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

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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 A₃ARs, while the 2'-deoxy modification reduced affinity and efficacy in a functional assay.

There are four subtypes of adenosine receptors (A₁, A_{2A}, A_{2B}, and A₃), 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 A₁ and A₃ 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 A₁ 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 A₁ versus A_{2A}/A₃ subtypes, and *N*⁶-benzyl substitutions favor selectivity for A₃ versus A₁/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.

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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 adenosine⁷ and P2Y receptors⁹ 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 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 N⁶-substituted adenosine agonists containing the (N)-methanocarba modification with either 5'-uronamide groups or the 2'-deoxy modification. The 2'-deoxy modification is 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 N⁶-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 tetroxide. 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 N⁶-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 earlier⁹ via the (N)-methanocarba analogue of 2,6-dichloropurine-2'-deoxyriboside, **28**, as an intermediate (Scheme 2).¹⁷

In binding assays at A₁, A_{2A}, and A₃ARs, 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 A₁/A₃AR selectivity. The (N)-methanocarba analogue, **5**, of the potent, nonselective agonist NECA (5'-N-ethyl-uronamidoadenosine) was slightly selective for A₁ and A₃ARs versus the A_{2A}AR. For this 6-NH₂ analogue, the 5'-uronamide, **5**, enhanced affinity at the A₁AR by 53-fold and at the A₃AR 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'-CH₂OH compounds, **6** and **7**, were selective in binding at the A₃ versus A₁AR by 12- and 39-fold, respectively. Compound **8** displayed a 4-fold increase in affinity at the A₃AR over **7**, and consequently 130-fold selectivity versus A₁AR. The 5'-uronamide-modified *N*⁶-(3-iodobenzyl) analogues, **14** and **16**, maintained affinity at A₁ and A₃ARs and, therefore, selectivity for A₃ receptors. With an *N*⁶-methyl substituent, the 5'-uronamide modification enhanced affinity at the A₃AR. 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 A₃AR.

Agonist efficacy of selected adenosine derivatives was determined in a functional assay consisting of stimulation of binding of [³⁵S]GTP-γ-S by activation of human A₁ and A₃ARs.^{7,18} EC₅₀ values for **10** and **12** at the A₁AR 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 A₁AR. EC₅₀ values (nM) at the A₃AR 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 A₁ (e.g., cyclopentyl) or A₃ARs (e.g., 3-iodobenzyl), retained the selectivity of the parent compound, especially at the A₃AR. As before, the present 'ribose-like' (N)-methanocarba analogues (2'-OH) had preserved or enhanced A₃AR affinity. For example, **5** was 6-fold more potent than the ribose equivalent at the A₃AR and slightly less potent at A₁ and A_{2A}ARs. The efficacy in present compound was reduced at A₁AR for 2'-deoxy analogues (**10** and **12**) and increased at A₃AR 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 A₃AR 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 A₃AR, 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 A₃AR-selective agonists.¹⁹ Compound **8** (MRS 2346) was 130-fold selective for the A₃ versus A₁AR, illustrating again that a bulky *N*⁶-substituent was not required to achieve A₃AR selectivity. Compound **16** (MRS 1898) was a potent and selective full agonist at the human A₃AR.

In conclusion, the (N)-methanocarba modification has provided new analogues having A₃AR selectivity, such as **8**, and mixed A₁/A₃AR selectivity. The pharmacological properties of these analogues as agonists or partial agonists of adenosine receptors may now be studied.

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14. General procedure for the 5'-carboxylic acid derivatives (**22–23**). To a solution of alcohol **20** (156 mg, 0.405 mmol) in CH₃CN/CHCl₃/H₂O (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 CH₂Cl₂ and H₂O, 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 CH₂Cl₂ and the combined organic layer was dried (MgSO₄), filtered and concentrated to dryness to give the acid **22** as colorless oil (151.8 mg, 97.3%). **22**: ¹H NMR (CD₃Cl, 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**: ¹H NMR (CD₃Cl, 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 CH₂Cl₂ (1 mL) was heated at 60 °C for 2 h. After removal of the excess solvent and reagent under N₂, the residue was suspended in anhyd CH₂Cl₂ 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 CH₂Cl₂. After usual workup, the residue was purified on preparative TLC (CH₃Cl/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 H₂O (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**: ¹H 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**: ¹H NMR (CD₃OD, 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**: ¹H NMR (CD₃OD, 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 (CH₃Cl/MeOH=15:1) to give the amide intermediate (10 mg, 49%). ¹H NMR (CD₃Cl, 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 acetamide group, 10% trifluoroacetic acid/MeOH (1 mL), and H₂O (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 (CH₃Cl/MeOH=12:1) to give the free amide **8** (3.8 mg, 85%). ¹H 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 drop-wise 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 CH₂Cl₂ (10 mL) and treated with 1 M BCl₃ in CH₂Cl₂ (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 CHCl₃ 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 CHCl₃ to furnish 0.023 g of product **12**: ¹H NMR (CDCl₃) 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 H₂ at atmospheric pressure. Solvent was removed, and the residue was purified by preparative TLC 10% MeOH in CHCl₃ to furnish 10 mg of **10**: ¹H NMR (CD₃OD) 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 CHCl₃ to furnish 0.03 g of **17**: ¹H NMR (CD₃OD) 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.
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1, 2E (North)

2, 3E (South)

Figure 1.

Scheme 1.

(a) (i) BCl_3 , CH_2Cl_2 , -78°C ; (ii) *p*-TsOH, DMP, acetone; (b) NaIO_4 , RuO_2 , K_2CO_3 , $\text{MeCN}/\text{CHCl}_3/\text{H}_2\text{O}=2:2:3$; (c) (i) EDAC, DMAP, MeNH_3 , $\text{CH}_2\text{Cl}_2/\text{DMF}=1:1$; (ii) 10% $\text{CF}_3\text{CO}_2\text{H}/\text{MeOH}$, H_2O ; (d) 3-I-benzylamine. HCl , TEA, MeOH; (e) (i) $(\text{COCl})_2$, 50°C , then MeNH_2 , CH_2Cl_2 ; (ii) 10% $\text{CF}_3\text{CO}_2\text{H}/\text{MeOH}$, H_2O ; (f) NH_3 /2-propanol, 90°C ; (g) (i) $(\text{COCl})_2$, 50°C , then EtNH_2 , CH_2Cl_2 ; (ii) 10% $\text{CF}_3\text{CO}_2\text{H}/\text{MeOH}$, H_2O , 60°C .

Scheme 2.

(a) (i) 2,6-Di-Cl-purine, DEAD, PPh₃, THF; (ii) BCl₃, CH₂Cl₂, 0 °C; (b) cyclopentylamine (for **12**) or 3-I-benzylamine·HCl, TEA (for **17**), MeOH; (C) H₂/Pd-C, MeOH.

Table 1

Affinities of methanocarba-adenosine analogues of the N-conformation and their 2'-deoxy analogues in radioligand binding assays at rat A₁,^a rat A_{2A},^b and human A₃ receptors,^c unless noted^d

Compd	R ¹	R ²	R ³	R ⁴	<i>K_i</i> (nM) or % displacement		
					rA ₁ ^a	rA _{2A} ^b	hA ₃ ^c
3	H	H	OH	CH ₂ OH	1680±80	22,500±100 (human)	404±70 ^e
4	H	Cl	OH	CH ₂ OH	273±36	1910±240	84.7±18.7
5	H	H	OH	CONHCH ₂ CH ₃	31.8±6.9	100±18	29.9±6.8
6	Me	H	OH	CH ₂ OH	1470±190	10% at 10 μM	126±18
7	Me	Cl	OH	CH ₂ OH	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 ₂ OH	5.06±0.51	6800±1800	170±51
10	CP	H	H	CH ₂ OH	5110±790	15% at 100 μM	2880±910
11	CP	Cl	OH	CH ₂ OH	8.76±0.81	3390±520	466±58
12	CP	Cl	H	CH ₂ OH	3600±780	45±5% at 100 μM	1090±190
13	IB	H	OH	CH ₂ OH	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 ₂ OH	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 ₂ OH	8730±370	25,400±3800	912±29

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

^bDisplacement of specific [³H]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 [³H]ZM241,385, unless noted.

^cDisplacement of specific [¹²⁵I]AB-MECA binding at human A₃ receptors expressed in CHO cells, in membranes, expressed as *K_i*±SEM (*n* = 3–4).

^dMe, methyl; CP, cyclopentyl; IB, 3-iodobenzyl.

^eMeasured in the absence of adenosine deaminase.