Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1333–1337

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Ring-Constrained (N)-Methanocarba Nucleosides as Adenosine Receptor Agonists: Independent 5′-Uronamide and 2′-Deoxy Modifications

Kyeong Lee,[a] Gnana Ravi,[a] Xiao-duo Ji,[a] Victor E. Marquez[b] and Kenneth A. Jacobson[a,*]

[a]*Molecular Recognition Section, LBC, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA*
[b]*Laboratory of Medicinal Chemistry, National Cancer Institute, Frederick, MD 21702, USA*

Received 12 January 2001; accepted 20 March 2001

Abstract—Novel methanocarba adenosine analogues, having the pseudo-ribose northern (N) conformation preferred at adenosine receptors (ARs), were synthesized and tested in binding assays. The 5′-uronamide modification preserved [N^6-(3-iodobenzyl)] or enhanced (N^6-methyl) affinity at A_3ARs, while the 2′-deoxy modification reduced affinity and efficacy in a functional assay. Published by Elsevier Science Ltd.

There are four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3), all of which are G protein-coupled receptors (GPCRs). Modulation of adenosine receptors by selective agonists and antagonists[1,2] has the potential for the treatment of a wide range of diseases, including those of the cardiovascular, inflammatory, and central nervous systems. For example, selective A_1 and A_3 receptor agonists protect cardiac myocytes from the damaging effects of ischemia.[3] Such agonists have also been shown to be protective in models of cerebral ischemia.[4] In general, adenosine acts as a protective local mediator, which responds to stress applied to a system as a negative feedback control, leading to either increased energy supply (usually via the A_{2A} receptor) to the organ or diminished energy demand (usually via the A_1 receptor). Recently, A_{2A} receptor agonists have been proposed as antiinflammatory agents and for use in ischemia reperfusion.[5]

Numerous structure–activity studies of adenosine derivatives as receptor agonists[2,6] conclude that selectivity may be provided by specific substitutions of the adenine ring. For example, N^6-cycloalkyl groups favor selectivity for A_1 versus A_{2A}/A_3 subtypes, and N^6-benzyl substitutions favor selectivity for A_3 versus A_1/A_{2A} subtypes. Selectivity for the A_{2A} receptor is often achieved through substitution at the 2-position. There are currently no selective agonists of the A_{2B} receptor. Modifications of the ribose moiety of adenosine agonists are less well tolerated and therefore less amenable to extensive modifications. However, small alkyl 5′-uronamide modification of the ribose often enhances affinity of adenosine derivatives at multiple subtypes.

We have recently examined conformational requirements of the ribose moiety in adenosine agonists.[7] In general, the ribose rings of nucleosides and nucleotides may adopt a range of conformations as described by the 'pseudorotational cycle'.[8] The northern [(N), 2′-*exo*] and southern [(S), 2′-*endo*] conformations are the most relevant to the biological activities observed for nucleosides and nucleotides in association with DNA, RNA, and various enzymes. We have defined a preference for the (N) conformation of ribose at both adenosine[7] and P2Y receptors[9] using methanocarba analogues in which a cyclopropane moiety constrains a pseudosugar (cyclopentane) ring of the nucleoside to either a (N)-, **1**, or (S)-, **2**, envelope conformation (Fig. 1). Such analogues

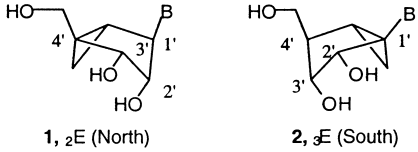


Figure 1.

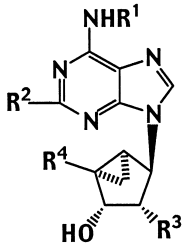
*Corresponding author. Tel.: +1-301-496-9024; fax: +1-301-480-8422; e-mail: kajacobs@helix.nih.gov

0960-894X/01/$ - see front matter Published by Elsevier Science Ltd.
PII: S0960-894X(01)00213-X

have helped to define the role of sugar puckering in stabilizing the active adenosine receptor-bound conformation, and thereby have allowed identification of the (N) conformation as the favored isomer.

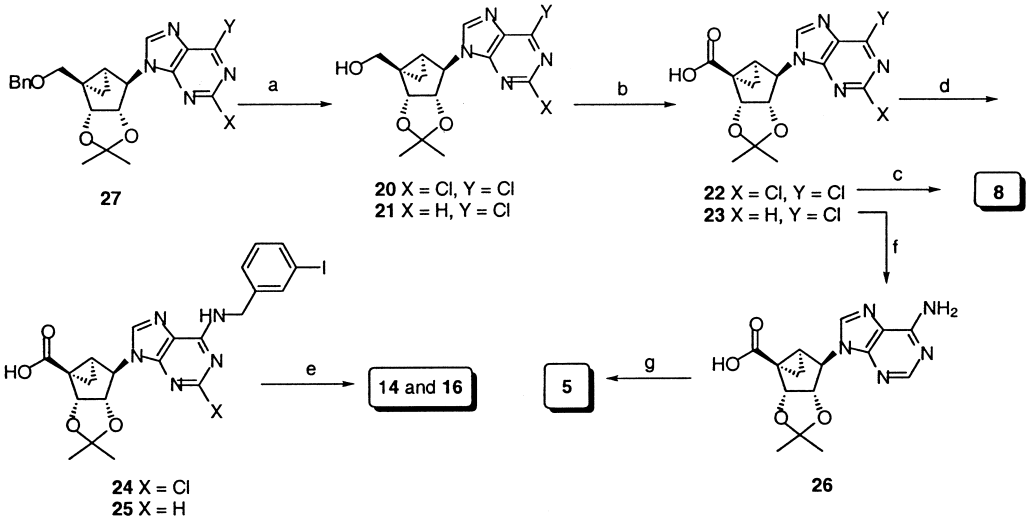
In the present study, we have combined N^6-substituted adenosine agonists containing the (N)-methanocarba modification with either 5′-uronamide groups or the 2′-deoxy modification. The 2′-deoxy modification is

Table 1. Affinities of methanocarba-adenosine analogues of the N-conformation and their 2′-deoxy analogues in radioligand binding assays at rat A_1,[a] rat A_{2A},[b] and human A_3 receptors,[c] unless noted[d]

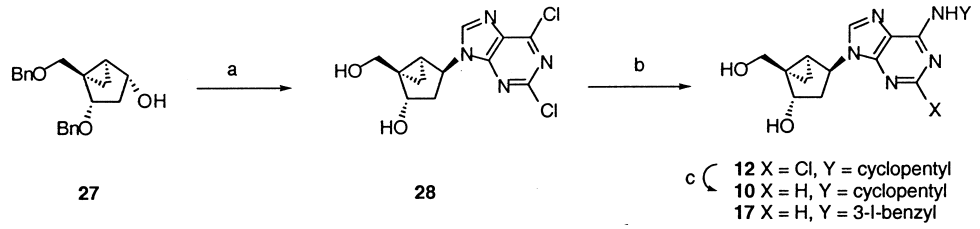


Compd	R^1	R^2	R^3	R^4	K_i (nM) or % displacement		
					rA_1[a]	rA_{2A}[b]	hA_3[c]
3	H	H	OH	CH_2OH	1680±80	22,500±100 (human)	404±70[e]
4	H	Cl	OH	CH_2OH	273±36	1910±240	84.7±18.7
5	H	H	OH	$CONHCH_2CH_3$	31.8±6.9	100±18	29.9±6.8
6	Me	H	OH	CH_2OH	1470±190	⩽10% at 10 μM	126±18
7	Me	Cl	OH	CH_2OH	884±99	⩽10% at 10 μM	22.5±7.4
8	Me	Cl	OH	$CONHCH_3$	805±197	⩽10% at 10 μM	6.19±0.42
9	CP	H	OH	CH_2OH	5.06±0.51	6800±1800	170±51
10	CP	H	H	CH_2OH	5110±790	15% at 100 μM	2880±910
11	CP	Cl	OH	CH_2OH	8.76±0.81	3390±520	466±58
12	CP	Cl	H	CH_2OH	3600±780	45±5% at 100 μM	1090±190
13	IB	H	OH	CH_2OH	69.2±9.8	601±236	4.13±1.76
14	IB	H	OH	$CONHCH_3$	52.7±5.2	548±115	2.39±0.54
15	IB	Cl	OH	CH_2OH	141±22	732±207	2.24±1.45
16	IB	Cl	OH	$CONHCH_3$	83.9±10.3	1660±260	1.51±0.23
17	IB	Cl	H	CH_2OH	8730±370	25,400±3800	912±29

[a]Displacement of specific [^{3}H]*R*-PIA binding to A_1 receptors in rat brain membranes, expressed as K_i±SEM (n=3–5).
[b]Displacement of specific [^{3}H]CGS 21680 binding to A_{2A} receptors in rat striatal membranes, expressed as K_i±SEM (n=3–6), and at A_{2B} receptors expressed in HEK-293 cells versus [^{3}H]ZM241,385, unless noted.
[c]Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in CHO cells, in membranes, expressed as K_i±SEM (n=3–4).
[d]Me, methyl; CP, cyclopentyl; IB, 3-iodobenzyl.
[e]Measured in the absence of adenosine deaminase.



Scheme 1. (a) (i) BCl_3, CH_2Cl_2, −78 °C; (ii) *p*-TsOH, DMP, acetone; (b) $NaIO_4$, RuO_2, K_2CO_3, MeCN/$CHCl_3$/H_2O=2:2:3; (c) (i) EDAC, DMAP, $MeNH_3$, CH_2Cl_2/DMF=1:1; (ii) 10% CF_3CO_2H/MeOH, H_2O; (d) 3-I-benzylamine·HCl, TEA, MeOH; (e) (i) $(COCl)_2$, 50 °C, then $MeNH_2$, CH_2Cl_2; (ii) 10% CF_3CO_2H/MeOH, H_2O; (f) NH_3/2-propanol, 90 °C; (g) (i) $(COCl)_2$, 50 °C, then $EtNH_2$, CH_2Cl_2; (ii) 10% CF_3CO_2H/MeOH, H_2O, 60 °C.



27 28

Scheme 2. (a) (i) 2,6-Di-Cl-purine, DEAD, PPh_3, THF; (ii) BCl_3, CH_2Cl_2, 0 °C; (b) cyclopentylamine (for **12**) or 3-I-benzylamine·HCl, TEA (for **17**), MeOH; (C) H_2/Pd–C, MeOH.

known to diminish agonist efficacy, to create partial agonists.[10]

The structures of (N)-methanocarba adenosine analogues (**3**–**17**) synthesized and tested in binding assays at three subtypes of adenosine receptors[11–13] are shown in Table 1. We combined the (N)-methanocarba modification of known potent adenosine agonists with either 5′-uronamide groups (**5**, **8**, **14**, and **16**) or a 2′-deoxy modification (**10**, **12**, and **17**). These adenosine agonists contain methyl (**6**–**8**), cyclopentyl (**9**–**12**), or 3-iodobenzyl (**13**–**17**) substitution at the N^6-position. Both 2-H and 2-Cl analogues are included. Several of the compounds, that is the triols **9**, **11**, **13**, and **15**, were reported to be selective adenosine agonists in a previous study.[7]

The synthetic methods used to prepare the new 5′-uronamide analogues are shown in Scheme 1. Nucleoside analogues, **20** and **21**, containing a 6-Cl and a 5′-OH group, and protected as the 2′,3′-acetonides, were oxidized using basic sodium periodate and ruthenium tetraoxide. The resulting carboxylic acid derivatives, **23**–**25**,[14] were substituted at the 6-position by amine treatment, converted to the acid chloride using oxalyl chloride, and reacted immediately with methyl- or ethylamine to form 5′-uronamides.[15] In the case of an N^6-methyl 5′-uronamide derivative, the substitution of the 6-Cl and the formation of the uronamide from **22** were carried out in a single step, using a carbodiimide condensing reagent, to yield, after deprotection, **8**.[16] 2′-Deoxy analogues were synthesized by the methods reported earlier[9] via the (N)-methanocarba analogue of 2,6-dichloropurine-2′-deoxyriboside, **28**, as an intermediate (Scheme 2).[17]

In binding assays at A_1, A_{2A}, and A_3ARs, the (N)-methanocarba analogue of 2-chloroadenosine, **4**, in comparison to the (N)-methanocarba adenosine, **3**, reported earlier,[7] showed substantial enhancement of affinity at all three subtypes and displayed mixed A_1/A_3AR selectivity. The (N)-methanocarba analogue, **5**, of the potent, nonselective agonist NECA (5′-*N*-ethyluronamidoadenosine) was slightly selective for A_1 and A_3ARs versus the A_{2A}AR. For this 6-NH_2 analogue, the 5′-uronamide, **5**, enhanced affinity at the A_1AR by 53-fold and at the A_3AR by only 14-fold in comparison to the triol, **3**.

In the series of N^6-methyl derivatives, binding at A_{2A}ARs was absent, and the simple 4′-CH_2OH compounds, **6** and **7**, were selective in binding at the A_3 versus A_1AR by 12- and 39-fold, respectively. Compound **8** displayed a 4-fold increase in affinity at the A_3AR over **7**, and consequently 130-fold selectivity versus A_1AR. The 5′-uronamide-modified N^6-(3-iodobenzyl) analogues, **14** and **16**, maintained affinity at A_1 and A_3ARs and, therefore, selectivity for A_3 receptors. With an N^6-methyl substituent, the 5′-uronamide modification enhanced affinity at the A_3AR. The 2′-deoxy modified N^6-cyclopentyl analogues, **10** and **12**, bound weakly to adenosine receptors and were nonselective. The 2′-deoxy modified N^6-(3-iodobenzyl) analogue, **17**, displayed greatly reduced affinity and selectivity for the A_3AR.

Agonist efficacy of selected adenosine derivatives was determined in a functional assay consisting of stimulation of binding of [^{35}S]GTP-γ-S by activation of human A_1 and A_3ARs.[7,18] EC_{50} values for **10** and **12** at the A_1AR were 2.89±0.14 μM (30±1% efficacy) and 2.28±0.99 μM (40±8% efficacy), respectively. Thus these two 2′-deoxy analogues, **10** and **12**, were weak, partial agonists at the A_1AR. EC_{50} values (nM) at the A_3AR were: 25.5±6.1 (**8**), 6.80±1.95 (**14**), 5.25±2.20 (**16**), and 303±93 (NECA), and all four derivatives reached full agonist efficacy.

As reported previously,[7] (N)-methanocarba analogues, such as **9**, **13**, and **15**, containing various N^6-substituents, in which the parent compounds were potent agonists at either A_1 (e.g., cyclopentyl) or A_3ARs (e.g., 3-iodobenzyl), retained the selectivity of the parent compound, especially at the A_3AR. As before, the present 'ribose-like' (N)-methanocarba analogues (2′-OH) had preserved or enhanced A_3AR affinity. For example, **5** was 6-fold more potent than the ribose equivalent at the A_3AR and slightly less potent at A_1 and A_{2A}ARs. The efficacy in present compound was reduced at A_1AR for 2′-deoxy analogues (**10** and **12**) and increased at A_3AR for 5′-uronamides (**14** and **16**).

In this study, we have introduced a new synthetic route for the oxidation of the 5′-carbon in the (N)-methanocarba series. This has allowed us to extend the SAR to include a modification that is generally potency-enhancing in the ribose series (i.e., 5′-uronamide). With a bulky N^6-substituent (3-iodobenzyl), the A_3AR affinity-enhancing effects of (N)-methanocarba and 5′-uronamide groups were not additive. Since the 5′-uronamide modification had either unchanged (for a large N^6-substituent) or enhanced (for a small N^6-substituent, methyl) affinity at the A_3AR, we may conclude that in

this series, requirements for N^6- and 5′-substitutions are interrelated (i.e., non-independent in receptor binding). Another small substituent at the N^6-position (MeONH–), when combined with modified 5′-groups, resulted in A_3AR-selective agonists.[19] Compound **8** (MRS 2346) was 130-fold selective for the A_3 versus A_1AR, illustrating again that a bulky N^6-substituent was not required to achieve A_3AR selectivity. Compound **16** (MRS 1898) was a potent and selective full agonist at the human A_3AR.

In conclusion, the (N)-methanocarba modification has provided new analogues having A_3AR selectivity, such as **8**, and mixed A_1/A_3AR selectivity. The pharmacological properties of these analogues as agonists or partial agonists of adenosine receptors may now be studied.

References and Notes

1. Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Dubyak, G. R.; Harden, T. K.; Jacobson, K. A.; Schwabe, U.; Williams, M. *Trends Pharmacol. Sci.* **1997**, *18*, 79.
2. Jacobson, K. A.; Knutsen, L. J. S. In *Handbook of Experimental Pharmacology*; Abbracchio, M. P., Williams, M., Eds.; Springer: Berlin, 2001; Vol. 151/I, pp 301–383.
3. Liang, B. T.; Jacobson, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6995.
4. Von Lubitz, D. K. J. E.; Lin, R. C.-S.; Popik, P.; Carter, M. F.; Jacobson, K. A. *Eur. J. Pharmacol.* **1994**, *263*, 59.
5. Okusa, M. D.; Linden, J.; Macdonald, T.; Huang, L. *Am. J. Physiol.* **1999**, *277*, F404.
6. Müller, C. E. *Curr. Med. Chem.* **2000**, *7*, 1269.
7. Jacobson, K. A.; Ji, X.-D.; Li, A. H.; Melman, N.; Siddiqui, M. A.; Shin, K. J.; Marquez, V. E.; Ravi, R. G. *J. Med. Chem.* **2000**, *43*, 2196.
8. Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739.
9. Nandanan, E.; Jang, S. Y.; Moro, S.; Kim, H.; Siddiqui, M. A.; Russ, P.; Marquez, V. E.; Busson, R.; Herderwijn, P.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2000**, *43*, 829.
10. Van Der Graaf, P. H.; Van Schaick, E. A.; Mathôt, R. A.; IJzerman, A. P.; Danhof, M. *Pharmacol. Exp. Ther.* **1997**, *283*, 809.
11. Schwabe, U.; Trost, T. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *313*, 179.
12. Jarvis, M. F.; Schutz, R.; Hutchison, A. J.; Do, E.; Sills, M. A.; Williams, M. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888.
13. Olah, M. E.; Gallo-Rodriguez, C.; Jacobson, K. A.; Stiles, G. L. *Mol. Pharmacol.* **1994**, *45*, 978.
14. General procedure for the 5′-carboxylic acid derivatives (**22**–**23**). To a solution of alcohol **20** (156 mg, 0.405 mmol) in $CH_3CN/CHCl_3/H_2O$ (14 mL, 2:2:3) were added sodium periodate (1.73 g, 8.1 mmol), ruthenium dioxide (40 mg), and potassium carbonate (40 mg), and the mixture was stirred for 24 h and filtered through filter paper. The filtrate was diluted with CH_2Cl_2 and H_2O, and the water layer was separated. The combined water layer was acidified with concd HCl to pH 5–6 at 0 °C. The mixture was extracted with CH_2Cl_2 and the combined organic layer was dried ($MgSO_4$), filtered and concentrated to dryness to give the acid **22** as colorless oil (151.8 mg, 97.3%). **22**: ^{1}H NMR (CD_3Cl, 300 MHz) δ 8.11 (s, 1H), 5.87 (d, J = 7.2 Hz, 1H), 4.98 (s, 1H), 4.72 (d, J = 6.9 Hz, 1H), 2.29 (s, 1H), 1.88 (m, 1H), 1.66 (m, 1H). MS (FAB) m/z 385 ($M^+ + 1$). **23**: ^{1}H NMR (CD_3Cl, 300 MHz) δ 8.74 (s, 1H), 8.21 (s, 1H), 5.91 (d, J = 6.6 Hz, 1H), 5.05 (s, 1H), 4.79 (d, J = 6.9 Hz, 1H), 2.35 (s, 1H), 1.86 (m, 1H), 1.67 (m, 1H). MS (NCI): m/z 350 (M^-).
15. General procedure for the 5′-uronamide derivatives **5**, **14**, and **16**. A stirred mixture of the acid **22** (76.3 mg, 0.131 mmol) and oxalyl chloride (1.4 mL) in CH_2Cl_2 (1 mL) was heated at 60 °C for 2 h. After removal of the excess solvent and reagent under N_2, the residue was suspended in anhyd CH_2Cl_2 and treated with methylamine (2 M in THF; in the case of **5**, 2 M ethylamine/THF). The resulting mixture was stirred at rt for 1 h and poured into ice-cold water, which was extracted with CH_2Cl_2. After usual workup, the residue was purified on preparative TLC (CH_3Cl/MeOH = 15:1) to provide the amide intermediate (52.6 mg, 67.5%). The mixture of amide intermediate (14.7 mg, 0.025 mmol) having an acetonide group, 10% trifluoroacetic acid/MeOH (3 mL), and H_2O (0.3 mL) was heated at 60 °C for 4 h. The solvent was removed and coevaporated with toluene. The residue was purified on silica gel to give the free amide **14** (12.2 mg, 89%). **14**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.21 (s, 1H), 8.08 (s, 1H), 7.74 (s, 1H), 7.58 (m, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.13 (t, J = 7.8 Hz, 1H), 4.93 (d, J = 5.7 Hz, 1H), 4.66 (s, 1H), 4.60 (d, J = 4.8 Hz, 1H), 3.89 (d, J = 6 Hz, 1H), 3.38 (m), 2.67 (d, J = 4.2 Hz, 3H), 1.82 (m, 1H), 1.60 (m, 1H), 1.29 (m, 1H). HRMS (FAB) calcd 555.0408, found 555.0418. **16**: ^{1}H NMR (CD_3OD, 300 MHz) δ 8.90 (t, 1H), 8.11 (s, 1H), 7.74 (s, 1H), 7.58 (m, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.13 (t, J = 7.8 Hz, 1H), 4.93 (d, J = 5.7 Hz, 1H), 4.66 (s, 1H), 4.60 (d, J = 4.8 Hz, 1H), 3.89 (d, J = 6 Hz, 1H), 3.38 (m), 2.67 (d, J = 4.2 Hz, 3H), 1.82 (m, 1H), 1.60 (m, 1H), 1.29 (m, 1H). HRMS (FAB) calcd 521.0798, found 521.0814. **5**: ^{1}H NMR (CD_3OD, 300 MHz) δ 8.31 (s, 1H), 8.23 (s, 1H), 5.15 (d, J = 6.6 Hz, 1H), 5.41–5.05 (m), 4.13 (d, J = 6.3 Hz, 1H), 2.19 (m, 1H), 1.97 (m, 1H), 1.80 (m, 1H), 1.30 (t, J = 7.2 Hz, 3H). UV (MeOH) λ_{max} 260.0 nm. HRMS (FAB) calcd 319.1519, found 319.1510.
16. Procedure for the preparation of the N^6-methylamino-5′-uronamide, **8**. A mixture of the acid **23** (19.9 mg, 0.052 mmol), DMAP (2.1 mg, 0.017 mmol), EDAC (1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide·HCl, 13 mg, 0.068 mmol), and methylamine (2 M in THF, 0.034 mL, 0.068 mmol) was stirred for 2.5 h at rt. The reaction mixture was concentrated to dryness and the residue was then washed with water. The organic layer was dried, filtered and dried to dryness, which was purified on preparative TLC (CH_3Cl/MeOH = 15:1) to give the amide intermediate (10 mg, 49%). ^{1}H NMR (CD_3Cl, 300 MHz) δ 7.70 (s, 1H), 6.91 (br s, 1H), 6.26 (br s, 1H), 5.66 (d, J = 7.5 Hz, 1H), 4.78 (m, 2H), 3.18 (d, 3H), 2.92 (d, J = 5.1 Hz, 3H), 2.93 (s, 3H), 2.91 (s, 3H). HRMS (FAB) calcd 393.1442, found 393.1446. A mixture of amide intermediate (5.2 mg, 0.013 mmol) having an acetonide group, 10% trifluoroacetic acid/MeOH (1 mL), and H_2O (0.1 mL) was heated at 60 °C for 4 h. The solvent was removed and coevaporated with toluene. The residue was purified on silica gel (CH_3Cl/MeOH = 12:1) to give the free amide **8** (3.8 mg, 85%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.06 (s, 1H), 5.43 (d, J = 4.5 Hz, 1H), 4.93 (t, 6.3 Hz, 1H), 4.82 (d, J = 7.8 Hz, 1H), 4.66 (s, 1H), 4.12 (m, 2H), 3.87 (m, 1H), 2.91 (d, 3H), 2.66 (d, J = 4.2 Hz, 3H), 1.81 (m, 1H), 1.60 (m, 1H), 1.35 (m, 1H). UV (MeOH) λ_{max} 271.0 nm. HRMS (FAB) calcd 353.1129, found 353.1139.
17. To a mixture of **27**[20] (0.25 g, 0.77 mmol), dichloropurine (0.29 g, 1.54 mmol), and triphenylphosphine (0.4 g, 1.54 mmol) in anhydrous THF (10 mL) was added DEAD dropwise at 0 °C with stirring for 6 h. Solvent was removed under vacuum and the residue obtained was purified using flash chromatography using 7:3 petroleum ether/ethyl acetate to furnish 0.25 g of protected product. This compound (0.19 g, 0.38 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated with

1 M BCl_3 in CH_2Cl_2 (1.14 mL, 1.14 mmol) at 0 °C and stirred for 15 min for complete reaction. Solvent was removed under vacuum and the residue obtained was purified by flash chromatography using 10% MeOH in $CHCl_3$ to furnish 0.13 g of product **28**. To a solution of **28** (0.025 g, 0.08 mmol) in MeOH (2 mL), was added cyclopentylamine (0.04 mL, 0.4 mmol) and the mixture was stirred at rt for 8 h. Solvent was removed under vacuum and the residue was purified by flash chromatography using 5% MeOH in $CHCl_3$ to furnish 0.023 g of product **12**: ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H, C-8), 6.22 (bs, 1H), 5.16 (t, 1H, J = 7.81 Hz), 4.58 (s, 1H), 4.25 (d, 1H, J = 11.72 Hz), 3.50 (d, 1H, J = 11.72 Hz), 2.17–2.10 (m, 3H), 1.95–1.85 (m, 1H), 1.76–1.52 (m, 7H), 1.0–0.74 (m, 3H). HRMS (FAB) calcd 364.1540, found 364.1549. To a solution of **12** in MeOH (10 mL) was added 10% Pd/C (4 mg) and stirred under H_2 at atmospheric pressure. Solvent was removed, and the residue was purified by preparative TLC 10% MeOH in $CHCl_3$ to furnish 10 mg of **10**: ^{1}H NMR (CD_3OD) δ 8.49 (s, 1H, C-8), 8.23 (s, 1H, C-2), 5.03 (d, 1H, J = 6 Hz) 4.58 (d, 1H J = 12 Hz), 4.29 (d, 1H, J = 11.53 Hz), 3.34 (d, 1H, J = 11.53 Hz), 2.2–1.98 (m, 4H), 1.92–1.6 (m, 8H), 1.1–1.0 (m, 1H), 0.98–0.75 (m, 1H). HRMS (FAB) calcd 330.1930, found 330.1941. To a solution of **28** (0.025 g, 0.08 mmol) in MeOH (2 mL) was added 3-iodobenzylamine hydrochloride (0.1 g, 0.4 mmol) and triethylamine (0.056 mL, 0.4 mmol), and the mixture was stirred at rt for 6 h. Solvent was removed under vacuum and the residue was purified using flash chromatography using 5% MeOH in $CHCl_3$ to furnish 0.03 g of **17**: ^{1}H NMR (CD_3OD) δ 8.46 (s, 1H, C-8), 7.77 (s, 1H), 7.59 (d, 1H, J = 7.81 Hz), 7.39 (d, 1H, J = 7.81 Hz), 7.09 (t, 1H, J = 7.81 Hz), 4.96 (d, 1H, J = 5.86 Hz), 4.7 (s, 2H), 4.27 (d, 1H, J = 11.72 Hz), 3.36 (d, 1H, J = 11.72 Hz), 2.1–1.96 (m, 1H), 1.9–1.7 (m, 1H), 1.7–1.6 (m, 1H), 1.04–1.01 (m, 1H), 0.82–0.74 (m, 1H). HRMS (FAB) calcd 512.0350, found 512.0358.

18. Lorenzen, A.; Guerra, L.; Vogt, H.; Schwabe, U. *Mol. Pharmacol*. **1996**, *49*, 915.
19. Mogensen, J. P.; Roberts, S. M.; Bowler, A. N.; Thomsen, C.; Knutsen, L. J. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1767.
20. Marquez, V. E.; Russ, P.; Alonso, R.; Siddiqui, M. A.; Hernandez, S.; George, C.; Nicklaus, M. C.; Dai, F.; Ford, H. *Helv. Chim. Acta* **1999**, *82*, 2119.