Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 20, pp. 2409-2412, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00420-3

PYRAZOLO[3,4-*d*]PYRIMIDINES; ADENOSINE RECEPTOR SELECTIVITY

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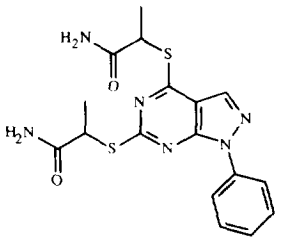
Abstract: Substitution of 1-phenylpyrazolo[3,4-*d*]pyrimidines at C6 (corresponding to C2 of xanthines/ adenosines) with thioethers containing amide moieties has resulted in compounds with A_1 and A_{2a} adenosine receptor selectivity. This further demonstrates that distal moieties can modify receptor selectivity.

Pyrazolo[3,4-*d*]pyrimidines were originally identified as adenosine antagonists during a study of a large number of nitrogen heterocycles, related to caffeine and theophylline, for activity as potential adenosine A_1 antagonists.[1,2] The most active compound was 4,6-bis-α-carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]-pyrimidine (**1**). At the time, it was not known how this group of compounds bound to adenosine receptors. The receptor topology was not known and there were not many pyrazolo[3,4-*d*]pyrimidines available to determine structure-activity relationships. Since that time, many pharmacophore models have been developed which try to explain how active agonists and antagonists bind to the adenosine receptors.[3-7] Molecular cloning has now established that adenosine receptors belong to the superfamily of seven transmembrane G protein-coupled membrane receptors and the physiological responses to adenosine are elicited through interaction with four major subtypes of adenosine receptors A_1, A_{2a}, A_{2b} and A_3.[8,9] This has now led to receptor modelling studies.[10,11]



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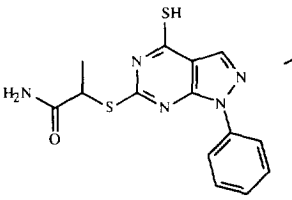

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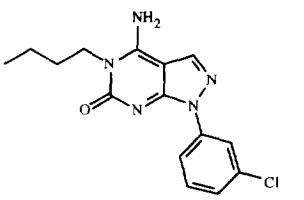
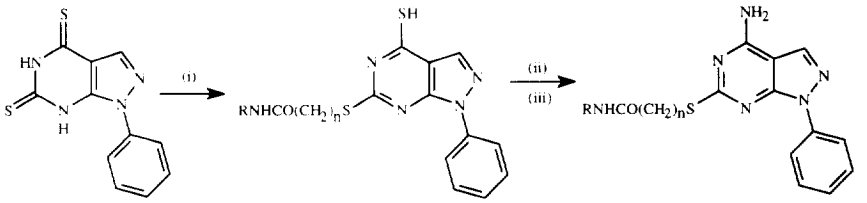


3

Further work on pyrazolo[3,4-*d*]pyrimidines indicated that the series may able to be modified to alter their adenosine receptor affinities. We have been able to demonstrate that α-(4-mercapto-1-phenylpyrazolo[3,4-*d*]-pyrimidin-6-ylthio) propionamide (**2**) had similar affinity for the A_1 receptor as **1** thereby demonstrating that the C4 amide side chain was not required for binding.[12] In other work, a series of pyrazolo[3,4-*d*]pyrimidine analogues of the naturally occurring adenosine agonist, 1-methylisoguanosine, were synthesised and tested for adenosine receptor affinity.[13-15] The study probed the effects of different alkyl and aryl substituents on N1 and

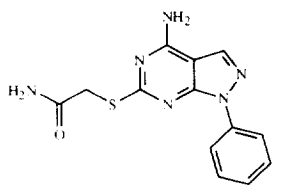
N5. The most active of this series was 4-amino-5-*N*-butyl-1-(3-chlorophenyl)1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-one (**3**) with an IC_{50} of 6.4 μM at the A_1 receptor and an IC_{50} of 19.2 μM at the A_{2a} receptor.

We now report the synthesis and receptor binding at A_1 and A_{2a} receptors of 1-phenylpyrazolo[3,4-*d*]-pyrimidines substituted at C6 with thioethers containing distal amide substituents. These compounds are analogues of **1** which was shown to be an A_1 antagonist. They lack the sugar moiety that has been a requisite for agonist activity. 1-Phenyl-5H,7H-pyrazolo[3,4-*d*]pyrimidine dithione (**4**) was monoalkylated with the corresponding bromoamides, alkylated with methyl iodide and converted to the 4-amino compounds by treatment with ethanolic ammonia in a sealed tube.[16]

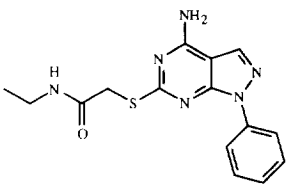


4 5 6

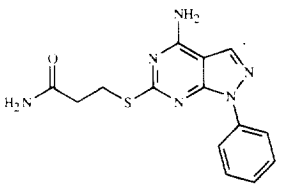
(i) $BrCH_2)_nCONHR$, R = H or Et, pyridine, rt; (ii) CH_3I, NaOH(aq), rt; (iii) $NH_3(g)$, EtOH, 100 °C



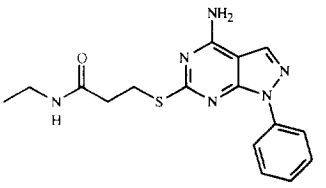
6a



6b



6c



6d

Table 1 Receptor binding at rat membrane adenosine A_1 and A_{2a} receptors.[17]

Compound	A_1 receptor K_i, nM	A_{2a} receptor K_i, nM	K_iA_{2a}/KiA_1
6a	28.5 ± 4.7	44.9 ± 17.2	1.6
6b	12.1 ± 4.5	131 ± 36	10.8
6c	428± 25	101 ± 26	0.24
6d	551 ± 81	1280 ± 170	2.3

The affinity of **1** had been reported only for the A_1 receptor, in our assay system **1** had a K_iA_1 of 229 ± 20nM and a K_i A_{2a} of 146 ±27 nM. The compounds **6a** and **6b** had increased A_1 affinity compared to **1** while

6a also had increased A_{2a} affinity. **6b** and **6c** 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 2'-(4-Amino-1-phenylpyrazolo[3,4-*d*] pyrimidin-6-ylthio)N-ethyl-ethanamide (**6b**) 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_{2a} receptor was 3'-(4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ylthio)propanamide (**6c**) 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 **6b** and **6c** 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 nM and 101 nM). In both cases N-ethyl substitution reduced A_{2a} affinity (44.9 to 131 nM for **6a/6b**, 101 to 1280 nM for **6c/6d**). In contrast N-ethyl substitution had little effect at the A_1 receptor (28.5 and 12.1 nM for **6a/6b**, 428 and 551 nM for **6c/6d**). 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 **6a/6c**, 12.1 to 551 nM for **6b/6d**) compared to the A_{2a} receptor (44.9 to 101 nM for **6a/6c**, 131 to 1280 nM for **6b/6d**). 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.

It may be worth exploring further substitutions in this series to define the non-hydrophobic binding domains. The changes observed with small structural variation at C6 indicating that further optimisation may be possible.

The pharmacophore model developed by ourselves (a C2-N6-C8 model)[9] and Peet (a N6-C8 model)[9] arose from the concept of commonality in hydrophobic residues in adenosine receptor ligands. Identification of a common hydrophobic binding site may lead to an assumption that modification of the hydrophobic domain is the best approach to design of selective compounds however a more correct conclusion is that the other regions could be exploited. This manuscript demonstrates that there is value in examining such other regions, specifically in pyrazolopyrimidines, and therefore in other compounds in order to develop selective adenosine receptor ligands.

Acknowledgment: We thank the National Health and Medical Research Council for support of this research.

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16. *Spectral data for 6a (2'-(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)ethanamide)* mp decomp. 262-272 °C. ^{1}H NMR (250.12 MHz, DMSO-d_6): δ 3.78 (s, 2H, SCH_2), 7.18 (br s, 1H, NH), 7.27 - 8.21 (m, 6H, 5CH_{arom}, 1NH), 7.92 (br s, 1H, NH), 8.20 (br s, 1H, NH), 8.26, (s, 1H, H_3). ^{13}C NMR (62.8 MHz, DMSO-d_6): δ 34.2 (t, SC_2H_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) ν_{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%.
Spectral data for 6b 2'-(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)N-ethyl-ethanamide mp 254-256 °C. ^{1}H NMR (250.12 MHz, DMSO-d_6): δ 0.92, (t, 3H, J = 7.2 Hz, CH_2CH_3), 3.03 (m, 2H, J = 7.1 Hz, 5.7 Hz, CH_2CH_3), 3.80 (s, 2H, SCH_2), 7.28 - 8.19 (m, 5H, CH_{arom}), 7.86 (br s, 1H, NH), 8.06 (br t, 2H, 1CONH, 1NH), 8.25, (s, 1H, H_3). ^{13}C NMR (62.8 MHz, DMSO-d_6): δ 14.5 (q, CH_2CH_3), 33.8 (t, CH_2CH_3), 34.4 (t, SC_2H_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) ν_{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%.
Spectral data for 6c (3'-(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)propanamide) mp 247.5-249 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.59 (t, 2H, J = 7.1 Hz, CH_2), 3.27 (t, 2H, J = 7.1 Hz, SCH_2), 6.91 (br s, 1H, CONH), 7.28 - 8.25 (m, 6H, 5CH_{arom}, 1CONH), 7.93 (br s, 1H, NH), 8.02 (br s, 1H, NH), 8.26, (s, 1H, H_3). ^{13}C NMR (62.8 MHz, DMSO-d_6): δ 26.0 (t, SC_3H_2), 35.0 (t, C_2H_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) ν_{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%.
Spectral data for 6d (3'-(4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-ylthio)N-ethyl-propanamide) mp 251-253.5 °C. ^{1}H NMR (250.12 MHz, DMSO-d_6): δ 1.01 (t, 3H, J = 7.2 Hz, CH_2CH_3), 2.59 (t, 2H, J = 7.1 Hz, CH_2), 3.10 (m, 2H, J = 7.3 Hz, 5.6 Hz, CH_2CH_3), 3.29 (t, 2H, J = 7.2 Hz, SCH_2), 7.32 - 8.24 (m, 5H, CH_{arom}), 7.56(br t, 2H, CONH, NH), 7.86 (br s, 1H, NH), 8.27 (s, 1H, H_3). ^{13}C NMR (62.8 MHz, DMSO-d_6): δ 14. 5 (q, CH_2CH_3), 26.1 (t, SC_3H_2), 33.2 (t, CH_2CH_3), 35.2 (t, C_2H_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) ν_{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%.

17. A_1 binding measured inhibition of [^{3}H]PIA binding to whole rat brain membranes at 37 °C.[18] Values are geometric means from two determinations, run in duplicate ± standard error. A_1 K_i values calculated using the Cheng-Prusoff equation[19], using the average K_d value of [^{3}H]PIA as 2.35 nM. A_{2a} binding measured inhibition of [^{3}H]CGS21680 binding to rat brain striatum membranes at 25 °C.[20] Values are geometric means from two determinations, run in duplicate ± standard error. A_2 K_i values calculated using the Cheng-Prusoff equation[19] and using the average K_d value of [^{3}H]CGS21680 as 14.9 nM.
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(*Received in USA* 3 *July* 1995; *accepted* 13 *September* 1995)