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# 1-Phenylpyrazolo[3,4-*d*]pyrimidines as Adenosine Antagonists: the Effects of Substituents at C4 and C6

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**Abstract**—Forty-two 1-phenyl-pyrazolo[3,4-*d*]pyrimidines substituted at C6 with thioethers containing distal amide substituents and substituted at C4 with thiol, thiomethyl or amino were synthesized and tested for adenosine $A_1$ and $A_{2a}$ receptor binding. Compared with a thiol at C4, both *S*-methylation and conversion to an amino resulted in increased affinity at both receptors with the C4 amino compounds having the highest affinity. The C-4 region of the receptor consists of an alkyl pocket containing a hydrogen-bonding site. The study established that for high affinity at both the $A_1$ and $A_{2a}$ adenosine receptors the distal amide should be separated from the C6 thiol by only one carbon. In this study, 2′-(4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-ethanamide (**4b**) had the highest affinity at the $A_1$ receptor with a $K_i$ of 12.1 nM while 2′-(4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)ethanamide (**4a**) had the highest affinty at the $A_{2a}$ receptor with a $K_i$ of 44.9 nM. 

## Introduction

Adenosine and many of its analogues exert physiological responses via G protein-coupled receptors, consisting of seven transmembrane helices.[1,2] Adenosine receptors can act through effectors other than adenylate cyclase, including potassium channels, calcium channels, phospholipase $A_2$ or C and guanylate cyclase. There are four major subtypes of adenosine receptors, $A_1$, $A_{2a}$, $A_{2b}$, $A_3$. Pyrazolo[3,4-*d*]pyrimidines were originally identified as adenosine $A_1$ antagonists during a study of a large number of nitrogen heterocycles related to caffeine and theophylline. 4,6-Bis-α-carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (**1**) was the most active in the series with a $K_i$ of $370 \pm 60$ nM at the $A_1$ receptor.[3,4] Figure 1 gives the structure of **1** and related compounds discussed below.

α-(1-Phenylpyrazolo[3,4-*d*]pyrimidin-4-ylthio)propanamide (**2**), containing the thiopropanamide substituent at C4, had reduced affinity at the adenosine $A_1$ receptors, while α-(4-mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)propanamide (**3**), containing the thiopropanamide substituent at C6, maintained similar affinity at the adenosine $A_1$ receptor as compound (**1**).[5] It is evident that the amide side chain at C4 is not required for binding while the amide side chain at C6 is required for binding at the adenosine receptors.[5]

We have recently reported the synthesis and receptor binding at $A_1$ and $A_{2a}$ receptors of 4-amino-1-phenylpyrazolo[3,4-d]pyrimidines (**4a–d**) substituted at C6 with thioethers containing distal amide substitutents.[6] The compounds **4a** and **b** had increased $A_1$ affinity compared with **1** while **4a** also had increased, $A_{2a}$ affinity. Compounds **4b** and **4c** had approximately the same affinity as **1** to $A_{2a}$. The most selective 1-phenylpyrazolo[3,4-*d*]pyrimidine for the $A_1$ receptor was

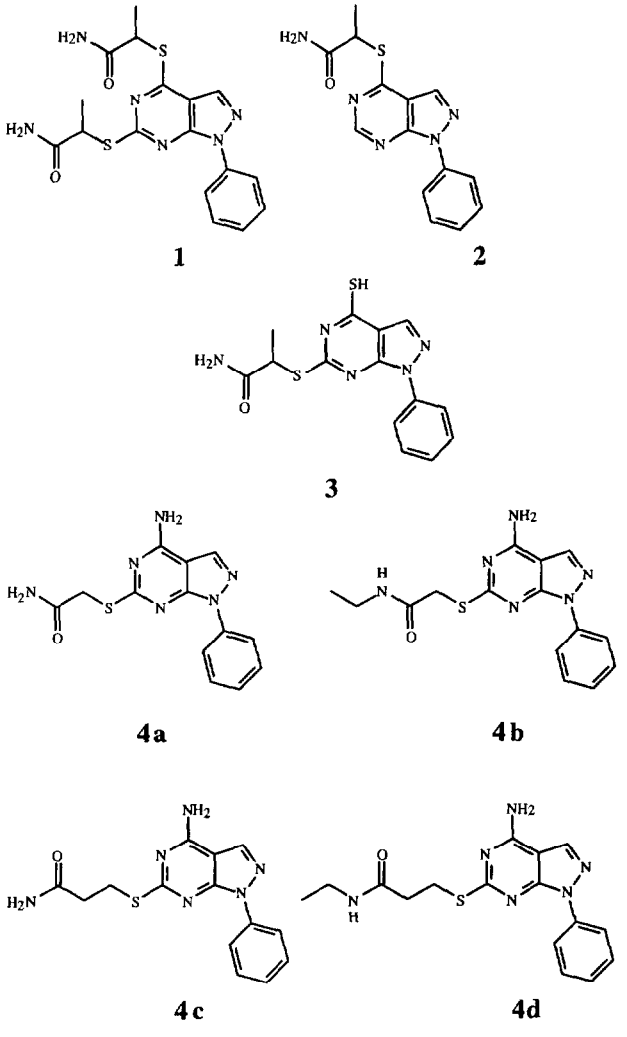



**Figure 1.**

2′-(4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-ethanamide (**4b**) with a $K_i$ $A_1$ of 12.1 ± 4.5 nM and a $K_i$ $A_{2a}$ of 131 ± 36 nM and is a modest 10.8 times more selective for this receptor. The most active and selective pyrazolo[3,4-*d*]pyrimidine for the $A_{2b}$ receptor was 3′-(4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)propanamide (**4c**) with a $K_i$ $A_1$ of 428 ± 25 nM and a $K_i$ $A_{2a}$ of 101 ± 26 and is a modest 4.2 times more selective for this receptor. Comparing **4b** and **4c** there was a 45-fold alteration in selectivity from 10.8-fold $A_1$ selective to 4.2-fold $A_{2a}$ selective. This was gained mainly by decreased $A_1$ affinity (12.1 nM to 428 nM) while $A_{2a}$ affinity remained relatively unaffected (131 and 101 nM). In both cases *N*-ethyl substitution reduced $A_{2a}$ affinity (44.9 to 131 nM for **4a/b**, 101 to 1280 nM for **4c/d**). In contrast *N*-ethyl substitution had little effect at the $A_1$ receptor (28.5 and 12.1 nM for **4a/b**, 428 and 551 nM for **4c/d**). An increase in the methylene bridge by one carbon resulted in slightly greater decreases in potency at the $A_1$ receptor (28.5 to 428 nM for **4a/c**, 12.1 to 551 nM for **4b/d**) compared with the $A_{2a}$ receptor (44.9 to 101 nM for **4a/c**, 131 to 1280 nM for **4b/d**). The $A_{2a}$ receptor had less tolerance for bulky substituents at C6 as all such changes decreased affinity. The $A_1$ receptor was more sensitive to the methylene bridge alteration.

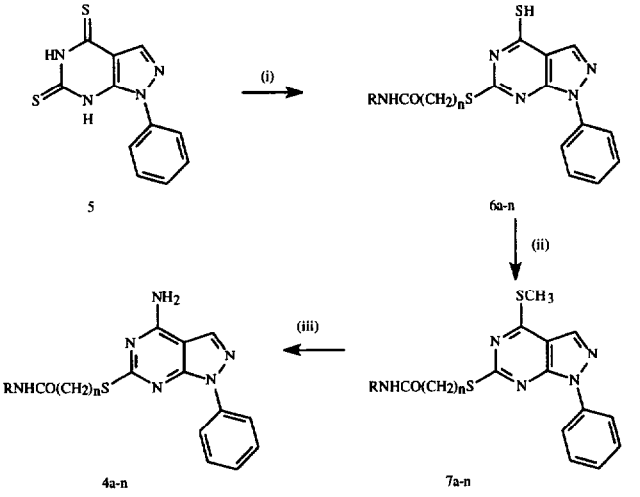
We have studied the effect of thiol and thiomethyl at C4 compared with amino and the effect of increasing the chain length between sulfur and the amide functionality at C6 for the three C4 substitutents, thiol, thiomethyl and amino.

## Results

1-Phenylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dithione (**5**) was monoalkylated with the corresponding bromo amide or the bromo *N*-ethylamide in pyridine (apart form the use of 5-chloropentanamide and 5-chloro-*N*-ethylpentanamide) to give the C6 thiol alkylated with distal amides or *N*-ethylamides (**6a–n**).[5] The corresponding C4 thiomethyl compounds (**7a–n**) were synthesized from compounds (**6a–n**) via methylation with iodomethane in aqueous sodium hydroxide. Aminolysis, using ammonia in ethanol at 100 °C for 72 h, of compounds **7a–n** gave the C4 amino compounds **4a–n**. The general synthetic scheme is outlined in Scheme 1.

$^1$H and $^{13}$C NMR spectra of compounds **6a–n**, **7a–n** and **4a–n** were assigned with the aid of 2-D NMR sequences COSY, correlation spectroscopy,[7] and HMQC, heteronuclear multiple quantum coherence,[8] on one compound in each chain length series, namely **4h**, **4i**, **6k**, and **6n** as in Table 1.

The potency of the pyrazolo[3,4-*d*]pyrimidines at the $A_1$ and $A_{2a}$ receptors were determined by standard radioligand binding procedures.[9–11] Compounds were first screened at 1 μM to determine the percentage inhibition of the compound at this concentration at both receptors. Compounds whose inhibition of $^3$H-PIA and $^3$H-CGS21680 was greater than 50% were further tested and the $IC_{50}$s and corresponding $K_i$s were determined using the experimentally obtained $K_d$ of $^3$H-PIA and $^3$H-CGS21680 of 2.35 nM and 14.9 nM,




Key: (i) $Br(CH_2)_nCONHR$, R = H or Et, pyridine, r.t.; (ii) $CH_3I$, NaOH(aq), r.t.; (iii) $NH_3$(g), EtOH, 100°C

**Scheme 1.** Synthesis of the compounds.

**Table 1.** Assignment of compounds **4h**, **4i**, **6k** and **6n** using the 2-D NMR sequences, COSY and HMQC

| Compound | Assigned protons and carbons of chain using HMQC, and COSY where required. δ (ppm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_2$-$H_2$ | $C_3$-$H_2$ | $C_4$-$H_2$ | $C_5$-$H_2$ | $C_6$-$H_2$ | $C_7$-$H_2$ | $C_8$-$H_2$ | $C_9$-$H_2$ | $C_{10}$-$H_2$ | $C_{11}$-$H_2$ | $C_{12}$-$H_2$ |
| **4h** | 2.08 | 1.68 | 1.68 | 3.05 | | | | | | | |
| $S(CH_2)_4CONHEt$ | 35.0 | 24.8 | 29.0 | 29.8 | | | | | | | |
| **4i** | 2.03 | 1.52 | 1.41 | 1.71 | 3.07 | | | | | | |
| $S(CH_2)_5CONH_2$ | 35.1 | 24.8 | 28.4 | 29.3 | 30.0 | | | | | | |
| **6k** | 2.01 | 1.45 | 1.24 | 1.37 | 1.42 | 1.70 | 3.17 | | | | |
| $S(CH_2)_7CONH_2$ | 35.1 | 25.1 | 28.4 | 28.5 | 28.7 | 28.9 | 30.4 | | | | |
| **6n** | 2.06 | 1.49 | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.35 | 1.76 | 3.18 |
| $S(CH_2)_{11}CONHEt$ | 35.4 | 25.2 | 28.4[a] 28.6 | 28.4[a] 28.6 | 28.4[a] 28.6 | 28.4[a] 28.6 | 28.4[a] 28.6 | 28.4[a] 28.6 | 28.7 | 28.8 | 30.3 |

[a]Interchangeable $^{13}$C assignments.

respectively, using the Cheng–Prusoff equation.[12] The $IC_{50}$'s were determined by competitive binding against $^3$H-PIA using rat brain membranes for $A_1$ receptors[11] and against $^3$H-CGS21680 using rat striatum membranes for $A_{2a}$ receptors.[10] Table 2 shows the binding results of the compounds.

At both the $A_1$ and $A_{2a}$ receptors, the C4 thiol compounds had the least affinity for the same C6 substituent. At both the $A_1$ and $A_{2a}$ receptors the introduction of a methyl group on sulfur in the C4 position resulted in increased affinity of between 51-fold (**6d** $K_i$ 47000 nM; **7d** $K_i$ 920 nM at $A_1$) and 6.4-fold (**6c** $K_i$ 15300 nM; **7c** $K_i$ 2390 nM at $A_1$). The most potent *S*-methyl compound at the $A_1$ receptor was **7a** with a $K_i$ of 52 nM while **7a** was also the most potent *S*-methyl compound at the $A_{2a}$ receptor with a $K_i$ of 443 nM

The conversion of the *S*-methyl to an amino group in the C4 position resulted in increased affinity in all cases. The largest increase in affinity was 12.1-fold (**7b** $K_i$ 146 nM; **4b** $K_i$ 12 nM at $A_1$) and the least 1.7-fold (**6d** $K_i$ 920 nM; **4d** 551 nM at $A_1$). The most potent amino compound at the $A_1$ receptor was **4b** with a $K_i$ of 12.1 nM while the most potent amino compound at the $A_{2a}$ receptor was **4a** with a $K_i$ of 44.9 nM. A significantly greater increase in affinity was achieved with the change from thiol to amino. The largest increase in affinity was 411-fold (**6b** $K_i$ 4940 nM; **4b** $K_i$ 12 nM at $A_1$) and the least 36-fold (**6c** $K_i$ 15300 nM; **4c** 428 nM at $A_1$).

The most potent compounds at both $A_1$ and $A_{2a}$ receptors were those with one methylene (Scheme 1, $n=1$) between the thiol and the distal amide or *N*-ethyl amide at C6. This was observed in all three series of C4 substitutents thiol (**6a–n**), thiomethyl (**7a–n**) and amino (**4a–n**). Increasing the length of the methylene bridge to $n=4$ and beyond resulted in decreased affinity at both receptors. The compounds with $n=2$ and 3 were less potent than the $n=1$ compounds, but in some cases the $n=3$ compounds were more potent than the $n=2$ compounds.

The previously reported compound **4b** had the highest affinity at the $A_1$ receptor while **4a**, also previously reported, had the highest affinity at the $A_{2a}$ receptor. The 10.8-fold $A_1$ selectivity of **4b** and the 4.2-fold $A_{2a}$ selectivity of **4c** were not improved upon in this study.

## Discussion

There was an increase in affinity at both receptors proceeding from thiol to thiomethyl to amino at C4 for the same substituent at C6. The observed increased affinity of the C4 *S*-methyl compounds indicates an alkyl binding pocket. Both receptors appear to have an alkyl pocket in this region. The observed increased affinity of the C4 amino compounds for the same substituents at C6 indicates a hydrogen-binding site located near the C4 position of 1-phenylpyrazolo[3,4-*d*]pyrimidines.

Increasing the chain length between sulfur and the amide functionality at C6 decreased affinity at both the $A_1$ and $A_{2a}$ receptors. With the C6 amino series, the amide (**4a**, Scheme 1, $n=1$) and the corresponding *N*-ethyl amide (**4b**, Scheme 1, $n=1$) were the most potent compounds with affinity in the nanomolar range. These compounds have solubility in water producing 270 μM (hot) and 38 μM (rt) solutions for **4a** and 91 μM (hot) and 13 μM (rt) solutions for **4b**. The amide (**4a**) was non-selective with $K_i$ of 28.5 nM and 44.9 nM at the $A_1$ and $A_{2a}$ receptors, respectively. The corresponding *N*-ethyl compound (**4b**) was 10.8-fold $A_1$ selective with a $K_i$ of 12.1 and 131 nM at the $A_1$ and $A_{2a}$ receptors, respectively. With the C6 thiomethyl series, the amide (**7a**) had a $K_i$ of 52 and 443 nM at the $A_1$ and $A_{2a}$ receptors respectively and the corresponding *N*-ethyl compound (**7b**) had a $K_i$ of 146 and 712 nM at the $A_1$ and $A_{2a}$ receptors respectively. With the C6 thiol series, the amide (**6a**) had a $K_i$ of 2070 and 4010 nM at the $A_1$ and $A_{2a}$ receptors, respectively, and the corresponding *N*-ethyl compound (**6b**) had a $K_i$ of 4940 and 5290 nM at the $A_1$ and $A_{2a}$ receptors, respectively.

While not identifying any compounds with higher potency or selectivity than previously reported, this study has revealed that C4 substituents in 1-phenylpyrazolo[3,4-*d*]pyrimidines can alter $A_1$ and $A_{2a}$ adenosine receptor affinity by interacting with alkyl pockets and hydrogen bonding sites in those regions of the two types of receptors. This study also established that for high affinity at both $A_1$ and $A_{2a}$ adenosine receptors the distal amide should be separated from the C6 thiol by only one carbon.

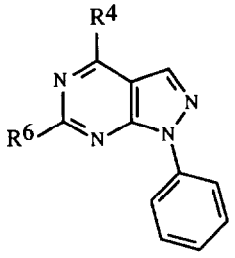
## Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. $^1$H and $^{13}$C NMR spectra were obtained on Bruker WM-250, Bruker CXP 300 and Varian Gemini-200. The type of carbon atom was assigned by using the DEPT pulse sequence obtained on a Varian Gemini-200; q = methyl, t = methylene, d = methine, and s = quaternary. COSYs and HMQCs were run on a Varian Unity 400 MHz instrument. Unless otherwise stated, DMSO-$d_6$ was used as a solvent and the solvent peak was used as the internal standard. IR spectra were recorded as KBr discs on a Jasco IR-810 or Perkin–Elmer FTIR 1700 spectrometers. Starting materials were obtained from Aldrich Pty Ltd. Ethanol was dried by reflux and distillation over magnesium turnings and a catalytic amount of iodine and was stored over 3 Å molecular sieves. Pyridine was dried by reflux and distilled from potassium hydroxide and stored over 4 Å molecular sieves. DMF was dried over BaO. Hexane was distilled prior to use.

Compounds **6k–n**, **7k–n** and **4k–n** had a small contamination of 1,3-dicyclohexylurea (DHU) which could not be removed by recrystallization. The compounds were not soluble in the eluting solvent

(1:1; hexane:ethyl acetate) and were applied by dissolving in ethyl acetate and the solution reduced in volume with silica gel (3 g/0.5 g of compound). The dried silica gel/compound was purified by eluting over

**Table 2.** Binding results of compounds **4**, **6** and **7**




| | $R^4$ | $R^6$ | R-$^3$H-N$^6$-PIA | | $^3$H-CGS21680 | | $A_{2a}K_i/A_1K_i$ |
|---|---|---|---|---|---|---|---|
| | | | $A_1K_i$ (nM)[a] | % inhibition[b] | $A_{2a}K_i$ (nM)[c] | % inhibition[d] | |
| **6a** | SH | $SCH_2CONH_2$ | 2070 ± 210 | | 4010 ± 510 | | 1.9 |
| **6b** | SH | $SCH_2CONHEt$ | 4940 ± 1500 | | 5290 ± 940 | | 1.1 |
| **6c** | SH | $S(CH_2)_2CONH_2$ | 15300 ± 2500 | | > 10000[e] | | |
| **6d** | SH | $S(CH_2)_2CONHEt$ | 47000 ± 3200 | | > 10000[e] | | |
| **6e** | SH | $S(CH_2)_3CONH_2$ | 1960 ± 550 | | > 1000[e] | | |
| **6f** | SH | $S(CH_2)_3CONHEt$ | 18400 ± 2800 | | > 10000[e] | | |
| **6g** | SH | $S(CH_2)_4CONH_2$ | | 0 | | 20 | |
| **6h** | SH | $S(CH_2)_4CONHEt$ | | 0 | | 0 | |
| **6i** | SH | $S(CH_2)_5CONH_2$ | | 0 | | 0 | |
| **6j** | SH | $S(CH_2)_5CONHEt$ | | 0 | | 0 | |
| **6k** | SH | $S(CH_2)_7CONH_2$ | | 0 | | 0 | |
| **6l** | SH | $S(CH_2)_7CONHEt$ | | 0 | | 4 | |
| **6m** | SH | $S(CH_2)_{11}CONH_2$ | | 0 | | 0 | |
| **6n** | SH | $S(CH_2)_{11}CONHEt$ | | 0 | | 3 | |
| **7a** | $SCH_3$ | $SCH_2CONH_2$ | 52.0 ± 7.8 | | 443 ± 85 | | 8.5 |
| **7b** | $SCH_3$ | $SCH_2CONHEt$ | 146 ± 29 | | 712 ± 110 | | 4.9 |
| **7c** | $SCH_3$ | $S(CH_2)_2CONH_2$ | 2390 ± 690 | | > 1000[e] | | |
| **7d** | $SCH_3$ | $S(CH_2)_2CONHEt$ | 920 ± 280 | | > 1000[e] | | |
| **7e** | $SCH_3$ | $S(CH_2)_3CONH_2$ | | 22 | | 15 | |
| **7f** | $SCH_3$ | $S(CH_2)_3CONHEt$ | | 15 | | 18 | |
| **7g** | $SCH_3$ | $S(CH_2)_4CONH_2$ | | 0 | | 0 | |
| **7h** | $SCH_3$ | $S(CH_2)_4CONHEt$ | | 5 | | 15 | |
| **7i** | $SCH_3$ | $S(CH_2)_5CONH_2$ | | 0 | | 0 | |
| **7j** | $SCH_3$ | $S(CH_2)_5CONHEt$ | | 3 | | 13 | |
| **7k** | $SCH_3$ | $S(CH_2)_7CONH_2$ | | 11 | | 3 | |
| **7l** | $SCH_3$ | $S(CH_2)_7CONHEt$ | | 0 | | 10 | |
| **7m** | $SCH_3$ | $S(CH_2)_{11}CONH_2$ | | 0 | | 0 | |
| **7n** | $SCH_3$ | $S(CH_2)_{11}CONHEt$ | | 0 | | 16 | |
| **4a** | $NH_2$ | $SCH_2CONH_2$ | 28.5 ± 4.7 | | 44.9 ± 17.2 | | 1.6 |
| **4b** | $NH_2$ | $SCH_2CONHEt$ | 12.1 ± 4.5 | | 131 ± 36 | | 10.8 |
| **4c** | $NH_2$ | $S(CH_2)_2CONH_2$ | 428 ± 25 | | 101 ± 26 | | 0.24 |
| **4d** | $NH_2$ | $S(CH_2)_2CONHEt$ | 551 ± 81 | | 1280 ± 170 | | 2.3 |
| **4e** | $NH_2$ | $S(CH_2)_3CONH_2$ | 311 ± 34 | | 319 ± 81 | | 1.0 |
| **4f** | $NH_2$ | $S(CH_2)_3CONHEt$ | 1220 ± 330 | | 535 ± 74 | | 0.44 |
| **4g** | $NH_2$ | $S(CH_2)_4CONH_2$ | 3070 ± 820 | | 1500 ± 480 | | 0.49 |
| **4h** | $NH_2$ | $S(CH_2)_4CONHEt$ | 1080 ± 310 | | 906 ± 250 | | 0.84 |
| **4i** | $NH_2$ | $S(CH_2)_5CONH_2$ | | 23 | | 20 | |
| **4j** | $NH_2$ | $S(CH_2)_5CONHEt$ | | 12 | | 22 | |
| **4k** | $NH_2$ | $S(CH_2)_7CONH_2$ | | 17 | | 31 | |
| **4l** | $NH_2$ | $S(CH_2)_7CONHEt$ | | 5 | | 14 | |
| **4m** | $NH_2$ | $S(CH_2)_{11}CONH_2$ | | 0 | | 0 | |
| **4n** | $NH_2$ | $S(CH_2)_{11}CONHEt$ | | 0 | | 7 | |

[a]All $A_1$ binding results used $^3$H-PIA as the competitive ligand in whole rat brain. Data was the average of two independent experiments in duplicate. $K_i$ values were obtained from the $K_d$ of PIA and was 2.35 nM.
[b]$A_1$ % inhibition of drug at a concentration of 1 μM.
[c]All $A_2$ binding results used $^3$H-CGS21680 as the competitive ligand in rat striata. Data was the average of two independent experiments in duplicate. $K_i$ values were obtained from the $K_d$ of CGS21680 and was 14.9 nM.
[d]$A_2$% inhibition of drug at a concentration of 1 μM.
[e]The lack of solubility of the compounds around the $IC_{50}$ resulted in obtaining inaccurate values.

silica gel, Merck (60 g/0.5 g; 4 cm thick column, Aldrich Cat. No. 22,719-6; grade 60, 230–400 mesh, 60 Å) with ethyl acetate and hexane (1:1) to remove DHU ($R_f$=0.3; viewed only when concentrated with pure DHU and by using $KMnO_4$ spray) and then the solvent polarity increased to ethyl acetate (100%) to elute the compounds. Some compounds containing the amine group on C4 also were purified in this way to remove small amounts of the thiomethyl compounds. $R_f$ values and solvent systems are indicated in those cases in which chromatographic purification was undertaken.

The starting compounds, (ethoxymethylene)malononitrile, 1-phenyl-5-amino-4-cyanopyrazole, and 1-phenylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dithione (**5**) were prepared following known methods.[5]

Straight-chain bromo acid chlorides (apart from the use of 5-chloropentoyl chloride) were converted to the corresponding amide and *N*-ethyl amide by reaction with ammonia or ethylamine respectively. For the longer amide side chains of compounds (**6k–n**), the corresponding bromo acids were treated with dicyclohexylcarbodiimide followed by bubbling ammonia or ethylamine through the reaction mixture at room temperature.

**2′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)amides (6).** The following general procedure was used apart from a variation in work-up for the synthesis of **6g–n** in which cases the pyridine was removed *in vacuo* and ethyl acetate added to give a yellow solid. 2-Bromoethanamide (0.5 mmol) was added to a solution of 1-phenylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dithione (**5**, 0.75 mmol) in pyridine (45 mL). The solution was stirred at room temperature for 3 h. Treatment with ethyl acetate resulted in precipitation of a white solid which was recrystallized from DMSO and water to give 2′-(4-*mercapto*-1-*phenylpyrazolo*[3,4-*d*]*pyrimidin*-6-*ylthio*)*ethanamide* (**6a**): Yield 84%; mp dec 249–251 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 3.92(t, 2H, $SCH_2$), 7.34–8.07 (m, 6H, $5CH_{arom}$, 1 NH), 7.65 (br s, 1H, NH), 8.46 (s, 1H, $H_3$), 14.14 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 34.2 (t, $SC_2H_2$), 116.4 (s, $C_{3a}$), 121.0 (d, $C_{2''}$, $C_{6''}$), 126.9 (s, $C_{4''}$), 129.4 (d, $C_{3''}$, $C_{5''}$), 138.1 (s, d, $C_{1''}C_3$), 146.6 (s, $C_{7a}$), 160.8 (s, $C_6$), 166.4 (s, C=O), 180.1 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3400, NH; 3200, NH; 1640 cm$^{-1}$, C—O; Anal. calcd for ($C_{13}H_{11}N_5OS_2$): C, 49.2; H, 3.5; N, 22.1. Found: C, 49.0; H, 3.7; N, 22.0%.

**2′-(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)-*N*-ethyl-ethanamide (6b).** Yield 80%; mp dec 260–265 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.92 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 3.02 (m, 2H, $J$ = 7.2 Hz, 5.5 Hz, $CH_2CH_3$), 3.95 (s, 2H, $SCH_2$), 7.35–8.05 (m, 5H, $CH_{arom}$), 8.21 (br t, 1H, $J$=5.5 Hz, CONH), 8.33 (s, 1H, $H_3$), 14.16 (br s, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.3 (q, $CH_2CH_3$), 33.9 (s, $CH_2CH_3$), 34.2 (t, $SC_2H_2$), 116.3 (s, $C_{3a}$), 121.1 (d, $C_{2''}$, $C_{6''}$), 127.0 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 138.1 (s, d, $C_{1''}$, $C_3$), 146.6 (s, $C_{7a}$), 160.5 (s, $C_6$), 169.8 (s, C=O), 180.7 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3300, NH; 1650, cm$^{-1}$, C—O; Anal. calcd for ($C_{15}H_{15}N_5OS_2$): C, 65.4; H, 5.5; N, 25.4. Found: C, 65.3; H, 5.9; N, 25.7%.

**3′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)propanamide (6c).** Yield 68%; mp dec 228–232 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 2.66 (t, 2H, $J$=6.8 Hz, $CH_2$), 3.39 (t, 2H, $J$=6.8 Hz, $SCH_2$), 7.03 (br s 1H, NH), 7.37–8.12 (m, 6H, $5CH_{arom}$, 1NH), 8.35 (s, 1H, $H_3$), 14.0 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 26.2 (q, $SC_3H_2$), 34.0 (t, $C_2H_2$), 116.3 (s, $C_{3a}$), 121.1 (d, $C_{2''}$, $C_{6''}$) 127.0 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 138.1 (s, d, $C_{1''}$, $C_3$) 146.6 (s, $C_{7a}$), 160.8 (s, $C_6$), 172.2 (s, C—O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3425, NH; 3200, NH; 3125, NH; 1660, C=O, 1600 cm$^{-1}$, C—C; Anal. calcd for ($C_{14}H_{13}N_5OS_2$·0.5DMSO): C, 48.6; H, 4.4; N, 18.9. Found: C, 48.9; H, 4.2; N, 19.2%.

**3′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-propanamide (6d).** Yield 70%; mp dec 229–233 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.01 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 2.63 (t, 2H, $J$=6.8 Hz, $CH_2$), 3.09 (m, 2H, $J$=7.2 Hz, 5.7 Hz, $CH_2CH_3$), 3.39 (t, 2H, $J$=6.8 Hz, $SCH_2$), 7.40–8.11 (m, 5H, $CH_{arom}$), 7.90 (br t, 1H, $J$=5.1 Hz, 5.5 Hz, NH), 8.35 (s, 1H, $H_3$), 14.03 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.5 (q, $CH_2CH_3$), 26.4 (t, $SC_3H_2$), 33.3 (t, $CH_2CH_3$), 34.2 (t, $C_2H_2$), 116.3 (s, $C_{3a}$), 121.1 (d, $C_{2''}$, $C_{6''}$), 127.0 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.5 (s, $C_{7a}$), 160.7 (s, $C_6$), 169.9 (s, C—O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3325, NH; 1640 cm$^{-1}$, C=O; Anal. calcd for ($C_{16}H_{17}N_5OS_2$): C, 53.5; H, 4.8; N, 19.5. Found: C, 53.7; H, 4.8; N, 19.6%.

**4′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)butanamide (6e).** Yield 78%; mp dec 229–230.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.96 (m, 2H, $J$=7.3 Hz, C3′$H_2$), 2.21 (t, 2H, $J$=7.3 Hz, $C_2H_2$), 3.19 (t, 2H, $J$=7.3 Hz, $SCH_2$), 6.81 (br s, 1H, NH), 7.35–8.07 (m, 6H, $5CH_{arom}$, 1NH), 8.30 (s, 1H, $H_3$), 14.04 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 24.4 (t, $C_3H_2$), 30.0 (t, $SC_4H_2$), 33.8 (t, $C_2H_2$), 116.3 (s, $C_{3a}$), 121.1 (d, $C_{2''}$, $C_{6''}$), 127.0 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.6 (s, $C_{7a}$), 160.8 (s, $C_6$), 173.4 (s, C=O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3430, NH; 3240, NH; 1670 cm$^{-1}$, C=O. Anal. calcd for ($C_{15}H_{15}N_5OS_2$): C, 52.2; H, 4.4; N, 20.3. Found: C, 52.1; H, 4.5; N, 20.0%.

**4′-(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)-*N*-ethylbutanamide (6f).** Yield 72%; mp decomp. 238.5–243 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.96 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 1.99 (quintet, 2H, $J$=6.7, 7.2 Hz, $C_3H_2$), 2.21 (t, 2H, $J$=7.3, 7.4 Hz, $C_2H_2$), 3.03 (m, 2H, $J$=5.5, 7.2 Hz, $CH_2CH_3$), 3.18 (t, 2H, $J$=6.81, 7.5 Hz, $SCH_2$), 7.33–8.06 (m, 5H, $CH_{arom}$), 7.84 (br t, 1H, CONH), 8.30 (s, 1H, $H_3$), 14.04 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.6 (q, $CH_3CH_2$), 24.6 (t, $C_3H_2$), 30.0 (t, $SC_4H_2$), 33.2 (t, $CH_2CH_3$), 34.1 (t, $C_2H_2$), 116.3 (s, $C_{3a}$), 121.1 (d, $C_{2''}$, $C_{6''}$), 127.0 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$),

138.1 (s, d, $C_{1''}$, $C_3$), 146.6 (s, $C_{7a}$), 160.8 (s, $C_6$), 170.8 (s, C=O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3330, NH; 3120, NH; 1640 $cm^{-1}$, C=O; Anal. calcd for ($C_{17}H_{19}N_5OS_2$): C, 54.7; H, 5.1; N, 18.8; Found: C, 55.0; H, 5.3; N, 18.5%.

**5′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)pentanamide (6g)**. Yield 76%; mp dec 200.5–202.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.70 (m, 4H, $J$=7.3 Hz, 7.0 Hz, 2×C$H_2$), 2.11 (t, 2H, $J$=6.9 Hz, $C_{2'}H_2$), 3.20 (t, 2H, $J$=7.0 Hz, SC$H_2$), 6.77 (br s, 1H, NH), 7.29 (br s, 1H, NH), 7.38–8.08 (m, 5H, $CH_{arom}$), 8.34 (s, 1H, $H_3$), 14.4 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 24.4 (t, $C_{3'}H_2$), 28.3 (t $C_{4'}H_2$), 30.0 (t, $SC_{5'}H_2$), 34.5 (t, $C_{2'}H_2$), 116.2 (s, $C_{3a}$), 121.2 (d, $C_{2''}$, $C_{6''}$), 127.1 (s, $C_{4'}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.6 (s, $C_{7a}$), 160.9 (s, $C_6$), 174.1(s, C=O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3375, NH; 3300, NH; 3175, NH; 1660 $cm^{-1}$, C=O. Anal. calcd for ($C_{16}H_{17}N_5OS_2$): C, 53.5; H, 4.8; N, 19.5. Found: C, 53.7; H, 4.8; N, 19.4%.

**5′-(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)-*N*-ethylpentanamide (6h).** Yield 53%; mp 193–195 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.96 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 1.65 (m, 4H, 2×C$H_2$), 2.07 (t, 2H, $J$=6.9 Hz, $C_{2'}H_2$), 3.02 (m, 2H, $J$=7.2 Hz, 5.5 Hz, $CH_2CH_3$), 3.16 (t, 2H, $J$=6.9 Hz, SC$H_2$), 7.37–8.04 (m, 5H, $CH_{arom}$), 7.76 (br t, 1H, $J$=5.1 Hz, 5.5 Hz, NH), 8.31 (s, 1H, $H_3$), 14.03 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.6 (q, $CH_2CH_3$), 24.6 (t, $C_{3'}H_2$), 28.8 (t, $C_{4'}H_2$), 30.0 (t, $SC_{5'}H_2$), 33.1 (t, $CH_2CH_3$), 34.8 (t, $C_{2'}H_2$), 116.2 (s, $C_{3a}$), 121.2 (d, $C_{2''}$, $C_{6''}$), 127.1 (s, $C_{4'}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.6 (s, $C_{7a}$), 160.9 (s, $C_6$), 171.5 (s, C=O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3325, NH; 3125, NH; 1640 $cm^{-1}$, C=O. Anal. calcd for ($C_{18}H_{21}N_5OS_2$): C, 55.8; H, 5.5; N, 18.1. Found: C, 56.0; H, 5.4; N, 18.3%.

**6′-(4-Mercapto-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)hexanamide (6i)**. Yield 66%; mp dec 212–215 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.40 (m, 2H, $J$=7.1 Hz, C$H_2$), 1.51 (m, 2H, $J$=7.0 Hz, C$H_2$), 1.72 (m, 2H, $J$=6.9 Hz, C$H_2$), 2.00 (t, 2H, $J$ =7.1 Hz, $C_{2'}H_2$), 3.18 (t, 2H, $J$=6.7 Hz, SC$H_2$), 6.65 (br s, 1H, NH), 7.19 (br s, 1H, NH), 7.36–8.08 (m, 5H, $CH_{arom}$), 8.32 (s, 1H, $H_3$), 13.99 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 24.5 (t, $C_{3'}H_2$), 28.0 (t, $C_{4'}H_2$), 28.5 (t, $C_{5'}H_2$), 30.1 (t, $SC_{6'}H_2$), 34.8 (t, $C_{2'}H_2$), 116.2 (s, $C_{3a}$), 121.2 (d, $C_{2''}$, $C_{6''}$), 127.1 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.6 (s, $C_{7a}$), 160.9 (s, $C_6$), 174.2 (s, C=O), 180.2 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3375, NH; 1650 $cm^{-1}$, C=O. Anal. calcd for ($C_{17}H_{19}N_5OS_2$): C, 54.7; H, 5.1; N, 18.8. Found: C, 54.9; H, 4.9; N, 18.5%.

**6′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-hexanamide (6j)**. Yield 81%; mp dec 190.5–191.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.96 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 1.37 (m, 2H, $J$=7.2 Hz, C$H_2$), 1.51 (m, 2H, $J$=7.3 Hz, C$H_2$), 1.71 (m, 2H, $J$=7.0 Hz, C$H_2$), 2.02 (t, 2H, $J$=7.2 Hz, $C_{2'}H_2$), 3.02 (m, 2H, $J$=7.2 Hz, 5.5 Hz, $CH_2CH_3$), 3.17 (t, 2H, $J$=7.3 Hz, SC$H_2$), 7.36–8.04 (m, 5H $CH_{arom}$), 7.76 (br t, 1H, NH), 8.29 (s, 1H, $H_3$), 13.99 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.3 (q, $CH_2CH_3$), 24.5 (t, $C_{3'}H_2$), 27.8 (t, $C_{4'}H_2$), 28.3 (t, $C_{5'}H_2$), 30.0 (t, $SC_{6'}H_2$), 33.0 (t, $CH_2CH_3$), 35.1 (t, $C_{2'}H_2$), 117.1 (s, $C_{3a}$), 122.1 (d, $C_{2''}$, $C_{6''}$) 128.0 (s, $C_{4''}$) 130.2 (d, $C_{3''}$, $C_{5''}$), 139.2 (d, s, $C_3$, $C_{1''}$), 147.9 (s, $C_{7a}$), 162.3 (s, $C_6$), 173.2 (s, C=O), 181.9 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3325, NH; 3150, NH; 1640 $cm^{-1}$, C=O. Anal. calcd for ($C_{19}H_{23}N_5OS_2$): C, 56.8; H, 5.8; N, 17.4. Found: C, 57.0; H, 5.8; N, 17.1%.

**8′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)octanamide (6k)**. $R_f$ in ethyl acetate = 0.52; yield 62%; mp 192–194 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 1.14–1.48 (m, 8H, 4×$J$=7.4 Hz, C$H_2$), 1.69 (m, 2H, C$H_2$), 2.01 (t, 2H, $J$=6.7 Hz, $C_{2'}H_2$), 3.17 (t, 2H, $J$=7.3 Hz, SC$H_2$), 6.66 (br s, 1H, NH), 7.21 (br s, 1H, NH), 7.39–8.06 (m, 5H, $CH_{arom}$), 8.31 (s, 1H, $H_3$), 13.97 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 25.1 (t, $C_{3'}H_2$), 28.4 (t, $C_{5'}H_2$), 28.5 (t, $C_{4'}H_2$), 28.7 (t, $C_{6'}H_2$), 28.9 (t, $C_{7'}H_2$), 30.4 (t, $SC_{8'}H_2$), 35.1 (t, $C_{2'}H_2$), 116.3 (s, $C_{3a}$), 121.3 (d, $C_{2''}$, $C_{6''}$), 127.2 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.2 (d, $C_3$), 146.6 (s, $C_{7a}$), 161.0 (s, $C_6$), 174.4 (s, C=O), 180.1 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3400, NH; 3235, NH; 3175, NH; 1660 $cm^{-1}$, C=O. Anal. calcd for ($C_{19}H_{23}N_5OS_2$): C, 56.8; H, 5.8; N, 17.4. Found: C, 56.8; H, 5.8; N, 17.0%.

**8′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-octanamide (6l)**. $R_f$ in ethyl acetate = 0.67; yield 58%; mp 171–172 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 1.01 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 1.09–1.68 (m, 8H, $J$=7.3 Hz, 4×C$H_2$), 1.76 (m, 2H, $J$=7.0 Hz, C$H_2$), 2.04 (t, 2H, $J$=7.3 Hz, $C_{2'}H_2$), 3.06 (m, 2H, $J$=7.2 Hz, 5.6 Hz, $CH_2CH_3$), 3.21 (t, 2H, $J$=7.4 Hz, SC$H_2$), 7.40–8.10 (m, 5H, $CH_{arom}$), 7.74 (br t, 1H, NH), 8.36 (s, 1H, $H_3$), 14.04 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 14.8 (q, $CH_3$), 25.2 (t, $C_{3'}H_2$), 28.3 (t, $C_{5'}H_2$), 28.4 (t, $C_{4'}H_2$), 28.6 (t, $C_{6'}H_2$), 28.9 (t, $C_{7'}H_2$), 30.3 (t, $C_{8'}H_2$), 33.2 (t, $CH_2CH_3$), 35.4 (t, $C_{2'}H_2$), 116.2 (s, $C_{3a}$), 121.2 (d, $C_{2''}$, $C_{6''}$), 127.1 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.6 (s, $C_{7a}$), 160.9 (s, $C_6$), 171.7 (s, C=O), 180.1 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3310, NH; 3125, NH; 1635 $cm^{-1}$, C=O. Anal. calcd for ($C_{21}H_{27}N_5OS_2$): C, 58.7; H, 6.3; N, 16.3. Found: C, 58.5; H, 6.3; N, 16.2%.

**12′-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)dodecanamide (6m)**. $R_f$ in ethyl acetate = 0.64; yield 60%; mp 165–167 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 1.10–1.49 (m, 18H, $J$=7.5 Hz, 7.2 Hz, 7.1 Hz, 9×C$H_2$), 1.70 (quintet, 2H, $J$=7.3 Hz, C$H_2$), 2.03 (t, 2H, $J$=7.4 Hz, $C_{2'}H_2$), 3.16 (t, 2H, 7.4 Hz, SC$H_2$), 6.72 (br s, 1H, NH), 7.24 (br s, 1H, NH), 7.35–8.05 (m, 5H, $CH_{arom}$), 8.31 (s, 1H, $H_3$), 13.99 (br s, 1H, $N_5HC_4$=S); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 25.1 (t, $C_{3'}H_2$), 28.4 (t, $C_{10'}H_2$), 28.5, 28.7 (t, $C_{9'}H_2$-$C_{4'}H_2$

indistinguishable), 28.9 (t, $C_{11'}H_2$), 30.4 (t, $SC_{12'}H_2$), 35.1 (t, $C_{2'}H_2$), 116.3 (s, $C_{3a}$), 121.3 (d, $C_{2''}$, $C_{6''}$), 127.2 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 138.0 (s, $C_{1''}$), 138.1 (d, $C_3$), 146.6 (s, $C_{7a}$), 161.0 (s, $C_6$), 174.2 (s, C=O), 180.1 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3425, NH; 3200, NH; 1640 and 1610 $cm^{-1}$, C=O. Anal. calcd for ($C_{23}H_{31}N_5OS_2$): C, 60.4; H, 6.8; N, 15.3. Found: C, 60.8; H, 6.7; N, 14.9%.

**12'-(4-Mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-dodecanamide (6n).** $R_f$ in ethyl acetate = 0.76; $R_f$ in hexane:ethyl acetate (1:1) = 0.23; Yield 68%; mp 150–154 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 1.05 (t, 3H, $J$ = 7.2 Hz, $CH_2CH_3$), 1.13–1.52 (m, 18H, $J$ = 6.7 Hz, 9 × $CH_2$), 1.76 (quintet, 2H, $J$ = 7.3 Hz, $CH_2$), 2.07 (t, 2H, $J$ = 7.2 Hz, $C_{2'}H_2$), 3.10 (m, 2H, $J$ = 7.2 Hz, 5.5 Hz, $CH_2CH_3$), 3.25 (t, 2H, $J$ = 7.2 Hz, $SCH_2$), 7.43–8.15 (m, 5H, $CH_{arom}$), 7.81 (br t, 1H, NH), 8.40 (s, 1H, $H_3$), 14.08 (br s, 1H, $N_5HC_4$=S). $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 14.7 (q, $CH_2CH_3$), 25.2 (t, $C_{3'}H_2$), 28.4 (t, $C_{10'}H_2$), 28.6, 28.7 (t, $C_{9'}H_2$-$C_{4'}H_2$ indistinguishable), 28.8 (t, $C_{11'}H_2$), 30.3 (t $SC_{12'}H_2$), 33.1 (t, $CH_2CH_3$), 35.4 (s, $C_{2'}H_2$), 116.3 (s, $C_{3a}$), 121.3 (d, $C_{2''}$, $C_{6''}$), 127.2 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 138.1 (s, $C_{1''}$), 138.2 (d, $C_3$), 146.7 (s, $C_{7a}$), 161.0 (s, $C_6$), 171.3 (s, C=O), 180.3 (s, $C_4$); IR (KBr disc): $\nu_{max}$ 3350, NH; 1640 $cm^{-1}$, C=O. Anal. calcd for ($C_{25}H_{35}N_5OS_2$): C, 61.8; H, 7.3; N, 14.4. Found: C, 61.5; H, 7.5; N, 14.3%.

**2'-(4-methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)amides (7).** The following general procedure was used: Compound **6a** (0.3 mmol) was added to a sodium hydroxide solution (20 mL, 1.5 M). Iodomethane (0.43 mmol) was added and the mixture stirred at room temperature for 30 min. A white solid precipitated and was recrystallized from DMSO and water to give *2'-(4-methylthio-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)ethanamide* (**7a**) Yield 80%; mp 235–237 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 2.69 (s, 3H, $SCH_3$), 3.91 (s, 2H, $SCH_2$), 7.24 (br s, 1H, NH), 7.34–8.18 (m, 5H, $CH_{arom}$), 7.64 (br s, 1H, NH), 8.48 (s, 1H, $H_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.5 (q, $SCH_3$), 34.7 (t, $SC_{2'}H_2$), 110.4 (s, $C_{3a}$), 120.7 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.4 (d, $C_{3''}$, $C_{5''}$), 133.8 (d, $C_3$), 138.3 (s, $C_{1''}$), 151.0 (s, $C_{7a}$), 165.6 (s, $C_4$), 168.2 (s, $C_6$), 169.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3310, NH; 1655 $cm^{-1}$, C=O. Anal. calcd for ($C_{14}H_{13}N_5OS_2$): C, 50.7; H, 4.0; N, 21.7. Found: C, 51.0; H, 4.0; N, 21.6%.

**2'-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-ethanamide (7b).** Yield 75%; mp decomp. 227–228.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.94 (t, 3H, $J$ = 6.9 Hz, $CH_3$), 2.68 (s, 3H, $SCH_3$), 3.05 (m, 2H, $J$ = 6.8 Hz, 6.1 Hz, $CH_2CH_3$), 3.94 (s, 2H, $SCH_2$), 7.34–8.16 (m, 6H, 5$CH_{arom}$, 1NH), 8.48 (s, 1H, $H_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.5 (q, $SCH_3$), 14.4 (q, $CH_3$), 33.8 (t, $CH_2CH_3$), 34.8 (s, $SC_{2'}H_2$), 110.5 (s, $C_{3a}$), 120.7 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$) 129.4 (d, $C_{3''}$ $C_{5''}$), 133.8 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.2 (s, $C_{7a}$), 161.6 (s, $C_4$), 165.7 (s, $C_6$), 166.7 (s, C=O); IR (KBr disc): $\nu_{max}$ 3310, NH; 1655 $cm^{-1}$, C=O. Anal. calcd for ($C_{16}H_{17}N_5OS_2$): C, 53.4; H, 4.8; N, 19.5. Found: C, 53.0; H, 4.9; N, 19.8%.

**3'-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)propanamide (7c).** Yield 60%; mp 202–204.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 2.62 (t, 2H, $J$ = 7.1 Hz, $CH_2$), 2.68 (s, 3H, $SCH_3$), 3.36 (t, 2H, $J$ = 7.1 Hz, $SCH_2$), 6.93 (br s, 1H, NH), 7.36–8.17 (m, 6H, 5$CH_{arom}$, 1NH), 8.47 (s, 1H, $H_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 26.5 (t, $SC_{3'}H_2$), 34.5 (t, $C_{2'}H_2$), 110.4 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 133.7 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.2 (s, $C_{7a}$), 165.6 (s, $C_4$), 168.4 (s, $C_6$), 172.4 (s, C=O); IR (KBr disc): $\nu_{max}$ 3425, NH; 3375, NH; 1665, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{15}H_{15}N_5OS_2$): C, 52.2; H, 4.4; N, 20.3. Found: C, 51.9; H, 4.7; N, 20.6%.

**3'-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-propanamide (7d).** Yield 57%; mp 177–178.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.99 (t, 3H, $J$ = 7.2 Hz, $CH_2CH_3$), 2.61 (t, 2H, $J$ = 7.0 Hz, $CH_2$), 2.68 (s, 3H, $SCH_3$), 3.08 (m, 2H, $J$ = 7.2 Hz, 5.5 Hz, $CH_2CH_3$), 3.37 (t, 2H, $J$ = 7.0 Hz, $SCH_2$), 7.36–8.17 (m, 5H, $CH_{arom}$), 7.87 (br t, 1H, NH), 8.46 (s, 1H, $H_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 26.5 (t, $SC_{3'}H_2$), 34.5 (t, $C_{2'}H_2$), 110.4 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 133.7 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.2 (s, $C_{7a}$), 165.6 (s, $C_4$), 168.4 (s, $C_6$), 172.4 (s, C=O); IR (KBr disc): $\nu_{max}$ 3325, NH; 1650, C=O; 1600 $cm^{-1}$, C=C; Anal. calcd for ($C_{17}H_{19}N_5OS_2$): C, 54.7; H, 4.6; N, 18.8. Found: C, 54.7; H, 4.8; N, 19.0%.

**4'-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)butanamide (7e).** Yield 55%); mp 173.5–174 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.96 (m, 2H, $J$ = 7.1 Hz, $C_{3'}H_2$), 2.24 (t, 2H, $J$ = 7.3 Hz, $C_{2'}H_2$), 2.67 (s, 3H, $SCH_3$), 3.19 (t, 2H, $J$ = 7.1 Hz, $SCH_2$), 6.80 (br s, 1H, NH), 7.34–8.15 (m, 6H, 5$CH_{arom}$, 1NH), 8.45 (s, 1H, $H_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 24.8 (t, $C_{3'}H_2$), 30.2 (t, $SC_{4'}H_2$), 33.9 (t, $C_{2'}H_2$), 110.4 (s, $C_{3a}$), 120.7 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 133.7 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.2 (s, $C_{7a}$), 165.5 (s, $C_4$), 168.5 (s, $C_6$), 173.5 (s, C=O); IR (KBr disc): $\nu_{max}$ 3425, NH; 3225, NH; 1670, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{16}H_{17}N_5OS_2$): C, 53.5; H, 4.8; N, 19.5. Found: C, 53.4; H, 4.6; N, 19.5%.

**4'-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-butanamide (7f).** Yield 59%; mp 173.5–175.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.98 (t, 3H, $J$ = 7.2 Hz, $CH_2CH_3$), 1.99 (m, 2H, $J$ = 7.2 Hz, $C_{3'}H_2$), 2.23 (t, 2H, $J$ = 7.3 Hz, $C_{2'}H_2$), 2.66 (s, 3H, $SCH_3$), 3.04 (m, 2H, $J$ = 7.1 Hz, 5.6 Hz, $CH_2CH_3$); 3.17 (t, 2H, $J$ = 7.3 Hz, $SCH_2$), 7.33–8.14 (m, 5H, $CH_{arom}$), 7.83 (br s, 1H, NH), 8.41 (s, 1H, $H_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 14.6 (q, $CH_3$), 25.0 (t, $C_{3'}H_2$), 30.2 (t, $SC_{4'}H_2$), 33.2 (t, $CH_2CH_3$), 34.3 (t, $C_{2'}H_2$), 110.4 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.3 (d, $C_{3''}$, $C_{5''}$), 133.7 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.2 (s, $C_{7a}$), 165.5 (s, $C_4$), 168.6 (s, $C_6$), 171.0 (s, C=O); IR

(KBr disc): $\nu_{max}$ 3325, NH; 1640, C=O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{18}H_{21}N_5OS_2$): C, 55.8; H, 5.5; N, 18.1. Found: C, 56.0; H, 5.2; N, 17.8%.

**5′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)pentanamide (7g).** Yield 74%; mp 141.5–143 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.71 (m, 4H, 2×$J$=6.9 Hz, C$\underline{H}_2$), 2.12 (t, 2H, $J$=6.9 Hz, C$_{2'}\underline{H}_2$), 2.69 (s, 3H, SC$\underline{H}_3$), 3.18 (t, 2H, $J$=7.0 Hz, SC$\underline{H}_2$), 6.77 (br s, 1H, N$\underline{H}$), 7.30 (br s, 1H, N$\underline{H}$), 7.35–8.16 (m, 5H, C$\underline{H}_{arom}$), 8.44 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$); 24.5 (t, $C_{3'}H_2$), 28.7 (t, $C_{4'}H_2$), 30.3 (t, $SC_{5'}H_2$), 34.6 (t, $C_{2'}H_2$), 110.3 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 133.6 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.1 (s, $C_{7a}$), 165.4 (s, $C_4$), 168.6 (s, $C_6$), 174.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3425, NH; 3225, NH; 1655, C=O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{17}H_{19}N_5OS_2$): C, 54.7; H, 5.1; N, 18.8. Found: C, 54.5; H, 4.9; N, 18.5%.

**5′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-pentanamide (7h).** Yield 80%; mp 143.0–145.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.95 (t, 3H, $J$=7.2 Hz, $CH_2C\underline{H}_3$), 1.70 (m, 4H, 2×C$\underline{H}_2$), 2.08 (t, 2H, $J$=6.7 Hz, C$_{2'}\underline{H}_2$), 2.68 (s, 3H, $SCH_3$), 3.02 (m, 2H, $J$=7.2 Hz, 5.6 Hz, C$\underline{H}_2$CH$_3$), 3.17 (t, 2H, $J$=6.8 Hz, SC$\underline{H}_2$), 7.34–8.14 (m, 5H, C$\underline{H}_{arom}$), 7.76 (br t, 1H, N$\underline{H}$), 8.46 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 14.6 (q, $CH_2CH_3$), 24.6 (t, $C_{3'}H_2$), 28.6 (t, $C_{4'}H_2$), 30.3 (t, $SC_{5'}H_2$), 33.1 (t, $CH_2CH_3$), 34.9 (t, $C_{2'}H_2$), 110.3 (s, $C_{3a}$), 120.7 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 133.7 (d, $C_3$) 138.3 (s, $C_{1''}$), 151.2 (s, $C_{7a}$), 165.5 (s, $C_4$), 168.6 (s, $C_6$), 171.5 (s, C—O); IR (KBr disc): $\nu_{max}$ 3325, NH; 1655, C=O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{19}H_{23}N_5OS_2$): C, 56.8; H, 5.8; N, 17.4. Found: C, 56.8; H, 5.9; N, 17.4%.

**6′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)hexanamide (7i).** Yield 66%; mp 153.5–154 °C; $^1$H NMR (250.12 MHz, DMSO-*d*6): δ 1.43 (m, 2H, $J$=6.1 Hz, C$\underline{H}_2$), 1.55 (m, 2H, $J$=7.3 Hz, C$\underline{H}_2$), 1.71 (m, 2H, $J$=7.3 Hz, C$\underline{H}_2$), 2.04 (t, 2H, $J$ =7.2 Hz, C$_{2'}\underline{H}_2$), 2.66 (s, 3H, $SCH_3$), 3.15 (t, 2H, $J$=7.2 Hz, $SCH_2$), 7.35–8.17 (m, 5H, C$\underline{H}_{arom}$), 7.6 (br t, 1H, N$\underline{H}$), 8.47 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 24.6 (t, $C_{3'}H_2$), 28.1 (t, $C_{4'}H_2$), 28.8 (t, $C_{5'}H_2$), 30.4 (t, $SC_{6'}H_2$), 34.9 (t, $C_{2'}H_2$), 110.1 (s, $C_{3a}$), 120.4 (d, $C_{2''}$, $C_{6''}$), 126.4 (s, $C_{4''}$), 129.0 (d, $C_{3''}$, $C_{5''}$), 133.4 (d, $C_3$), 138.1 (s, $C_{1''}$), 151.0 (s, $C_{7a}$), 165.2 (s, $C_4$), 168.4 (s, $C_6$), 171.4 (s, C—O); IR (KBr disc): $\nu_{max}$ 3375, NH; 3175, NH; 1655, C—O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{18}H_{21}N_5OS_2$): C, 55.8; H, 5.5; N, 18.1. Found: C, 56.0; H, 5.5; N, 17.9%.

**6′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-hexanamide (7j).** Yield 58%; mp 138.5–139.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.97 (t, 3H, $J$=7.2 Hz, $CH_2CH_3$), 1.40 (m, 2H, $J$=6.3 Hz, C$\underline{H}_2$), 1.55 (m, 2H, $J$=7.2 Hz, C$\underline{H}_2$), 1.71 (m, 2H, $J$=7.3 Hz, 6.5 Hz, C$\underline{H}_2$), 2.03 (t, 2H, $J$=7.1 Hz, C$_{2'}\underline{H}_2$), 2.64 (s, 3H, SC$\underline{H}_3$), 3.03 (m, 2H, $J$=7.1 Hz, 5.5 Hz, C$\underline{H}_2$CH$_3$); 3.12 (t, 2H, $J$=7.4 Hz, SC$\underline{H}_2$), 7.31–8.13 (m, 5H, C$H_{arom}$), 7.75 (br t, 1H, N$\underline{H}$), 8.39 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 14.6 (q, $CH_2CH_3$), 24.7 (t, $C_{3'}H_2$), 28.0 (t, $C_{4'}H_2$), 28.7 (t, $C_{5'}H_2$), 30.3 (t, $SC_{6'}H_2$), 33.1 (t, $CH_2CH_3$), 35.2 (t, $C_{2'}H_2$), 110.1 (s, $C_{3a}$), 120.4 (d, $C_{2''}$, $C_{6''}$), 126.4 (s, $C_{4''}$) 129.0 (d, $C_{3''}$, $C_{5''}$), 133.4 (d, $C_3$) 138.1 (s, $C_{1''}$), 151.0 (s, $C_{7a}$), 165.2 (s, $C_4$), 168.4 (s, $C_6$), 171.4 (s, C=O); IR (KBr disc): $\nu_{max}$ 3300, NH; 1650, C=O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{20}H_{25}N_5OS_2$): C, 57.8; H, 6.1; N, 16.9. Found: C, 57.8; H, 6.0; N, 17.2%.

**8′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)octanamide (7k).** $R_f$ in ethyl acetate = 0.58; Yield 52%; mp 145.5–148 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 1.25–1.52 (m, 8H, 4×C$\underline{H}_2$), 1.76 (quintet, 2H, $J$=7.1 Hz, C$\underline{H}_2$), 2.03 (t, 2H, $J$=7.4 Hz, C$_{2'}\underline{H}_2$), 2.71 (s, 3H, SC$\underline{H}_3$), 3.20 (t, 2H, $J$=7.5 Hz, SC$\underline{H}_2$), 6.70 (br s, 1H, N$\underline{H}$), 7.23 (br s, 1H, N$\underline{H}$), 7.55–8.12 (m, 5H, C$\underline{H}_{arom}$), 8.43 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 24.9 (t, $C_{3'}H_2$), 28.2 (t, $C_{5'}H_2$), 28.3 (t, $C_{4'}H_2$), 28.5 (t, $C_{6'}H_2$), 29.0 (t, $C_{7'}H_2$), 30.4 (t, $SC_{8'}H_2$), 34.9 (t, $C_{2'}H_2$), 110.2 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.5 (s, $C_{4''}$), 129.0 (d, $C_{3''}$, $C_{5''}$), 133.5 (d, $C_3$) 138.1 (s, $C_{1''}$), 151.0 (s, $C_{7a}$), 165.3 (s, $C_4$), 168.5 (s, $C_6$), 174.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3375, NH; 3200, NH; 1660, C—O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{20}H_{25}N_5OS_2$): C, 57.8; H, 6.1; N, 16.9. Found: C, 57.8; H, 6.2; N, 16.6%.

**8′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-octanamide (7l).** $R_f$ in ethyl acetate = 0.65; Yield 48%; mp 124–126 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 0.99 (t, 3H, $J$=7.2 Hz, $CH_2C\underline{H}_3$), 1.06–1.46 (m, 16H, 8×C$\underline{H}_2$), 1.74 (quintet, 2H, $J$=7.3 Hz, C$\underline{H}_2$), 2.00 (t, 2H, $J$=7.3 Hz, C$_{2'}\underline{H}_2$), 2.69 (s, 3H, SC$\underline{H}_3$), 3.06 (m, 2H, $J$=7.2 Hz, 5.5 Hz, C$\underline{H}_2$CH$_3$), 3.19 (t, 2H, $J$=7.4 Hz, SC$\underline{H}_2$), 7.35–8.17 (m, 5H, C$\underline{H}_{arom}$), 7.64 (br t, 1H, N$\underline{H}$), 8.46 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 24.9 (t, $C_{3'}H_2$), 28.2 (t, $C_{5'}H_2$), 28.3 (t, $C_{4'}H_2$), 28.5 (t, $C_{6'}H_2$), 29.0 (t, $C_{7'}H_2$), 30.4 (t, $SC_{8'}H_2$), 34.9 (t, $C_{2'}H_2$), 110.2 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.5 (s, $C_{4''}$), 129.0 (d, $C_{3''}$, $C_{5''}$), 133.5 (d, $C_3$), 138.1 (s, $C_{1''}$), 151.0 (s, $C_{7a}$), 165.3 (s, $C_4$), 168.5 (s, $C_6$), 174.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3310, NH; 1650, C=O; 1600 cm$^{-1}$, C=C. Anal. calcd for ($C_{22}H_{28}N_5OS_2$): C, 59.7; H, 6.4; N, 15.8. Found: C, 59.6; H, 6.6; N, 15.8%.

**12′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)dodecanamide (7m).** $R_f$ in ethyl acetate = 0.65; Yield 55%; mp 131–134 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 1.14–1.44 (m, 16H, 8×C$\underline{H}_2$), 1.73 (quintet, 2H, $J$=6.5 Hz, C$\underline{H}_2$), 2.01 (t, 2H, $J$=7.3 Hz, C$_{2'}\underline{H}_2$), 2.68 (s, 3H, SC$\underline{H}_3$), 3.17 (t, 2H, $J$=7.2 Hz, SC$\underline{H}_2$), 6.66 (br s, 1H, N$\underline{H}$), 7.20 (br s, 1H, N$\underline{H}$), 7.38–8.16 (m, 5H, C$\underline{H}_{arom}$), 8.46 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): δ 11.4 (q, $SCH_3$), 25.0 (t, $C_{3'}H_2$), 28.4 (t, $C_{10'}H_2$), 28.5, 28.6, 28.7, 28.8 (t, $C_{9'}H_2$-$C_{4'}H_2$, indistinguishable), 29.0 (t, $C_{11'}H_2$), 30.5 (t, $C_{12'}H_2$), 35.1 (t, $C_{2'}H_2$), 110.3 (s, $C_{3a}$), 120.6 (d, $C_{2''}$, $C_{6''}$), 126.6 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 133.7 (d, $C_3$), 138.4 (s,

$C_{1''}$), 151.2 (s, $C_{7a}$), 165.5 (s, $C_4$), 168.8 (s, $C_6$), 174.5 (s, C=O); IR (KBr disc): $\nu_{max}$ 3400, NH; 3200, NH; 1650, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{24}H_{33}N_5OS_2$): C, 61.1; H, 7.1; N, 14.9. Found: C, 61.0; H, 7.1; N, 14.6%.

**12′-(4-Methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-dodecanamide (7n).** $R_f$ in ethyl acetate = 0.80, $R_f$ in hexane:ethyl acetate (1:1) = 0.29; Yield 60%; mp 124–125 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 0.99 (t, 3H, $J$ = 7.2 Hz, $CH_2C\underline{H}_3$), 1.06–1.46 (m, 16H, 8 × $C\underline{H}_2$), 1.74 (quintet, 2H, $J$ = 7.2 Hz, $C\underline{H}_2$), 2.01 (t, 2H, $J$ = 7.3 Hz, $C_{2'}\underline{H}_2$), 2.70 (s, 3H, $SC\underline{H}_3$), 3.10 (m, 2H, $J$ = 7.1 Hz, 5.9 Hz, $C\underline{H}_2CH_3$); 3.19 (t, 2H, $J$ = 7.3 Hz, $SC\underline{H}_2$), 7.35–8.17 (m, 5H, $C\underline{H}_{arom}$), 7.64 (br s, 1H, N$\underline{H}$), 8.46 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$, −60°C): δ 11.2 (q, $SCH_3$), 14.4 (q, $CH_2\underline{C}H_3$), 25.0 (t, $C_{3'}H_2$), 28.1 (t, $C_{10'}H_2$), 28.2, 28.3, 28.5 (t, $C_{9'}H_2$-$C_{4'}H_2$, indistinguishable), 28.8 (t, $C_{11'}H_2$), 30.6 (t, $C_{12'}H_2$), 32.7 (t, $\underline{C}H_2CH_3$), 35.2 (t, $C_{2'}H_2$), 110.1 (s, $C_{3a}$), 120.5 (d, $C_{2''}$, $C_{6''}$), 126.3 (s, $C_{4''}$), 128.7 (d, $C_{3''}$, $C_{5''}$), 133.2 (d, $C_3$) 138.0 (s, $C_{1''}$), 150.1 (s, $C_{7a}$), 165.4 (s, $C_4$), 168.5 (s, $C_6$), 171.4 (s, C=O); IR (KBr disc): $\nu_{max}$ 3300, NH; 1640, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{26}H_{37}N_5OS_2$): C, 62.5; H, 7.5; N, 14.0. Found: C, 62.7; H, 7.6; N, 13.8%.

**2′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)amides (4).** The following general procedure was used: Compound **7a** (0.1 mmol) was added to a saturated solution of ammonia in ethanol (15 mL). The mixture was heated at 100 °C for 72 h in a bomb. The solvent was removed to yield a white solid recrystallized from DMSO and water to give *2′-(4-amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)ethanamide* (**4a**): yield 75%; mp dec 262–272 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 3.78 (s, 2H, $SC\underline{H}_2$), 7.18 (br s, 1H, N$\underline{H}$), 7.27–8.21 (m, 6H, 5$C\underline{H}_{arom}$, 1N$\underline{H}$), 7.92 (br s, 1H, N$\underline{H}$), 8.20 (br s, 1H, N$\underline{H}$), 8.26, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 34.2 (t, $SC_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.3 (d, $C_{2''}$, $C_{6''}$), 125.9 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.6 (s, $C_{7a}$), 157.4, (s, $C_4$), 168.8 (s, $C_6$), 169.7 (s, C=O); IR (KBr disc): $\nu_{max}$ 3450, NH; 3400, NH; 3300, NH; 3150, NH; 1650 $cm^{-1}$, C=O. Anal. calcd for ($C_{13}H_{12}N_6OS$): C, 52.0; H, 4.0; N, 28.0. Found: C, 52.1; H, 4.1; N, 27.6%.

**2′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethylethanamide (4b).** $R_f$ in ethyl acetate = 0.58; (yield 82%); mp 254–256 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.92, (t, 3H, $J$ = 7.2 Hz, $CH_2C\underline{H}_3$); 3.03 (m, 2H, $J$ = 7.1 Hz, 5.7 Hz, $C\underline{H}_2CH_3$), 3.80 (s, 2H, $SC\underline{H}_2$), 7.28–8.19 (m, 5H, $C\underline{H}_{arom}$), 7.86 (br s, 1H, N$\underline{H}$), 8.06 (br t, 2H, 1CON$\underline{H}$, 1N$\underline{H}$), 8.25, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.5 (q, $CH_2\underline{C}H_3$), 33.8 (t, $\underline{C}H_2CH_3$), 34.4 (t, $SC_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.2 (d, $C_{2''}$, $C_{6''}$), 125.9 (s, $C_{4''}$), 129.2 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 138.9 (s, $C_{1''}$), 153.5 (s, $C_{7a}$), 157.3 (s, $C_4$), 167.2 (s, $C_6$), 168.6 (s, C=O); IR (KBr disc): $\nu_{max}$ 3500, NH; 3315, NH; 3230, NH; 1660, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{15}H_{16}N_6OS$): C, 54.9; H, 4.9; N, 25.6. Found: C, 54.8; H, 4.9; N, 25.4%.

**3′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)propanamide (4c).** $R_f$ in ethyl acetate = 0.47; Yield 50%; mp 247.5–249 °C; $^1$H NMR (200 MHz, DMSO-$d_6$): δ 2.59 (t, 2H, $J$ = 7.1 Hz, $C\underline{H}_2$), 3.27 (t, 2H, $J$ = 7.1 Hz, $SC\underline{H}_2$), 6.91 (br s, 1H, CON$\underline{H}$), 7.28–8.25 (m, 6H, 5$C\underline{H}_{arom}$, 1CON$\underline{H}$), 7.93 (br s, 1H, N$\underline{H}$), 8.02 (br s, 1H, N$\underline{H}$), 8.26, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 26.0 (t, $SC_{3'}H_2$), 35.0 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.0 (d, $C_{2''}$, $C_{6''}$), 125.8 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.2 (d, $C_3$), 138.9 (s, $C_{1''}$), 153.6 (s, $C_{7a}$), 157.4 (s, $C_4$), 169.1 (s, $C_6$), 176.2 (s, C=O); IR (KBr disc): $\nu_{max}$ 3485 NH; 3425, NH; 3300, NH; 3175, NH; 3100, NH; 1700 and 1660, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{14}H_{14}N_6OS$): C, 53.5; H, 4.5; N, 26.7. Found: C, 53.6; H, 4.5; N, 26.7%.

**3′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethylpropanamide (4d).** Yield 55%; mp 251–253.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.01 (t, 3H, $J$ = 7.2 Hz, $CH_2C\underline{H}_3$), 2.59 (t, 2H, $J$ = 7.1 Hz, $C\underline{H}_2$), 3.10 (m, 2H, $J$ = 7.3 Hz, 5.6 Hz, $C\underline{H}_2CH_3$), 3.29 (t, 2H, $J$ = 7.2 Hz, $SC\underline{H}_2$), 7.32–8.24 (m, 5H, $C\underline{H}_{arom}$), 7.56 (br t, 2H, CON$\underline{H}$, N$\underline{H}$), 7.86 (br s, 1H, N$\underline{H}$), 8.27 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14. 5 (q, $CH_2\underline{C}H_3$), 26.1 (t, $SC_{3'}H_2$), 33.2 (t, $\underline{C}H_2CH_3$), 35.2 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.1 (d, $C_{2''}$, $C_{6''}$), 125.8 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.6 (s, $C_{7a}$), 157.5 (s, $C_4$), 169.2 (s, $C_6$), 170.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3475, NH; 3325, NH; 3100; NH; 1660 and 1645 $cm^{-1}$, C=O. Anal. calcd for ($C_{16}H_{18}N_6OS$): C, 56.1; H, 5.3; N, 24.5. Found: C, 56.2; H, 5.2; N, 24.6%.

**4′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)butanamide (4e).** Yield 86%; mp 265–267 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.93 (m, 2H, $J$ = 7.2 Hz, $C_{3'}\underline{H}_2$), 2.23 (t, 2H, $J$ = 7.4 Hz, $C_{2'}\underline{H}_2$), 3.09 (t, 2H, $J$ = 7.3 Hz, $SC\underline{H}_2$), 6.79 (br s, 1H, CON$\underline{H}$), 7.28–8.21 (m, 6H, 5$C\underline{H}_{arom}$, 1N$\underline{H}$), 7.83 (br s, 1H, N$\underline{H}$), 8.01 (br s, 1H, N$\underline{H}$), 8.25 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-*d6*): δ 25.2 (t, $C_{3'}H_2$), 29.8 (t, $SC_{4'}H_2$), 34.2 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.0 (d, $C_{2''}$, $C_{6''}$), 125.8 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.2 (d, $C_3$), 138.9 (s, $C_{1''}$), 153.6 (s, $C_{7a}$), 157.3 (s, $C_4$), 169.2 (s, $C_6$), 173.5 (s, C=O); IR (KBr disc): $\nu_{max}$ 3450, NH; 3325, NH; 3200, NH; 3100, NH; 1660, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{15}H_{16}N_6OS$): C, 54.9; H, 4.9; N, 25.6. Found: C, 55.0; H, 5.0; N, 25.8%.

**4′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-butanamide (4f).** $R_f$ in ethyl acetate = 0.60; yield 65%; mp 247–248 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.97 (t, 3H, $J$ = 7.2 Hz, $CH_2CH_3$), 1.96 (m, 2H, $J$ = 7.3 Hz, $C_{3'}\underline{H}_2$), 2.22 (t, 2H, $J$ = 7.3 Hz, $C_{2'}\underline{H}_2$), 3.05 (m, 4H, $J$ = 7.2 Hz, 5.6 Hz, $C\underline{H}_2CH_3$, $SC\underline{H}_2$), 7.30–8.21 (m, 5H, $C\underline{H}_{arom}$), 7.84 (br s, 2H, CON$\underline{H}$, N$\underline{H}$), 8.0 (br s, 1H, N$\underline{H}$), 8.25 (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.6 (q, $CH_2\underline{C}H_3$), 25.3 (t, $C_{3'}H_2$), 29.7 (t, $SC_{4'}H_2$), 33.1 (t, $\underline{C}H_2CH_3$), 34.4 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.1 (d, $C_{2''}$, $C_{6''}$), 125.9 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.8 (s, $C_{7a}$), 157.5 (s, $C_4$), 169.3 (s, $C_6$), 171.1 (s, C=O); IR

(KBr disc): $\nu_{max}$ 3450, NH; 3325, NH; 3075, NH; 1650, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{17}H_{20}N_6OS$): C, 57.3; H, 5.7; N, 23.6. Found: C, 57.4; H, 5.6; N, 23.6%.

**5′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)pentanamide (4g).** $R_f$ in ethyl acetate = 0.42; Yield 70%; mp dec 229–233 °C; $^1$H NMR (250.12 MHz, *DMSO-d6*): δ 1.66 (m, 4H, 2 × C$\underline{H}_2$), 2.08 (t, 2H, $J$ = 6.8 Hz, $C_{2'}\underline{H}_2$), 3.09 (t, 2H, $J$ = 6.7 Hz, S$\underline{CH}_2$), 6.71 (br s, 1H, CON$\underline{H}$), 7.25 (br s, 1H, CON$\underline{H}$), 7.28–8.20 (m, 5H, C$\underline{H}_{arom}$), 7.84 (br s, 1H, N$\underline{H}$), 7.99 (br s, 1H, N$\underline{H}$), 8.25, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 24.6 (t, $C_{3'}H_2$), 29.0 (s, $C_{4'}H_2$), 29.7 (s, $SC_{5'}H_2$), 34.6 (s, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.2 (d, $C_{2''}$, $C_{6''}$), 125.9 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.8 (s, $C_{7a}$), 157.4 (s, $C_4$), 169.4 (s, $C_6$), 174.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3450, NH; 3425, NH; 3400, NH; 3275, NH; 3075, NH; 1650 $cm^{-1}$, C=O. Anal. calcd for ($C_{16}H_{18}N_6OS$): C, 56.1; H, 5.3; N, 24.5. Found: C, 56.4; H, 5.3; N, 24.5%.

**5′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-pentanamide (4h).** Yield 68%; mp decomp. 201.5–204.5 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.96 (t, 3H, $J$ = 7.2 Hz, $CH_2C\underline{H}_3$), 1.68 (m, 4H, 2 × $CH_2$), 2.08 (t, 2H, $J$ = 6.6 Hz, $C_{2'}\underline{H}_2$), 3.05 (m 4H, $J$ = 7.1 Hz, 5.8 Hz, $C\underline{H}_2CH_3$, $SC\underline{H}_2$), 7.27–8.20 (m, 5H, C$\underline{H}_{arom}$), 7.79 (br t, 2H, CON$\underline{H}$, N$\underline{H}$), 8.01 (br s, 1H, N$\underline{H}$), 8.25, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$) δ 14.6 (q, $CH_2\underline{C}H_3$), 24.8 (t, $C_{3'}H_2$), 29.0 (t, $C_{4'}H_2$), 29.8 (t, $SC_{5'}H_2$), 33.1 (t, $\underline{C}H_2CH_3$), 35.0 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.2 (d, $C_{2''}$, $C_{6''}$), 125.9 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.1 (s, $C_{1''}$), 153.8 (s, $C_{7a}$), 157.5 (s, $C_4$), 169.5 (s, $C_6$), 171.7 (s, C=O); IR (KBr disc): $\nu_{max}$ 3425, NH; 3325, NH; 3125, NH; 1655 and 1625 $cm^{-1}$, C=O. Anal. calcd for ($C_{18}H_{22}N_6OS$): C, 58.4; H, 6.0; N, 22.7. Found C, 58.6; H, 6.0; N, 22.6%.

**6′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-hexanamide (4i).** $R_f$ in ethyl acetate = 0.44; Yield 55%; mp 215–217 °C; $^1$H NMR (200.0 MHz, DMSO-$d_6$): δ 1.50 (m, 4H, $J$ = 7.3 Hz, 6.7 Hz, 2 × C$\underline{H}_2$) 1.74 (m, 2H, $J$ = 7.4 Hz, $C_{5'}\underline{H}_2$), 2.07 (t, 2H, $J$ = 7.0 Hz, $C_{2'}\underline{H}_2$), 3.11 (t, 2H, $J$ = 7.1 Hz, S$\underline{CH}_2$), 6.73 (br s, 1H, CON$\underline{H}$), 7.25 (br s, 1H, CON$\underline{H}$), 7.29–8.23 (m, 5H, C$\underline{H}_{arom}$), 7.95 (br s, 2H, N$\underline{H}_2$), 8.27, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (200.0 MHz, DMSO-$d_6$): δ 24.8 (t, $C_{3'}H_2$), 28.4 (t, $C_{4'}H_2$), 29.3 (t, $C_{5'}H_2$), 30.0 (t, $C_{6'}H_2$), 35.1 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.1 (d, $C_{2''}$, $C_{6''}$), 126.0 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.1 (s, $C_{1''}$), 153.7 (s, $C_{7a}$), 157.4 (s, $C_4$), 169.4 (s, $C_6$), 174.1 (s, C=O); IR (KBr disc): $\nu_{max}$ 3470, NH; 3425, NH; 3320, NH; 3225, NH; 3125, NH; 1650, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{17}H_{20}N_6OS$): C, 57.3; H, 5.7; N, 23.6. Found C, 57.2; H, 5.7; N, 23.4%.

**6′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-hexanamide (4j).** Yield 60%; mp 205–207 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 0.96 (t, 3H, $J$ = 7.2 Hz, $CH_2C\underline{H}_3$), 1.40 (m, 2H, $J$ = 6.9 Hz, $C\underline{H}_2$), 1.53 (m, 2H, $J$ = 7.0 Hz, $C\underline{H}_2$), 1.70 (quintet, 2H, $J$ = 7.2 Hz, $C_{5'}\underline{H}_2$), 2.03 (t, 2H, $J$ = 7.3 Hz, $C_{2'}\underline{H}_2$), 3.04 (m 4H, $J$ = 7.2 Hz, 5.6 Hz, $C\underline{H}_2CH_3$, $SC\underline{H}_2$), 7.27–8.19 (m, 5H, C$\underline{H}_{arom}$), 7.79 (br t, 2H, CON$\underline{H}$, N$\underline{H}$), 8.00 (br s, 1H, N$\underline{H}$), 8.24, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (62.8 MHz, DMSO-$d_6$): δ 14.7 (q, $CH_2\underline{C}H_3$), 24.9 (t, $C_{3'}H_2$), 28.2 (t $C_{4'}H_2$), 29.2 (t, $C_{5'}H_2$), 29.9 (t, $SC_{6'}H_2$), 33.2 (t, $\underline{C}H_2CH_3$), 35.4 (t, $C_{2'}H_2$), 99.2 (s, $C_{3a}$), 120.1 (d, $C_{2''}$, $C_{6''}$), 125.8 (s, $C_{4''}$), 129.0 (d, $C_{3''}$, $C_{5''}$), 134.2 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.7 (s, $C_{7a}$), 157.3, (s, $C_4$), 169.4 (s, $C_6$), 171.7 (s, C=O); IR (KBr disc): $\nu_{max}$ 3435, NH; 3310, NH; 3075, NH; 1660 and 1650 $cm^{-1}$, C=O; 1600 $cm^{-1}$, C=C. Anal. calcd for ($C_{19}H_{24}N_6OS$): C, 59.4; H, 6.3; N, 21.9. Found C, 59.0; H, 6.3; N, 21.7%.

**8′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-octanamide (4k).** Yield 50%; mp 183–187 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.20–1.45 (m, 8H, 4 × C$\underline{H}_2$) 1.70 (m, 2H, $J$ = 6.9 Hz, $C\underline{H}_2$), 2.00 (t, 2H, $J$ = 7.2 Hz, $C_{2'}\underline{H}_2$), 3.09 (t, 2H, $J$ = 7.5 Hz, S$\underline{CH}_2$), 6.65 (br s, 1H, CON$\underline{H}$), 7.20 (br s, 1H, CON$\underline{H}$), 7.28–8.20 (m, 5H, C$\underline{H}_{arom}$), 7.94 (br s, 2H, N$\underline{H}_2$), 8.25, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 25.1 (t, $C_{3'}H_2$), 28.5 (t, $C_{5'}H_2$), 28.6 (t, $C_{4'}H_2$), 28.7 (t, $C_{6'}H_2$), 29.5 (t, $C_{7'}H_2$), 30.1 (t, $C_{8'}H_2$), 35.1 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.2 (d, $C_{2''}$, $C_{6''}$), 126.0 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.7 (s, $C_{7a}$), 157.4 (s, $C_4$), 169.5 (s, $C_6$), 174.3 (s, C=O); IR (KBr disc): $\nu_{max}$ 3500, NH; 3450, NH; 3350, NH; 3250, NH; 3125, NH; 1660, C=O; 1595 $cm^{-1}$, C=C. Anal. calcd for ($C_{19}H_{24}N_6OS$): C, 59.4; H, 6.3; N, 21.9. Found C, 59.7; H, 6.2; N, 21.6%.

**8′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-octanamide (4l).** $R_f$ in ethyl acetate = 0.66; Yield 45%; mp 171–172 °C; $^1$H NMR (200 MHz, DMSO-*d6*) δ 0.98 (t, 3H, $J$ = 7.2 Hz, $CH_2C\underline{H}_3$), 1.26–1.50 (m, 8H, 4 × $C\underline{H}_2$), 1.68 (quintet, 2H, $J$ = 7.5 Hz, $C\underline{H}_2$), 2.01 (t, 2H, $J$ = 7.3 Hz, $C_{2'}\underline{H}_2$), 3.03 (m, 4H, $J$ = 7.2 Hz, 5.5 Hz, $C\underline{H}_2CH_3$, $SC\underline{H}_2$), 7.28–8.22 (m, 5H, C$\underline{H}_{arom}$), 7.71 (br t, 1H, CON$\underline{H}$), 7.90 (br s, 2H, N$\underline{H}_2$), 8.24, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 14.8 (q, $CH_2\underline{C}H_3$), 25.3 (t, $C_{3'}H_2$), 28.5 (t, $C_{5'}H_2$), 28.6 (t, $C_{4'}H_2$), 28.7 (t, $C_{6'}H_2$), 29.5 (t, $C_{7'}H_2$), 30.1 (t, $C_{8'}H_2$), 33.2 (t, $\underline{C}H_2CH_3$), 35.4 (t, $C_{2'}H_2$), 99.3 (s, $C_{3a}$), 120.2 (d, $C_{2''}$, $C_{6''}$), 125.9 (s, $C_{4''}$), 129.1 (d, $C_{3''}$, $C_{5''}$), 134.3 (d, $C_3$), 139.0 (s, $C_{1''}$), 153.7 (s, $C_{7a}$), 157.4 (s, $C_4$), 169.5 (s, $C_6$), 171.7 (s, C=O); IR (KBr disc): $\nu_{max}$ 3440, NH; 3310, NH; 3075, NH; 1655 and 1630, C=O; 1595 $cm^{-1}$, C=C. Anal. calcd for ($C_{21}H_{28}N_6OS$): C, 61.1; H, 6.8; N, 20.4. Found C, 61.0; H, 6.8; N, 20.0%.

**12′-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-dodecanamide (4m).** $R_f$ in ethyl acetate = 0.54; Yield 40%; mp 170–172 °C; $^1$H NMR (250.12 MHz, DMSO-$d_6$): δ 1.11–1.45 (m, 18H, 9 × C$\underline{H}_2$) 1.67 (m, 2H, $J$ = 7.3 Hz, $C\underline{H}_2$), 1.99 (t, 2H, $J$ = 7.4 Hz, $C_{2'}\underline{H}_2$), 3.08 (t, 2H, $J$ = 7.2 Hz, S$\underline{CH}_2$), 6.64 (br s, 1H, CON$\underline{H}$), 7.18 (br s, 1H, CON$\underline{H}$), 7.27–8.21 (m, 5H, C$\underline{H}_{arom}$), 7.84 (br s, 2H, N$\underline{H}_2$), 8.25, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 24.9 (t, $C_{3'}H_2$), 28.3 (t, $C_{10'}H_2$), 28.4, 28.5, 28.7 (t, $C_{9'}H_2$-$C_{4'}H_2$, indistinguishable), 29.2 (t, $C_{11'}H_2$), 29.4 (t,

$C_{12'}H_2$), 34.9 (t, $C_{2'}H_2$), 99.2 (s, $C_{3a}$), 119.9 (d, $C_{2''}$, $C_{6''}$), 125.6 (s, $C_{4''}$), 128.7 (d, $C_{3''}$, $C_{5''}$), 134.0 (d, $C_3$), 139.3 (s, $C_{1''}$), 153.6 (s, $C_{7a}$), 157.2 (s, $C_4$), 169.3 (s, $C_6$), 174.0 (s, C=O); IR (KBr disc): $\nu_{max}$ 3375, NH; 3275, NH; 3075, NH; 1660, C=O; 1595 $cm^{-1}$, C=C. Anal. calcd for ($C_{23}H_{32}N_6OS$): C, 62.7; H, 7.3; N, 19.1. Found C, 62.5; H, 7.6; N, 18.8%.

**12'-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)-*N*-ethyl-dodecanamide (4n).** $R_f$ in ethyl acetate = 0.75; Yield 30%; mp 179–180.5 °C; $^1$H NMR (200 MHz, DMSO-$d_6$) δ 1.05 (t, 3H, $J$ = 7.3 Hz, $CH_2C\underline{H}_3$), 1.21–1.55 (m, 18H, $9\times C\underline{H}_2$), 1.78 (quintet, 2H, $J$ = 6.6 Hz, $C\underline{H}_2$), 2.07 (t, 2H, $J$ = 7.4 Hz, $C_{2'}\underline{H}_2$), 3.13 (m, 4H, $J$ = 7.3 Hz, 5.6 Hz, $C\underline{H}_2CH_3$, $SC\underline{H}_2$), 7.35–8.30 (m, 5H, $C\underline{H}_{arom}$), 7.80 (br t, 1H, CONH), 7.95 (br s, 2H, $N\underline{H}_2$), 8.33, (s, 1H, $\underline{H}_3$); $^{13}$C NMR (50.3 MHz, DMSO-$d_6$): δ 14.6 (q, $CH_2\underline{C}H_3$), 25.0 (t, $C_{3'}H_2$), 28.4 (t, $C_{10'}H_2$), 28.5, 28.6, 28.7 (t, $C_{9'}H_2$-$C_{4'}H_2$, indistinguishable), 29.2 (t, $C_{11'}H_2$), 29.9 (t, $C_{12'}H_2$), 33.0 (t, $\underline{C}H_2CH_3$), 35.3 (t, $C_{2'}H_2$), 99.1 (s, $C_{3a}$), 120.0 (d, $C_{2''}$, $C_{6''}$), 125.8 (s, $C_{4''}$), 128.8 (d, $C_{3''}$, $C_{5''}$), 134.6 (d, $C_3$), 138.9 (s, $C_{1''}$), 153.4 (s, $C_{7a}$), 157.2 (s, $C_4$), 169.3 (s, $C_6$), 171.5 (s, C=O); IR (KBr disc): $\nu_{max}$ 3460, NH; 3325, NH; 3050, NH; 1655 and 1645, C=O; 1595 $cm^{-1}$, C=C. Anal. calcd for ($C_{25}H_{36}N_6OS$): C, 64.1; H, 7.7; N, 17.9. Found C, 64.2; H, 7.9; N, 17.7%.

### Preparation of membranes from rat cerebral cortex and rat cerebellum

This followed a simplified version of the Gray and Whittaker method.[5,13] Whole brains from Wistar rats (25 rats, 300–350 g) were immersed in 0.32 M ice-cold sucrose. The striata were explanted and placed in 10 mL ice-cold buffer (50 mM TrisHCl (Sigma), 10 mM $MgCl_2$, pH 7.4), to be used for the preparation of $A_{2a}$ adenosine receptors. The cerebellum and remainder of the cerebral cortex was placed in 15 mL of ice-cold buffer (50 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.4). The tissue was homogenized (Brinkman polytron) and the volume adjusted to 48.0 g. Homogenates were centrifuged (1000 g for 10 mins at 4 °C) and the supernatant ($S_1$) recentrifuged (35000 g for 15 min at 4 °C). The supernatant ($S_2$) was discarded and every eight pellets ($P_2$) pooled and resuspended in ice-cold distilled water (30 mL) and the volume was adjusted to 48.00 g. The homogenates were recentrifuged (35000 g for 15 min at 4 °C). The pellets ($P_3$) were pooled and resuspended in ice-cold buffer (60 mL of 50 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.4). When stored at −30 °C, binding to the synaptosomal membranes remained unchanged for 1 month. The protein concentration was estimated using Pierce BCA protein assay reagent using a modified Lowry protein assay.[14] Tissue samples were solubilized using 10 μL of 10% SDS and were made up to 100 μL. The standards contained 0 to 120 mg of protein. Five dilutions of tissue from 4 to 75% were sampled. The absorbance was read using a Titertek Multiscan MC Microtiter Plate Reader at 540 nM absorbance. The average concentration of $A_1$ tissue was 2.63 μg/μL.

### Preparation of membranes from rat striata

The buffer was drained from the striata and weighed. The striata were placed in 20 mL ice-cold buffer [50 mM Tris-HCl (Sigma), 10 mM $MgCl_2$, pH 7.4] and homogenised using the polytron, placed into centrifuge tubes and the volume adjusted to 48.00 g, and centrifuged (35000 g for 15 min at 4 °C). The supernatant was discarded and the pellet resuspended in ice-cold buffer (10 mL of 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4). The homogenate was recentrifuged (35000 g for 15 min at 4 °C). The supernatant was discarded and the pellets pooled and resuspended in ice-cold buffer (1 g of striata/10 mL of 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4). This was stored at −30 °C and remained unchanged for 1 month. The protein concentration was estimated using Pierce BCA protein assay reagent. The average concentration of $A_{2a}$ tissue was 3.78 μg/μL.

### $^3$H-(*R*)-$N^6$-(phenylisopropyl)adenosine binding assay[11]

Aliquots of $A_1$ tissue were thawed and preincubated with adenosine deaminase (1 mg/mL for 20 min at 37 °C; ADA, EC 3.5.4.4, Sigma) to remove endogenous adenosine. Between 0.3–0.5 mg of protein per replicate was incubated with 2 nM $^3$H-(*R*)-$N^6$-(phenylisopropyl)adenosine (60 Ci/mmol, 1 mCi/mL, New England Nuclear) for 1 h at 37 °C in an incubation volume of 0.5 mL. Radioligand binding assays were performed over 10 concentrations in duplicates. Each compound was tested in two different experiments. The total volume of the $A_1$ assay was 0.5 mL. The compounds proved insoluble in buffer and hence the stock concentrations were made up in DMSO. The final concentration of DMSO in the assay was 1%. The assay was monitored against a control of total binding and non-specific binding each containing 1% DMSO. Non-specific binding was determined using cold CPA (0.1 mM final concentration). Reactions were terminated by the addition of 3 mL of ice-cold incubation buffer. This was filtered over Whatman GF/B Glass fiber filters and washed with two more 3 mL of ice-cold buffer washes. The filters were transferred to vials, thoroughly shaken with 3 mL of BCS scintillant fluid. Samples were equilibrated in the dark for a minimum of 6 h before counting for 1 min in a Packard Tricarb 2000CA series liquid scintillation Analyser at 40% efficiency.

The results from concentration–inhibition studies were analyzed with non-linear-least-squares curve-fitting program.[15] $A_1K_i$ values were calculated using the Cheng–Prusoff equation[12] using the average $K_d$ value of $^3$H-PIA as 2.35 nM and a final ligand concentration of 2 nM. $A_1K_i$ values are geometric means from two determinations, run in duplicate ± standard error.

### $^3$H-CGS21680 binding assay[10]

Aliquots of $A_{2a}$ tissue were thawed and preincubated with adenosine deaminase (1 mg/mL for 20 min at 37 °C; ADA, EC 3.5.4.4) to remove endogenous adenosine. Between 0.3–0.5 mg of protein per replicate was

incubated with 5 nM $^3$H-CGS21680 (48.6 Ci/mmol, 1 mCi/mL, New England Nuclear) for 95 min at room temperature. Radioligand binding assays were performed over 10 concentrations in duplicates. Each compound was tested in two different experiments. Total volume of the $A_{2a}$ assay was 0.5 mL. The compounds proved insoluble in buffer and hence the stock concentrations were made up in DMSO. The final concentration of DMSO in the assay was 1%. The assay was monitored against a control of total binding and non-specific binding each containing 1 percent DMSO. Non-specific binding was determined using cold 2-CADO (0.1 mM final concentration). Reactions were terminated by the addition of 3 mL of ice-cold incubation buffer. This was filtered over presoaked (6 h) Whatman GF/B Glass fiber filters and washed with two more 3 mL of ice-cold buffer washes. The filters were transferred to vials, thoroughly shaken with 3 mL of BCS scintillant fluid. Samples were equilibrated in the dark for a minimum of 6 h before counting for 1 min in a Packard Tricarb 2000CA series liquid scintillation Analyser at 40% efficiency.

The results from concentration–inhibition studies were analyzed with non-linear-least-squares curve-fitting program.[15] $A_{2a}K_i$ values were calculated using the Cheng–Prusoff equation[12] using the average $K_d$ value of $^3$H-CGS21680 as 14.9 nM and a final ligand concentration of 5 nM. $A_{2a}K_i$ values are geometric means from two determinations, run in duplicate $\pm$ standard error.

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**References**

1. Collis, M. G.; Hourani, S. M. O. *Trends Pharmacol. Sci.* **1993**, *14*, 360.

2. Müller, C. E.; Scior, T. *Pharm. Acta Helv.* **1993**, *68*, 77.

3. Davies, L. P.; Brown, D. J.; Chow, S. C.; Johnston, G. A. R. *Neurosci. Lett.* **1983**, *41*, 189.

4. Davies, L. P.; Chow, S. C.; Skerritt, J. H.; Brown, D. J.; Johnston, G. A. R. *Life Sci.* **1984**, *34*, 2117.

5. Quinn, R. J.; Scammells, P. J.; Tucker, D. J. *Aust. J. Chem.* **1991**, *44*, 753.

6. Chebib, M.; Quinn, R. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2409.

7. Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: Oxford, 1987.

8. Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285.

9. Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. *Biochem. Pharmacol.* **1986**, *35*, 2467.

10. Jarvis, M. F.; Schulz, R.; Hutchison, A. J.; Do, U. H.; Sills, M. A.; Williams, M. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888.

11. Schwabe, U.; Trost, T. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *313*, 179.

12. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

13. Gray, E. G.; Whittaker, V. P. *J. Anatom.* **1962**, *96*, 79.

14. Peterson, G. L. *Anal. Biochem.* **1977**, *83*, 346.

15. McPherson, G. A. *A Collection of Radioligand Binding Analysis Programs*; Elsevier-Biosoft: Amsterdam, 1985.