A Novel Synthesis of Xanthines: Support for a New Binding Mode for Xanthines with Respect to Adenosine at Adenosine Receptors

A comparison of adenosine receptor binding affinities of substituted xanthine derivatives with adenosine derivatives bearing the same substituents has led to the suggestion that different binding modes are operative for these two classes of adenosine receptor agents.[1-5] In this communication we provide cooroboration for this suggestion and propose a specific new binding mode for xanthines with respect to adenosines. This new binding mode is supported with partial atomic charge comparisons, molecular modeling, and a novel synthesis of xanthines that allows the introduction of chiral recognition units at the 8-position.

It is evident that substitution at the C^6N position of adenosine parallels substitution at the C^8 position of xanthines for A_1 receptors as displayed in Table I, wherein cycloalkyl groups give the highest potencies, followed by phenyl and benzyl.[2,6-13] Adenosine A_1 receptors from a number of different tissues display the same order of decreasing potency with this set of substituents with few exceptions. A similar correlation and potency order is also observed for this set of substituents for C^6N-substituted adenosine derivatives on A_2 receptors from dog coronary artery,[9,14] human platelets,[10] and rat pC12 cells,[10] and for 8-substituted 1,3-dipropylxanthine derivatives on A_2 receptors from human VA13 fibroblasts[5] and human platelets.[12] In addition, the large functionalized chains that have been incorporated (functionalized congener approach) at the C^6N and C^8 positions of adenosine and xanthines, respectively, give compound sets with parallel binding potencies at A_1 receptors.[15-17]

On the basis of these correlations and the data reported herein, we propose a new receptor binding mode for theophylline with respect to adenosine (Figure 2). In

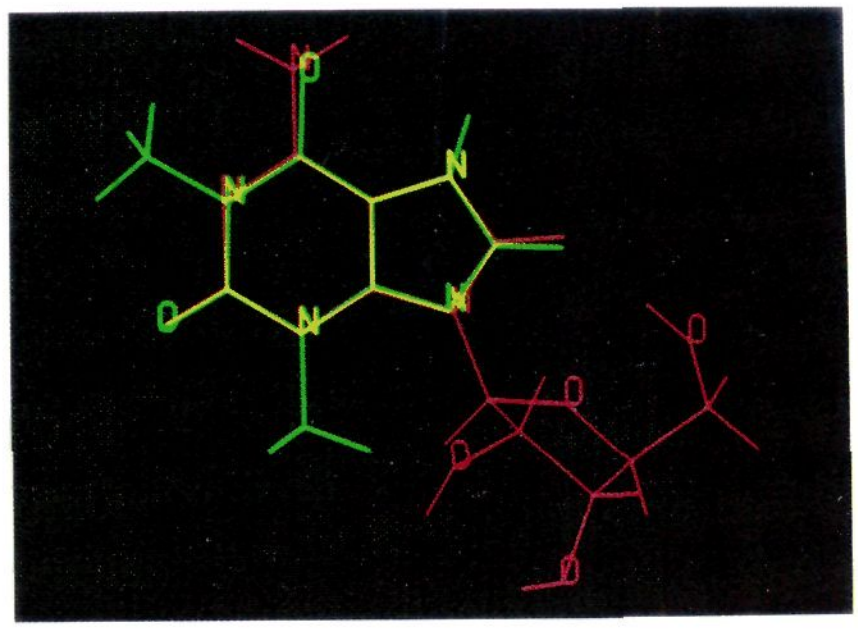

Figure 1. Standard binding mode of theophylline relative to adenosine.

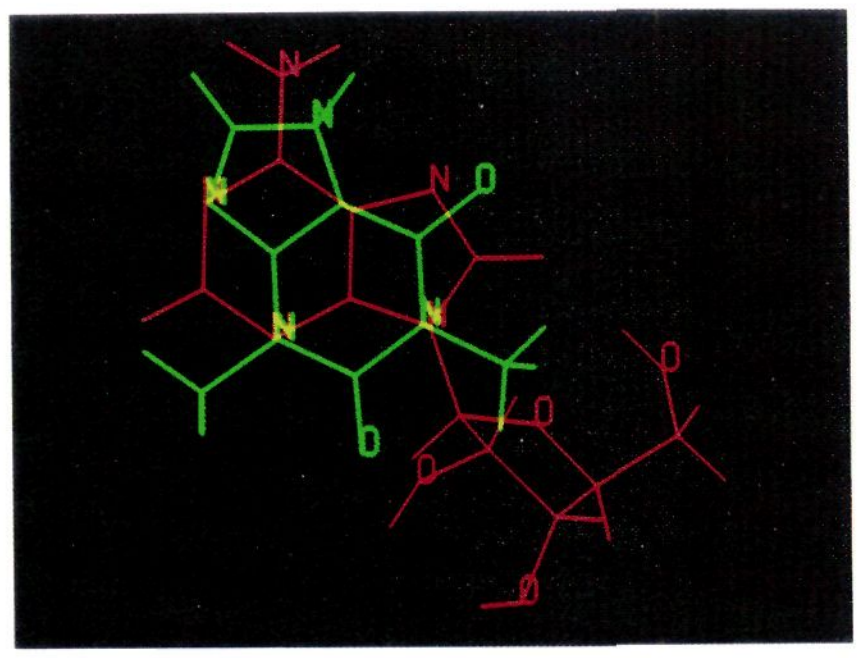

Figure 2. New proposed binding mode for theophylline relative to adenosine.

Figure 1 is shown what we refer to as the standard overlay of these two receptor agents, where all ring nitrogen atoms are perfectly overlaid. In Figure 2 theophylline, with respect to its position in Figure 1, has been flipped over and rotated to assume its position. This overlay was achieved by using a SYBYL[18] root mean square (RMS) fit procedure for N^1, N^3, and N^9 of adenosine and N^9, N^3, and N^1 of theophylline, respectively. With this superimposition, note the close proximity of not only the above-mentioned three pairs of nitrogen atoms but also the N^7 nitrogen atom and a C^6 amino hydrogen atom of adenosine with the C^6 carbonyl oxygen atom and the N^7 hydrogen atom of theophylline, respectively. In the standard binding mode of Figure 1, the N–H groups in the two molecules are not in proximity.

The new proposed binding mode of theophylline relative to adenosine was corroborated with comparative charge localization studies that were done on the two modes. Five pairs of atoms considered to be important for receptor binding were selected from these modes, as shown in Table II. Three different methods of charge calculation were employed. Each is routinely used for point charge calculations; the AM1 charges were calculated by using AMPAC (QCPE 506, a general-purpose semiempirical quantum

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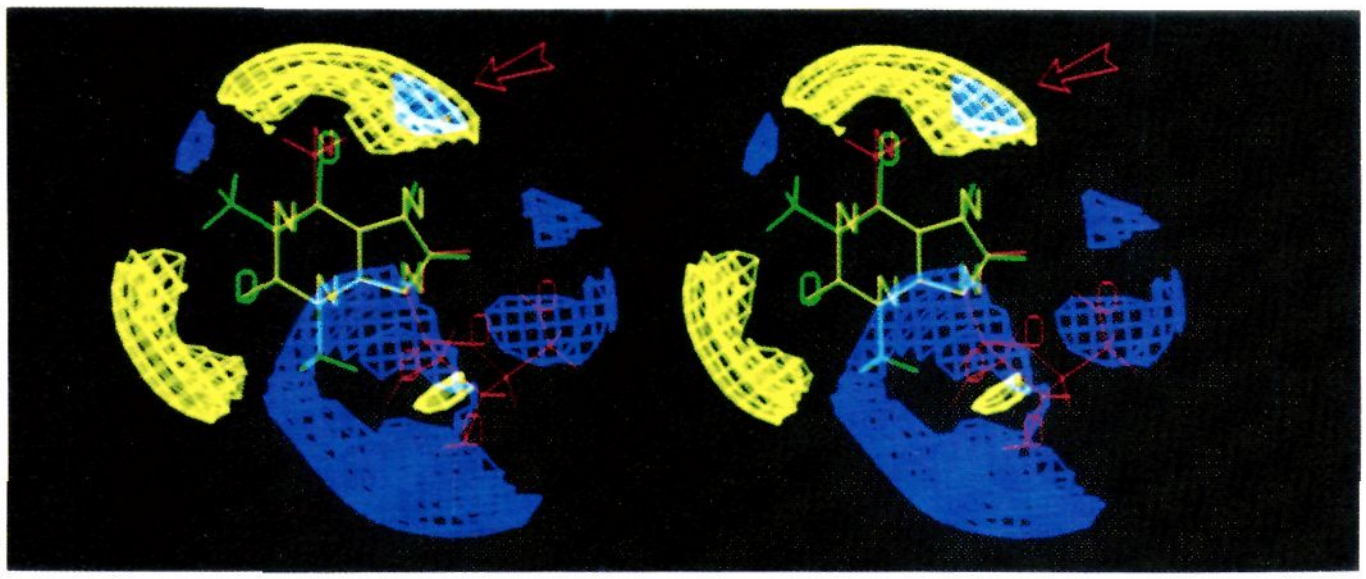

Figure 3. GRID energy contours of water probes for the standard overlay of theophylline (green with yellow contour at −3.0 kcal/mol) with adenosine (red with blue contour at −4.0 kcal/mol). The arrow indicates the region of contour overlap with both contours approximately 4 kcal/mol above the global minimum.

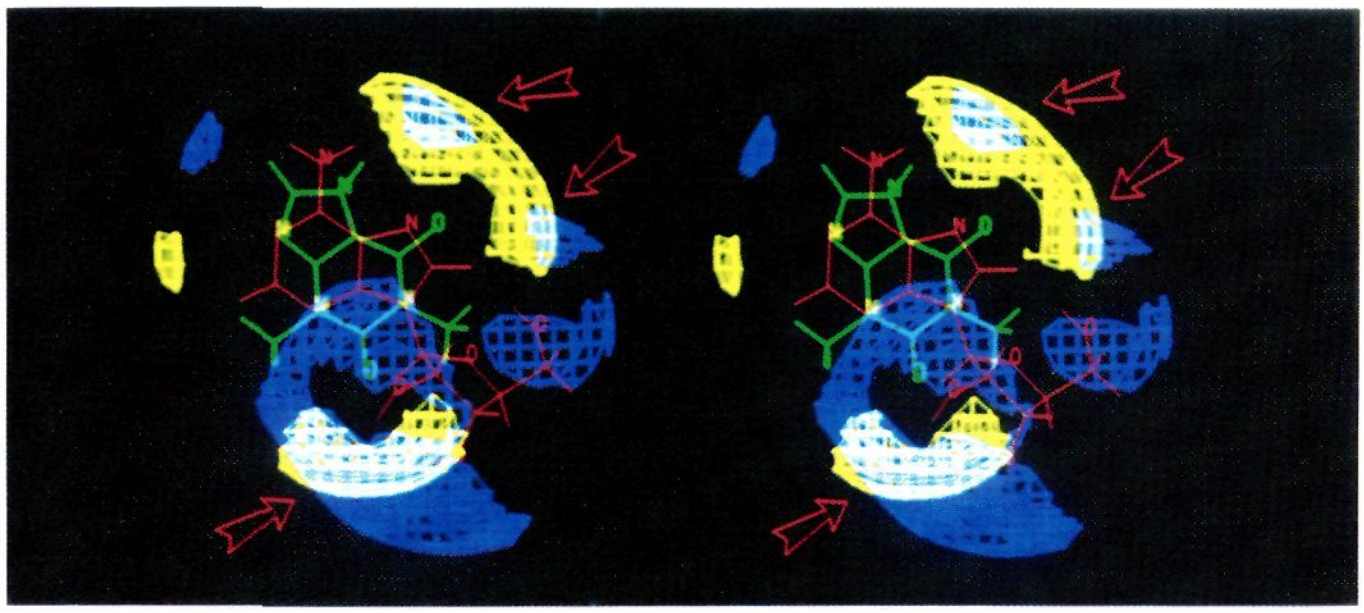

Figure 4. GRID energy contours of water probes for the overlay of theophylline (green with yellow contour at −3.0 kcal/mol) and adenosine (red with blue contour at −4.0 kcal/mol) in the new proposed binding mode. The arrows indicate regions of contour overlap with both contours approximately 4 kcal/mol above the global minimum.

package encompassing MINDO/3,[19] MNDO,[20] and AM1[21] Hamiltonians). The absolute differences using any of the methods for the selected pairs of atoms are similar, which suggests that the new proposed binding mode is reasonable.

Three-dimensional energy contour surfaces for a water probe at different energy levels were also constructed for adenosine and theophylline to compare the interactive sites of these molecules for both the standard and new proposed binding modes. The program GRID (Goodford[22]) was used to develop the contour presentations in Figures 3 and 4 with the water probe. GRID employs a potential energy function for estimating nonbonded interactions. Thus, the contours represent areas of favorable interaction between the probe and the host molecule. Figure 3 shows the GRID energy contours for adenosine and theophylline overlaid in the standard binding mode, where there is one area of contour overlap indicated by the red arrow. In Figure 4 is shown the GRID energy contours for adenosine and theophylline overlaid in the new proposed binding mode. Three areas of contour overlap are indicated by the red arrows. Although the sugar moiety is flexible, we feel that

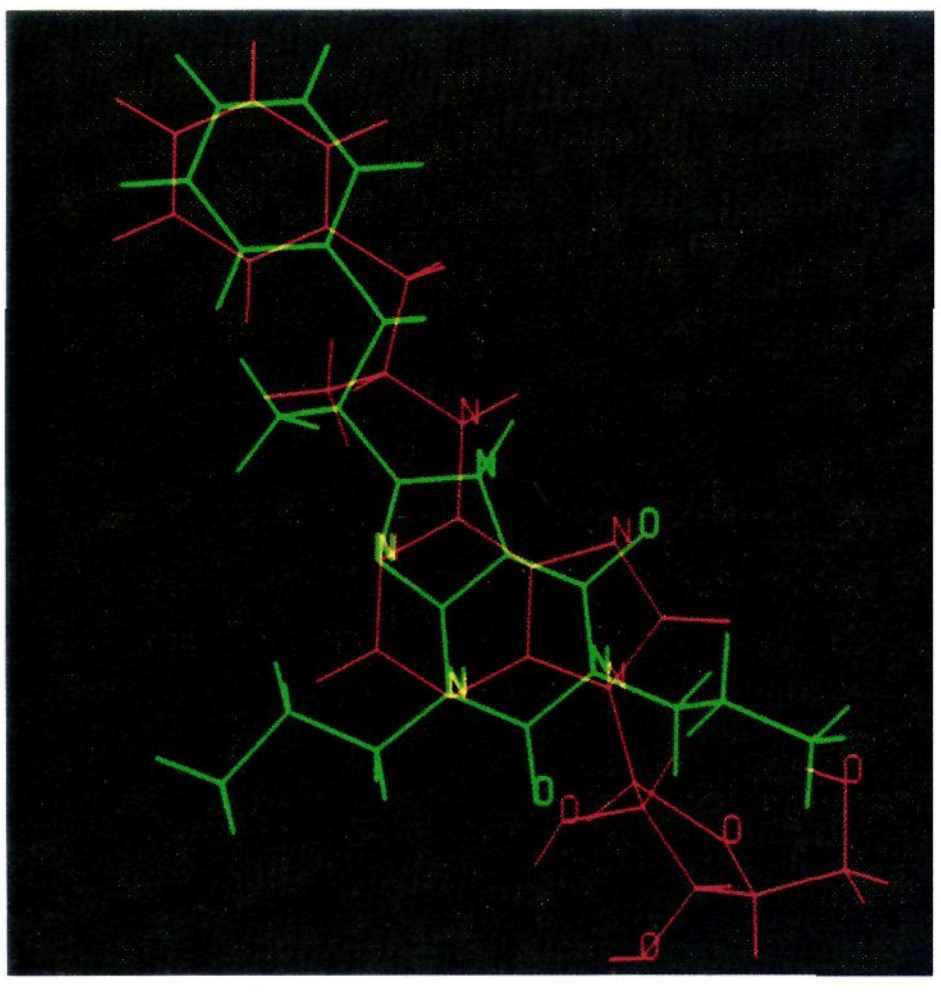

Figure 5. Overlay of **6** with R-PIA.

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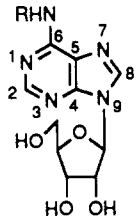
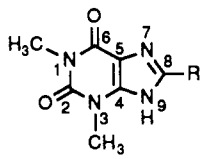
Table I. Effects of Adenosine C^6N Substituents and Theophylline C^8 Substituents on Binding and Functional Assays for A_1 Receptors

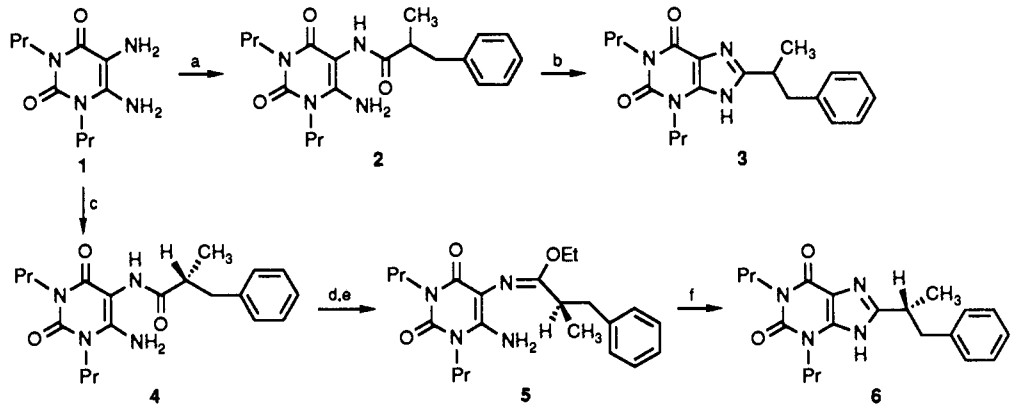




R	[^{3}H]CHA binding to rat membrane:[a] K_i, nM	[^{3}H]R-PIA binding to gp membranes:[b] K_i, nM	adenylate cyclase activity in rat fat cells:[c] IC_{50}, nM	[^{3}H]CHA binding to rat membranes:[d] K_i, nM	[^{3}H]R-PIA binding to gp membranes:[b] K_i, nM	adenylate cyclase activity in rat fat cells:[e] IC_{50}, nM
cyclopentyl	0.32	1.1	19.2	0.46	3.9	0.47
cyclohexyl	0.85	2.4	27		4.7	2.5
cyclopropyl	2.1					42
C_6H_5	3.2	110	190	13	20.9	37
$C_6H_5CH_2$	125	350	2200			890
H	5.1			700	1310	940

[a] Assay measures inhibition of [^{3}H]CHA binding to rat cerebral cortical membranes (ref 9). Value for adenosine is taken from ref 10. [b] Assay measures inhibition of [^{3}H]R-PIA binding to guinea pig cerebral cortical membranes (ref 2). [c] Functional assay measures inhibition of adenylate cyclase in rat adipocytes (ref 10). [d] Assay measures inhibition of [^{3}H]CHA binding to rat cerebral cortical membranes (refs 8 and 13). [e] Functional assay measures reversal of adenylate cyclase inhibition by R-PIA in rat adipocytes (ref 12).

Scheme I. Synthesis of 8-Substituted Xanthines[a]



[a] Reagents: (a) *N*-methylmorpholine, isobutyl chloroformate, 2-benzylpropionic acid, CH_3CN, −20 °C; (b) KOH, H_2O–EtOH, reflux, 2 h; (c) *N*-methylmorpholine, isobutyl chloroformate, (*R*)-2-benzylpropionic acid, CH_3CN, −20 °C; (d) triethyloxonium tetrafluoroborate, benzene, 50 °C, 15 h; (e) silica gel chromatography; (f) dry benzene, reflux, 2 h.

the conformation shown for adenosine is important to receptor interaction, on the basis of rigid molecules that have been reported, wherein the sugar is fixed in anti, syn, and other positions in between.[23]

To further test the hypothetical new binding mode for xanthines with respect to adenosine, racemic and enantiomeric 8-(phenylisopropyl)xanthines were required. A traditional preparation of 8-substituted xanthines involves acylation at the 5-amino group of a 5,6-diaminouracil, followed by cyclization of the resulting amide with strong aqueous base.[6,8] This procedure was used for the preparation of racemic 1,3-dipropyl-8-(phenylisopropyl)xanthine (**3**). Since an optically active version of **2** would presumably racemize using these cyclization conditions, we developed a new xanthine synthesis. Coupling of **1** with (*R*)-2-benzylpropionic acid, whose synthesis we have recently described,[24] using the isobutyl chloroformate coupling procedure gave chiral amide **4** in 64% yield. Alkylation of **4** with Meerwein's reagent followed by chromatography[25] provided a 40% yield of imino ether **5**, which thermally cyclized to chiral[26] xanthine **6**, mp 141–142 °C, in 80% yield. In the same fashion was prepared the *S* enantiomer[27] (**7**; mp 151–152 °C), using (*S*)-2-benzylpropionic acid.[24]

The A_1 and A_2 adenosine receptor binding data for **3**, **6**, and **7** in rat brain is displayed in Table III. It is clear that the *R* and *S* enantiomers have significantly different affinities for both A_1 and A_2 receptors, with the *R* enantiomer being more potent. Similarly, N^6-[(*R*)-1-methyl-2-phenylethyl]adenosine (R-PIA) is more potent than N^6-[(*S*)-1-methyl-2-phenylethyl]adenosine (S-PIA) at both A_1 and A_2 rat brain receptors.[28] Thus, the *marked stereochemical requirement* for receptor affinity shown by **6** and **7** supports the hypothesis that the C^8 substituent of a xanthine antagonist is binding in the same region as is the C^6N substituent of an adenosine derivative agonist.

In Figure 5 is shown an overlay of **6** and R-PIA derived from a SYBYL Multifit procedure, wherein the structures

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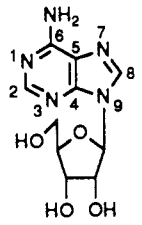
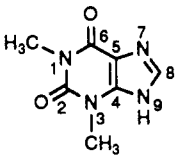
(24) Lentz, N. L.; Peet, N. P. *Tetrahedron Lett.* **1990**, *31*, 811.
(25) Chromatography was necessary to remove a component that caused partial racemization in the subsequent cyclization step.
(26) For *R* enantiomer **6**: $[\alpha]_D^{20} = -42°$ (c = 0.75, $CHCl_3$); >95% enantiomeric excess (HPLC using Chiralcel OK column with 98:2 v/v pentane/methanol mobile phase).
(27) For *S* enantiomer **7**: $[\alpha]_D^{20} = +38.5°$ (c = 0.69, $CHCl_3$); 96% enantiomeric excess (HPLC using Chiralcel OK column with 98:2 v/v pentane/methanol mobile phase).
(28) Moos, W. H.; Szotek, D. S.; Bruns, R. F. *J. Med. Chem.* **1983**, *28*, 1383.

Tabel II. Absolute Charge Differences between Selected Adenosine and Theophylline Atoms



adenosine

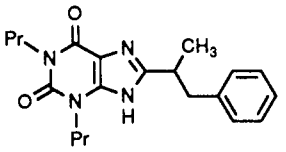


theophylline

binding mode	atoms: adenosine–theophylline	absolute charge differences[a] Gasteiger[b]	Mulliken[c]	AM1[d]
standard	N^1–N^1	0.00	0.13	0.08
standard	N^3–N^3	0.02	0.10	0.04
standard	N^7–N^7	0.07	0.10	0.01
standard	N^9–N^9	0.02	0.11	0.04
standard	C^6N–C^6=O	0.07	0.00	0.09
new	N^1–N^9	0.00	0.11	0.10
new	N^3–N^3	0.02	0.10	0.04
new	N^9–N^1	0.02	0.13	0.14
new	C^6NH–N^7H	0.01	0.08	0.07
new	N^7–C^6=O	0.04	0.14	0.14

[a] Charge calculations were performed with SYBYL (ref 18) software; the results were displayed on the Evans and Sutherland PS-390 Picture System. [b] Marsili, M.; Gasteiger, J. *Croat. Chem. Acta* **1981**, *53*, 601; *Chem. Abstr.* **1981**, *94*, 208209y. [c] Clementi, E. *J. Phys. Chem.* **1980**, *84*, 2122. [d] References 18–20.

Table III. Binding Constants for 8-(Phenylisopropyl)xanthines at A_1 and A_2 Adenosine Receptors



compd	stereochem	A_1 receptor K_i,[a] nM	A_2 receptor K_i,[b] nM
6	*R*	6.9 ± 1.6	157 ± 27
3	racemic	32.6 ± 4.6	644 ± 209
7	*S*	60.7 ± 5.3	848 ± 99

[a] Binding of [^{3}H]CHA in whole rat brain membranes was measured at 25 °C. Values are geometric means ± standard error, n = 3 separate determinations. See: Goodman, R.; Cooper, M.; Gavish, M.; Snyder, S. *Mol. Pharmacol.* **1982**, *21*, 329. [b] Binding of [^{3}H]NECA was measured in rat brain striatum at 25 °C. Values are geometric means ± standard error, n = 3 separate determinations. See: Bruns, R, R.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.

were simultaneously fitted and energy minimized. It is clear from this overlay that the phenylisopropyl recognition units of each molecule can occupy the same space.

Several additional 8-substituted xanthines that are in preparation using the novel synthetic route described here will be the subject of a future report.

Supplementary Material Available: A listing of coordinates with AM1 charges for the fit versions of adenosine and theophylline and a figure showing compound **6** with assigned atom identification numbers (4 pages). Ordering information is given on any current masthead page.

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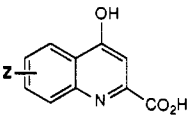
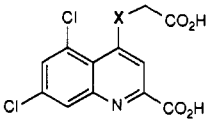
4-[(Carboxymethyl)oxy]- and 4-[(Carboxymethyl)amino]-5,7-dichloroquinoline-2-carboxylic Acid: New Antagonists of the Strychnine-Insensitive Glycine Binding Site on the *N*-Methyl-D-aspartate Receptor Complex

Recent research efforts have suggested that overstimulation of the *N*-methyl-D-aspartate (NMDA) receptor plays a critical role in the neuropathology of disease states including epilepsy, Huntington's chorea, and anoxic conditions, such as stroke.[1] A large body of work has shown that the NMDA receptor complex is composed of several distinct binding domains including the glutamate agonist site,[2] the strychnine-insensitive glycine site,[2] the receptor gated ion channel,[2] a zinc ion site,[2] and a recently described polyamine site.[3] The glycine site, which recognizes glycine and certain analogs[4] as agonists, was originally thought to be a modulatory site; however, recent work has proven that glycine is obligatory for L-glutamic acid to activate this receptor complex.[5] These findings suggest that potent, selective glycine site antagonists of the NMDA receptor complex would find utility in therapeutic areas, such as epilepsy and stroke.

The earliest reported glycine site antagonist, kynurenic acid (**1**), an endogenous product of the tryptophan metabolism pathway, has micromolar binding affinity for both the glycine site[6] and the L-glutamate site[7] (IC_{50} = 16 μM versus [^{3}H]glycine, IC_{50} = 71 μM versus [^{3}H]CPP,[8] respectively).[9] Subsequently, the chloro derivatives 7-chlorokynurenic acid[10] (**2**) (IC_{50} = 0.4 μM versus [^{3}H]-glycine; IC_{50} = 162 μM versus [^{3}H]CPP) and 5,7-dichlorokynurenic acid[11] (**3**) (IC_{50} = 0.08 μM versus [^{3}H]-glycine; IC_{50} = 37 μM versus [^{3}H]CPP) were found to be more potent and selective than kynurenic acid. In this communication we describe the synthesis and evaluation of two new potent, selective glycine site antagonists: 4-[(carboxymethyl)oxy]-5,7-dichloroquinoline-2-carboxylic acid (**4**) and 4-[(carboxymethyl)amino]-5,7-dichloroquinoline-2-carboxylic acid (**5**).



1: Z = H
2: Z = 7-Cl
3: Z = 5,7-di-Cl



4: X = O
5: X = NH

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