

Synthesis and Structure–Activity Relationship of Pyrazolo[3,4-*d*]pyrimidines: Potent and Selective Adenosine A₁ Receptor Antagonists

Sally-Ann Poulsen and Ronald J. Quinn*

Queensland Pharmaceutical Research Institute, Griffith University, Brisbane 4111, Australia

Received January 16, 1996[®]

A series of 12 substituted 1-phenylpyrazolo[3,4-*d*]pyrimidines were synthesized and evaluated for rat brain adenosine A₁ and A_{2a} receptor binding affinity. Substituents at C-4 and C-6 were varied in order to define these regions in terms of molecular recognition by the receptor subtypes. At C-4, the effects of a mercapto, methylthio, and amino substituent were evaluated, while at C-6, amides with varying alkyl groups extending from the α -carbon were examined. This study identified both potent and selective adenosine A₁ receptor antagonists. The most potent of the 12 compounds was α -[(4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl)thio]hexanamide (**14**); with an A₁ K_i of 0.939 nM and an A_{2a} K_i of 88.3 nM, this compound is 94-fold A₁ selective. The most selective of the 12 compounds was α -[[4-(methylthio)-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl]thio]hexanamide (**10**); with an A₁ K_i of 6.81 nM and an A_{2a} K_i > 40 000 nM, this compound is >5900-fold A₁ selective. The structure–activity relationships for the complete series has identified discrete structural differences between the A₁ and A_{2a} receptors with respect to the binding of pyrazolo[3,4-*d*]pyrimidines. This study resulted in prediction that increased A₁ affinity could be achieved by incorporation of NH-alkyl substituents at C-4. This was confirmed by synthesis of α -[[4-(methylamino)-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl]thio]hexanamide (**15**) which was found to have an A₁ K_i of 0.745 nM.

Introduction

Adenosine is an endogenous nucleoside which mediates a variety of important physiological responses by interaction with specific adenosine receptors.^{1–3} These receptors consist of seven transmembrane α -helices and are G-protein coupled.³ There are four major adenosine receptor subtypes which have been identified and cloned from various mammalian tissue types: the A₁, A_{2a}, A_{2b}, and A₃ subtypes.⁴ Despite the enormous therapeutic potential for drugs interacting with these adenosine receptor subtypes, new drugs which do so have not been forthcoming.^{1–3,5} This situation is partly a consequence of undesirable side effects due to the nonselectivity of potential drug candidates for a unique adenosine receptor subtype. Structure–activity studies leading to the development of potent and subtype selective agonists and antagonists of adenosine should allow delineation of the specific requirements for binding to the individual receptor subtypes. Such ligands would be valuable as ‘molecular tools’, probing the structural requirements of the individual receptor subtype binding sites.

4,6-Bis[(α -carbamoylthio)-1-phenylpyrazolo[3,4-*d*]pyrimidine (**1**) was identified as a novel adenosine A₁ receptor antagonist, antagonizing adenosine-stimulated cyclic adenosine monophosphate generation in guinea pig brain slices.^{6,7} It has a K_i of 370 $\pm$ 60 nM at the A₁ receptor and so is moderately potent. 1-Phenylpyrazolo[3,4-*d*]pyrimidine has been modified at C-4 with mercapto, methylthio, and amino substituents in order to probe for hydrogen-bonding sites and steric tolerance. At C-6, thioethers containing distal amides with varying branched and linear alkyl groups extending from the α -carbon were examined. This allowed exploration of the steric and hydrophobic tolerance of the A₁ and A_{2a} receptor subtypes. The structure–activity relationships

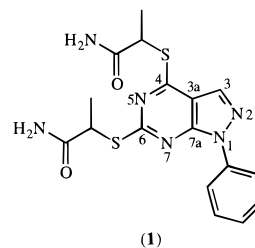


Figure 1. 4,6-Bis[(α -carbamoylthio)-1-phenylpyrazolo[3,4-*d*]pyrimidine, the lead compound for this study.

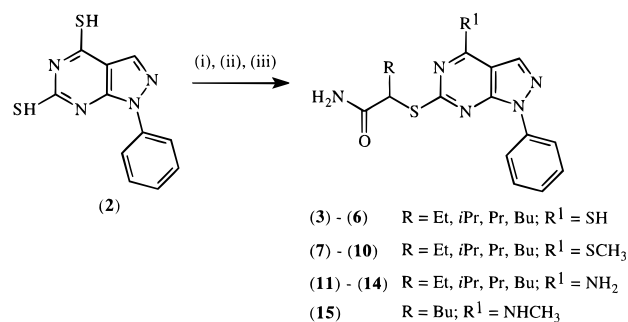
for these compounds have highlighted discrete differences in pyrazolo[3,4-*d*]pyrimidines with respect to the A₁ and A_{2a} receptor binding, thus having implications in the future design of more potent and more selective ligands.

Chemistry

The synthetic route to the target pyrazolo[3,4-*d*]pyrimidines is outlined in Scheme 1. Alkylation of 1-phenyl-5*H*,7*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-dithione (**2**) at the C-6 sulfur was achieved by stirring with a 1 mol equiv of bromoalkylamide in pyridine at room temperature (rt). The products (**3–6**) were collected by removal of solvent and recrystallized from Me₂SO and water. The bromoalkylamides were prepared from the bromo acid bromides or bromo acid chlorides by reaction with ammonia. The site of alkylation of the monoalkylated product, i.e., the sulfur at the C-4 or C-6 position, has previously been determined using NMR spectroscopy experiments to be at C-6.⁸

The C-4 methylthio series of compounds (**7–10**) were synthesized by alkylation of the corresponding mercapto series with iodomethane. A slight molar excess of iodomethane and the required monoalkylated mercapto compound were stirred in 1.5 M NaOH at room temperature. The reaction products precipitated from solu-

[®] Abstract published in *Advance ACS Abstracts*, September 1, 1996.

Scheme 1. Synthetic Route to 1-Phenyl-4,6-disubstituted-pyrazolo[3,4-*d*]pyrimidines^a

^a Reaction conditions: (i) BrCHRCONH₂, pyridine; (ii) CH₃I, NaOH (aq); (iii) NH₃ (g), EtOH, 110 °C or CH₃NH₂ (g), EtOH, 110 °C.

tion within 20 min, and the crude products were recrystallized from Me₂SO and water.

Aminolysis of the methylthio compounds to give compounds **11–14** was achieved by heating with ethanolic ammonia and to give compound **15** by heating with ethanolic methylamine to 110 °C in a pressure-sealed flask for 72 h. Workup of the reaction was achieved by either the addition of ice cold water to precipitate the products or removal of solvent. The crude products were recrystallized from Me₂SO and water.

Structural confirmation of target compounds was achieved by NMR spectroscopy. ¹H and ¹³C NMR spectra were assigned with the aid of DEPT (distortionless enhancement by polarization transfer), HMQC (heteronuclear multiple-quantum coherence), and HMBC (heteronuclear multiple-bond coherence) NMR experiments on a representative compound from each series.

Radioligand Binding

The adenosine A₁ and A_{2a} receptor binding affinities of the pyrazolo[3,4-*d*]pyrimidines were determined using standard radioligand binding assay procedures.^{9,10} Competitive binding assays against (R)-[³H]-N⁶-(phenylisopropyl)adenosine ([³H]-N⁶-PIA) using rat brain membranes for A₁ receptors and against [³H]-2-[[*p*-(2-carboxyethyl)phenethyl]amino]-5'-ethanecarboxamidoadenosine ([³H]CGS 21680) using rat striatal membranes for A_{2a} receptors were used to determine a full concentration–inhibition curve for each compound. Analysis of the results allowed calculation of an IC₅₀ and corresponding K_i value for each compound. These calculations used K_d values of 1 and 14.9 nM for [³H]-N⁶-PIA and [³H]CGS 21680, respectively, in the Cheng–Prusoff equation.¹¹ The results for the receptor binding assays are given in Table 1.

Results

The structure–activity analysis of the series of 12 initial pyrazolo[3,4-*d*]pyrimidines **3–14** showed that the C-4 and C-6 substituent effects were tightly linked. The overall activity and subtype selectivity of each compound reflected this interdependency and has enabled us to identify discrete differences in the A₁ and A_{2a} receptors in terms of binding of the pyrazolo[3,4-*d*]pyrimidines.

At the A₁ receptor the effects of the C-4 and C-6 substituents were additive in terms of biological activity. At C-4 the order of potency was consistently amino > methylthio > mercapto, while for the C-6 α-alkyl side

Table 1. Receptor Binding Results for 1-Phenyl-4,6-disubstituted pyrazolo[3,4-*d*]pyrimidines Binding to Rat A₁ and A_{2a} Receptors

compd	R ⁴	R ⁶	A ₁ K _i (nM) ^a	A _{2a} K _i (nM) ^b or % inhibtn (nM) ^c	A _{2a} K _i /A ₁ K _i
3	SH	Et	155 ± 5	8750 ± 1100	56
4	SH	<i>i</i> -Pr	256 ± 4	44% (20 000)	≥ 78
5	SH	Pr	56.3 ± 4.5	4450 ± 790	79
6	SH	Bu	37.2 ± 0.8	52% (10 000)	≥ 270
7	SCH ₃	Et	8.40 ± 0.32	796 ± 135	95
8	SCH ₃	<i>i</i> -Pr	15.7 ± 0.6	37% (20 000)	≥ 1300
9	SCH ₃	Pr	7.55 ± 2.32	4380 ± 410	580
10	SCH ₃	Bu	6.81 ± 0.61	12% (40 000)	> 5900
11	NH ₂	Et	1.56 ± 0.09	44.5 ± 12.6	29
12	NH ₂	<i>i</i> -Pr	2.73 ± 0.12	147 ± 17	54
13	NH ₂	Pr	1.08 ± 0.30	35.3 ± 2.2	33
14	NH ₂	Bu	0.939 ± 0.341	88.3 ± 3.8	94
15	NHCH ₃	Bu	0.745 ± 0.045	247 ± 42	332

^a A₁ receptor binding data utilizing competitive displacement of specific [³H]-N⁶-PIA binding from A₁ receptors in whole rat brain membranes. Data are the average of at least two independent experiments performed in duplicate and expressed as K_i ± SEM. K_d of [³H]-N⁶-PIA was 1 nM. ^b A_{2a} receptor binding data utilizing competitive displacement of specific [³H]CGS 21680 binding from A_{2a} receptors in rat striatal membranes. Data are the average of at least two independent experiments performed in duplicate and expressed as K_i ± SEM. K_d of [³H]CGS 21680 was 14.9 nM. ^c Lack of solubility at high concentrations precluded determination of IC₅₀.

chain the order was consistently α-butyl > α-propyl > α-ethyl > α-isopropyl. The most potent compound was **14** which combined amino at C-4 with a α-butyl side chain at C-6; it has an A₁ K_i of 0.939 nM. The least potent compound was **4**, combining mercapto at C-4 with an α-isopropyl side chain at C-6, and it has an A₁ K_i of 256 nM.

The structure–activity relationships exhibited by these compounds at the A_{2a} receptor are different from those exhibited at the A₁ receptor. At C-4 the order of potency was amino > methylthio > mercapto (as it was at the A₁ receptor) where the C-6 side chain is α-ethyl, α-isopropyl, and α-propyl. For the α-butyl side chain this relationship did not hold, and the order of potency was amino > mercapto > methylthio. The most potent A_{2a} compound was **13**, with amino at C-4 and a α-propyl side chain at C-6, and it has an A_{2a} K_i of 35.3 nM. The least potent A_{2a} compound was **10**, with methylthio at C-4 and a α-butyl side chain at C-6, and it has a A_{2a} K_i of > 40 000 nM.

At both receptor subtypes the amino substituent at C-4 is significantly more potent than the mercapto substituent for all α-alkyl side chains at C-6. At the A₁ receptor the increase in potency ranges from 40-fold for the α-butyl side chain at C-6 to 99-fold for the α-ethyl side chain at C-6. At the A_{2a} receptor the increase in potency is even more pronounced than at the A₁ receptor, increasing from 113-fold for the α-butyl side chain at C-6 to 197-fold for the α-ethyl side chain at C-6.

The methylthio substituent at C-4 is more potent than the mercapto substituent for all α-alkyl side chains at C-6 at the A₁ receptor and all but the α-butyl side chain

at the A_{2a} receptor. At the A₁ receptor the increase is 18.5-fold for α -ethyl, 16.3-fold for α -isopropyl, 7.5-fold for α -propyl, and 5.5-fold for α -butyl side chains at C-6. At the A_{2a} receptor the improvement in potency is 11.0-fold for the α -ethyl side chain; with the α -propyl and α -isopropyl side chains the potency does not increase significantly, while with the α -butyl side chain there is a >4-fold drop in potency.

Discussion

Considering the C-4 position, the increase, of up to 2 orders of magnitude, in affinity at both receptor subtypes of the amino substituent at C-4 compared to the mercapto substituent at C-4 indicates a favorable interaction between the amino substituent and the receptor binding sites. As the ability to form hydrogen bonds is the key difference between the amino and mercapto substituents, the superiority of the amino substituents potency most likely identifies a hydrogen-bonding interaction with the receptor binding sites. The structure–activity relationships between mercapto and methylthio indicate the existence of a hydrophobic pocket in both the A₁ and A_{2a} receptor binding sites which accommodates the methyl group of the C-4 methylthio substituent. The favorable occupancy of this pocket by the methyl group does however depend on the nature of the alkyl group on the C-6 substituent.

Considering the C-6 position, the structure–activity relationships exhibited by increasing the length of a linear carbon chain extending from the α -carbon of the amide substituent at C-6 provided information on steric and hydrophobic tolerance of the A₁ and A_{2a} receptor binding sites. The increase in length of the linear carbon side chain from two to three to four carbons was favorably tolerated at the A₁ receptor for each C-4 substituent. At the A_{2a} receptor the three-carbon α -propyl side chain was the favored group for mercapto and amino at C-4, while increasing beyond this to the four-carbon α -butyl side chain caused a reduction in potency of 2.2- and 2.5-fold, respectively. For methylthio at C-4 the two-carbon α -ethyl was the favored side chain, as the three-carbon α -propyl side chain led to a 5.5-fold reduction in potency and the four-carbon α -butyl side chain produced a further >9.1-fold reduction, in total a >50.2-fold decrease.

Comparison of the α -ethyl and α -isopropyl side chains showed that both the A₁ and A_{2a} receptor do not favorably tolerate the increase in steric bulk introduced by the β -methyl of the α -isopropyl substituent in place of the β -hydrogen of the α -ethyl substituent. The reduction in potency caused by introduction of the β -branching ranges from 1.7- to 1.9-fold at the A₁ receptor and from 2.3- to 25-fold at the A_{2a} receptor.

The interdependency of the C-4 and C-6 substituent effects in the structure–activity analysis of this series of pyrazolo[3,4-*d*]pyrimidines is clearly evident as it is a combination of the substituents that determines the overall affinity of the ligands. Tolerance of the various substituent changes is not the same for the A₁ and A_{2a} receptor subtypes, and this influences the subtype selectivity for each ligand. For the A₁ receptor an amino group is highly favored at C-4; however, there is also room to favorably accommodate the methyl group of the methylthio substituent, and hence it is likely that improved potency can be achieved by incorporation of

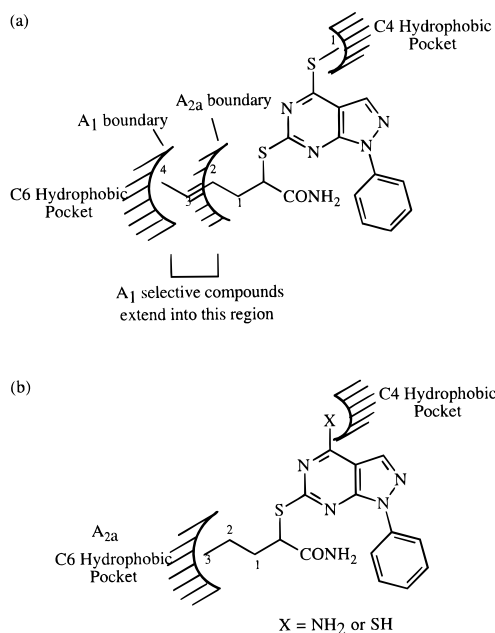


Figure 2. Hypothetical C-4 and C-6 hydrophobic pockets of adenosine A₁ and A_{2a} receptors. The combined C-4/C-6 hydrophobic pocket tolerance of alkyl carbons: (a) at the A₁ receptor, five, one at C-4 and four at C-6; at the A_{2a} receptor, three, one at C-4 and two at C-6 (for the methylthio substituent) or, alternatively, (b) at the A_{2a} receptor, three, zero at C-4 and three at C-6 (for the mercapto or amino substituent).

NH-alkyl substituents at C-4. The size of the hydrophobic pocket at the α -carbon of the C-6 substituent is sufficient to accommodate the four carbons of an α -butyl side chain. At the A_{2a} receptor the amino group is also highly favored at C-4; again there is a small hydrophobic pocket that can also accommodate the methyl of the methylthio substituent. The alkyl carbon chain length tolerated in the C-6 hydrophobic pocket is smaller than at the A₁ receptor. It can accommodate only three carbons for the amino and mercapto groups at C-4 and only two carbons for the C-4 methylthio series. The hydrophobic pockets identified at C-4 and C-6 appear not to be independent, and *together* they tolerate an overall total alkyl carbon number, Figure 2. At the A₁ receptor this is at least five carbons (one at C-4 and four at C-6), and at the A_{2a} receptor it is three carbons (zero at C-4 and three at C-6 or one at C-4 and two at C-6).

The steric tolerance of the A_{2a} receptor binding site has been pushed to the outer limit by the variable C-6 α -alkyl side chains, and going beyond this limit leads to A₁ selective compounds. The least selective compounds are those where the *total* alkyl carbon number is three or less (compounds **3–5**, **7**, **8**, and **11–13**) as these can bind favorably to either receptor subtype. The compounds with four or five *total* alkyl carbons (compounds **6**, **9**, **10**, and **14**) are the most A₁ selective as these bind favorably to the A₁ receptor and unfavorably to the A_{2a} receptor, Figure 2. The most selective compound was **10** with methylthio at C-4 and an α -butyl side chain at C-6, and it has a total of five alkyl carbons and is >5900-fold A₁ selective, i.e., 4 orders of magnitude selective. The least selective compound is **11** with amino at C-4 and an α -ethyl side chain at C-6, and it has only two alkyl carbons (and so is tolerated by both receptor subtypes) and is 29-fold A₁ selective. The amino substituent of **11** also contributes to this low selectivity. This substituent is highly favored at both

receptor subtypes but more so at the A_{2a} receptor (discussed above), and hence the A_{2a}/A₁ selectivity ratio becomes even less. This is true for all the compounds with amino at C-4: They exhibit lower selectivity than the corresponding compounds with mercapto or methylthio at C-4.

The structure–activity relationships exhibited by **3–14** and the combined C-4/C-6 hydrophobic tolerance proposed in Figure 2 suggested that **15**, with methylamino at C-4 and α -butyl side chain at C-6, would exhibit improved A₁ receptor affinity. The basis for introduction of a methylamino substituent at C-4 in **15** was the identification of both a hydrogen-bonding interaction and a small hydrophobic pocket within the A₁ receptor binding site. The combined C-4/C-6 hydrophobic pocket tolerance at the A₁ receptor has been proposed as five alkyl carbons (Figure 2), and the α -butyl side chain at C-6 combined with the methylamino at C-4 would conform to this requirement, thereby providing an additional test for the proposal of Figure 2. The corresponding compounds **10** and **14** with an α -butyl side chain at C-6 were the most potent compounds at the A₁ receptor in the C-4 methylthio and C-4 amino series, respectively. Compound **15** with a methylamino at C-4 and an α -butyl side chain at C-6 was synthesized and evaluated.¹² **15** has an A₁ K_i of 0.745 nM compared to an A₁ K_i of 6.81 nM for the corresponding C-4 methylthio compound **10** and an A₁ K_i of 0.939 nM for the corresponding C-4 amino compound **14**. Consistent with Figure 2, **15** had decreased A_{2a} affinity and was 332-fold A₁ selective. Both the increased A₁ affinity and the increased A₁ selectivity provide further evidence for the combined C-4/C-6 hydrophobic pocket tolerance (Figure 2).

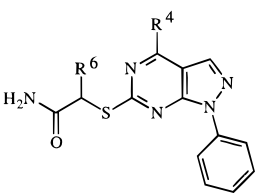
Conclusions

This study has identified new compounds which are highly potent (subnanomolar, e.g., **14** and **15**) and highly selective (4 orders of magnitude A₁ selective, e.g., **10**) for adenosine A₁ receptors over adenosine A_{2a} receptors. These new compounds were generated by the successful optimization of the potency and selectivity exhibited by the lead compound **1**. The structure–activity relationships for the complete series of compounds has highlighted clear differences with respect to the A₁ and A_{2a} receptor binding of substituted pyrazolo[3,4-d]pyrimidines. In this context, the compounds of this study have proved valuable in probing discrete structural differences in the A₁ and A_{2a} receptor binding sites. In particular, the differing steric tolerance of the hydrophobic pocket identified at C-6 and the hydrogen-bonding requirements of the C-4 substituent have potential for exploitation in the future design of more potent and more selective pyrazolo[3,4-d]pyrimidines, allowing a greater understanding of the structural parameters of the A₁ and A_{2a} receptor subtype binding sites.

Experimental Section

All chemicals were obtained from Aldrich. Cylinders of anhydrous ammonia were obtained from BOC gases. Dry ethanol was prepared by refluxing distilled ethanol over magnesium turnings and a catalytic quantity of iodine followed by distillation and storage over 4 Å molecular sieves. Dry pyridine was prepared by refluxing over sodium and storage over potassium hydroxide. Dry hexane was prepared by

Table 2. Chemical Data for Compounds **3–14**



compd	R ⁴	R ⁶	yield (%)	mp (°C)	formula	anal.
3	SH	Et	73	233–251 dec	C ₁₅ H ₁₅ N ₅ OS ₂	C, H, N, S
4	SH	<i>i</i> -Pr	69	221–240 dec	C ₁₆ H ₁₇ N ₅ OS ₂	C, H, S; N ^a
5	SH	Pr	91	222–232 dec	C ₁₆ H ₁₇ N ₅ OS ₂	C, H; N ^b
6	SH	Bu	80	221–241 dec	C ₁₇ H ₁₉ N ₅ OS ₂	C, H, S; N ^c
7	SCH ₃	Et	86	223–224	C ₁₆ H ₁₇ N ₅ OS ₂	C, H, N, S
8	SCH ₃	<i>i</i> -Pr	67	227–228	C ₁₇ H ₁₉ N ₅ OS ₂	C, H, N, S
9	SCH ₃	Pr	69	222–224	C ₁₇ H ₁₉ N ₅ OS ₂	C, H; N ^d
10	SCH ₃	Bu	59	219–221	C ₁₈ H ₂₁ N ₅ OS ₂	C, H, N, S
11	NH ₂	Et	69	250–258 dec	C ₁₅ H ₁₆ N ₆ OS	C, H, S; N ^e
12	NH ₂	<i>i</i> -Pr	70	240–254 dec	C ₁₆ H ₁₈ N ₆ OS	C, H, S; N ^f
13	NH ₂	Pr	70	235–244 dec	C ₁₆ H ₁₈ N ₆ OS	C, H, N
14	NH ₂	Bu	85	234–247 dec	C ₁₇ H ₂₀ N ₆ OS	C, H, N, S
15	NHCH ₃	Bu	74	198–199	C ₁₈ H ₂₂ N ₆ OS	C, H, S; N ^g

^a N: calcd, 19.49; found, 18.9. ^b N: calcd, 19.49; found, 18.8. ^c N: calcd, 18.75; found, 18.0. ^d N: calcd, 18.75; found, 18.1. ^e N: calcd, 25.59; found, 25.0. ^f N: calcd, 24.55; found, 23.6. ^g N: calcd, 22.69; found, 21.3.

refluxing over sodium and storage under nitrogen. Dry DMF was prepared by stirring over barium oxide, under an atmosphere of nitrogen for 24 h, followed by high vacuum distillation and storage over 4 Å molecular sieves. All other solvents were analytical grade or distilled prior to use.

Melting points were determined on a Gallenkamp digital melting point apparatus and are uncorrected. ¹H, ¹³C, and DEPT NMR spectra were recorded using a Bruker WM250 or Varian Gemini-200 spectrometer. HMBC and HMQC experiments were recorded with a Varian Unity-400 spectrometer. IR spectra were recorded on a Perkin Elmer FTIR instrument, with solid samples examined in KBr disks. Solvents were removed under reduced pressure with a Buchi rotary evaporator, DMF and Me₂SO removal requiring high-vacuum apparatus.

Microanalytical data were obtained from the Australian Microanalytical Service. Compound purity was ascertained by NMR, TLC, and electrospray mass spectrometry. High-resolution mass spectra were obtained from the Organic Mass Spectrometry Facility, University of Tasmania, Hobart, Tasmania, Australia.

1-Phenyl-5H,7H-pyrazolo[3,4-d]pyrimidine-4,6-dithione (**2**) was prepared by known methods.⁸ A three-step procedure involving synthesis of (ethoxymethylene)malononitrile and its reaction with phenylhydrazine to give 5-amino-4-cyano-1-phenylpyrazole followed by cyclization of this with potassium O-ethylxanthogenate afforded **2**.

α -(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]butanamide (3**). Method A:** To a solution of **2** (0.500 g, 1.92 mmol) in dry pyridine (10 mL) was added 1 mol equiv of 2-bromobutanamide (0.318 g, 1.92 mmol) in small amounts over 20 min. The reaction mixture was stirred at room temperature, and after 2 h a cream solid precipitated. Solvent was removed from the reaction mixture under reduced pressure; the cream solid that remained was collected by suction filtration and washed with ice cold water. This crude product was refluxed in ethanol and filtered while hot to remove unreacted starting material, leaving a white solid. Recrystallization of this solid from Me₂SO and water afforded pure **3** as a white solid (yield 73%): mp 233–251 °C dec; ¹H NMR (200 MHz, Me₂SO-*d*₆) δ 0.97 (t, 3H, *J* = 7.2 Hz, CH₃), 1.95 (m, 2H, CH₂), 4.32 (t, 1H, *J* = 7.2 Hz, CH), 7.42 (br s, 1H, NH), 7.43 (t, 1H, *J* = 7.6 Hz, H-4'), 7.55 (dd, 2H, *J* = 7.6 Hz, H-3', H-5'), 7.89 (br s, 1H, NH), 8.05 (d, 2H, *J* = 7.6 Hz, H-2', H-6'), 8.34 (s, 1H, H-3), 14.10 (br s, 1H, SH); ¹³C NMR (50 MHz, Me₂SO-*d*₆) δ 11.6 (CH₃), 25.8 (CH₂), 50.8 (CH), 116.4

(C-3a), 121.3 (C-2', C-6'), 127.2 (C-4'), 129.4 (C-3', C-5'), 138.0 (C-1'), 138.2 (C-3), 146.5 (C-7a), 160.3 (C-6), 171.0 (C=O), 180.2 (C-4); IR (KBr disk) 3393, 3289 (primary amide NH), 1657 (C=O), 1593 cm^{-1} (C=C).

α -[(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thiol]- β -methylbutanamide (4). Method A was used to prepare **4** (yield 69%): mp 221–239 °C dec; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 1.02 (d, 3H, J = 6.8 Hz, CH_3), 1.03 (d, 3H, J = 6.8 Hz, CH_3), 2.33 (m, 1H, βCH), 4.38 (d, 1H, J = 5.6 Hz, CH), 7.38 (br s, 1H, NH), 7.40 (t, 1H, J = 7.6 Hz, H-4'), 7.53 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.80 (br s, 1H, NH), 8.07 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.34 (s, 1H, H-3), 14.10 (br s, 1H, SH); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 19.7 (CH_3), 19.7 (CH_3), 30.5 (βCH), 56.0 (CH), 116.4 (C-3a), 121.3 (C-2', C-6'), 127.1 (C-4'), 129.4 (C-3', C-5'), 138.0 (C-1'), 138.2 (C-3), 146.4 (C-7a), 160.5 (C-6), 170.7 (C=O), 180.0 (C-4); IR (KBr disk) 3392, 3171 (primary amide NH); 1657 (C=O); 1573 cm^{-1} (C=C); HRMS (chemical ionization, CI) 360.0952, calcd for $\text{C}_{16}\text{H}_{17}\text{N}_5\text{OS}_2\text{H}^+$ 360.0953.

α -[(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thiol]pentanamide (5). Method A was used to prepare **5** (yield 91%): mp 222–232 °C dec; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.92 (t, 3H, J = 7.2 Hz, CH_3), 1.41 (m, 2H, γCH_2), 1.94 (m, 2H, βCH_2), 4.40 (t, 1H, J = 7.2 Hz, CH), 7.37 (br s, 1H, NH), 7.43 (t, 1H, J = 7.6 Hz, H-4'), 7.53 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.87 (br s, 1H, NH), 8.09 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.36 (s, 1H, H-3), 14.10 (br s, 1H, SH); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 13.7 (CH_3), 20.3 (γCH_2), 34.6 (βCH_2), 49.2 (CH), 116.4 (C-3a), 121.4 (C-2', C-6'), 127.2 (C-4'), 129.3 (C-3', C-5'), 138.0 (C-1'), 138.2 (C-3), 146.4 (C-7a), 160.2 (C-6), 171.0 (C=O), 180.0 (C-4); IR (KBr disk) 3386, 3177 (primary amide NH), 1657 (C=O), 1572 cm^{-1} (C=C); HRMS (CI) 360.0945, calcd for $\text{C}_{16}\text{H}_{17}\text{N}_5\text{OS}_2\text{H}^+$ 360.0953.

α -[(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thiol]hexanamide (6). Method A was used to prepare **6** (yield 80%): mp 221–241 °C dec; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.85 (t, 3H, J = 6.7 Hz, CH_3), 1.33 (m, 4H, $\delta\text{CH}_2\gamma\text{CH}_2$), 1.94 (m, 2H, βCH_2), 4.40 (t, 1H, J = 7.2 Hz, CH), 7.38 (br s, 1H, NH), 7.44 (t, 1H, J = 7.6 Hz, H-4'), 7.57 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.89 (br s, 1H, NH), 8.09 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.37 (s, 1H, H-3), 14.10 (br s, 1H, SH); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 13.6 (CH_3), 21.8 (δCH_2), 29.0 (γCH_2), 32.2 (βCH_2), 49.3 (CH), 116.4 (C-3a), 121.4 (C-2', C-6'), 127.2 (C-4'), 129.3 (C-3', C-5'), 138.0 (C-1'), 138.2 (C-3), 146.5 (C-7a), 160.3 (C-6), 171.2 (C=O), 181.0 (C-4); IR (KBr disk) 3381, 3164 (primary amide NH), 1658 (C=O), 1570 cm^{-1} (C=C); HRMS (CI) 374.1106, calcd for $\text{C}_{17}\text{H}_{19}\text{N}_5\text{OS}_2\text{H}^+$ 374.1109.

α -[(4-(Methylthio)-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]butanamide (7). Method B: Compound **3** (0.152 g, 0.44 mmol) was dissolved in 1.5 M NaOH (10 mL). A 1.5 mol excess of iodomethane (0.094 g, 0.66 mmol) was added and the reaction mixture stirred at room temperature. After 10–20 min a fine white precipitate had formed. The reaction mixture was left stirring for 1 h. The precipitate was collected by suction filtration and recrystallized from Me_2SO and water, producing fine white needles of pure **7** (yield 86%): mp 223–224 °C; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 1.00 (t, 3H, J = 7.2 Hz, CH_3), 1.95 (m, 2H, CH_2), 2.68 (s, 3H, SCH_3), 4.36 (t, 1H, J = 6.9 Hz, CH), 7.26 (br s, 1H, NH), 7.38 (t, 1H, J = 7.6 Hz, H-4'), 7.56 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.79 (br s, 1H, NH), 8.14 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.49 (s, 1H, H-3); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 11.7 (SCH_3 or CH_3), 11.8 (SCH_3 or CH_3), 25.9 (CH_2), 50.5 (CH), 110.5 (C-3a), 120.9 (C-2', C-6'), 126.8 (C-4'), 129.4 (C-3', C-5'), 133.9 (C-3), 138.2 (C-1'), 150.9 (C-7a), 165.6 (C-4), 168.1 (C-6), 171.7 (C=O); IR (KBr disk) 3346, 3166 (primary amide NH), 1687 (C=O), 1593 cm^{-1} (C=C).

β -Methyl- α -[(4-(methylthio)-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]butanamide (8). Method B was used to prepare **8** (yield 67%): mp 227–228 °C; ^1H NMR (250 MHz, $\text{Me}_2\text{SO}-d_6$) δ 1.04 (d, 3H, J = 7.0 Hz, CH_3), 1.07 (t, 3H, J = 7.0 Hz, CH_3), 2.31 (m, 1H, βCH), 2.70 (s, 3H, SCH_3), 4.41 (d, 1H, J = 5.8 Hz, CH), 7.25 (br s, 1H, NH), 7.38 (t, 1H, J = 7.6 Hz, H-4'), 7.57 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.70 (br s, 1H, NH), 8.16 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.48 (s, 1H, H-3); ^{13}C NMR (62.8 MHz, $\text{Me}_2\text{SO}-d_6$) δ 11.6 (SCH_3), 19.6 (CH_3), 20.1

(CH_3), 30.5 (CH_2), 55.8 (CH), 110.5 (C-3a), 120.8 (C-2', C-6'), 126.7 (C-4'), 129.4 (C-3', C-5'), 133.8 (C-3), 138.3 (C-1'), 150.9 (C-7a), 165.6 (C-4), 168.5 (C-6), 171.6 (C=O); IR (KBr disk) 3376, 3180 (primary amide NH), 1682 (C=O), 1596 cm^{-1} (C=C).

α -[(4-(Methylthio)-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]pentanamide (9). Method B was used to prepare **9** (yield 69%): mp 222–224 °C; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.94 (t, 3H, J = 7.2 Hz, CH_3), 1.45 (m, 2H, γCH_2), 1.93 (m, 2H, βCH_2), 2.73 (s, 3H, SCH_3), 4.45 (t, 1H, J = 7.0 Hz, CH), 7.25 (br s, 1H, NH), 7.42 (t, 1H, J = 7.6 Hz, H-4'), 7.59 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.78 (br s, 1H, NH), 8.18 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.53 (s, 1H, H-3); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 11.7 (SCH_3), 13.8 (CH_3), 20.4 (γCH_2), 34.7 (βCH_2), 48.9 (CH), 110.5 (C-3a), 120.9 (C-2', C-6'), 126.8 (C-4'), 129.4 (C-3', C-5'), 133.9 (C-3), 138.2 (C-1'), 151.0 (C-7a), 165.6 (C-4), 168.1 (C-6), 171.8 (C=O); IR (KBr disk) 3355, 3166 (primary amide NH), 1687 (C=O), 1593 cm^{-1} (C=C); HRMS (electron impact, EI) 373.1040, calcd for $\text{C}_{17}\text{H}_{19}\text{N}_5\text{OS}_2$ 373.1031.

α -[(4-(Methylthio)-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]hexanamide (10). Method B was used to prepare **10** (yield 59%): mp 219–221 °C; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.87 (t, 3H, J = 6.9 Hz, CH_3), 1.26 (m, 4H, $\delta\text{CH}_2\gamma\text{CH}_2$), 1.93 (m, 2H, βCH_2), 2.72 (s, 3H, SCH_3), 4.43 (t, 1H, J = 7.0 Hz, CH), 7.25 (br s, 1H, NH), 7.42 (t, 1H, J = 7.6 Hz, H-4'), 7.59 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.78 (br s, 1H, NH), 8.18 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.53 (s, 1H, H-3); ^{13}C NMR (62.8 MHz, $\text{Me}_2\text{SO}-d_6$) δ 11.5 (SCH_3), 13.7 (CH_3), 21.9 (δCH_2), 29.2 (γCH_2), 32.2 (βCH_2), 49.0 (CH), 110.5 (C-3a), 120.9 (C-2', C-6'), 126.8 (C-4'), 129.4 (C-3', C-5'), 133.9 (C-3), 138.3 (C-1'), 151.0 (C-7a), 165.8 (C-4), 168.2 (C-6), 172.0 (C=O); IR (KBr disk) 3383, 3197 (primary amide NH), 1669 (C=O), 1619 cm^{-1} (C=C).

α -[(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]butanamide (11). Method C: Compound **7** (0.150 g, 0.46 mmol) was added to 20 mL of ethanolic ammonia, prepared by saturating ethanol at 0 °C with ammonia gas. The solution was placed in a bomb, sealed, and heated in an oil bath preheated at 110 °C for 72 h. The reaction product precipitated on cooling; ice cold water was added to the bomb to aid any further precipitation of product. The crude product was collected by suction filtration and recrystallized from ethanol and water (yield 69%): mp 250–258 °C dec; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 1.01 (t, 3H, J = 7.2 Hz, CH_3), 1.94 (m, 2H, CH_2), 4.26 (t, 1H, J = 7.0 Hz, CH), 7.19 (br s, 1H, NH), 7.35 (t, 1H, J = 7.6 Hz, H-4'), 7.55 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.68 (br s, 1H, NH), 7.95 (br s, 1H, NHamine), 8.06 (br s, 1H, NHamine), 8.22 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.28 (s, 1H, H-3); ^{13}C NMR (62.8 MHz, $\text{Me}_2\text{SO}-d_6$) δ 11.8 (CH_3), 25.7 (CH_2), 49.5 (CH), 99.4 (C-3a), 120.4 (C-2', C-6'), 126.0 (C-4'), 129.2 (C-3', C-5'), 134.4 (C-3), 139.0 (C-1'), 153.6 (C-7a), 157.5 (C-4), 169.0 (C-6), 172.3 (C=O); IR (KBr disk) 3490 (NH); 3379, 3171 (primary amide NH), 1652 (C=O), 1588 cm^{-1} (C=C); HRMS (EI) 328.1106, calcd for $\text{C}_{15}\text{H}_{16}\text{N}_6\text{OS}$ 328.1106.

α -[(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]- β -methylbutanamide (12). Method C was used to prepare **12** (yield 70%): mp 240–254 °C dec; ^1H NMR (250 MHz, $\text{Me}_2\text{SO}-d_6$) δ 1.03 (d, 6H, J = 6.5 Hz, $2 \times \text{CH}_3$), 2.23 (m, 1H, βCH), 4.27 (d, 1H, J = 6.7 Hz, CH), 7.15 (br s, 1H, NH), 7.32 (t, 1H, J = 7.6 Hz, H-4'), 7.52 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.56 (br s, 1H, NH), 7.92 (br s, 1H, NHamine), 8.08 (br s, 1H, NHamine), 8.20 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.25 (s, 1H, H-3); ^{13}C NMR (62.8 MHz, $\text{Me}_2\text{SO}-d_6$) δ 19.8 (CH_3), 20.0 (CH_3), 30.2 (CH), 54.8 (CH), 99.4 (C-3a), 120.4 (C-2', C-6'), 126.0 (C-4'), 129.2 (C-3', C-5'), 134.3 (C-3), 139.0 (C-1'), 153.6 (C-7a), 157.4 (C-4), 169.2 (C-6), 172.2 (C=O); IR (KBr disk) 3494 (NH), 3383, 3167 (primary amide NH), 1669 (C=O), 1594 cm^{-1} (C=C); HRMS (EI) 342.1276, calcd for $\text{H}_{18}\text{N}_6\text{OS}$ 342.1263.

α -[(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]pentanamide (13). Method C was used to prepare **13** (yield 70%): mp 235–244 °C dec; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.94 (t, 3H, J = 7.2 Hz, CH_3), 1.42 (m, 2H, γCH_2), 1.87 (m, 2H, βCH_2), 4.33 (t, 1H, J = 7.2 Hz, CH), 7.16 (br s, 1H, NH), 7.35 (t, 1H, J = 7.6 Hz, H-4'), 7.54 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.65 (br s, 1H, NH), 7.93 (br s, 1H, NHamine), 8.02 (br s, 1H, NHamine), 8.21 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.29

(s, 1H, H-3); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 13.8 (CH_3), 20.5 (βCH_2), 34.8 (γCH_2), 47.9 (CH), 99.4 (C-3a), 120.4 (C-2', C-6'), 126.1 (C-4'), 129.1 (C-3', C-5'), 134.4 (C-3), 139.0 (C-1'), 153.6 (C-7a), 157.4 (C-4), 168.9 (C-6), 172.4 (C=O); IR (KBr disk) 3492 (NH), 3388, 3180 (primary amide NH), 1651 (C=O), 1586 cm^{-1} (C=C).

α -[(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl)thio]hexanamide (14). Method C was used to prepare **14** (yield 85%): mp 234–247 °C dec; ^1H NMR (250 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.84 (t, 3H, J = 6.7 Hz, CH_3), 1.31 (m, 4H, $\delta\text{CH}_2\gamma\text{CH}_2$), 1.86 (m, 2H, βCH_2), 4.29 (t, 1H, J = 7.1 Hz, CH), 7.16 (br s, 1H, NH), 7.33 (t, 1H, J = 7.6 Hz, H-4'), 7.51 (dd, 2H, J = 7.6 Hz, H-3', H-5'), 7.67 (br s, 1H, NH), 7.92 (br s, 1H, NHamine), 8.07 (br s, 1H, NHamine), 8.18 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.26 (s, 1H, H-3); ^{13}C NMR (62.8 MHz, $\text{Me}_2\text{SO}-d_6$) δ 13.8 (CH_3), 21.9 (βCH_2), 29.3 (γCH_2), 32.3 (δCH_2), 48.0 (CH), 99.4 (C-3a), 120.5 (C-2', C-6'), 126.1 (C-4'), 129.2 (C-3', C-5'), 134.4 (C-3), 139.0 (C-1'), 153.7 (C-7a), 157.6 (C-4), 169.0 (C-6), 172.5 (C=O); IR (KBr disk) 3464 (NH), 3352, 3158 (primary amide NH), 1668 (C=O), 1587 cm^{-1} (C=C).

α -[[4-(Methylamino)-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl]thio]hexanamide (15). α -[[4-(Methylthio)-1-phenylpyrazolo[3,4-d]pyrimidin-6-yl]thio]hexanamide (**10**) (0.190 g, 0.490 mmol) was added to ethanolic methylamine (15 mL, prepared by saturating ethanol at 0 °C with methylamine gas). The solution was placed in a bomb, sealed, and heated in an oil bath at 110 °C. After 72 h the bomb was cooled to 0 °C. The product precipitated on cooling, ice cold water was added, and the crude product was collected and recrystallized from Me_2SO and water affording pure **15** as a white solid (yield 74%): mp 198.3–199.3 °C dec; ^1H NMR (200 MHz, $\text{Me}_2\text{SO}-d_6$) δ 0.87 (t, 3H, J = 7.2 Hz, CH_3), 1.37 (m, 4H, $\delta\text{CH}_2\gamma\text{CH}_2$), 1.90 (m, 2H, βCH_2), 3.00 (d, 3H, J = 4.6 Hz, NHCH_3), 4.36 (t, 1H, J = 7.2 Hz, CH), 7.16 (br s, 1H, NH), 7.35 (t, 1H, J = 7.6 Hz, H-4'), 7.54 (dd, 2H, J = 7.6, 7.6 Hz, H-3', H-5'), 7.68 (br s, 1H, NH), 8.20 (d, 2H, J = 7.6 Hz, H-2', H-6'), 8.27 (s, 1H, H-3), 8.54 (q, 1H, J = 4.6 Hz, NHCH_3); ^{13}C NMR (50 MHz, $\text{Me}_2\text{SO}-d_6$) δ 14.0 (CH_3), 22.1 (δCH_2), 27.0 (NCH_3), 29.5 (γCH_2), 32.6 (βCH_2), 48.5 (CH), 99.8 (C-3a), 120.4 (C-2', C-6'), 126.1 (C-4'), 129.1 (C-3', C-5'), 133.8 (C-3), 138.9 (C-1'), 153.0 (C-7a), 156.0 (C-4), 168.9 (C-6), 172.4 (C=O); IR (KBr disk) 3300 (NH), 3379, 3180 (primary amide NH), 1670 (C=O), 1598 cm^{-1} (C=C); HRMS (EI) 370.1567, calcd for $\text{C}_{18}\text{H}_{22}\text{N}_6\text{OS}$ 370.1576. Anal. Calcd ($\text{C}_{18}\text{H}_{22}\text{N}_6\text{OS}$): C, 58.35; H, 6.00; N, 22.69; S, 8.65. Found: C, 58.7; H, 6.0; N, 21.3; S, 8.8.

Adenosine A_1 Receptor Binding Assay. Compounds were assessed for their ability to inhibit binding of the A_1 selective agonist radioligand [^3H]- N^6 -PIA to membranes from rat whole brain using a literature procedure modified for automation. Receptor binding assays were carried out in 96-well microtiter plates in an assay volume of 200 μL . Each assay contained membrane (100 μg , pretreated with 0.1 U/mg adenosine deaminase for 10 min at 37 °C), 1 nM (R)-[^3H]- N^6 -PIA (Amersham; 61 Ci/mmol), incubation buffer (50 mM Tris-HCl, pH 7.4), and test compound (at least 12 concentrations) in Me_2SO giving a final Me_2SO concentration of 1%, 2%, or 5%. Both 1% and 2% Me_2SO did not decrease control binding, while 5% Me_2SO decreased control binding by 17%. Nonspecific binding was determined in the presence of 10 μM 2-chloroadenosine. The assay was incubated for 90 min at 25 °C and then filtered using a cell harvester (Tomtec Harvester 96) onto untreated glass fiber B filtermats. All assays were performed a minimum of two times with duplicate determinations. Known compounds were used to characterize this assay procedure. Data were fitted to optimized models by nonlinear regression analysis (Graphpad Inplot IV, San Diego, CA).

Analysis of the results allowed calculation of an IC_{50} and corresponding K_i value for each compound using a K_d value of 1 nM for [^3H]- N^6 -PIA and the Cheng-Prusoff equation.

Adenosine A_{2a} Receptor Binding Assay. Compounds were assessed for their ability to inhibit the binding of the A_{2a} agonist radioligand [^3H]CGS 21680 to rat striatal membranes. The assay volume was 250 μL . Each assay contained striatal membrane (150 μg , pretreated as above), 5 nM [^3H]CGS 21680 (New England Nuclear; 48.6 Ci/mmol), incubation buffer (50 mM Tris-HCl, 10 mM MgCl_2 , pH 7.4), and test compound (at least 12 concentrations) in Me_2SO giving a final Me_2SO concentration of 1%, 2%, or 5%. Both 1% and 2% Me_2SO decreased control binding by 9%, while 5% Me_2SO decreased control binding by 24%. Nonspecific binding was determined in the presence of 20 μM 2-chloroadenosine. The assay was incubated for 120 min at 25 °C. Subsequent harvesting of the assay was identical with the A_1 assay. Results from concentration-response curves were analyzed with Graphpad Inplot IV, as above. Analysis of the results allowed calculation of an IC_{50} and corresponding K_i value for each compound using a K_d value of 14.9 nM for [^3H]CGS 21680 and the Cheng-Prusoff equation.

Acknowledgment. Support of this work by the National Health and Medical Research Council and the bequest of the late Thomas Patrick Hinch is gratefully acknowledged. We acknowledge the award of an Australian Postgraduate Award to S.P. We thank Roger Moni for advice on radioligand binding assays.

References

- (1) Müller, C. E.; Scior, T. Adenosine receptors and their modulators. *Pharm. Acta Helv.* **1993**, *68*, 77–111.
- (2) Collis, M. G.; Hourani, S. M. O. Adenosine receptor subtypes. *TIPS* **1993**, *14*, 360–366.
- (3) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine receptors: pharmacology, structure-activity relationships, and therapeutic potential. *J. Med. Chem.* **1992**, *35*, 407–422.
- (4) Jacobson, M. Cloning and expression of human adenosine receptor subtypes. In *Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology*; Bellardinelli, L., Pelleg, A., Eds.; Kluwer: Norwell, MA, 1995; pp 5–13.
- (5) Erfurth, A.; Schmauss, M. Treating neuropsychiatric disorders with adenosinergic drugs-new perspective. *Fortschr. Neurol., Psychiatr.* **1995**, *63*, 93–98.
- (6) Davies, L. P.; Brown, D. J.; Chow, S. C.; Johnston, G. A. R. Pyrazolo [3,4-d]pyrimidines, a new class of adenosine antagonists. *Neurosci. Lett.* **1983**, *41*, 189–193.
- (7) Davies, L. P.; Chow, S. C.; Skerritt, J. H.; Brown, D. J.; Johnston, G. A. R. Pyrazolo[3,4-d]pyrimidines as adenosine antagonists. *Life Sci.* **1984**, *34*, 2117–2128.
- (8) Quinn, R. J.; Scammells, P. J.; Tucker, D. J. Mono- α -carbamoylthio-substituted pyrazolo[3,4-d]pyrimidines: the position of substitution. *Aust. J. Chem.* **1991**, *44*, 753–757.
- (9) Schwabe, U.; Trost, T. Characterization of adenosine receptors in rat brain by (-)[^3H] N^6 -phenylisopropyladenosine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *313*, 179–188.
- (10) Jarvis, M. F.; Schulz, R.; Hutchison, A. J.; Do, U. H.; Sills, M. A.; Williams, M. [^3H]CGS21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888–893.
- (11) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibition which causes 50 percent inhibition (IC_{50}) of enzyme reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
- (12) Poulsen, S. A.; Quinn, R. J. Pyrazolo[3,4-d]pyrimidines: C4, C6 Substitution leads to Adenosine A_1 receptor selectivity. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 357–360.

JM960052S