shida and his colleagues for the biological assays.

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N^6-(2,2-Diphenylethyl)adenosine, a Novel Adenosine Receptor Agonist with Antipsychotic-like Activity

Sir:

Schizophrenia is a serious mental illness present in approximately 1% of the world population.[1] Available antipsychotic drugs are brain dopamine receptor antagonists, and although they reduce some schizophrenic symptoms, they are not completely efficacious and frequently induce serious neurological side effects, including acute Parkinsonism and tardive dyskinesia.[2] Because of these problems, we have sought to identify potential antipsychotic agents that do not act through dopamine receptor blockade. One such mechanism that we have pursued is the activation of brain adenosine receptors. This paper describes N^6-(2,2-diphenylethyl)adenosine (CI-936, **11**), an adenosine agonist that produces potent and selective effects in preclinical tests predictive of antipsychotic efficacy.

Adenosine is an endogenous purine that in addition to a role in intermediary metabolism may act as a local hormone involved in regulation of energy supply and demand.[3] The actions of adenosine occur via at least two types of extracellular receptors, which were originally defined by their opposing effects on adenylate cyclase. Activation of A_1 (R_i) receptors inhibits adenylate cyclase, whereas activation of A_2 (R_a) receptors stimulates adenylate cyclase.[4,5] The two receptors also differ in their structure–activity relationships.[5]

A growing body of evidence suggests that adenosine is a neuromodulator in the central nervous system.[6] Adenosine agonists such as N^6-[(*R*)-1-methyl-2-phenylethyl]adenosine (*R*-PIA) produce sedation in rodents, an effect that appears to be mediated via central adenosine receptors.[7] Our initial studies indicated that *R*-PIA inhibited dark-stimulated motor activity at doses somewhat below those that produced ataxia as measured by an inverted screen test,[8] a profile of activity that is characteristic of antipsychotic drugs and one that has proven useful in our laboratories for distinguishing antipsychotics from drugs of other therapeutic classes.[9] Similar results have been reported for N^6-cyclohexyladenosine.[10] On the basis of the results with *R*-PIA, we examined a series of adenosine analogues and discovered that an antipsychotic-like profile (ED_{50} ataxia > ED_{50} motor activity inhibition) is also seen with other adenosine agonists including N^6-cyclopentyl-, N^6-cyclohexyl-, 2-chloro-, and 5′-(*N*-ethylamino)carbonyl-substituted adenosines.[8] Among reference agents, the largest separation between doses inhibiting motor activity and those producing ataxia was obtained with 2-(phenylamino)adenosine (CV-1808), a compound developed as an antianginal agent.[11] Development of an A_2-receptor binding assay[12] revealed that CV-1808, unlike most other adenosine reference agonists, has greater relative affinity for A_2 as compared with A_1 adenosine receptors. Additional evidence suggesting a specific link between antipsychotic activity and A_2 receptors comes from the regional distributions of A_1 and A_2 receptors in the brain: A_1 receptors are widely distributed, whereas high-affinity A_2 receptors are localized to the striatum, nucleus accumbens, and olfactory tubercle,[12,13] areas that have been implicated in antipsychotic drug action.

On the basis of these results, we initiated a program to synthesize adenosine agonists with appreciable affinity for the A_2 receptor (either A_2 selective or balanced in A_1/A_2 affinity) as potential antipsychotic agents. Among the N^6-substituted adenosines, most reference agonists are quite A_1 selective, possessing high affinity for A_1 but low affinity for A_2 adenosine receptors. However, the N^6-aralkyl derivative *R*-PIA, although very A_1 selective, has higher affinity for A_2 receptors than the N^6-alkyl and N^6-cycloalkyl adenosines.[12,14] Therefore, we examined the effect of aryl substitution at the N^6-position. A series of N^6-phenylalkyl-substituted adenosines were synthesized by refluxing the corresponding amines with 6-chloropurine riboside in ethanol containing triethylamine[15,16] (Table I). The monophenyl compounds provided high A_1-receptor[12,17] affinity with the exception of the N^6-benzyl derivative **2**. Maximal affinity for A_2 receptors was achieved with the phenethyl side chain **3**. Although the absolute A_2 affinity of **3** is slightly less than that of its methylated analogue *R*-PIA (**6**), the phenethyl compound has greater relative affinity for A_2 receptors, the K_i ratio of A_2/A_1 being 12.7 vs. 103 for R-PIA. An examination of methylation with three other phenethyl side chains (**7–9**) revealed similarly deleterious effects on either A_2 potency or relative A_2 affinity. However, addition of a second aryl group, as in the diphenylalkyl series (**10–14**), revealed a clear maximum for A_2 binding affinity at the ethyl side chain (**11**, CI-936). Compound **11** has high affinity for both A_1 and A_2 receptors, with a marked enhancement in A_2 affinity as compared to the phenethyl compound. In agreement with its high A_2 affinity, **11** has been reported to possess moderately potent activity at the dog coronary artery A_2 recep-

(1) Baldessarini, R. J. *N. Engl. J. Med.* **1977**, *297*, 998.
(2) Tarsy, D.; Baldessarini, R. In *Clinical Neuropharmacology*; Klawans, H., Ed.; Raven: New York, 1976; Vol. 1.
(3) Sparks, H. V., Jr.; Bardenheuer, H. *Circ. Res.* **1986**, *58*, 193.
(4) Van Calker, D.; Muller, M.; Hamprecht, B. *J. Neurochem.* **1979**, *33*, 999.
(5) Londos, C.; Cooper, D. M. F.; Wolff, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2551.
(6) Daly, J. W.; Bruns, R. F.; Snyder, S. H. *Life Sci.* **1981**, *28*, 2083.
(7) Vapaatalo, H.; Onken, D.; Neuvonen, P.; Westermann, E. *Arzneim-Forsch.* **1975**, *25*, 407.
(8) Heffner, T. G.; Downs, D. A.; Bristol, J. A.; Bruns, R. F.; Harrigan, S. E.; Moos, W. H.; Sledge, K. L.; Wiley, J. N. *Pharmacologist* **1985**, *27*, 293.
(9) McLean, J. R.; Parker, R. B.; Coughenour, L. L. *Pharmacol. Biochem. Behav.* **1978**, *8*, 97.
(10) Geyer, H. M.; Wilker, J.; Spaulding, T.; Cornfeldt, M.; Brugger, S.; Huger, F.; Novick, W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 1071.
(11) Kawazoe, K.; Matsumoto, N.; Tanabe, M.; Fujiwara, S.; Yanagimoto, M.; Hirata, M.; Kikuchi, K. *Arzneim.-Forsch.* **1980**, *30*, 1083.
(12) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
(13) Premont, J.; Perez, M.; Blanc, G.; Tassin, J.-P.; Thierry, A.-M.; Herve, D.; Bockaert, J. *Mol. Pharmacol.* **1979**, *16*, 790.
(14) Kusachi, S.; Thompson, R. D.; Bugni, W. J.; Yamada, N.; Olsson, R. A. *J. Med. Chem.* **1985**, *28*, 1636.
(15) Fleysher, M. H. *J. Med. Chem.* **1972**, *15*, 187.
(16) Bristol, J. A.; Moos, W. H.; Trivedi, B. K. Eur. Patent Application 175-465-A, 1984; U.S. Patent 4 657 898, 1987.
(17) Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. *Biochem. Pharmacol.* **1986**, *35*, 2467.

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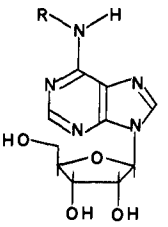
Table I. Adenosine Receptor Binding Affinities[a] and Effects on Motor Activity[b] (MA) and Ataxia (ATX) of N^6-Aralkyladenosines



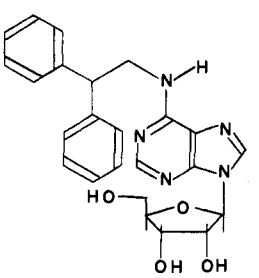
no.	side chain R	formula[c]	mp, °C	binding: K_i ± SE, nM		ED_{50}, mg/kg ip	
				A_1	A_2	MA	ATX
1	Ph	$C_{16}H_{17}N_5O_4$	194–195	4.6 ± 0.2	660 ± 80	25	30
2	$PhCH_2$	$C_{17}H_{19}N_5O_4$	184–186	120 ± 20	280 ± 9	22	>100
3	$PhCH_2CH_2$	$C_{18}H_{21}N_5O_4$	166–168	12.7 ± 0.2	161 ± 3	15	>100
4	$PhCH_2CH_2CH_2$	$C_{19}H_{23}N_5O_4$	124–128	23	420	19	>100
5	$PhCH_2CH_2CH_2CH_2$	$C_{20}H_{25}N_5O_4$	125–127	15.9	1230	32	>100
6	(*R*)-$PhCH_2CH(CH_3)$	$C_{19}H_{23}N_5O_4$	143–145	1.2 ± 0.2	124 ± 9	0.8	2
7	(*S*)-$PhCH_2CH(CH_3)$	$C_{19}H_{23}N_5O_4$	105–112	49 ± 2	1720 ± 380	16	75
8	(*RS*)-$PhCH(CH_3)CH_2$	$C_{19}H_{23}N_5O_4$	94–98	2.4	98	0.5	7
9	$PhC(CH_3)_2CH_2$	$C_{20}H_{25}N_5O_4$	92–99	16.2	320	6.7	30
10	Ph_2CH	$C_{23}H_{23}N_5O_4$	162–171	480	3800	80	>100
11	Ph_2CHCH_2 (CI-936)	$C_{24}H_{25}N_5O_4$	106–108	6.8 ± 0.2	25 ± 3	1.3	145
12	$Ph_2CHCH_2CH_2$	$C_{25}H_{27}N_5O_4$	115–122	79	290	33	>100
13	$Ph_2CHCH_2CH_2CH_2$	$C_{26}H_{29}N_5O_4$	102–108	290	4700	32	>100
14	$Ph_2CHCH_2CH_2CH_2CH_2$	$C_{27}H_{31}N_5O_4$	78–82	146	2200	30	>100
15	$Ph_2C(CH_3)CH_2$	$C_{25}H_{27}N_5O_4$	109–114	37 ± 3	30 ± 2	7	>100
16	Ph_3CCH_2	$C_{30}H_{29}N_5O_4$	123–125	14300	26000	80	>100

[a] Binding of [^{3}H]-N^6-cyclohexyladenosine to A_1 adenosine receptors in rat whole brain membranes and binding of [^{3}H]NECA (1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide) to A_2 receptors in rat striatal membranes was performed as described.[12] Affinities for compounds 1–3, 6, 7, 11, and 15 are averages of three or more independent experiments; values for other compounds are from single determinations. Values for 1, 2, 6, and 7 are taken (with permission) from ref 12. A_1 binding results for 1–8, 10, and 11 are in good agreement with published results.[17] [b] Motor activity and ataxia were determined as previously described.[8] [c] All compounds had NMR, IR, and MS fully in accord with the assigned structures, and all new compounds had correct elemental analyses.

tor.[18] Addition of a benzylic methyl group to **11** produced a compound, **15**, that is slightly A_2 selective, whereas addition of a third phenyl group (**16**) reduces both A_1 and A_2 binding.

Compound **11** inhibits exploratory activity in mice, with a potency similar to that for *R*-PIA; however, **11** is 50-fold less potent than *R*-PIA for producing ataxia (Table I). This selectivity of sedative action in mice is greater than that obtained with a variety of dopamine antagonist antipsychotics, including haloperidol, chlorpromazine, and thioridazine (Table II). Compound **11** retains potency and selectivity of sedative action upon oral administration in rats (Table II). Furthermore, it shows an antipsychotic-like profile in inhibiting continuous unsignaled (Sidman) avoidance responding in rats (Table II) without impairing the ability of animals to respond. For example, a 4 mg/kg dose of **11** produced a 90% inhibition of avoidance responding, but reduced shock termination responding by only 7%.[19] Selective blockade of Sidman avoidance responding, an effect produced by all known antipsychotic drugs, is widely used as a preclinical predictor of antipsychotic efficacy.[20] Although the mildly A_2 selective agonist, **15**, also showed a good, reasonably potent profile in Sidman avoidance (ED_{50} 5.6 mg/kg), it had a very short duration of action in that test (≤2 h) in contrast to **11**, which retained good activity throughout the duration of the test (6 h). The behavioral effects of **11** appear to be

Table II. Comparison of 11 with Haloperidol and Thioridazine in the Motor Activity (MA) and Ataxia (ATX) Test and in Sidman Conditioned Avoidance[a]



11

compd	mouse: ED_{50}, mg/kg ip		rat: ED_{50}, mg/kg po		
	MA	ATX	MA	ATX	Sidman
11	1.3	145	2.2	31	2.5
haloperidol	0.27	12	0.24	>1	0.3
thioridazine	3.3	13	13.3	>30	19.7

[a] Behavioral tests were performed as described.[8,18]

mediated by adenosine receptors in that the locomotor inhibition caused by this agent (2.5 mg/kg ip) is completely reversed in a dose-dependent manner by the adenosine receptor antagonist theophylline (1–10 mg/kg ip). Its lack of appreciable affinity for dopamine or other neurotransmitter receptors (L. Coughenour, personal communication) suggests that **11** should not share the side-effect profile of available antipsychotics.

In summary, we have identified **11** as a novel agent that produces selective antipsychotic-like effects in animals by a heretofore unexplored mechanism of action. As the first adenosine agonist to be developed for a CNS indication,

(18) Kusachi, S.; Thompson, R. D.; Yamada, N.; Daly, D. T.; Olsson, R. A. *J. Med. Chem.* **1986**, *29*, 989. This report appeared after the completion of the work described in the present paper.
(19) Wise, L. D.; Butler, D. E.; DeWald, J. A.; Lustgarten, D.; Coughenour, L. L.; Downs, D. A.; Heffner, T. G.; Pugsley, T. A. *J. Med. Chem.* **1986**, *29*, 1628.
(20) Niemegeers, C. J. E.; Verbruggen, F. J.; Janssen, P. A. J. *Psychopharmacology (Berlin)* **1969**, *16*, 175.

11 may provide an improved profile for the treatment of schizophrenia.

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Enantiomers of 1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid: Preparation and Biological Activity

Sir:

We have recently reported the synthesis of a new quinolone antibacterial agent, 1-ethyl-7-[3-[(ethylamino)-methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, CI-934 (**1**), which is unusually active against a wide spectrum of aerobic and anaerobic bacteria, especially against streptococcus and staphylococcus species, and is an effective inhibitor of bacterial gyrase.[1,2] We have also demonstrated that this unique activity against the Gram-positive strains (streptococci and staphylococci) is directly related to the 3-(aminomethyl)pyrrolidinyl side chain **2**.[1]

Virtually all of the significant quinolones reported to date either are achiral or are being developed as racemic mixtures.[3] Recently, however, several cases have been reported in which the enantiomers of certain quinolones were separated, and a substantial difference in potency was observed between the chiral forms.[3c] In particular, flumequine (**3**),[4] ofloxacin (**4**),[3c,5] and S-25930 (**5**)[6] all have asymmetry at the methyl-substituted carbon in the benzoxazine or quinolizine rings. In all cases, most if not all of the activity (10–100-fold!) was present in just one enantiomer. Since fewer quinolones contain chiral side chains, much less has been reported on the influence of side-chain asymmetry on antibacterial activity. In just one case, the enantiomeric quinolones with 2-substituted pyrrolidinyl side chains were synthesized. Once again, one isomer (**6**) possessed a substantial share of the potency (10–60-fold).[7] Because of the excellent antibacterial properties of **1** and the asymmetric center present in its *N*-ethyl-3-pyrrolidinemethanamine side chain, we report the synthesis and biological activity (in vivo, in vitro, and at the enzyme level) of the pure enantiomeric forms of this agent.

Attempts at classical resolution of 5-oxo-1-(phenylmethyl)-3-pyrrolidinecarboxylic acid,[8] a key intermediate in the reported synthesis of **1**,[1] were tedious and only partially successful (80% enantiomeric excess achievable). Instead, we turned our attention to the use of (*R*)-(+)-α-methylbenzylamine as both a resolving agent and a protecting group for the chiral synthesis. The chiral benzylamine **8** (Scheme I) was added to either itaconic acid or its dimethyl ester **7** to produce a near 50:50 mixture of the acids **9ab** or the esters **10ab**, respectively. The acids and esters were readily interconvertible under standard conditions.[9] The diastereomeric esters **10ab** were separated cleanly by column chromatography on silica gel using ethyl acetate–pentane. The 3*S* isomer **10b** was isolated as a white solid, while the epimeric 3*R* isomer **10a** was a thick syrup. The properties and reaction conditions of these and the other products in Scheme I are given in Table I. Prior to chromatography the isomer **10b** could be seeded and crystallized in pure form from the 50:50 mixture in ~14% yield.

To establish the absolute configuration at the 3-position, **10a** and **10b** were each reduced to the alcohols **12a** and **12b**. Deprotection of the pyrrolidine nitrogen gave the (*R*)-(+)-**15a** and *S*)-(−)-**15b** pyrrolidinemethanols. The absolute configuration of the (*S*)-(−)-pyrrolidinemethanol

(1) Domagala, J. M.; Heifetz, C. L.; Mich, T. F.; Nichols, J. B. *J. Med. Chem.* **1986**, *29*, 445.
(2) Cohen, M. A.; Griffin, T. J.; Bien, P. A.; Heifetz, C. L.; Domagala, J. M. *Antimicrob. Agents Chemother.* **1985**, *28*, 766.
(3) (a) Bergan, T. In *Antimicrobial Agents Annual 1*; Peterson, P. K., Verhoef, J., Eds.; Elsevier Science: 1986. (b) Wolfson, J. S.; Hooper, D. C. *Antimicrob. Agents Chemother.* **1985**, *28*, 581 and 716. (c) Mitscher, L. A. International Symposium on the Chemistry and Biology of the Quinolone Antiinfective Agents, American Institute of Chemists, Chicago, IL, Sept 26–28,1986.
(4) Gerster, J. F.; Rohlfing, S. R.; Winandy, R. M. North America Medicinal Chemistry Symposium, Toronto, Canada, June 1982; Abstract 109.
(5) (a) Imamura, M.; Shibamura, S.; Hayakawa, I.; Osada, Y. *Antimicrob. Agents Chemother.* **1986**, *29*, 163. (b) Imamura, M.; Shibamura, S.; Hayakawa, I.; Osada, Y. *Antimicrob. Agents Chemother.* **1986**, *31*, 325.
(6) Gerster, J. F.; Rolfing, S. R.; Pecore, R. M.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, MN, Sept 29–Oct 2, 1985; Abstract 134.
(7) Cooper, C. S.; Chu, D. T.; Fernandes, P. B.; Pihuleac, E.; Pernet, A. 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, MN, Sept 29–Oct 2, 1985; Abstract 130.
(8) Fles, D.; Ghyczy, T. *Croat. Chem. Acta* **1964**, *36*, 27.
(9) Scarborough, H. C.; Minielli, J. L.; Laws, B. C.; Lobeck, W. G.; Corrigan, J. R.; Wu, Y. H. *J. Org. Chem.* **1961**, *26*, 4955.
(10) (a) Heifetz, C. L.; Chodubski, J. A.; Pearson, I. A.; Silverman, C. A.; Fisher, M. W. *Antimicrob. Agents Chemother.* **1974**, *6*, 124. (b) Miller, L. C.; Tainter, M. L. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261.
(11) (a) Sugino, A.; Peebles, C. L.; Kreuzer, K. N.; Cozzarelli, N. R. *Proc. Natl. Acad. Sci U.S.A.* **1977**, *74*, 4767. (b) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Itoh, T.; Tomizawa, J. *Proc. Natl. Acad. Sci U.S.A.* **1977**, *74*, 4772. (c) Cozzarelli, N. R. *Science (Washington, D.C.)* **1980**, *207*, 953.
(12) (a) Otter, R.; Cozzarelli, N. R. *Methods Enzymol.* **1983**, *100*, 171. (b) Higgins, P. N.; Peebles, C. L.; Sugino, A.; Cozzarelli, N, R. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1773.
(13) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. *J. Med Chem.* **1986**, *29*, 394.
(14) High-performance liquid chromatography (HPLC) was carried out by using an LKB 2150 pump, Rheodyne 7125 injector with 20-μL loop, Supelco LC18-DB column (5-μm particle size, 250 × 4.6 mm i.d.), Perkin-Elmer LC-95 UV absorbance detector, and Hewlett-Packard 3390 integrator.

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