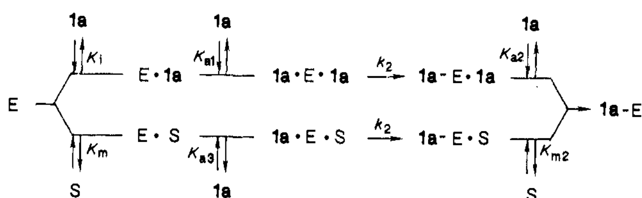
J. Med. Chem. 1987, 30, 1706-1709

Scheme II



zyme activity in the presence of 15, 30, and 100 μM of substrate. The observed rates of enzyme inactivation and the calculated k_2 were unaffected by the presence of substrate (Table I). Given the affinity of the substrate for the active site (K_m = 6.4 μM),[4] a significant decrease in rate[6] would have been observed if either **1a** caused inactivation at the active site or the substrate also had affinity at the site of inactivation.

Given the unique structural specificity for binding and reaction at the site of inactivation, the chemical precursor to compound **1a**, 5-(1,4-dimethoxy-3-methyl-2-naphthyl)-2′-deoxyuridine 5′-phosphate (**2a**), was examined in this assay. Compound **2a** has reasonably high affinity for the active site (K_i = 2.4 μM) and does not inactivate thymidylate synthase.[4] The structural similarity to **1a** suggests that compound **2a** also could have affinity at the site of inactivation. However, the presence of 6 and 30 μM of compound **2a** in the assay for enzyme inactivation by **1a** failed to decrease the rate of inactivation (Table I).

It is recognized that active-site binding of substrate or substrate analogues requires the presence of the 5-phosphate group. Examination of the corresponding nucleoside **1b** in the same assay at a higher concentration (50 μM) showed the rate of enzyme loss was the same as the control ($k_{obsd} = 5 \times 10^{-5}$ s^{-1}). The chemical reactivity of the nucleoside **1b** is the same as the nucleotide **1a**, and since the former is inactive, a nonspecific bimolecular mechanism for the enzyme inactivation afforded by **1a** is unlikely. Furthermore, binding to the site of inactivation by **1a** also requires the 5′-phosphate group.

In summary, the kinetic results satisfy the proposal that (a) the rate of inactivation is dependent on the concentration of the binary noncovalent complex formed from E and **1a**, (b) the rate-determining step is k_2, and (c) there is no detectable substrate affinity at the alternate site. Both **1a** and substrate have high affinity for the active site. At the concentrations of **1a** and substrate used, an initial complex of E·**1a** (K_i) or, in the presence of substrate, E·S (K_m) are formed by association at the active site as shown in the kinetic model in Scheme II. Furthermore, since the inactivation appears to take place at an alternate site, the inactivation is dependent on the concentration of the ternary complex of **1a**·E·**1a** or, in the presence of substrate, **1a**·E·S, according to Scheme II. The determined K_a could be a composite of K_{a1} and K_{a2}. However, k_2 is identical with or without substrate. The association values of the inactivated enzyme with either **1a** or substrate cannot be determined with the current data.

Dialysis studies with quinone nucleotides that inactivate thymidylate synthase have shown that active-site inactivation by the dimethylbenzoquinone[4] **3a** was reversible and by the benzoquinone **4a** was irreversible.[5] Dialysis studies using enzyme inactivated with 30 μM of **1a** to give 18% of the original activity showed that a maximum of 55% of the control activity could be recovered by dialysis in 50 mM 2-mercaptoethanol at 2 °C for 16 h.

One potential chemical mechanism for inactivation of the enzyme by **1a** is addition of a nucleophile to carbon 6 of the pyrimidine ring to form a covalent complex. The lack of reaction at this site in chemical model studies[4] and the absence of inactivation by the nucleoside **1b** argue against this as the mechanism. Model studies[4] did show that the naphthoquinone **1b** was an effective oxidizing agent for thiols, and a similar oxidation of the enzyme could lead to inactivation. If this were the case, the trimethylquinone **5a** would have inactivated the enzyme at a faster rate since both nucleotides have high affinity and the rate of oxidation of a model thiol by the trimethylquinone **5b** was over 10 times faster than oxidation by the naphthoquinone **1b**.

Given the above results, it appears that inactivation of the enzyme requires the formation of a complex. One possible explanation that remains to be verified is that **1a** binds and reacts at the cofactor binding site. If this were the case, **1a** would have shown noncompetitive inhibition in the initial studies with the substrate. An unprecedented proposal is that inactivation results from reaction of **1a** with the enzyme at an alternate binding site that apparently has affinity for the substrate analogue **1a**, but not for substrate. The affinity of **1a** for this alternate site is considerably less than that of **1a** for the active site. However, formation of the complex at the alternate site leads to inactivation that is unaffected by the presence of substrate. Such an alternate affinity site that does not appear to bind substrate has not been recognized for thymidylate synthase. Further studies could uncover regulatory or control roles for this region of the enzyme.[7]

Note Added in Proof: Two additional inactivation experiments were completed with the conditions reported in Table I that offer further support for the proposed mechanism. After subtraction of the appropriate control rates of inactivation, incubation of the enzyme with 6 μM of compound **1a** afforded the same rate of inactivation of thymidylate synthase in the absence and presence of 220 μM *dl*-H_4folate. In the second experiment incubation of the enzyme with 23 μM of the nucleoside, compound **1b**, in the absence or presence of 30 μM of substrate did not cause enzyme inactivation.

Acknowledgment. This work was supported by a grant (CA 7522) from the National Cancer Institute of the National Institutes of Health.

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Diketopiperazines as a New Class of Platelet-Activating Factor Inhibitors

Sir:

Recent hypothesis of the involvement of platelet-activating factor (PAF) in allergic, inflammatory, and other disease states has generated considerable impact in

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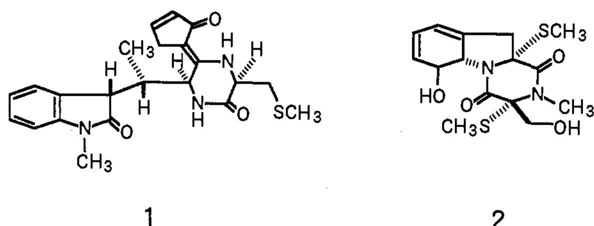
Table I. Inhibition of PAF-Induced Platelet Aggregation by Diketopiperazine Derivatives[a]

compd	IC_{50}, M	compd	IC_{50}, M
6a	2.5×10^{-4}	**6g**	3.6×10^{-6}
6b	3.6×10^{-5}	**6h**	1.8×10^{-7}
6c	1.4×10^{-4}	**6i**	1.9×10^{-6}
6d	7.9×10^{-6}	**1**	3.7×10^{-7}
6e	2.3×10^{-6}	CV3988[b]	2.5×10^{-6}
6f	7.0×10^{-7}	L-652,731[c]	3.2×10^{-7}

[a] Inhibitory activity was measured according to the method reported by Okamoto et al.[3] Compounds were dissolved in Me_2SO and diluted with saline. [b] Reference 2a. [c] Reference 2b.

searching for PAF inhibitors (or antagonists) which are likely to have therapeutic effects in these diseases.[1,2] It has recently been found in our laboratories that FR900452 (**1**), isolated as a metabolite of a *Streptomyces*, possesses



potent, specific PAF-inhibitory activity.[3] The structure of **1** is unique in its incorporation of an unusual vinylogous diketopiperazine moiety, 5-(2-oxocyclopent-3-en-1-ylidene)-2-oxopiperazine.[4] It is intriguing to us to speculate that this moiety may contribute to the ability of the molecule to bind to PAF receptor sites.[5] In order to address this point, we were interested in preparing structurally simpler analogues incorporating the diketopiperazine skeleton. This approach might be quite reasonable in light of the fact that glyotoxin analogue **2**, isolated as a PAF inhibitor from microbial origin, possesses a diketopiperazine function.[6] Herein we report the synthesis and PAF-inhibitory activity of compounds of this new prototype.

We anticipated that the oxindole moiety of **1** could be replaced by other similar nuclei, since we assumed that this part might interact with a hydrophobic area in the receptor site. Therefore we replaced the oxindole nucleus of **1** with the structurally simpler *N*-methylindole and α-naphthalene. All the stereoisomers **6a–d** with the *N*-methylindole nucleus were prepared,[7] respectively, by condensation of N^{α}-Boc-1-methyl-L- and -D-tryptophans (**3a** and **3b**) and ethyl *S*-methyl-L- and -D-cysteinates (**4a** and **4b**) using the DCC method, followed by removal of the Boc protecting group (HCl/AcOEt) and subsequent cyclization (NH_3/EtOH) (Scheme I). Via same procedure, the methionine derivative **6e** was prepared[7] as one of the chemical modifications of the *S*-methylcysteine moiety. To gain an understanding of the significance of the β-methyl group in the tryptophan residue of **1**, we synthesized[7-9] compounds **6f** (7*R*) and **6g** (7*S*) with the 3*R*,6*R* configuration (corresponding to D,D) by starting from *dl-erythro-* and *dl-threo-*N^{α}-Boc-1,β-dimethyltryptophans (**3c** and **3d**).[10]

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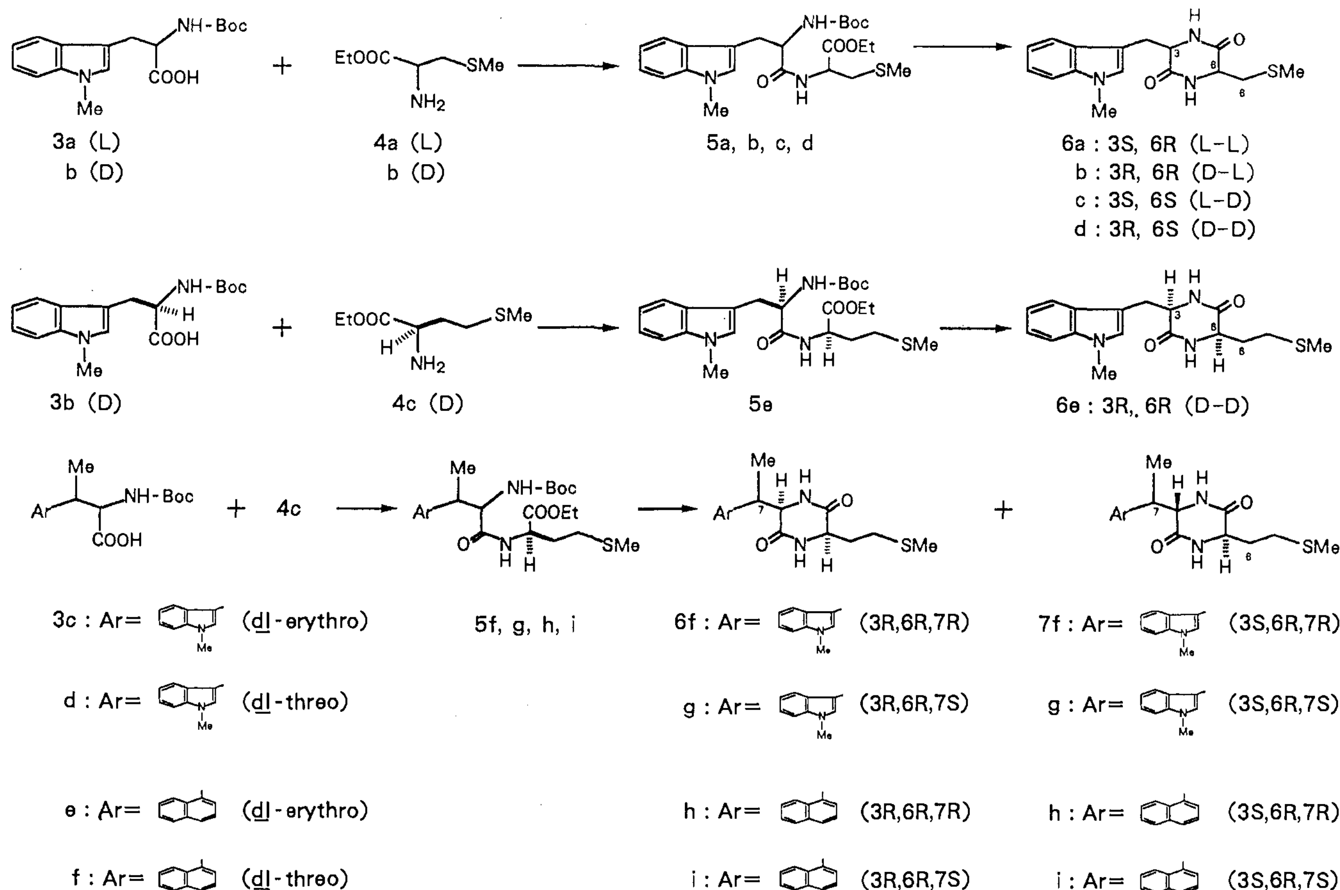
(7) Physical data of the compounds prepared in this study (TLC, silica gel 60 F_{254} (E. Merck AG), solvent $CHCl_3$–MeOH (10:1); elemental analyses (C, H, N) within ±0.4% of the calculated values): **6a**, mp >250 °C, $[\alpha]_D$ −85.3° (*c* 0.14, Me_2SO), TLC R_f 0.43, 72.5% from N^{α}-Boc-1-methyl-L-tryptophan and ethyl *S*-methyl-L-cysteinate; **6b**, mp 244–247 °C, $[\alpha]_D$ −7.0° (*c* 0.12, Me_2SO), TLC R_f 0.50, 66.4% from N^{α}-Boc-1-methyl-D-tryptophan and ethyl *S*-methyl-L-cysteinate; **6c**, mp 245–248 °C, $[\alpha]_D$ +7.7° (*c* 0.17, Me_2SO), TLC R_f 0.50, 68.3% from N^{α}-Boc-1-methyl-L-tryptophan and ethyl *S*-methyl-D-cysteinate; **6d**, mp >250 °C, $[\alpha]_D$ +88.8° (*c* 0.17, Me_2SO), TLC R_f 0.43, 71.4% from N^{α}-Boc-1-methyl-D-tryptophan and ethyl *S*-methyl-D-cysteinate; **6e**, mp 229–231 °C, $[\alpha]_D$ +27.2° (*c* 0.16, Me_2SO), TLC R_f 0.43, 70.1% from N^{α}-Boc-1-methyl-D-tryptophan and ethyl D-methioninate; **6f**, mp 279–280 °C, $[\alpha]_D$ +9.3° (*c* 0.13, Me_2SO), TLC R_f 0.40, 20.3% from *dl-erythro-*N^{α}-Boc-1,β-dimethyltryptophan and ethyl D-methioninate; **6g**, mp 264–267 °C, $[\alpha]_D$ +104.2° (*c* 0.14, Me_2SO), TLC R_f 0.45, 25.5% from *dl-threo-*N^{α}-Boc-1,β-dimethyltryptophan and ethyl D-methioninate; **6h**, mp 265–268 °C, $[\alpha]_D$ −99.3° (*c* 0.14, Me_2SO), TLC R_f 0.36, 26.1% from *dl-erythro-N*-Boc-β-methyl(1-naphthyl)alanine and ethyl D-methioninate; **6i**, mp 290–293 °C, $[\alpha]_D$ +62.1° (*c* 0.10, Me_2SO), TLC R_f 0.50, 16.2% from *dl-threo-N*-Boc-β-methyl(1-naphthyl)alanine and ethyl D-methioninate.

(8) Separation of compounds **6** from the corresponding diastereoisomers **7** was performed at the stage of the intermediates **5** by silica gel chromatography ($CHCl_3$–MeOH): **7f**, mp 265–266 °C, TLC (see ref 7) R_f 0.45; **7g**, mp 217–218 °C, TLC R_f 0.50; **7h**, mp 290–292 °C, TLC R_f 0.51; **7i**, mp >250 °C, TLC R_f 0.55.

(9) The structural assignment of compounds **6f–i** was based on the ^{1}H NMR (Me_2SO-d_6) and TLC data. The 8-methylene protons of these cis compounds always resonated in a higher field region than those of the corresponding trans isomers **7f–i** (anisotropic effects of the indole and naphthalene nuclei): **6f**, δ 0.49 (m, 1 H), 1.00 (m, 1 H); **7f**, δ 1.82 (m, 2 H); **6g**, δ 1.11 (m, 1 H), 1.44 (m, 1 H); **7g**, δ 1.66 (m, 2 H); **6h**, δ 0.37 (m, 1 H), 1.02 (m, 1 H); **7h**, δ 2.74 (m, 2 H); **6i**, δ 1.30 (m, 1 H), 1.55 (m, 1 H); **7i**, δ 2.76 (m, 2 H). The cis isomers were all more polar on TLC than the corresponding trans isomers: see ref 7 and 8.

(10) Prepared by methylation (MeI/liquid NH_3) of *dl-erythro-* and *dl-threo-*β-methyltryptophans, followed by acylation with $(Boc)_2O$ (dioxane–H_2O), respectively. For the synthesis of β-methyltryptophan, see: Gould, S.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 707.

Scheme I



The corresponding naphthalene derivatives **6h** and **6i** were also similarly prepared[7-9] from *dl-erythro-* and *dl-threo-N*-Boc-β-methyl(1-naphthyl)alanines (**3e** and **3f**).[11]

Each of the compounds prepared above was evaluated in vitro for inhibition of PAF-induced rabbit platelet aggregation, and the results were given in comparison with those of **1**, CV3988,[2a] and L-652,731[2b] in Table I. Of the four structurally simplest stereoisomers **6a–d**, none were as potent as **1**, but **6d** did show a reasonable inhibitory activity, with an IC_{50} of 7.9×10^{-6} M, while the other diastereoisomers were considerably less potent or substantially inactive. It is notable that the D,D configuration (*R,S* in the *S*-methylcysteine and *R,R* in the methionine series, respectively) is indispensable for activity in this diketopiperazine series, and this stereochemistry is opposite to that observed in **1**.

Interestingly, the methionine derivative **6e** was more potent than **6d** (by ca. 3-fold). There is still further room for optimization of this part of the molecule, which is a prospective subject in this research program.

The introduction of the methyl group β to the tryptophan residue of **6e** exerted a remarkable influence on activity. Thus, the 7(*R*)-methyl derivative **6f** exhibited an increased potency over **6e** (by ca. 3-fold) and, on the contrary, the 7(*S*)-methyl counterpart **6g** showed a somewhat decreased potency.[12] This trend was also observed in the activity of the naphthalene derivatives. Indeed, the 7(*R*)-methyl compound **6h** showed a high potency, with an IC_{50} of 1.8×10^{-7} M (ca. 2-fold more potent than **1**), being the most active compound in the present study, while the 7(*S*)-methyl compound **6i** was considerably less potent than **6h**.[12] This data shows that the biological activity is also affected by the stereochemistry of the tertiary carbon (C-7) bonded to the aromatic nucleus.

In this study, we have found a new class of PAF inhibitors, the diketopiperazines, one member of which, **6h**, showed activity of the same order as or rather superior to that of natural product **1**[13] and the reference compounds (CV3988 and L-652,731). Compound **6h** showed no inhibitory activity in platelet aggregation induced by arachidonic acid and ADP (both $>2.3 \times 10^{-4}$ M), proving that it is a PAF-specific inhibitor. As pointed out above, the D,D stereochemistry of the diketopiperazine methine carbons is important for the biological activity in this series in contrast to the opposite stereochemistry of **1** at the corresponding asymmetric carbons. Further chemical variations of this new prototype for optimal PAF-inhibitory activity as well as more detailed biological evaluations of compound **6h** are in progress.

Acknowledgment. We are grateful to Dr. Keizo Yo-

(11) A mixture of *dl-erythro-* and *dl-threo-β*-methyl(1-naphthyl)-alanines, prepared from 1-acetylnaphthalene according to the method for the synthesis of β-ethyl(1-naphthyl)alanine, was converted to the *N*-Boc derivatives ($(Boc)_2O$/dioxane–H_2O) and separated by silica gel chromatography. For the synthesis of β-ethyl(1-naphthyl)alanine, see: Horner, L.; Schwahn, H. *Justus Liebigs. Ann. Chem.* **1955**, *591*, 99.

(12) Compounds **7f–i** were considerably less active than **6f–i** or substantially inactive: **7f**, $IC_{50} > 2.3 \times 10^{-4}$ M; **7g**, $IC_{50} > 2.3 \times 10^{-4}$ M; **7h**, $IC_{50} > 2.3 \times 10^{-4}$ M; **7i**, $IC_{50} = 1.8 \times 10^{-5}$ M.

(13) In an assay method measuring inhibition of PAF-induced increase of vascular permeability in guinea pigs, compounds **6f** and **6h** showed the following biological data (ip administration): **6f**, 1 mg/kg 34%, 10 mg/kg 30%; **6h**, 1 mg/kg 10%, 10 mg/kg 40% (**1**, 1 mg/kg 39%, 10 mg/kg 59%; CV3988, 10 mg/kg 26%; L-652,731, 10 mg/kg 26%).

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_1 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-

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