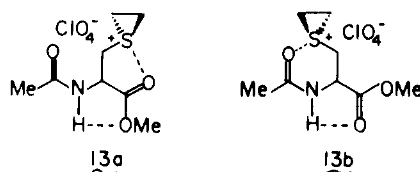

which the positive sulfur and carbonyl oxygen were within 2.3 Å of one another, with neither having any apparent severe steric strain. Such interactions, if present, would be expected to decrease the effective positive charge on the sulfur, stabilizing the episulfonium ring and rendering it less susceptible to nucleophilic attack. The existence of such an unexpected conformational distribution is presently being investigated.

Several conclusions can be drawn from this preliminary study. First, **7** has a half-life on the order of hours in strongly acidic water at ambient temperature. This is in contrast to simple aliphatic ESIs which often cannot even be isolated at room temperature. Second, ESIs with relatively acidic β protons are likely to undergo elimination as well as nucleophilic addition under physiological conditions. Since **4** and **6** also have the necessary structural requirements to undergo elimination, this decomposition route may be relevant for them as well. Such a route could play a role in the toxicological profile of the DHEs. Third, if significant intramolecular stabilization is present in **7**, as indicated (but not proven) by the NMR and modeling data, it would support increased stability for the putative ultimate carcinogens **4** and **6**, which also have suitably positioned carbonyls. The cysteine-based ESI **5** is predicted to be less stable by this argument. This hypothesis is presently being tested and further studies on these and compounds based on the parent molecules (GSH and MA) will be reported in a subsequent paper.

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Registry No. 7, 114301-30-3; 7·$HClO_4$, 114301-33-6; 8, 7652-46-2; 9, 77109-48-9; 10, 114301-31-4; 11, 35356-70-8; 12, 114301-32-5; $BrCH_2CH_2OH$, 540-51-2; $HSCH_2CH_2OH$, 60-24-2.

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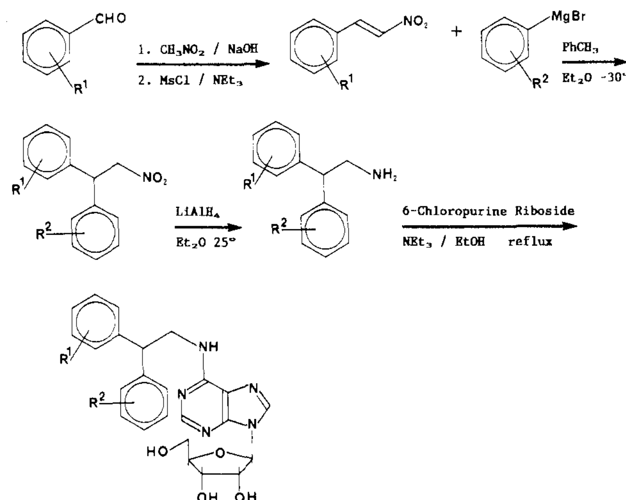
N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine and Its Uronamide Derivatives. Novel Adenosine Agonists with Both High Affinity and High Selectivity for the Adenosine A_2 Receptor

Sir:

Extracellular adenosine acts as a local hormone, operating through two major subclasses of membrane-bound adenosine receptors, called A_1 and A_2, which are distinguished by their structure–activity relationships.[1-3] Relative affinities of compounds for these adenosine receptor subtypes can be determined by using specific A_1 and A_2 receptor binding assays.[4] Many potent and selective A_1 agonists have been found, typified by *R*-PIA (**1**)[5] and CPA (**2**)[6] (Table I and Chart I). However, progress toward potent and selective A_2 agonists has been much slower. For instance, NECA (**3**),[7] although very potent at A_2 receptors,[8] is slightly A_1 selective in the A_1 and A_2 binding assays.[4] Among the few known A_2-selective agonists are 2-(phenylamino)adenosine (CV-1808, **4**)[8] and N^6-(1-naphthalenylmethyl)adenosine (**5**).[9-11] Although the former compound has the greatest A_2 selectivity yet reported, its selectivity is only 5-fold and its binding affinity is 1 order of magnitude lower than that of NECA.[4] In this communication we wish to report the discovery of a series of agonists with 2–4-fold stronger A_2 affinity than NECA, which show up to a 40-fold selectivity for the A_2 receptor.

Scheme I. Synthesis of N^6-(2,2-Diarylethyl)adenosines



Recently we reported[12] that N^6-(2,2-diphenylethyl)-adenosine (CI-936, **6**) is a moderately potent A_2 agonist with 25 nM A_2 affinity, a nearly balanced receptor binding profile, and an excellent profile in behavioral tests predictive of antipsychotic-like activity. Simply bridging the phenyl rings, to give N^6-(9-fluorenylmethyl)adenosine (**7**),[11] markedly increases the affinity at the A_2 receptor, to give a balanced agonist with 5 nM affinities at both receptors. Results such as these convinced us that a detailed examination of this series might be fruitful in a search for potent, A_2-selective adenosine agonists.

Several different conceptual approaches to the modification of the 2,2-diphenylethyl side chain of **6** were examined. As alluded to above, bridging of the two phenyl

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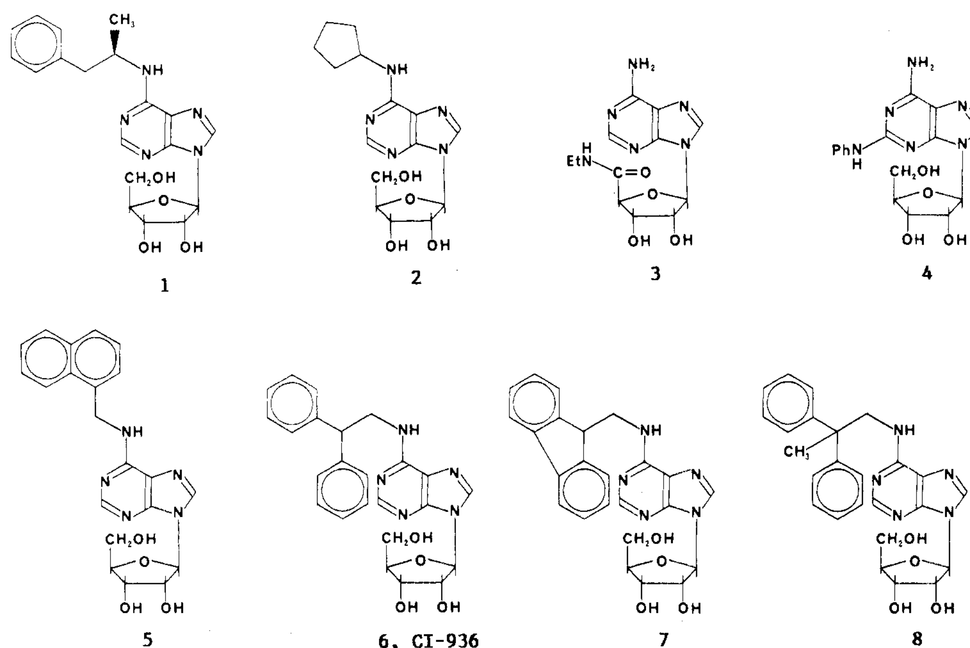
Table I. Receptor Binding Affinities of Adenosine Agonists

compd	formula[b]	mp, °C	K_i (nM)[a] ± SE		K_i ratio: A_2/A_1
			A_1	A_2	
1	$C_{19}H_{23}N_5O_4$	143–145	1.2 ± 0.2	124 ± 9	106
2	$C_{15}H_{21}N_5O_4$	123–128	0.59 ± 0.02	460 ± 15	780
3	$C_{12}H_{16}N_6O_4$	153–155	6.3 ± 0.5	12 ± 1.5	1.87
4	$C_{16}H_{18}N_6O_4$	242–244	600 ± 27	116 ± 10	0.19
5	$C_{21}H_{21}N_5O_4$	145–150	24 ± 4	9.4 ± 1.1	0.38
6	$C_{24}H_{25}N_5O_4$	106–108	6.8 ± 0.2	25 ± 3	3.61
7	$C_{24}H_{23}N_5O_4$	139–142	5.2 ± 0.5	4.9 ± 0.5	0.95
8	$C_{25}H_{27}N_5O_4$	109–114	37 ± 2.6	30 ± 1.9	0.79
9	$C_{26}H_{29}N_5O_4$	99–106	9.3 ± 0.3	18.8 ± 3.4	2.03
10	$C_{24}H_{23}Cl_2N_5O_4$	102–108	2.6 ± 0.1	6.0 ± 1.0	2.31
11	$C_{26}H_{29}N_5O_4$	112–124	229 ± 13	62 ± 15	0.27
12	$C_{24}H_{23}Cl_2N_5O_4$	102–112	61 ± 5	19 ± 0.6	0.31
13	$C_{24}H_{23}Cl_2N_5O_4$	110–115	18.4 ± 1.0	6.4 ± 0.12	0.34
14	$C_{26}H_{29}N_5O_6$	100–106	30.2 ± 2.5	6.1 ± 0.6	0.20
15	$C_{28}H_{33}N_5O_6$	107–117	140 ± 31	19.5 ± 2.2	0.14
16	$C_{27}H_{31}N_5O_6$	183–184	142 ± 23	4.4 ± 0.2	0.031
16A (+) isomer	$C_{27}H_{31}N_5O_6$	168–169	183 ± 31	6.2 ± 0.3	0.033
16B (−) isomer	$C_{27}H_{31}N_5O_6$	195–197	118 ± 17	3.1 ± 0.1	0.027
17	$C_{29}H_{34}N_6O_6$	103–112	207 ± 29	5.6 ± 0.6	0.027
18	$C_{28}H_{32}N_6O_6$	122–127	1010 ± 79	43 ± 1.8	0.042
19	$C_{30}H_{34}N_6O_6$	117–126	232 ± 45	8.7 ± 1.1	0.038

[a] A_1 binding was carried out with [^{3}H]-N^6-cyclohexyladenosine in rat brain membranes, and A_2 binding was carried out with [^{3}H]NECA binding in rat striatal membranes in the presence of 50 nM unlabeled CPA.[4] Values are means ± interexperimental standard errors of three or more independent determinations. [b] All compounds had NMR, IR, and MS fully in accord with the assigned structures, and all new compounds had correct elemental analyses.

Chart I



groups produced the balanced agonist **7**, and methylation of the 2-position of the ethyl side chain gave an agonist **8**,[12] which was slightly A_2 selective. Despite these results, we felt that success was most likely to come in the large number of compounds available to us through substitution of the aromatic rings. Therefore, we set out to synthesize ring substituted 2,2-diarylethylamines as precursors of the desired derivatives of **6**.

Because none of the extant syntheses[13,14] met our requirements of flexibility, overall yields, and speed, we developed the simple preparation of 2,2-diarylethylamines shown in Scheme I, which allowed us to synthesize a large number of such compounds. In this synthesis an aryl aldehyde is condensed with nitromethane[15] and dehydrated by mesyl chloride to the corresponding nitrostyrene.[16] Michael addition of an aryl Grignard reagent, followed by $LiAlH_4$ reduction, provides the amines in 30–70% overall yields in most cases. The amines can be converted into the desired adenosines by reaction with 6-chloropurine ribonucleoside and NEt_3 in refluxing EtOH.[17]

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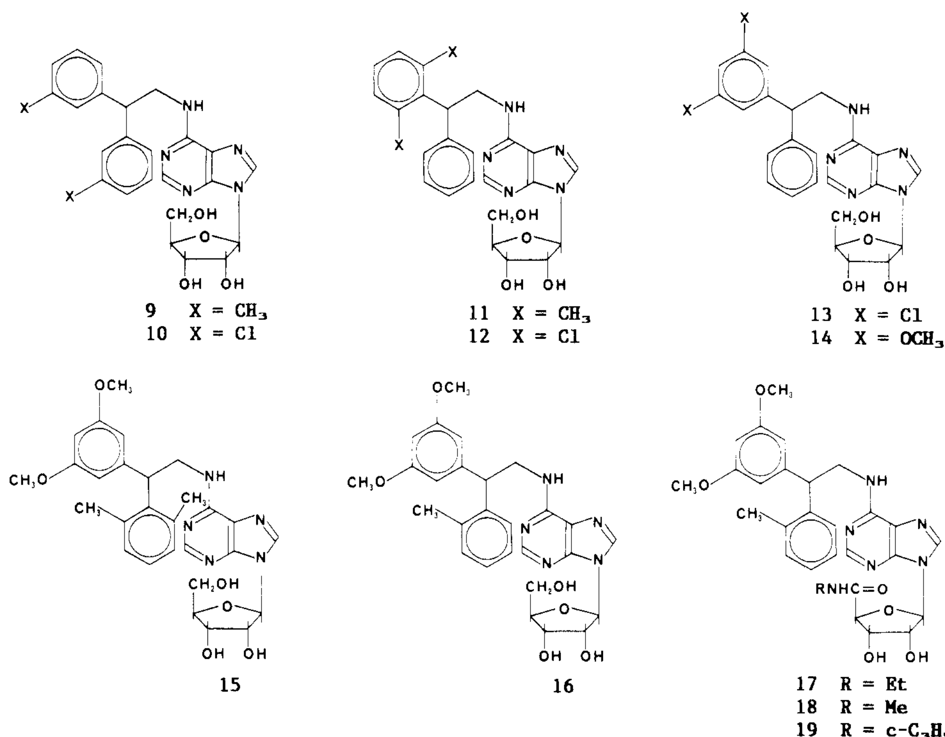
Chart II



Monosubstitution of one of the phenyl rings at the ortho or meta positions generally improved the receptor binding of the agonist, but usually by similar amounts at both receptors. Disubstitution proved more interesting.[18] When a single meta substituent was put in each ring, agonists such as **9** and **10** (Chart II), with rather similar affinities to the monosubstituted analogues were obtained. If, however, the two substituents were in the same ring, potent agonists with 3–6-fold A_2 selectivity were obtained, as exemplified by the bis-ortho-substituted agonists **11** and **12**, and the bis-meta agonists **13** and **14** (Table I). Initial attempts to increase the A_2 selectivity by placing a meta substituent in the unsubstituted phenyl ring of **13** or **14** were unsuccessful, as the single meta substituent either enhanced A_1 affinity or dramatically decreased A_2 affinity.

Binding results obtained from several of the above compounds were used to refine a pharmacophore model of the N^6 binding region of the A_2 receptor, which will be reported on in detail elsewhere.[19] Combining results generated by this model with the observed deleterious effects of ortho and di-meta substitution on A_1 receptor affinity, suggested that the best A_2 selectivity and affinity would be obtained with agonists that combined ortho substitution in one ring with 3,5-disubstitution in the other ring. The tetrasubstituted analogue **15** fulfilled our expectations of weak A_1 affinity, with a K_i of 140 nM, but the A_2 affinity had also deteriorated 3-fold, resulting in an agonist with moderate affinity and only 7-fold A_2 selectivity. However, the combination of a single *o*-methyl substituent on one ring with 3,5-dimethoxy substitution on the other ring (**16**) proved to be very successful, as the A_1 affinity was still as strongly attenuated as in **15**, but the A_2 affinity was slightly potentiated, giving a very potent agonist with 4.4 nM A_2 affinity and 32-fold A_2 selectivity.

Compound **16** is a 1:1 mixture of diastereoisomers, because differential substitution on the aromatic rings generates a chiral center at the benzylic position of the diarylethylamines, which were prepared as racemic mixtures. The diastereoisomers of **16** were separated by semipreparative HPLC using a stationary phase of cyclodextrin bonded to silica. (This system recognizes only the chiral center in the side chain, as it is equally effective in separating the enantiomers of the aglycon of **16**.) Both diastereoisomers of **16** had good A_2 affinity, with the more potent of the two isomers **16B** now having a K_i of 3.1 nM for the A_2 receptor and 37.5-fold A_2 selectivity. As this profile appeared to be suitable for a ligand for use in A_2 receptor studies, a sample of **16B** was tritiated.[20] Unfortunately, although **16B** showed good binding to the A_2 receptor, it also showed high nonspecific binding in the rat striatal membrane homogenates used in the standard binding assays, and thus was not superior to [^{3}H]NECA in this application.

Adenosine shows a dramatic increase in A_2 potency when the 5′-hydroxymethyl group is converted to an *N*-ethylcarboxamide (NECA) group.[7] However, the 300-fold increase in potency observed is in large part due to the stability of NECA to adenosine deaminase and adenosine kinase; if 2-chloroadenosine is taken as a metabolically stable proxy for adenosine, the potency increase for NECA over adenosine is only 1.5-fold in A_1 binding and 6-fold in A_2 binding.[4] It has been observed[21] that a similar enhancement of A_2 affinity by 5′-NECA modification is also seen for A_1-selective N^6-substituted agonists, but is much less dramatic for agonists that have balanced receptor affinities. Compound **17**, the NECA analogue of **16**, proved to have slightly weaker A_2 affinity than **16**, but a slightly greater loss of A_1 affinity improved the A_2 selectivity to that of **16B**. The *N*-methyl and cyclopropyl uronamides

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18 and 19 were also prepared. As would be expected from the parent N^6-substituted NECA analogue,[7] 19 showed nearly as high a selectivity ratio as 17, with less than a 2-fold loss of affinity, but 18, unlike in the parent series,[4,22] was somewhat less A_2 selective than 17, and showed a 10-fold loss in binding affinity.

In summary, we have identified a series of N^6-substituted adenosine derivatives that show substantial selectivity for the A_2 receptor. Development of a detailed model of the N^6 binding region of the A_2 receptor allowed us to refine the series, producing several agonists with 3–6 nM affinity and 20–40-fold binding selectivity for the A_2 receptor. This potency and selectivity should make these compounds very useful probes for adenosine pharmacology.

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Articles

Nonisomerizable Analogues of (*Z*)- and (*E*)-4-Hydroxytamoxifen. Synthesis and Endocrinological Properties of Substituted Diphenylbenzocycloheptenes

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Substituted 8,9-diphenyl-6,7-dihydro-5*H*-benzocycloheptenes 6–8, which are ring-fused analogues of (*Z*)-*trans*-4-hydroxytamoxifen, (*E*)-*cis*-tamoxifen, and (*E*)-*cis*-4-hydroxytamoxifen, were synthesized from 7-methoxy-1-benzosuberone. The hydroxy compounds 6 and 8 were individually prepared via a common synthetic intermediate from which either the perfluoro-*p*-tolyl or the methyl ether functions could be cleaved specifically. Compounds were assayed for binding affinity to estrogen receptors in cytosol and in MCF-7 whole cells and for growth inhibition of MCF-7 cells in vitro and rat uteri in vivo. The endocrinological properties of the cyclic analogues 5–7 paralleled those of the corresponding derivatives of tamoxifen although in the MCF-7 assay 6 was slightly less effective than 4-hydroxytamoxifen at 10^{-6} and 10^{-7} M. The compound 8 analogous to *cis*-4-hydroxytamoxifen antagonized the growth stimulation by estradiol of MCF-7 cell or rat uterus growth, and it is therefore an antiestrogen, but its potency was somewhat less, both as an antiestrogen and an estrogen, than reported for *cis*-4-hydroxytamoxifen attributable to modification of the biochemical properties of the latter by isomerization to the more potent trans isomer. Curiously, in the absence of estradiol, compound 8 stimulated MCF-7 cell growth at low concentration (10^{-8} M) but inhibited growth at higher concentration. In contrast, compound 7, which lacked the hydroxy function, was a full estrogen in the rat uterine growth assay. These compounds should be ideal for further structure–activity studies of triarylethylene-based antiestrogens without complications caused by isomerization.

(*Z*)-*trans*-4-Hydroxytamoxifen (1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-phenyl-1-butene) (2) is an important metabolite of the nonsteroidal antiestrogen tamoxifen (1) in patients undergoing treatment for advanced breast cancer.[1] It has attracted considerable interest since it has a much greater antiestrogenic potency in vitro than the parent drug.[2,3] However, although the pure isomers of 4-hydroxytamoxifen can be prepared,[4] they undergo a facile isomerization to give a mixture of isomers,[5,6] a process that has been shown to occur during cell culture experiments;[7,8] for instance, when MCF-7 cells are grown in a medium containing (*E*)-*cis*-4-hydroxytamoxifen, it is the trans isomer that preferentially accumulates in the cells.[8] (Trans and cis are used in this paper to refer to the relative positions of the aryl ring bearing the basic side chain and alkyl function on the

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