actinomycin D, bleomycin, and liblomycin) show minimal cross resistance (log CR ≈ 0). This warrants further consideration. Although numerous papers on MDR appear almost daily, none of the work which has appeared so far, except that in Table II, has included a great enough range of drugs to address this most unusual finding. While our rationalization (eq 5) of the mechanism is probably not the last word on this complex and extremely important problem, it is a starting point which must be considered in the design of drugs against resistant neoplasms.

Finally there are implications for multidrug chemotherapy. Although one does not expect a panacea since there are many ways by which cells acquire resistance, it might be worthwhile to study the simultaneous use of drugs at the extreme ends of the molecular weight and hydrophobicity scales. The study of MDR with a well-designed set of cytotoxic probes could yield new insights into membrane alterations and could eventually lead to improvements in current cancer chemotherapy regimens.

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2-(Arylalkylamino)adenosin-5′-uronamides: A New Class of Highly Selective Adenosine A_2 Receptor Ligands

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The synthesis and receptor-binding profiles at adenosine receptor subtypes for a series of 2-(arylalkylamino)-adenosin-5′-uronamides is described. Halogenated 2-phenethylamino analogues such as **3e** show greater than 200-fold selectivity for the A_2 receptor subtype on the basis of rat brain receptor binding. The general structure–activity relationship of this series of compounds is discussed both in terms of potency at A_2 receptors as well as receptor subtype selectivity. It is possible to introduce a hydrophilic carboxyalkyl substituent to this series such as in CGS 21680A (**3h**) and still retain good potency and selectivity for A_2 receptors. In addition, functional data in a perfused working rat heart model shows that these compounds possess full agonist properties at A_2 receptors with **3h** having a greater than 1500-fold separation between A_2 (coronary vosadilatory) and A_1 (negative chronotropic) receptor mediated events.

The purine nucleoside adenosine has been extensively studied as a modulator of cardiovascular function since it was shown to have potent hypotensive and bradycardic activity some 60 years ago by Drury and Szent-Gyorgyi.[1] The hypotensive actions of adenosine occur via several mechanisms among which are direct regulation of blood flow via vasodilation of the peripheral vasculature, including the coronary arteries.[2] Adenosine also produces sinus bradycardia and prolongation of impulse conduction in the atrioventricular node (AVN).[3] In addition, adenosine has the ability to inhibit neutrotransmitter release[4] and possesses potent central nervous system depressant and anticonvulsant activity.[5]

The vasodilator and conduction effects of adenosine are mediated through different receptor subtypes. In the heart, A_1 receptors present on nodal cells and cardiac myocytes are responsible for the negative dromo-, chrono-, and inotropic actions of adenosine.[3] Activation of A_2 receptors located on coronary smooth muscle results in vasodilation.[2] The potential use of an adenosine agonist as an antihypertensive agent has been limited by this spectrum of actions, nonselective agonists producing vasodilation that can be associated with cardiac depression as well as marked angina.[6] Selective A_2 receptor agonists may provide more viable agents as potential therapeutic candidates possessing effective vasodilatory hypotensive actions without the detrimental effects on cardiac conduction and renal function observed with currently available agonists. Whereas many highly selective agonists for the A_1 receptor have been described,[7] the prototypical A_2 agonist NECA (**1**) (*N*-ethyladenosine-5′-uronamide)[8] show little or no A_2 selectivity (see Table I). Until recently, the most selective A_2 agonist described was CV 1808 (**2**) ((2-phenylamino)adenosine), being approximately 5–10-fold selective for the A_2 receptor.[8] More recently, several N-6 substituted purine ribosides and NECA analogues with about 40-fold selectivity for the A_2 receptor have been described.[9] However, most of these analogues still possess reasonably potent A_1 receptor affinity (K_i ~ 200 nM). In the present paper, the synthesis and receptor-binding profiles at adenosine receptor subtypes for a series of 2-(arylalkylamino)adenosin-5′-uronamides are described. Some of the analogues described possess as much as 200-fold selectivity for the A_2 receptor on the basis

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Table I. Physical and Analytical Data for the Analogues 3a–k

no.	R_2R_3[a]	R_1	yield,[b] %	MP, °C	formula	anal.[c]
3a	$R_2 = (CH_2)_2Ph$	Me	30	188–190	$C_{19}H_{23}N_7O_4$	C,H,N
3b	$R_2 = (CH_2)_2Ph$	Et	47	115–118	$C_{20}H_{25}N_7O_4$	C,H,N
3c	$R_2 = (CH_2)_2Ph$	c-C_3H_5	26	145–147[d]	$C_{21}H_{26}ClN_7O_4$	C,H,N[e]
3d	$R_2 = CH_2Ph$	Et	39	132–137[d]	$C_{19}H_{24}ClN_7O_4$	C,H,N
3e	$R_2 = p$-$ClC_6H_4(CH_2)_2$	Et	16	130–131[d]	$C_{20}H_{25}Cl_2N_7O_4$	C,H,N
3f	$R_2 = p$-$FC_6H_4(CH_2)_2$	Et	37	110–114	$C_{20}H_{24}FN_7O_4$	C,H,N
3g	$R_2 = (CH_2)_2Ph$, R_3 = Me	Et	57	115–119[d]	$C_{21}H_{27}ClN_7O_4$	C,H,N
3h	$R_2 = p$-$[HOOC(CH_2)_2]C_6H_4(CH_2)_2$	Et	49	200–203[d]	$C_{23}H_{30}ClN_7O_6$	C,H,N
3i	$R_2 = p$-$(HOOCCH_2O)C_6H_4(CH_2)_2$	Et	24	137–141[d]	$C_{22}H_{28}ClN_7O_7$	C,H,N
3j	$R_2 = p$-$(HOOCCH_2)C_6H_4(CH_2)_2$	Et	34	140–145[d]	$C_{22}H_{28}ClN_7O_6$	C,H,N
3k	$R_2 = p$-$[MeOOC(CH_2)_2]C_6H_4(CH_2)_2$	Et	32	90–95	$C_{24}H_{31}ClN_7O_6$	C,H,N

[a] R_3 = H unless otherwise noted. [b] Overall yields from the acid 5. [c] Combustion analysis were within ±0.4% of the theoretical value except where noted otherwise. [d] As the HCl salt. [e] N: calcd, 20.59; found, 20.16.

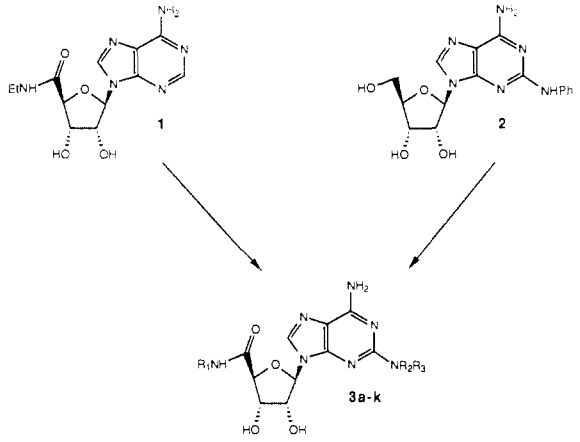


Figure 1.

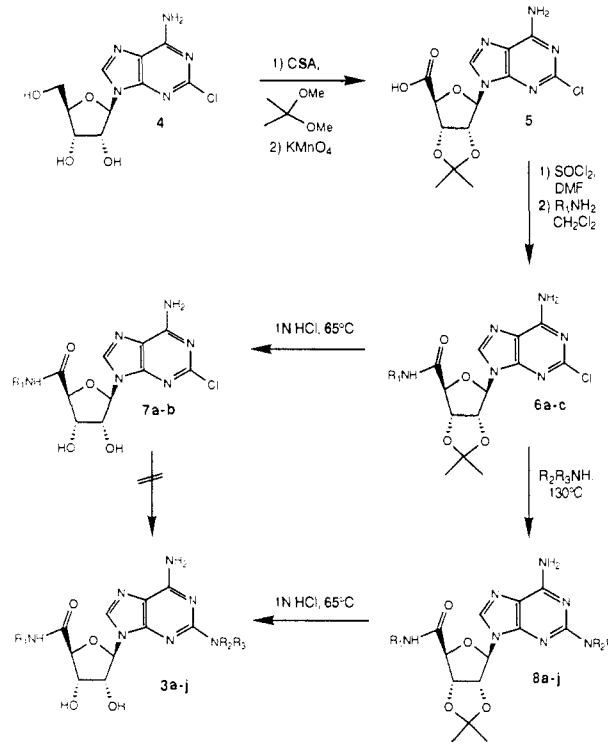
of rat brain receptor binding and in addition have negligible affinity for A_1 receptors (>1 μM) in an absolute sense. In addition, functional data in a perfused working rat heart model[10,11] for several of these selective ligands is presented that shows them to possess full agonist properties at A_2 receptors as well as greater than 2000-fold separation between A_2 (coronary vasodilatory) and A_1 (negative chronotropic) receptor mediated events.

The geneology of the 2-(arylalkylamino)adenosin-5′-uronamides is shown in Figure 1. Since at the initiation of this work the most A_2-selective agonist described was CV1808 (2) and NECA (1) showed high affinity for A_2 receptors, we sought to combine the structural features of both series to generate the general structure **3a–k**. Optimization of R_1, R_2, and R_3 in this series led to a number of highly potent and selective A_2 receptor ligands among which CGS 21680A (**3h**) was selected for extensive biological evaluation[12] as well as tritiation[13] for use as a A_2-selective ligand for receptor binding.

Chemistry

The general synthetic route employed for the preparation of the 2-(arylalkylamino)adenosin-5′-uronamides **3a–k** is outlined in Scheme I. The acetonide of 2-chloroadenosine was prepared in 58% yield from 2-CADO (4) by reaction with 2,2-dimethoxypropane in acetone with 1 equiv of camphorsulfonic acid as catalyst. This material

Scheme I



was oxidized with $KMnO_4$ in aqueous base under high dilution conditions to afford the carboxylic acid **5** in 74% yield. Running this reaction at higher concentrations resulted in much lower yields of the desired product. The acid **5** was converted to the corresponding acid chloride with $SOCl_2$ and a catalytic amount of DMF, and the crude acid chloride reacted with methyl-, ethyl-, or cyclopropylamine to afford the amides **6a–c** in good overall yields. These procedures are all modifications of methods found in the patent literature.[14]

Cleavage of the acetonides of **6a,b** with 1 N HCl at 65 °C afforded the NECA analogues **7a,b** in excellent yields. We initially attempted to react these derivatives with phenethylamine under a variety of conditions but unfortunately in no case could any of the desired substitution products **3a,b** be isolated in a pure state. However, when we reacted the acetonides **6a–c** with these same amines in excess at 130 °C, the corresponding substituted derivatives **8a–j** could be isolated in moderate to excellent yields. Unfortunately this methodology failed for preparation of anilino analogues (R_2 = aryl) such as the NECA analogue of CV 1808 (2) but works well for unhindered

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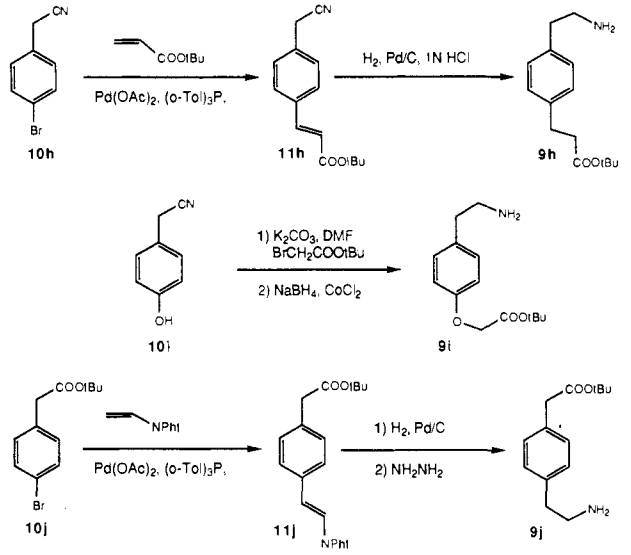
Scheme II



primary and secondary amines. Treating the acetonides **8a–j** with 1 N HCl as before afforded the desired analogues **3a–j** in good overall yields from the acetonides **6a–c**. In order to prepare the analogues **3h–j**, all of which possess a carboxylic acid moiety, it was necessary to prepare the required amino acids as their corresponding *tert*-butyl esters. The use of *tert*-butyl rather than ethyl esters in amino esters **9h–j** is essential because the ethyl esters corresponding to **9h–j** all spontaneously polymerize even at room temperature whereas the *tert*-butyl esters are at least somewhat stable, although they always were reacted immediately after preparation. The *tert*-butyl ester protecting group of the derivatives **8h–j** was simultaneously cleaved during acetonide removal in the conversion of **8h–j** to **3h–j**. Finally the analogue **3k** was prepared by esterification of the free base derived from **3h** with diazomethane in THF in 66% yield.

The preparation of the required arylalkylamines **9h–j** is outlined in Scheme II. Amine **9h** was prepared via a Heck[15] reaction between *p*-bromophenylacetonitrile (**10h**) and *tert*-butyl acrylate to afford the acrylate **11h**. Simultaneous hydogenation of the nitrile and acrylate double bond of **11h** with 10% Pd/C in 2-propanol with 1 equiv of 1 N HCl afforded the amine **9h** in an overall yield of 65%. Amine **9i** was prepared by alkylation of *p*-hydroxyphenylacetonitrile (**10i**) with *tert*-butyl bromoacetate in DMF with K_2CO_3 as base followed by reduction of the nitrile with $NaBH_4/CoCl_2$.[16] Finally the amine **9j** was prepared via a Heck reaction between *tert*-butyl *p*-bromophenylacetate (**10j**) and *n*-vinylphthalimide[17] to afford the phthalimidostyrene **11j**, which was hydrogenated over Pd/C catalyst in ethanol followed by treatment with ethanolic hydrazine to afford the amine **9j** in an overall yield of **55%**. The substitution patterns, overall yield of preparation from the acid **5** and the physical and analytical data for analogues **3a–k** and **7a,b** are collected in Table I.

Results and Discussion

The A_1 and A_2 receptor binding affinities for the analogues **7a,b** and **3a–k** along with relevant standards are collected in Table II. A_1 binding was measured in adenosine deaminase (ADA) pretreated rat cortical membranes with use of [^{3}H]cyclohexyladenosine in the presence of 2-chloroadenosine (2-CADO) to define specific binding as previously reported.[18] Binding to A_2 receptors was measured in ADA-pretreated rat striatal membranes with [^{3}H]CGS 21680.[13]

Table II. A_1 and A_2 Receptor Binding Affinities for the Analogues **7a,b** and **3a–k**

compd	A_1 binding:[a,b] K_i, nM ± SEM	A_2 binding:[a,c] K_i, nM ± SEM	A_1/A_2 ratio
CPA	1.3 ± 0.5	636 ± 53	0.002
NECA (1)	5.8 ± 0.3	11.7 ± 0.6	0.50
MECA	101 ± 4	71 ± 0.2	0.91
CV1808 (2)	689 ± 31	71 ± 0.2	9.7
7a	70 ± 9	229 ± 11	0.31
7b	12 ± 0.8	18 ± 1	0.67
3a	5350 ± 300	77 ± 4	69
3b	473 ± 18	9.7 ± 1	49
3c	502 ± 14	14 ± 11	36
3d	8604 ± 169	1130 ± 53	7.6
3e	1505 ± 81	7.5 ± 1	201
3f	1184 ± 32	7.7 ± 0.2	154
3g	12100 ± 700	71 ± 2	170
3h	1408 ± 63	19 ± 2	74
3i	4250 ± 81	42 ± 2	101
3j	4880 ± 240	45 ± 2	109
3k	1257 ± 97	9 ± 0.4	140

[a] Compounds were run at 5–10 concentrations in triplicate. IC_{50}'s were determined ± SEM's and converted to K_i's by using the Cheng–Prusoff equation assuming all inhibition of binding was competitive. [b] [^{3}H]CPA binding.[13] [c] [^{3}H]CGS 21680 binding.[11]

As shown in Table II, NECA (**1**) and MECA are virtually equipotent at A_1 and A_2 receptors in these assay systems whereas CV1808 (**2**) is about 10-fold selective for A_2 receptors. However NECA is about 7-fold more potent at A_2 receptors than CV1808. The 2-chloro analogues **7a** and **7b** showed comparable potency and selectivity to MECA and NECA, respectively, and hence show no superiority over these latter two compounds. However, the corresponding 2-phenethylamino analogues **3a** (R_1 = Me) and **3b** (R_2 = Et) showed enhanced affinity at A_2 receptors (K_i = 77 and 9.7 nM, respectively) while being only weakly active at A_1 receptors (K_i = 5350 and 473 nM, respectively). The 2-phenethylamino cyclopropyl amide **3c** (R_1 = c-C_3H_5) showed no enhancement of either potency or selectivity over **3b** and therefore the R_1 = Et series was chosen for a more extensive optimization of R_2 and R_3 since the R_1 = Et substitution showed the highest affinity for A_2 receptors of the analogues **3a–c**.

The corresponding benzyl analogue of **3b**, analogue **3d**, was virtually inactive at A_2 receptors and addition of an N-2 methyl group to analogue **3b**, giving analog **3g**, results in an approximately 10-fold loss in A_2 receptor affinity. However, introduction of substituents on the aromatic ring of **3b** could be utilized to enhance the potency and selectivity in this series. In particular the *p*-chlorophenyl analogue **3e** and the corresponding *p*-fluoro analogue **3f** show 150–200-fold selectivity for the A_2 receptor subtype as well as greater affinity than NECA ($K_i \sim 7.5$ nM). Compound **3e** currently represents the most selective ligand for the A_2 receptor yet described on the basis of receptor binding.

Having solved the problem of A_2 receptor affinity and selectivity, we sought to introduce substituents onto **3b** that would increase hydrophilicity and hence limit blood–brain barrier penetration as well as limiting po-

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Table III. Effects of 3b and 3j in a Perfused Working Heart Model

compd	perfused working heart model[a] A_2: EC_{25} CF,[b,e] nM	A_1: EC_{25} HR,[c,e] nM	A_1/A_2 ratio[d]
NECA (1)	5 (4–6)	60 (20–190)	12
2	110 (40–310)	>3000	>28
3b	3 (2–5)	>3000	>1000
3h	2 (1–3)	>3000	>1500

[a] Model description in the Experimental Section.[15,16] [b] Concentration required to produce a 25% increase in coronary flow (95% confidence limits). [c] Concentration required to produce a 25% decrease in heart rate. [d] Ratio of EC_{25} HR/EC_{25} CF. [e] n = 5–10.

tential intracellular actions of these compounds. One such successful stategy was the introduction of a carboxylic acid moiety to the template **3b** to afford the analogues **3h–j** among which **3h** (CGS 21680A) shows the highest affinity for A_2 receptors (K_i = 15 nM) as well as possessing excellent (74-fold) selectivity. The methyl ester of this compound, **3k**, shows even higher affinity and selectivity for the A_2 receptor subtype but is considerably more lipophilic.

Intrinsic activity data for analogues **3b** and **3h** in a perfused working heart model are shown in Table III along with comparable data on **2** and NECA (**1**).[11] Heart rate and coronary flow were measured as described by Neely et al.[10] in hearts obtained from male Sprague–Dawley rats. The relative negative chronotropic or vasodilatory potency of each agonist was expressed as the EC_{25} value, the mean concentration of agonist that produced a 25% decrease in heart rate or a 25% increase in coronary flow as described in the Experimental Section.

In this model, analogue **3h** effectively increased coronary flow with an ED_{25} value of 2 nM. The corresponding value for **3b** was 3 nM while that for CV 1808 (**2**) was 110 nM. The EC_{25} for eliciting bradycardia for all three compounds was >3000 nM. In general for the limited number of analogues tested the functional data tended to parallel the binding data both in terms of potency and selectivity. However all of the compounds tested showed even greater A_2 selectivity in the functional assay. The effects of all three compounds could be reversed by treatment with the xanthine adenosine antagonist, 8-[4-[[[[(2-aminoethyl)-amino]carbonyl]methyl]oxy]phenyl]-1,3-di-*n*-propylxanthine (XAC). Both compounds were full agonists at the rat coronary A_2 receptor relative to NECA.[11] Thus the analogue **3h** showed greater than 1500-fold separation between A_2 and A_1 receptor mediated effects. On the basis of this data coupled with its high hydrophilicity and lack of effects on the adenosine transport system,[19] the analogue **3h** was selected as an ideal compound for extensive exploration of A_2 receptor pharmacology. The structure–activity relationships derived for the 2-substituted ribose uronamides would indicate that certain bulky substituents in the 2-position are effective in reducing A_1 receptor affinity while retaining very potent A_2 receptor affinity and efficacy as agonists.

In conclusion, it has been shown that 2-(arylalkylamino)adenosine uronamides such as **3b** and **3h** are potent and extremely selective agonists for the adenosine A_2 receptor subtype and analogue **3e** represents the most selective ligand for the A_2 receptor reported to date.

Experimental Section

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer. All chemical shifts are expressed in ppm relative to a TMS internal standard. All reactions were carried out under a nitrogen atmosphere.

2-Chloro-2′,3′-*O*-isopropylideneadenosine. A mixture of 2-CADO (**4**) (10.0 g, 33.1 mmol), 2,2-dimethoxypropane (62.1 g, 0.6 mol), camphorsulfonic acid (7.68 g, 33.1 mmol), and acetone (300 mL) was stirred at room temperature for 16 h. The solvent was removed in vacuo, and the residue was chromatographed on silica gel with 5% MeOH in CH_2Cl_2 as the eluent to afford 6.6 g (58%) of the acetonide of the title compound melting at 235–236 °C after trituration with ether.[14] Anal. ($C_{13}H_{16}ClN_5O_4$) C, H, N.

2-Chloro-2′,3′-*O*-isopropylideneadenosine-5′-carboxylic Acid (5). To a stirred mixture of 2-chloro-2′,3′-*O*-isopropylideneadenosine (6.5 g, 19 mmol) and a solution of KOH pellets (3.2 g, 57 mmol) in water (1250 mL) was added $KMnO_4$ (9.0 g, 57 mmol) dissolved in water (250 mL). The resulting mixture was vigorously stirred for 3 days. After quenching of the reaction by addition of approximately 65 mL of 7.5% H_2O_2 solution, the reaction mixture was filtered through Celite and concentrated in vacuo to about 100 mL which the temperature was maintained below 40 °C. The reaction was acidified to pH 4 with 3 N HCl, and the resulting precipitated product was collected and dried in vacuo to afford 5.03 g (74%) of the acid **5** melting at 250–253 °C (lit.[14] mp 262–263 °C). Anal. ($C_{13}H_{14}ClN_5O_5 \cdot {}^1/_2H_2O$) C, N; H: calcd, 4.11; found, 3.69. ^{1}H NMR (CD_3OD): δ 1.42 (3 H, s), 1.54 (3 H, s), 4.74 (1 H, d, J = 1 Hz), 5.48 (1 H, d, J = 7 Hz), 5.65 (1 H, dd, J = 1, 7 Hz), 6.30 (1 H, d, J = 1 Hz), 8.20 (1 H, s).

2-Chloro-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide (6b). A stirred mixture of the acid **5** (5.03 g, 14.2 mmol), DMF (0.4 mL) and $SOCl_2$ (16 mL) was heated at 50 °C for 2 h. After removal of excess reagents in vacuo, toluene (50 mL) was added and the solvent was again removed in vacuo. This material was suspended in CH_2Cl_2 (30 mL), cooled to 0 °C, and treated with ethylamine (16 mL) in a dropwise fashion. After 1 h at room temperature, the reaction mixture was poured onto water. The organic layer was separated and the aqueous phase was extracted two additional times with CH_2Cl_2. After drying over $MgSO_4$, the solvent was removed in vacuo and the residue was triturated with ether to afford 5.0 g (92%) of the compound **6b** melting at 206–209 °C. Anal. ($C_{15}H_{19}ClN_6O_4$) C, H, N. ^{1}H NMR (CD_3OD): δ 0.72 (3 H, t, J = 8 Hz), 1.40 (3 H, s), 1.58 (3 H, s), 2.92 (2 H, m), 4.62 (1 H, d, J = 1 Hz), 5.40 (1 H, dd, J = 1, 7 Hz), 5.69 (1 H, dd, J = 1, 7 Hz), 6.30 (1 H, d, J = 1 Hz), 8.20 (1 H, s).

2-[[4-[2-(*tert*-Butoxycarbonyl)ethyl]phenethyl]amino]-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide (8h). A mixture of the chloro compound **6b** (4.0 g, 10.4 mmol) and the amine **9h** (14.0 g, 56.2 mmol) was heated with stirring at 130 °C for 3 h. After cooling, the reaction mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ solution, and dried over $MgSO_4$. After removal of the solvent, the residue was subjected to flash chromatography on silica gel with 5% MeOH in CH_2Cl_2 as the eluent to afford 3.4 g (55%) of the compound **8h** melting at 180–182 °C after trituration with ether. Anal. ($C_{30}H_{41}N_7O_6$) C, H, N.

2-[[4-(2-Carboxyethyl)phenethyl]amino]adenosine-5′-*N*-ethylcarboxamide Hydrochloride (3h). A mixture of the acetonide **8h** (2.5 g, 4.2 mmol) and 1 N HCl (35 mL) was heated at 65 °C with stirring for 1 h. After the mixture cooled to room temperature, the resulting solid was collected and washed with water and EtOAc to afford 2.16 g (96%) of the compound **3h** melting at 200–203 °C. Anal. ($C_{23}H_{30}ClN_7O_6$) C, H, N. ^{1}H NMR (CD_3OD) of the free base: δ 1.02 (3 H, t J = 7 Hz), 2.58 (2 H, t, J = 8 Hz), 2.85 (4 H, m), 3.18 (2 H, m), 3.56 (2 H, m), 4.40 (1 H, d, J = 2 Hz), 4.58 (1 H, m), 4.99 (1 H, dd, J = 7 Hz), 5.92 (1 H, d, J = 7 Hz), 7.18 (4 H, s), 8.00 (1 H, s).

2-[[4-(2-Carbomethoxyethyl)phenethyl]amino]-adenosine-5′-*N*-ethylcarboxamide Hydrochloride (3k). To a suspension of the free base derived from the acid **3h** (200 mg, 0.40 mmol), which was prepared from the hydrochloride by treatment with propylene oxide in EtOH in 40 mL of THF, was added 0.66 M ethereal diazomethane (4 mL, 2.64 mmol). After the mixture was stirred for 3 h at room temperature, the solvent and excess reagent were removed in vacuo, and the residue was subjected to flash chromatography on silica gel with 5% MeOH

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in CH_2Cl_2 as the eluent to afford 135 mg (66%) of the compound **3k** melting at 90–95 °C after trituration with ether. Anal. ($C_{24}H_{31}N_7O_6$) C, H, N.

***tert*-Butyl 4-(Cyanomethyl)cinnamate (11h).** A mixture of *p*-bromophenylacetonitrile (**10h**) (100 g, 510 mmol), *tert*-butyl acrylate (92 mL, 816 mmol), $Pd(OAc)_2$ (1.14 g, 5.1 mmol), and tri-*o*-tolylphosphine (6.2 g, 20 mmol) in Et_3N (240 mL) was refluxed with stirring for 6 h. The reaction mixture was poured onto ice and acidified with 10% HCl. The product was extracted with EtOAc, the organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was crystallized from ether/hexane to afford 105 g of 11h melting at 73–78 °C. Anal. ($C_{15}H_{17}NO_2$) C, H, N.

***tert*-Butyl 3-[4-(2-Aminoethyl)phenyl]propionate (9h).** A mixture of the nitrile **11h** (24.3 g, 100 mmol), 1 N HCl (117 mL, 117 mmol), 10% Pd/C catalyst (4.05 g), and 2-propanol (400 mL) was hydrogenated at 50 psi at room temperature for 7 h. After removal of the catalyst by filtration through Filter-cel, the solvent was removed in vacuo. The residue was partitioned between $NaHCO_3$ solution and ether, the ethereal layer was dried over K_2CO_3, and the solvent was removed in vacuo to afford 21 g (84%) of **9h** as a colorless oil, which was reacted immediately with **6b**. NMR ($CDCl_3$): δ 1.42 (9 H, s), 1.80 (2 H, bs), 2.52 (2 H, t, J = 8 Hz), 2.74 (2 H, t, J = 7 Hz), 2.88 (2 H, t, J = 8 Hz), 2.96 (2 H, t, J = 7 Hz), 7.12 (4 H, s).

***tert*-Butyl 4-(2-Aminoethyl)phenoxyacetate (9i).** A mixture of 4-hydroxybenzyl cyanide (**10i**) (2.03 g, 15.3 mmol), powdered K_2CO_3 (4.3 g, 31.2 mmol), *tert*-butyl bromoacetate (3.3 mL, 20.4 mmol), and DMF (25 mL) was vigorously stirred at 40 °C for 16 h. The reaction mixture was poured onto water and the product was extracted with ether. After drying over $MgSO_4$, the solvent was removed in vacuo and the residue was dissolved in THF (70 mL) and MeOH (40 mL). To this was added a solution of $CoCl_2$ (4.8 g, 37.2 mmol) in water (70 mL) in a dropwise manner followed by the portionwise addition of $NaBH_4$ (1.7 g, 45.9 mmol). After 10 min the reaction was filtered through Celite and the solvent was removed in vacuo. The residue was subjected to flash chromatography on silica gel with 7.5% ammonia saturated MeOH in CH_2Cl_2 as the eluent to afford 1.8 g (47% overall) of **9i** as a colorless oil, which was immediately reacted with **6b**.

4-[(*tert*-Butoxycarbonyl)methyl]-β-*N*-phthalimidostyrene (11j). A mixture of *tert*-butyl 4-bromophenylacetate (**10j**)[20] (12 g, 44.3 mmol), *N*-vinylphthalimide (7.7 g, 44.5 mmol), $Pd(OAc)_2$ (290 mg, 1.29 mmol), tri-*o*-tolylphosphine (1.4 g, 4.6 mmol), diisopropylethylamine (10 mL), and acetonitrile (12 mL) was refluxed with stirring for 16 h. After cooling to room temperature, the product was collected and dissolved in CH_2Cl_2 treated with activated charcoal and the solvent was removed in vacuo after filtration through Celite. The residue was crystallized from CH_2Cl_2/MeOH to afford 14 g (87%) of **11j** melting at 135–137 °C. Anal. ($C_{22}H_{21}NO_4$) C, H, N.

***tert*-Butyl 4-(2-Aminoethyl)phenylacetate (9j).** A mixture of the styrene **11j** (6.2 g, 17.1 mmol), 10% Pd/C catalyst (2.3 g), and EtOH (200 mL) is hydrogenated at 50 psi at room temperature for 7 h. After filtration through Celite, the solvent is removed in vacuo and the residue is treated with hydrazine hydrate (6.0 mL, 122 mmol) in EtOH (60 mL). After 90 min at 80 °C, the reaction mixture was cooled and filtered and the solvent was removed in vacuo. The residue was subjected to flash chromatography on silica gel with 5% ammonia saturated MeOH in CH_2Cl_2 as the eluent to afford 3.3 g (82% overall) of **9j** as a colorless oil, which was immediately reacted with **6b**.

Binding Studies. Evaluation of compounds for their ability to bind to rat brain A_1 receptors employed the previous published methodology. A_1 binding was measured in adenosine deaminase (ADA) pretreated rat cortical membranes with use of [^{3}H]-cyclohexyladenosine (CHA; specific activity 25 Ci/mmol) in the presence of 10 μM 2-chloroadenosine (2-CADO) to define specific binding.[18] Assays were run at 23 °C for 2 h with 100–200 μg of protein of ADA-treated tissue in a final volume of 1 mL of 50 mM Tris–HCl buffer, pH 7.4; [^{3}H]CHA was included at a final concentration of 1 nM. Bound radioactivity was isolated by vacuum filtration over Whatman GF/B filters and unbound radioactivity removed with 2 × 5 mL washes of ice-cold buffer. After equilibration in 4 mL of Aquassure scintillation cocktail (Du Pont-NEN, Boston, MA), radioactivity was determined by conventional liquid scintillation spectrometry at an efficency of 50%.

Binding to A_2 receptors was measured in ADA-pretreated rat striatal membranes with use of [^{3}H]CGS 21680 (specific activity 30–80 Ci/mmol)[13] by a modification of the method previously described for [^{3}H]NECA[8]. Rat striatum was homogenized with use of a Brinkmann polytron (setting 6, for 20 s) in 20 volumes of ice-cold 50 mM Tris-HCl, pH 7.4, containing 10 mM $MgCl_2$. This membrane homogenate was then centrifuged at 48000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 IU/mL adenosine deaminase (Boehringer Mannheim) to 20 mg/mL original tissue weight and incubated at 37 °C for 30 min to inactivate endogenous adenosine. The membrane homogenate was recentrifuged and the final pellet was frozen at −70 °C until the time of assay.

Routine assays were carried out in triplicate in 12 × 75 mm polypropylene test tubes containing an aliquot of striatal membranes (100–200 μg protein/mL) in incubation buffer (50 mM Tris–HCl and 10 mM $MgCl_2$, pH 7.4) with approximately 5 nM [^{3}H]CGS 21680 and/or inhibitor in a final volume of 1 mL. All assays were conducted at 23 °C for 90 min. Nonspecific binding was defined in the presence of 20 μM 2-CADO. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure with a Brandel Cell Harvester (Gaithersburg, MD). Filters were washed twice with ice-cold buffer (5 mL) and placed in scintillation vials, and bound radioactivity was determined by using conventional liquid scintillation spectroscopy techniques at an efficiency of 40–50%.

In the saturation studies, striatal membranes were incubated in 10–20 concentrations of [^{3}H]CGS 21680 ranging from 0.1 to 270 nM. For the competition studies, 9–18 concentrations of inhibitor were included in the incubation buffer. All data represented the mean ± the SEM for a minimum of three separate observations. IC_{50} values were determined by using a nonlinear, least-squares analysis program[21] and converted to K_i values with use of the Cheng–Prusoff[22] equation. In all cases the data was best represented as a one-site fit.

Pharmacological Assays: Perfused Working Heart Model. Heart rate and coronary flow were measured as described by Neely et al.[10] in hearts obtained from male Sprague–Dawley rats (Tac:N(SD)fBF; weight 275–350 g; Taconic Inc., Germantown, NY). The hearts were perfused with a Krebs-Henseleit bicarbonate buffer supplemented with 5 mM glucose and oxygenated with a gas mixture consisting of 95% O_2 and 5% CO_2 to maintain O_2 saturation at a pH of 7.4. Hearts were equilibrated for 30 min at a constant perfusion pressure from a reservoir placed at 15 cm above the left atrium. In this perfusion system, the hearts ejected fluid against a constant pressure head equal to 100 cm H_2O. Aortic pressure was continuously monitored with a Statham P23 ID pressure transducer connected to a side arm immediately above the cannulated portion of the ascending aorta. Recordings were made with a Beckman R 611 recorder and heart rate monitored by calculating the peak-to-peak intervals between successive aortic pressure pulses. Coronary flow was measured volumetrically by collecting coronary effluent from hearts electrically paced at 270 bpm. After equilibration, control values for heart rate and coronary flow were recorded. Compound **3h** and other adenosine agonists[11] were then perfused for 10 min at increasing concentrations (1 nM–3 μM) and aortic and coronary flow measurements made at the end of the initial 5 min of the perfusion period while the heart was paced. Intrinsic heart rate was then monitored during the second 5-min period with the electrical pacemaker stopped. Five concentrations of a single agonist were tested in one heart. Changes in heart rate and coronary flow were expressed as percentages of their respective control values.

The relative negative chronotropic or vasodilatory potency of each agonist was expressed as the EC_{25} value, the mean concentration of agonist that produced a 25% decrease in heart rate or

(20) Prepared via sulfuric acid catalyzed esterification of the acid with isobutylene in ether in 60% yield.

(21) RS/1; BBN, Boston, MA.

(22) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

a 25% increase in coronary flow. The EC_{25} was derived logarithmically from nonlinear regression logit analysis of concentration-response data for each heart with 95% confidence limits in parentheses.[23] The results summarized in Table III are the mean of 5-10 individual experiments.

Logarithms of the EC_{25} values were subjected to a Neuman-Keul multiple comparison test at the 0.05 significance level[24] to assess differences between the compounds tested.

Registry No. **3a**, 120225-61-8; **3b**, 120225-53-8; **3c**·HCl, 127258-32-6; **3d**·HCl, 127258-35-9; **3e**·HCl, 127258-37-1; **3f**, 120225-58-3; **3g**·HCl, 127258-40-6; **3h**·HCl, 124431-80-7; **3i**·HCl, 127258-42-8; **3j**·HCl, 127258-44-0; **3k**·HCl, 127258-46-2; **4**, 146-77-0; 4 acetonide derivative, 24639-06-3; **5**, 72209-19-9; **6a**, 127258-29-1; **6b**, 120225-75-4; **6c**, 127258-33-7; **7a**, 127258-30-4; **7b**, 72209-22-4; **8a**, 127258-31-5; **8b**, 120225-77-6; **8c**, 127258-34-8; **8d**, 127258-36-0; **8e**, 127258-38-2; **8f**, 127258-39-3; **8g**, 127258-41-7; **8h**, 120225-76-5; **8i**, 127258-43-9; **8j**, 127258-45-1; **9h**, 120225-79-8; **9i**, 124499-19-0; **9j**, 124499-20-3; **10h**, 16532-79-9; **10i**, 14191-95-8; **10j**, 33155-58-7; 11h, 120225-74-3; 11j, 116856-62-3.

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5-Hydroxytryptamine (5-HT_3) Receptor Antagonists. 1. Indazole and Indolizine-3-carboxylic Acid Derivatives

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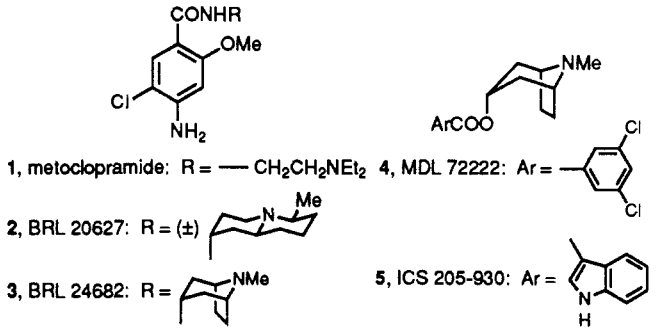
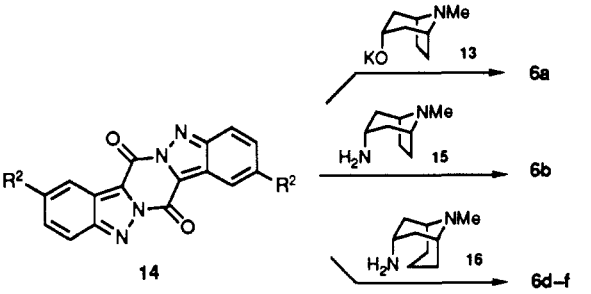
Metoclopramide (1) is a gastric motility stimulant and a weak dopamine and 5-HT_3 receptor antagonist. Conformational restriction of the (diethylamino)ethyl side chain of 1 in the form of the azabicyclic tropane gave **3**, a very potent gastric motility stimulant and 5-HT_3 receptor antagonist but devoid of significant dopamine receptor antagonist properties. Subsequent alteration of the aromatic nucleus led to the identification of indazoles **6a-h**, and 1- and 3-indolizines **7b-d** and **8**, and imidazo[1,5-*a*]pyridines **9** and **10**, as potent 5-HT_3 receptor antagonists devoid of either dopamine antagonist or gastric motility stimulatory properties. Further conformational restriction of the side chain identified quinuclidine **11** and isoquinuclidine **12** as potent 5-HT_3 receptor antagonists which mimic the distorted chair conformation of the tropane with, in the case of **11**, the *N*-methyl group axial. From these series, **6g** (BRL 43694) was found to be both potent and selective and has been shown to be a very effective antiemetic agent against cytotoxic drug induced emesis both in the ferret and in man.

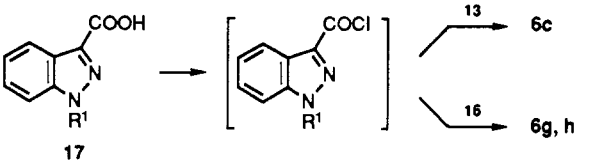
Metoclopramide (**1**) is a gastric prokinetic benzamide which is also a dopamine receptor antagonist.[1] In addition, **1** has been shown to be a relatively weak 5-hydroxytryptamine 5-HT_3 receptor antagonist.[2] We have recently correlated the 5-HT_3 receptor antagonist activity with the effectiveness of high-dose metoclopramide at inhibiting emesis evoked by cytotoxic agents used in cancer chemotherapy.[3] Antagonism of 5-HT_3 receptors has also been implicated for the treatment of migraine,[4] schizophrenia,[5] and anxiety.[6]



Scheme I. Synthesis of Indazoles **6a,b,d-f**



Scheme II. Synthesis of Indazoles **6c,g,h**



In earlier publications we showed that selectivity of action could be achieved by restricting the conformational freedom of the (diethylamino)ethyl side chain of **1**.[7,8] In particular **2** (BRL 20627) was identified as a selective stimulant of upper gastrointestinal motility.[7] Subsequently, tropane **3** (BRL 24682), was identified as both a potent gastric motility stimulant (lowest active dose 0.1 mg/kg sc in rat, method of McClelland et al.[9]) and a potent

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