among the nucleoside analogues synthesized were derivatives of hypoxanthine, guanosine, and xanthine. For inosine^{5,6} and xanthine-7-riboside⁷ analogues, a 5'-methyluronamide modification (nucleoside numbering) known to enhance the A₃ AR affinity was included.^{3,5} In comparison, xanthine-7-riboside derivatives **3** and **4** bound to and activated the rat A₃ AR with partial or full efficacy, respectively,^{7,8} and

*Corresponding author. Tel.: +1-301-496-9024; fax: +1-301-480-8422; kajacobs@helix.nih.gov.

were used as reference compounds. In other analogues, phosphate groups were introduced at the 5'-position with the aim of targeting P2 receptors,⁹ G proteins (in the case of a guanosine 5'-triphosphate analogue)¹⁰ or enzymes involved in purine metabolism (such as IMP dehydrogenase).¹¹



The structures of (N)-methanocarba purine analogues (**5–12**) synthesized for testing in binding and other biochemical assays are shown in Table 1. We introduced the (N)-methanocarba modification of known potent adenosine agonists having equivalents of either 5'-hydroxymethyl (**5**, **8**, and **10**) or 5'-uronamide groups (**6** and **11**). These 6-oxopurine bases were coupled at either the 7-(**10–12**) or 9-position (**5–9**) to the pseudoribose moiety, according to well-characterized alkylation patterns of purine bases.^{7,12} A 5'-monophosphate derivative, **7a**, and several 5'-triphosphate derivatives were included (**9** and **12**).

The synthetic methods used to prepare the new analogues are outlined in Schemes 1–3.^{13–15} 6-Monosubstituted derivatives were prepared from the 6-chloropurine derivative³ as a common intermediate. Refluxing **13** in the presence of 2-mercaptoethanol and NaOMe in MeOH gave the protected inosine derivative, **14**, which was converted into triol **5** in acidic conditions or to the 5'-uronamide, **6**, via a 5'-carboxylic acid, **15**. Treatment of **14** with RuCl₃, NaIO₄, followed by C₁₈ column chromatography gave **15**, which condensed with MeNH₂ in the presence of BOP-Cl [*N,N*-bis(2-oxo-3-oxazolidinyl)-phosphinic chloride] to yield **6**. Use of RuCl₃ instead of RuO₂ produced a cleaner reaction. The 5'-monophosphate derivative **7a** was prepared by reacting **14** with di-*t*-butyl-*N,N*-diethylphosphoramidite in the presence of tetrazole and then with mCPBA at –78 °C, followed by the treatment with Dowex resin. Attempted synthesis of the corresponding triphosphate by treating with 1,1'-carbonyldiimidazole followed by tetrabutylammonium pyrophosphate failed, and instead a dimer, **7b**, was obtained.

Guanosine derivative **8** was prepared by a Mitsunobu reaction of **16** and 2-amino-6-chloropurine to furnish **17**, which was heated with 2N NaOH in dioxane at 100 °C to yield the guanosine derivative, **18**. Debonylation at the 5' position using Pd to give compound **19**, followed by TFA deprotection provided the guanosine derivative, **8**. Compound **19** was converted into monophosphate **20** by the phosphoramidite method. This monophosphate was then converted to phosphorimidazolidate by treatment with 1,1'-carbonyldiimidazole

followed by 5% Et₃N/MeOH, and the phosphorimidazolidate reacted with tetrabutylammonium pyrophosphate to provide the triphosphate, **9b**.

Xanthine derivatives were prepared using a Mitsunobu coupling of **16** and 1,3-dibutylxanthine to furnish **21**, which was deprotected with BCl₃ to yield the xanthine methanocarba nucleoside, **10**. Compound **21** was debenzylated to furnish **22**, which was converted to the monophosphate **25** and to the triphosphate **12** by the above phosphorylation procedures. Compound **22** was oxidized using RuO₂, NaIO₄, K₂CO₃ to furnish the acid **23**, which was condensed with MeNH₂ using BOP-Cl to yield intermediate **24** and finally the deprotected uronamide, **11**.

The nucleoside analogues were evaluated in binding at three subtypes of ARs. While inosine itself displayed a measurable affinity at the A₃ AR and was even suggested to be an endogenous ligand for this AR,⁶ the corresponding (N)-methanocarba analogue, **5**, bound weakly. An effort to enhance the affinity in the inosine series through the introduction of 5'-methyluronamide group slightly increased the affinity of **6** (*K*_i 29 μM). The inosine-5'-monophosphate analogue, **7a**, was inactive at the adenosine A₃ AR. Curiously, the dimer **7b** displaced binding at the A₃ AR with a *K*_i value of 22 μM. Thus, this was an example of dimerization of an inactive ligand to increase the affinity, perhaps through crosslinking two sites on a receptor or by bridging two proximal receptor molecules. It is unusual for such a highly anionic molecule to bind to an AR, which may be the result of an enhancing effect of the (N)-methanocarba ring on A₃ affinity.² It will also be interesting to evaluate **7b** at P2X receptors, at which diinosine polyphosphates are potent antagonists.¹⁶

A receptor for guanosine was proposed,¹⁷ based on neuroprotective effects observed for this nucleoside. However, the guanosine analogues here were examined only for interaction with ARs. The (N)-methanocarba analogue, **8**, bound weakly, but selectively to the A₃ AR with a *K*_i value of 21 μM. The corresponding triphosphate, **9**, of interest for its potential interaction with G proteins, was inactive in binding to the A₃ AR.

The (N)-methanocarba analogue of 1,3-dibutylxanthine-7-riboside, **10**, was equipotent at A₁ and A₃ ARs and inactive at the A_{2A} AR. If proven to be an agonist at both AR subtypes, this profile of selectivity would be desirable for a cardioprotective agent.¹⁸ Introduction of the 5'-*N*-methyluronamide group, in **11**, decreased A₁ affinity by 8-fold and increased A₁ affinity by 2-fold. A comparison of compounds **3** and **4**⁷ with the corresponding (N)-methanocarba analogues, **10** and **11**, respectively, showed that rat A₁ affinities were the same, and rat A₃ affinities of the ribosides were roughly similar to *K*_i values at the human A₃ AR found in this study. The xanthine-riboside-5'-triphosphate analogue, **12**, was inactive at the A₃ AR.

The phosphate-containing analogues were tested for effects on intracellular calcium concentration in HL-60 leukemia cells, known to express several P2 receptor subtypes.¹⁹ Thus, a P2 receptor agonist would be expected to cause a rapid rise in intracellular calcium. Compounds **7a** and **7b** were essentially inactive at a concentration of 100 μM, either at stimulating calcium accumulation or inhibiting the rise in calcium produced by 1 μMUT P (acting at P2Y₂ receptors). Compound **9** (100 μM) stimulated a rise in intracellular calcium

equivalent to 53% of the rise produced by 1 μ MUT P, suggesting that it may activate a P2Y receptor.

In conclusion, we have identified non-adenosine analogues containing the (N)-methanocarba modification which bound weakly, and in some cases selectively, at the human A₃ AR. Since selectivity has been achieved in these simple derivatives, this could provide the basis for further development of more potent ligands. The phosphate analogues must also be evaluated at a range of enzymatic and receptor activities. The full evaluation of the biological activity of the new analogues is in progress.

References and Notes

- Nandan E, Jang SY, Moro S, Kim H, Siddiqi MA, Russ P, Marquez VE, Busson R, Herdewijn P, Harden TK, Boyer JL, Jacobson KA. *J. Med. Chem.* 2000; 43:829. [PubMed: 10715151]
- Jacobson KA, Ji X-d, Li AH, Melman N, Siddiqi MA, Shin KJ, Marquez VE, Ravi RG. *J. Med. Chem.* 2000; 43:2196. [PubMed: 10841798]
- Lee K, Ravi RG, Ji X-d, Marquez VE, Jacobson KA. *Bioorg. Med. Chem. Lett.* 2001; 11:1333. [PubMed: 11392549]
- Marquez VE, Siddiqui MA, Ezzitouni A, Russ P, Wang J, Wagner RW, Matteucci MD. *J. Med. Chem.* 1996; 39:3739. [PubMed: 8809162]
- van Galen PJM, van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, IJzerman AP, Stiles GL, Jacobson KA. *Mol. Pharmacol.* 1994; 45:1101. [PubMed: 8022403]
- Jin X, Shepherd RK, Duling BR, Linden J. *J. Clin. Invest.* 1997; 100:2849. [PubMed: 9389751]
- Kim HO, Ji X-d, Melman N, Olah ME, Stiles GL, Jacobson KA. *J. Med. Chem.* 1994; 37:4020. [PubMed: 7966162]
- Park KS, Hoffmann C, Kim HO, Padgett WL, Daly JW, Brambilla R, Motta C, Abbracchio MP, Jacobson KA. *Drug Dev. Res.* 1998; 44:97. [PubMed: 23487508]
- Williams M, Jarvis MF. *Biochem. Pharmacol.* 2000; 59:1173. [PubMed: 10736418]
- Zor T, Andorn R, Sofer I, Chorev M, Selinger Z. *FEBS Lett.* 1998; 433:326. [PubMed: 9744820]
- Yalowitz JA, Jayaram HN. *Anticancer Res.* 2000; 20:2329. [PubMed: 10953293]
- Simons, C. *Nucleoside Mimetics*. Amsterdam: Gordon and Breach; 2001.
- Procedures in Scheme 1. A solution of **13** (40 mg, 0.119 mmol), 2-mercaptoethanol (0.03 mL, 0.475 mmol), 0.5 N NaOMe/MeOH (0.95 mL, 0.475 mmol) in MeOH (1 mL) was heated at 90 °C for 3 h and the reaction mixture was neutralized by glacial AcOH. The resulting mixture was concentrated and purified on preparative TLC (CHCl₃/MeOH = 6:1) to provide the hypoxanthine derivative **14** (37 mg, 98%). ¹H NMR (CD₃OD) δ 8.06 (s, 1H), 7.96 (s, 1H), 5.34 (d, *J* = 7.1 Hz, 1H), 5.04 (s, 1H), 4.69 (d, *J* = 6.9 Hz, 1H), 3.97 (d, *J* = 11.5 Hz, 1H), 3.52 (d, *J* = 11.5 Hz, 1H), 1.72 (m, 1H), 1.51 (s, 3H), 1.24 (s, 3H), 1.16 (m, 1H), 0.97 (m, 1H). FAB *m/z* 319 (MH⁺). **15**: A mixture of **14** (37 mg, 0.116 mmol), RuCl₃ (10 mg, 0.048 mmol), NaIO₄ (181 mg, 0.847 mmol) in CH₃CN/CHCl₃/H₂O = 2:2:3 was vigorously stirred for 6 h at rt. Solvents were removed and the residue was subjected to reverse phase C₁₈ column chromatography using H₂O/MeOH (30:1–7:1) as eluents to afford the acid **15** (20.6 mg, 53.4%). ¹H NMR (D₂O) δ 8.15 (s, 1H), 8.11 (s, 1H), 5.74 (d, *J* = 7.4 Hz, 1H), 5.03 (s, 1H), 4.87 (d, *J* = 7.1 Hz, 1H), 1.22 (m, 1H), 1.61 (m), 1.55 (s, 3H), 1.34 (m, 1H), 1.28 (s, 3H). FAB *m/z* 333 (MH⁺). **5** (1*R*,2*R*,3*S*,4*R*,5*S*)-4-[6-oxo-9*H*-purin-9-yl]-1-(hydroxymethyl)bicyclo-[3.1.0]hexane-2,3-diol: ¹H NMR (CD₃OD) δ 8.51 (s, 1H), 8.06 (s, 1H), 7.91 (s, 1H), 4.82 (s, 1H), 4.76 (d, *J* = 6.6 Hz, 1H), 4.24 (d, *J* = 11.8 Hz, 1H), 4.88 (d, *J* = 6.8 Hz, 1H), 1.60 (m, 1H), 1.53 (m, 1H), 0.74 (m, 1H). FAB *m/z* 279 (MH⁺). HR-MS (FAB) calcd 279.1093, found 279.1084. Acetonide intermediate of **6**: BOP-Cl (26.5 mg, 0.104 mmol) was added to a solution of **15** (11.5 mg, 0.035 mmol) and Et₃N (0.015 mL, 0.104 mmol) in CH₂Cl₂/DMF (2 mL). To the suspension 2.0 M MeNH₂/THF (0.03 mL, 0.06 mmol) was added and the resulting mixture was stirred for 24 h at rt. Water was added and the solvent was removed. The residue was purified on silica gel (CHCl₃/MeOH = 6:1) to give the protected amide (8.1 mg, 68.6%). ¹H NMR (CD₃OD) δ 8.11 (s, 1H), 8.01 (s, 1H), 5.74 (d, *J* = 6.8 Hz, 1H), 5.03 (s, 1H),

2.79 (s, 3H), 2.17 (m, 1H), 1.55 (m, 1H), 1.52 (s, 3H), 1.42 (m, 1H), 1.27 (s, 3H). FAB m/z 346 (MH⁺). **6**: ¹H NMR (D₂O) δ 8.18 (s, 1H), 8.10 (s, 1H), 5.08 (d, J = 6.3 Hz, 1H), 4.96 (s, 1H), 4.17 (d, J = 6.6 Hz, 1H), 2.85 (s, 3H), 2.26 (m, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.51 (m, 1H), 1.51 (m, 1H). FAB m/z 306 (MH⁺). HR-MS (FAB) calcd 306.1202, found 306.1211. **7a** *t*-butyl intermediate. ¹H NMR (CD₃OD) δ 8.45 (s, 1H), 8.20 (s, 1H), 4.92 (s, 1H), 4.44 (dd, J = 5.2, 12 Hz, 1H), 4.04 (d, J = 6.3, 1H, 2H), 4.70 (dd, J = 5.7, 11.1 Hz, 1H), 1.86 (m, 1H), 1.49 (m, 1H), 0.97 (t, J = 6.7 Hz, 1H), 1.34–1.25 (m) 1.09 (m, 1H). FAB m/z 510 (MH⁺). **7a**: ¹H NMR (D₂O) δ 8.18 (s, 1H), 8.10 (s, 1H), 5.08 (d, J = 6.3 Hz, 1H), 4.96 (s, 1H), 4.17 (d, J = 6.6 Hz, 1H), 2.85 (s, 3H), 2.26 (m, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.51 (m, 1H), 1.51 (m, 1H). ³¹P NMR (D₂O) δ 0.51. FAB m/z 357 ((M–1)[–]). HR-MS (FAB[–]) calcd 357.0600, found 357.0607. **7b**: ¹H NMR (D₂O) δ 8.17 (s, 1H), 8.05 (s, 1H), 4.60 (d, J = 10.7 Hz, 1H), 3.93 (d, J = 6.6 Hz, 1H), 3.80 (d, J = 11.0, 1H), 1.88 (s, 1H), 1.53 (t, J = 4.7 Hz, 1H), 0.97 (t, J = 7.1 Hz, 1H). ³¹P NMR (D₂O) δ –10.96. FAB m/z 697 ((M–1)[–]). HR-MS (FAB[–]) calcd 697.1173, found 697.1166.

14. Procedures in Scheme 2. To a mixture of **16** (0.2 g, 0.69 mmol), 2-amino-6-chloropurine (0.234 g, 1.38 mmol) and triphenylphosphine (0.361 g, 1.38 mmol) was added DIAD (diisopropyl azodicarboxylate, 0.27 mL, 1.38 mmol) dropwise at 0 °C. The mixture was warmed up to rt and stirred for 6 h. The solvent was evaporated under vacuum and the residue was purified by flash chromatography using 6:4 petroleum ether/ethyl acetate to furnish **17** as a solid (0.2 g, 67%). ¹H NMR (CDCl₃) δ 8.30 (s, 1H), 7.36 (s, 5H), 5.29 (d, J = 7.15 Hz, 1H), 5.18 (bs, 2H), 4.96 (s, 1H), 4.69–4.53 (ABq, 2H), 4.48 (d, J = 7.15 Hz, 1H), 3.92 (d, J = 10.16 Hz, 1H), 3.28 (d, J = 10.16 Hz, 1H), 1.65–1.60 (m, 1H), 1.55 (s, 3H), 1.28–1.20 (m, 1H), 1.24 (s, 3H), 0.96–0.88 (m, 1H). **18**: To a solution of **17** (0.1 g, 0.23 mmol) in dioxane (2 mL) was added 2 N NaOH (8 mL) and heated at 90 °C for 3 h. The mixture was concentrated to dryness under vacuum and purified by flash chromatography using 10% MeOH in CHCl₃ to furnish **18** as a solid (0.08 g, 82%). ¹H NMR (CDCl₃) δ 7.96 (s, 1H), 7.37 (s, 5H), 6.29 (bs, 2H), 5.27 (d, J = 6.87 Hz, 1H), 4.89 (s, 1H), 4.67–4.50 (m, 3H), 3.92 (d, J = 10.16 Hz, 1H), 3.32 (d, J = 10.16 Hz, 1H), 1.70–1.60 (m, 1H), 1.55 (s, 3H), 1.25 (s, 4H), 0.96–0.86 (m, 1H). **19**: To a solution of **18** (0.06 g, 0.14 mmol) in 5% HCOOH/MeOH (5 mL) was added Pd black (0.06 g) and stirred at rt for 8 h. The reaction mixture was filtered through Celite and concentrated and purified by flash chromatography using 10% MeOH/CHCl₃ to furnish **19** as a solid (0.04 g, 86%). ¹H NMR (D₂O) δ 7.89 (s, 1H), 7.83 (s, 1H), 5.28 (d, J = 6.87 Hz, 1H), 4.77 (s, 1H), 4.59 (d, J = 6.87 Hz, 1H), 3.90 (d, J = 10.54 Hz, 1H), 3.44 (d, J = 10.54 Hz, 1H), 1.64–1.54 (m, 1H), 1.43 (s, 3H), 1.18 (s, 3H), 1.10–1.00 (m, 1H), 0.94–0.82 (m, 1H). **8**: Substrate **19** (0.02 g, 0.06 mmol) was dissolved in 5% TFA/MeOH (3 mL) and H₂O (1 mL) and stirred at rt for 6 h. The reaction mixture was concentrated to dryness under vacuum and purified by preparative TLC using 15% MeOH/CHCl₃ to furnish **8** (0.012 g, 68%). ¹H NMR (D₂O) δ 9.20 (s, 1H), 4.69 (d, J = 6.32 Hz, 1H), 4.25 (d, J = 11.54 Hz, 1H), 3.92 (d, J = 6.32 Hz, 1H), 3.23 (d, J = 11.54 Hz, 1H), 1.66–1.48 (m, 2H), 0.82–0.70 (m, 1H). **20**: To a solution of **19** (0.02 g, 0.06 mmol) in anhydrous THF (2 mL) was added tetrazole (0.013 g, 0.18 mmol) and di-*t*-butyl-*N,N*-diethylphosphoramidite (0.025 mL, 0.09 mmol) and stirred at rt for 12 h. The reaction mixture was cooled to –78 °C and was added a solution of mCPBA (0.018 g) and warmed to rt. Methanol (2 mL) was added to the reaction mixture and concentrated under vacuum and purified by preparative TLC using 10% MeOH/CHCl₃ to furnish 0.022 g product. This compound (0.02 g, 0.038 mmol) was dissolved in 5% TFA/MeOH (3 mL) and H₂O (1 mL) and stirred at rt for 6 h. The reaction mixture was concentrated to dryness under vacuum to furnish 0.013 g of **20**, which was used directly for the further reaction. ¹H NMR (D₂O) δ 8.15 (s, 1H), 4.71 (s, 1H), 4.43 (dd, J = 4.94, 11.26 Hz, 1H), 4.02 (d, J = 6.59 Hz, 1H), 3.70 (dd, J = 4.94, 11.26 Hz, 1H), 1.88–1.78 (m, 1H), 1.54–1.42 (m, 1H), 1.12–0.92 (m, 1H). ³¹P NMR (D₂O) δ 0.87. **9**: To a suspension of monophosphate (0.008 g, 0.02 mmol) in DMF (1 mL) was added 1,1'-carbonyldiimidazole (0.016 g, 0.1 mmol) and stirred for 12 h. A reaction mixture was treated with a solution of 5% Et₃N/MeOH and stirred for 4 h. This mixture was concentrated to dryness under vacuum and were added tributylammonium pyrophosphate (0.055 g, 0.012 mmol) and DMF (1 mL) and stirred for 3 days. Triethylammonium bicarbonate buffer (3 mL) was added to the reaction mixture followed by water (2 mL) and lyophilized. The crude material obtained was purified on a Sephadex column using 0.5 M ammonium bicarbonate and water to furnish 3.5 mg of **9**. ¹H NMR (D₂O) δ 8.12 (s, 1H), 4.69 (s, 1H), 4.54–4.44 (m, 1H), 4.07 (d, J = 6.32 Hz, 1H), 3.92–3.82 (m, 1H), 1.84–1.78 (m, 1H), 1.46–1.38 (m, 1H), 1.02–0.94 (m, 1H). ³¹P NMR (D₂O) δ –8.29, –10.69, –22.26.

15. Procedures in Scheme 3: To a mixture of **16** (0.1 g, 0.34 mmol), 1,3-dibutylxanthine (0.18 g, 0.68 mmol) and triphenylphosphine (0.178 g, 0.68 mmol) was added DEAD (diethyl azodicarboxylate, 0.1 mL, 0.68 mmol) dropwise at 0 °C. The mixture was warmed up to rt and stirred for 6 h. The solvent was evaporated under vacuum and the residue was purified by flash chromatography using 4:1 petroleum ether/ethyl acetate to furnish **21** (0.16 g, 88%). ¹H NMR (CDCl₃) δ 8.25 (s, 1H), 7.40–7.28 (m, 5H), 5.55 (s, 1H), 5.21 (d, *J* = 7.14 Hz, 1H), 4.64 (d, *J* = 12.4 Hz, 1H), 4.58 (d, *J* = 12.4 Hz, 1H), 4.49 (d, *J* = 7.14 Hz, 1H), 4.16–3.84 (m, 5H), 3.16 (d, *J* = 9.89 Hz, 1H), 1.82–1.54 (m, 4H), 1.52 (s, 3H), 1.48–1.28 (m, 6H), 1.23 (s, 3H), 1.02–0.84 (m, 7H). **10**: To a solution of **21** (0.025 g, 0.047 mmol) in anhydrous CH₂Cl₂ (1 mL) was added 1 M BCl₃/CH₂Cl₂ (0.23 mL, 0.23 mmol) at 0 °C and the mixture warmed to rt and stirred for 15 min. MeOH (1 mL) was added to the reaction mixture and concentrated under vacuum. **10** was purified by preparative TLC using 10% MeOH/CHCl₃ (12 mg, 63%). ¹H NMR (CD₃OD) δ 8.40 (s, 1H), 5.09 (s, 1H), 4.55 (d, *J* = 6.32 Hz, 1H), 4.17 (d, *J* = 11.54 Hz, 1H), 4.06–3.82 (m, 5H), 3.18 (d, *J* = 11.54 Hz, 1H), 1.70–1.40 (m, 5H), 1.40–1.18 (m, 5H), 0.88 (t, *J* = 7.14 Hz, 6H), 0.72–0.62 (m, 1H). **22**: To a solution of **21** (0.07 g, 0.13 mmol) in 5% HCOOH/MeOH (10 mL) was added Pd black (0.07 g) and stirred at rt for 8 h. The reaction mixture was filtered through Celite and concentrated and purified by flash chromatography using 5% MeOH/CHCl₃ to furnish **22** as a solid (0.05 g, 86%). ¹H NMR (CDCl₃) δ 7.88 (s, 1H), 5.46 (d, *J* = 7.14 Hz, 1H), 4.96 (s, 1H), 4.78 (d, *J* = 7.14 Hz, 1H), 4.25–3.99 (m, 5H), 3.26 (d, *J* = 11.54 Hz, 1H), 1.77–1.58 (m, 5H), 1.53 (s, 3H), 1.44–1.30 (m, 5H), 1.27 (s, 3H), 1.10–0.89 (m, 7H). **25**: To a solution of **22** (0.026 g, 0.058 mmol) in anhydrous THF (2 mL) was added tetrazole (0.012 g, 0.18 mmol) and di-*t*-butyl-*N,N*-diethylphosphoramidite (0.024 mL, 0.09 mmol) and stirred at rt for 12 h. The reaction mixture was cooled to –78 °C and was added a solution of mCPBA (0.017 g) and warmed to rt. Methanol (2 mL) was added to the reaction mixture and concentrated under vacuum and purified by preparative TLC using 10% MeOH/CHCl₃ to furnish 0.022 g product **25**. ¹H NMR (CDCl₃) δ 8.02 (s, 1H), 5.48 (s, 1H), 5.20 (d, *J* = 6.87 Hz, 1H), 4.54 (d, *J* = 6.87 Hz, 1H), 4.31–3.96 (m, 6H), 1.82–1.23 (m, 34H), 1.10–0.89 (m, 7H). Compound **25** was treated with DOWEX in MeOH/H₂O at 90°C for 2 h and filtered and concentrated to furnish 0.011 g of monophosphate. To a suspension of this monophosphate (0.011 g, 0.023 mmol) in DMF (1 mL) was added 1,1'-carbonyldiimidazole (0.019 g, 0.12 mmol) and stirred for 12 h. The reaction mixture was treated with a solution of 5% Et₃N/MeOH and stirred for 4 h. This mixture was concentrated to dryness under vacuum, treated with tributylammonium pyrophosphate (0.063 g, 0.014 mmol) and DMF (1 mL) and stirred for 3 days. Triethylammonium bicarbonate buffer (3 mL) was added to the reaction mixture followed by water (2 mL). After lyophilization, the crude material obtained was purified on a Sephadex column using 0.5 M ammonium bicarbonate and water to furnish 3.5 mg of **12**. ¹H NMR (D₂O) δ 8.20 (s, 1H), 5.18 (s, 1H), 4.80–4.73 (m, 2H), 4.09–3.77 (m, 6H), 1.80–1.22 (m, 10H), 1.04–0.84 (m, 7H). **23**: A mixture of **22** (0.03 g, 0.067 mmol), RuO₂ (0.18 g, 1.34 mmol), NaIO₄ (0.286 g, 1.34 mmol), K₂CO₃ (0.184 g, 1.34 mmol) in CH₃CN/CHCl₃/H₂O = 2:2:3 (10 mL) was stirred at rt vigorously for 20 h. Reaction mixture was filtered through Celite. Organic layer was separated and the aqueous layer was acidified with HCl and extracted with EtOAc, dried and concentrated under vacuum to furnish 0.012 g product. ¹H NMR (CDCl₃) δ 7.64 (s, 1H), 5.69 (d, *J* = 7.14 Hz, 1H), 5.30 (s, 1H), 4.74 (d, *J* = 7.14 Hz, 1H), 4.09 (t, *J* = 7.42 Hz, 2H), 4.00 (t, *J* = 7.42 Hz, 2H), 1.85–1.59 (m, 6H), 1.55 (s, 3H), 1.44–1.33 (m, 4H), 1.28 (s, 3H), 1.02–0.86 (m, 7H). **11**: A mixture of **23** (0.01 g, 0.022 mmol), BOP-Cl (0.011 g, 0.044 mmol), CH₃NH₂•HCl (0.006 g, 0.088 mmol), Et₃N (0.012 mL, 0.088 mmol) in THF (2 mL) was stirred at rt for 8 h. Solvent was removed under vacuum and the crude material was purified by preparative TLC using 10% MeOH/CHCl₃. The pure material was treated with 1 M BCl₃/CH₂Cl₂ (0.1 mL, 0.1 mmol) at 0 °C for 10 min. Methanol was added to the reaction mixture and the solvent was evaporated and the crude material was purified by preparative TLC using 15% MeOH/CHCl₃ to furnish 0.002 g product **11**. ¹H NMR (CDCl₃) δ 8.29 (s, 1H), 5.14 (s, 1H), 4.87 (s, 1H), 4.72 (s, 1H), 4.18–3.94 (m, 4H), 3.67 (s, 3H), 1.84–1.22 (m, 10H), 1.00–0.82 (m, 7H).
16. Dunn PM, Liu M, Zhong Y, King BF, Burnstock G. *Br. J. Pharmacol.* 2000; 130:1378. [PubMed: 10903979]
17. Rathbone MP, Middlemiss PJ, Gysbers JW, Andrew C, Herman MA, Reed JK, Ciccarelli R, Di Iorio P, Caciagli F. *Prog. Neurobiol.* 1999; 59:663. [PubMed: 10845757]
18. Jacobson KA, Xie R, Young L, Chang L, Liang BT. *J. Biol. Chem.* 2000; 275:30272. [PubMed: 10887176]

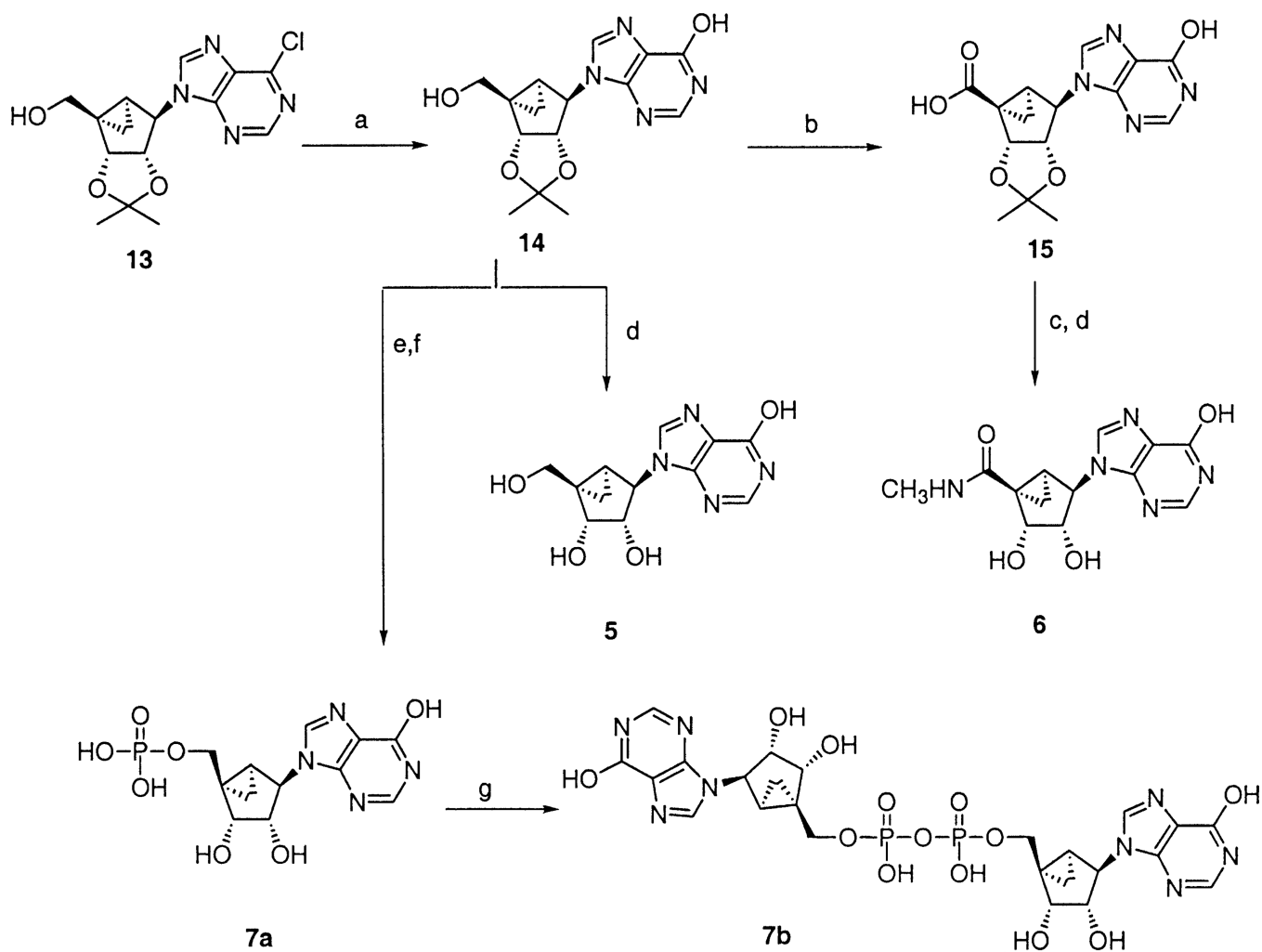
19. Communi D, Janssens R, Robaye B, Zeelis N, Boeynaems JM. FEBS Lett. 2000; 475:39.
[PubMed: 10854854]

Author Manuscript

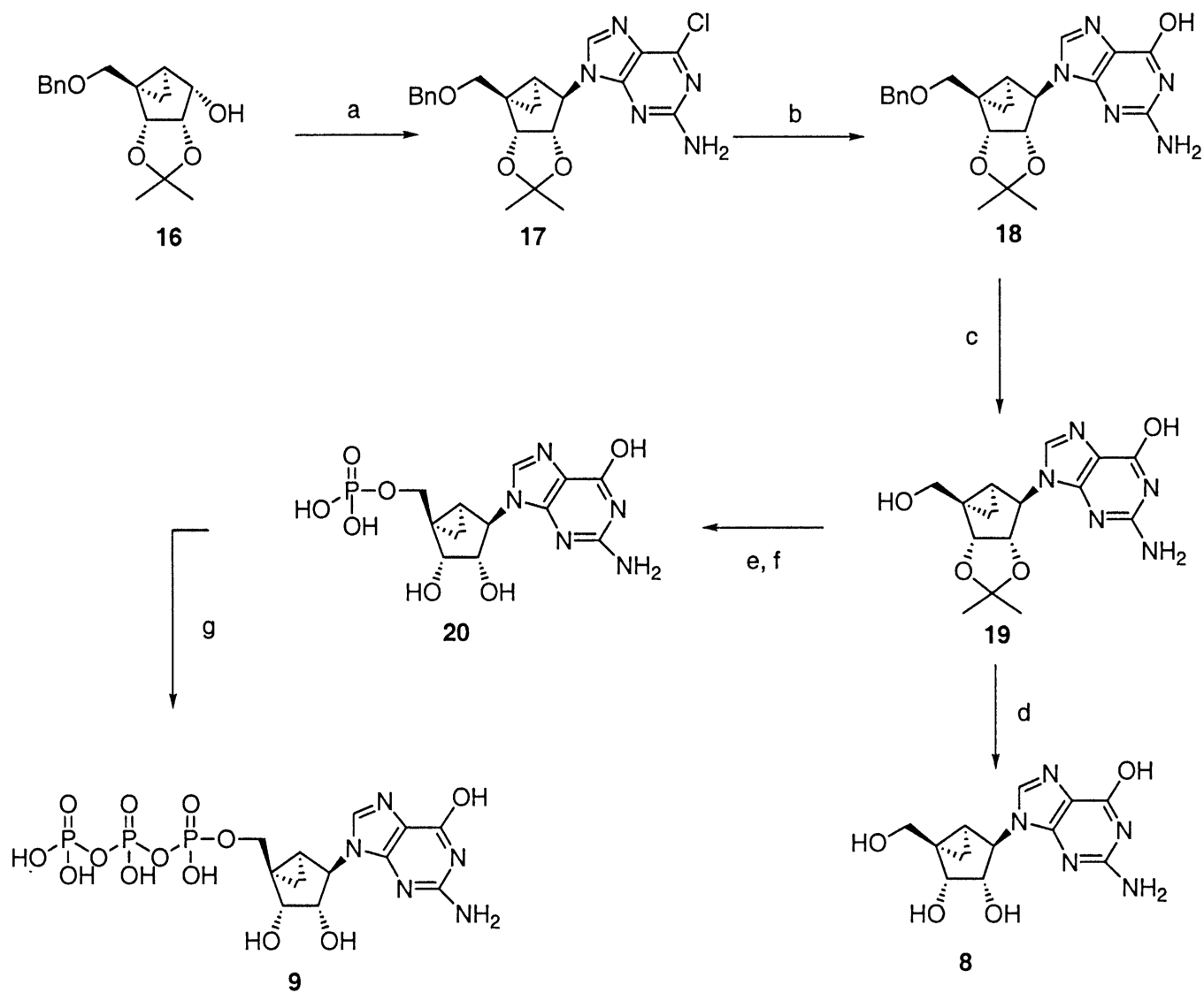
Author Manuscript

Author Manuscript

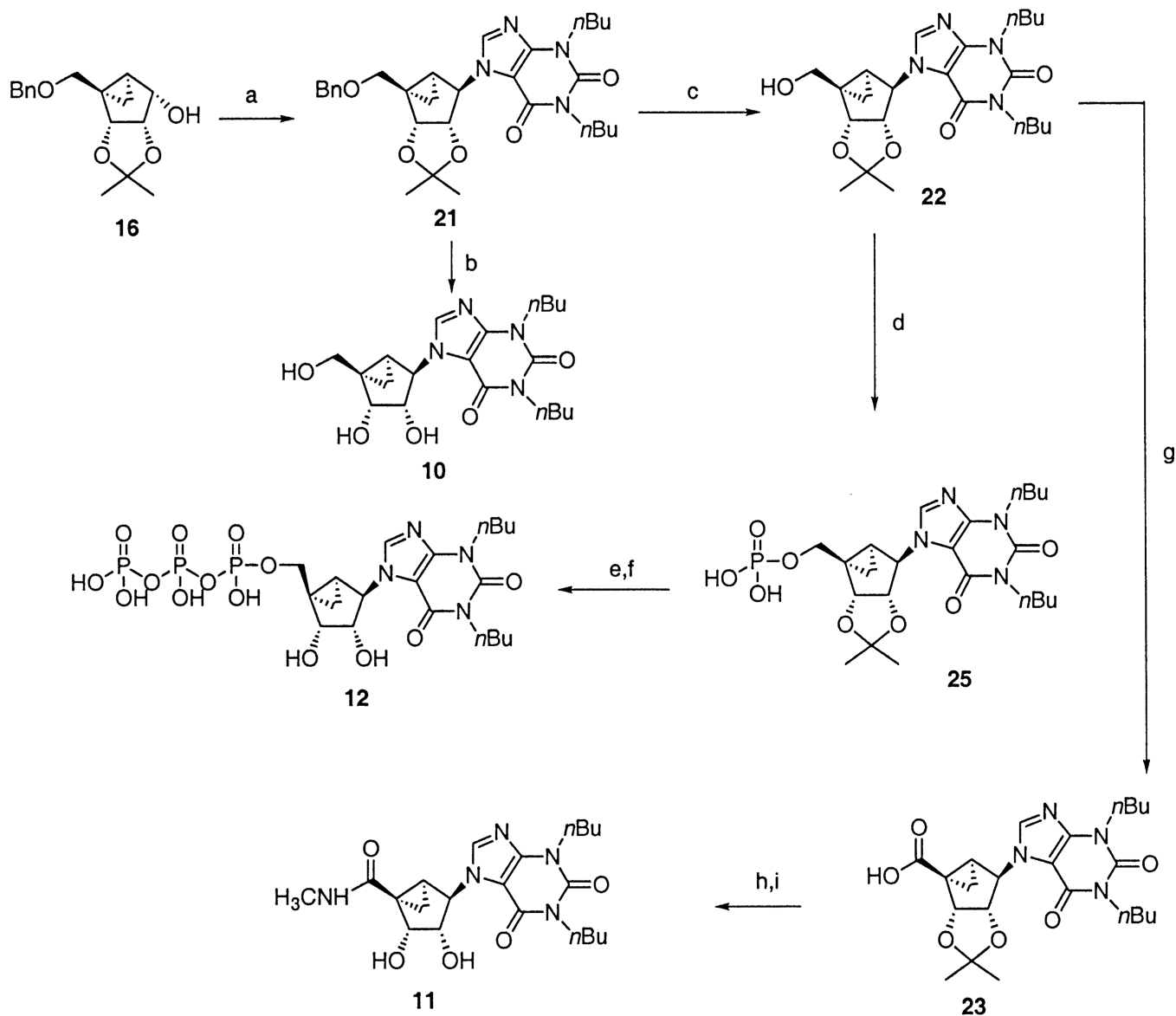
Author Manuscript

**Scheme 1.**

- (a) HSCH₂CH₂OH, NaOMe, MeOH, reflux; (b) RuCl₃, NaIO₄, CH₃CN/CHCl₃/H₂O=2:2:3; (c) BOP-Cl, Et₃N, MeNH₂ (2 M/THF), CH₂Cl₂/DMF=1:1; (d) CF₃CO₂H, wet MeOH, 60 °C; (e) (i) 1*H*-tetrazole, di-*t*-butyl *N,N*-diethylphosphoramidite, THF; (ii) mCPBA, CH₂Cl₂; (f) DOWEX 50×8–200, MeOH/H₂O=2:1; (g) (i) 1,1'-carbonyldiimidazole, DMF; (ii) 5% Et₃N/MeOH; (iii) tetrabutylammonium pyrophosphate.

**Scheme 2.**

(a) 2-Amino-6-chloropurine, DIAD, Ph_3P , THF; (b) 2N NaOH, dioxane; (c) Pd black, 10% HCO_2H in MeOH; (d) TFA/MeOH/ H_2O ; (e) (i) tetrazole, di-*t*-butyl-*N,N*-diethylphosphoramidite, THF; (ii) mCPBA, CH_2Cl_2 ; (f) TFA/MeOH/ H_2O (g) (i) 1,1'-carbonyldiimidazole, DMF; (ii) 5% Et_3N /MeOH; (iii) tetrabutylammonium pyrophosphate.

**Scheme 3.**

(a) 1,3-Dibutylxanthine, DEAD, Ph_3P , THF; (b) BCl_3 , CH_2Cl_2 ; (c) Pd black, 10% HCO_2H in MeOH; (d) (i) tetrazole, di-*t*-butyl-*N,N*-diethylphosphoramidite, THF; (ii) mCPBA, CH_2Cl_2 ; (e) DOWEX 50 \times 8–200, MeOH/ H_2O =1:1; (f) (i) 1,1'-carbonyldiimidazole, DMF; (ii) 5% Et_3N /MeOH; (iii) tetrabutylammonium pyrophosphate; (g) RuO_2 , NaIO_4 , K_2CO_3 , $\text{H}_2\text{O}/\text{CH}_3\text{CN}/\text{CHCl}_3$; (h) $\text{CH}_3\text{NH}_2^+ \text{HCl}$, Et_3N , BOP-Cl; (i) $\text{BCl}_3/\text{CH}_2\text{Cl}_2$

Affinities of (N)-methanocarba analogues of 6-oxypurine ribosides in radioligand binding assays at rat A₁,^a rat A_{2A},^b and human A₃ receptors,^c unless noted, and in other biochemical assay systems^d

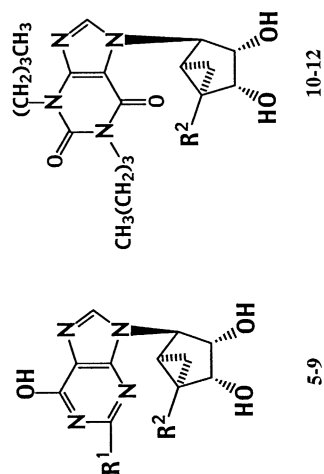
Table 1

Compound	R ¹	R ²	K _i (μM) or % displacement at 100 μM		
			rA ₁ ^a	rA _{2A} ^b	hA ₃ ^c
5 MRS 1957	H	CH ₂ OH	< 10%	< 10%	30 ± 10%
6 MRS 1997	H	CONHCH ₃	< 10%	< 10%	28.8 ± 5.2
7a MRS 2397 ^d	H	CH ₂ O-phosphate	< 10%	< 10%	11 ± 1%
7b MRS 2398 ^d	H	CH ₂ O-phosphate ₂ dimer	< 10%	< 10%	21.6 ± 0.4
8 MRS 1941	NH ₂	CH ₂ OH	< 10%	< 10%	20.5 ± 2.1
9 MRS 2351 ^d	NH ₂	CH ₂ O-triphosphate	< 10%	< 10%	< 10%
10 MRS 1971	—	CH ₂ OH	2.97 ± 0.15	< 10%	4.35 ± 0.40
11 MRS 1998	—	CONHCH ₃	24.4 ± 4.8	< 10%	1.86 ± 0.23
12 MRS 2396	—	CH ₂ O-triphosphate	< 10%	< 10%	< 10%

^aDisplacement of specific [³H]R-PIA binding to A₁ receptors in rat brain membranes, expressed as K_i ± SEM or % (*n* = 3–5).

^bDisplacement of specific [³H]CGS 21680 binding to A_{2A} receptors in rat striatal membranes, expressed as K_i ± SEM or % (*n* = 3–6).

^cDisplacement of specific [¹²⁵I]AB-MECA binding at human A₃ receptors expressed in HEK-293 cells, in membranes, expressed as K_i ± SEM or % (*n* = 3–5).



Selected compounds were examined in HL-60 cells for effects on calcium mobilization. Compounds **7a** and **7b** (100 μ M) were inactive in raising intracellular calcium, while compound **9** (100 μ M) caused a rise in intracellular calcium equivalent to $53 \pm 18\%$ of the effect of 1 μ M UTP ($n = 3$).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript