Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1979–1984

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DISCOVERY OF FR166124, A NOVEL WATER-SOLUBLE PYRAZOLO-[1,5-*a*]PYRIDINE ADENOSINE A_1 RECEPTOR ANTAGONIST

Satoru Kuroda,[a]* Atsushi Akahane,[a] Hiromichi Itani,[a] Shintaro Nishimura,[b] Kieran Durkin,[a] Takayoshi Kinoshita,[b] Yoshiyuki Tenda,[c] and Kazuo Sakane[a]

[a]*Medicinal Chemistry Research Laboratories,* [b]*Basic Research Laboratories and* [c]*Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*

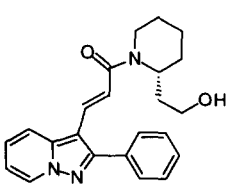
Received 17 March 1999; accepted 27 May 1999

Abstract: Novel 3-(2-cycloalkyl and cycloalkenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]-pyridines were synthesized and evaluated for their adenosine A_1 receptor binding activities. In this series, FR166124 (**3**) was found to be the most potent and selective adenosine A_1 receptor antagonist, and the double bond of the cyclohexenyl acetic acid group was essential for selectivity of A_1 receptor binding. Furthermore, the solubility in water of the sodium salt of FR166124 was high.

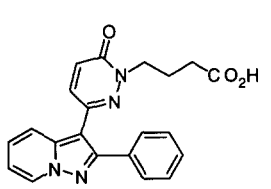
Introduction

In the past 15 years, various xanthine derivatives and non-xanthine heterocyclic compounds were synthesized as selective adenosine A_1 receptor antagonists. On the basis of pharmacological studies, it has been suggested that selective adenosine A_1 receptor antagonists are potential therapeutic agents.[1-5] In spite of considerable effort searching for potent and selective adenosine A_1 receptor antagonists, pharmacological studies have been limited because of their low water solubility.[3,6] Although several xanthine derivatives are known to be selective and water-soluble adenosine A_1 receptor antagonists,[7,8] it is desirable to search for more potent and selective adenosine A_1 receptor antagonists which have high water solubility.

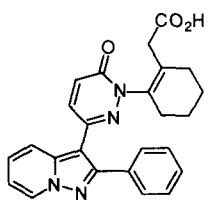
We recently reported the discovery and diuretic activity of FK453 (**1**)[9-13] and FK838 (**2**),[14-17] potent and selective non-xanthine adenosine A_1 receptor antagonists. Examination of the chemical properties of FK453 indicated photochemical *trans-cis* isomerization at the acryloylamide moiety[9] and solubility in water was low (11.9 μg/mL).[14] On the other hand, the photochemical stability and solubility in water of the sodium salt of FK838 are satisfactory (10 mg/mL).[14] Although the diuretic activity of FK838 was more potent than that of FK453, FK838 had lower binding affinity and poorer selectivity for receptor binding than that of FK453.[14] The discovery of FK453 and FK838 resulted from the evaluation of *in vivo* diuretic activities, additional *in vitro* tests indicated that the mechanism of the action of FK453 and FK838 was adenosine A_1 receptor antagonism.



FK453 (**1**)



FK838 (**2**)



FR166124 (**3**)

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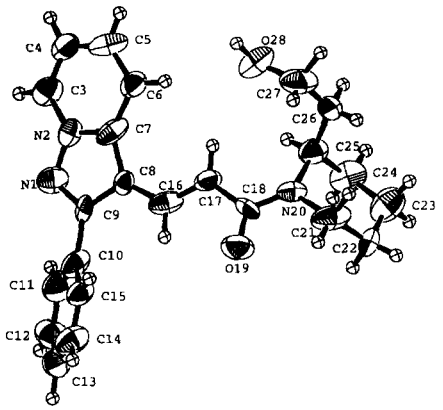


Figure 1. Ortep plot of FK453 (**1**). Selected bond angles (°): C(8)-C(16)-C(17)=128(1), C(16)-C(17)-C(18)=121(1), C(17)-C(18)-N(20)=120(1), C(18)-N(20)-C(21)=119(1), C(18)-N(20)-C(25)=126(1), N(20)-C(25)-C(26)=106(1).

Therefore, we considered that more potent and selective adenosine A_1 receptor antagonists derived from FK838 had the possibility of leading to strong diuretics.

It was noted that the presence of the (2*R*)-2-(2-hydroxyethyl)piperidine ring on the acryloylamide of FK453 was a significant conformationally limiting factor for adenosine A_1 receptor binding[9] and the pyridazinone ring of FK838 was a bioisostere of the acryloylamide of FK453.[14] In order to understand the difference in stereochemistry between FK453 and FK838, the X-ray crystal structure of FK453 was investigated (Figure 1).[14,18] It is clear that the piperidine acryloylamide part of FK453 had a planar orientation and that the hydroxyethyl group of the piperidine ring was oriented approximately perpendicular to that plane. On the other hand, it can be assumed that the butyric acid group of FK838 shows no conformational similarity to the piperidine amide group of FK453. We postulated that introduction of a ring structure by connecting an alkyl chain between the C-3 and C-4 positions of the butyric acid group of FK838 as a confomationally limiting factor, may lead to more potent and selective adenosine A_1 receptor antagonists than FK838. In order to search for more potent and water-soluble adenosine A_1 receptor antagonists, we thus introduced a range of cycloalkyl and cycloalkenyl acetic acid groups in place of the butyric acid group at the 2-position of the pyridazinone ring of FK838 (Figure 2).

In this paper, we wish to disclose the synthesis and adenosine A_1 receptor binding assay of these analogs, the finding that the best compound FR166124 (**3**) had remarkably increased potency and selectivity for the adenosine A_1 receptor over the A_{2A} receptor compared to FK838, and the high water solubility of the sodium salt of FR166124.

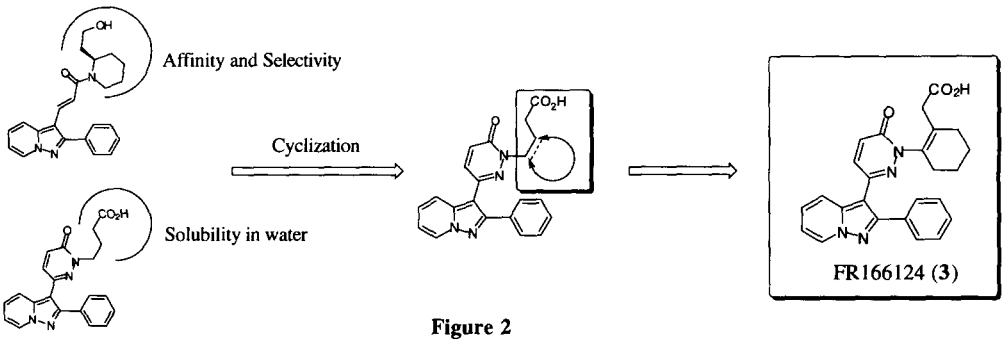


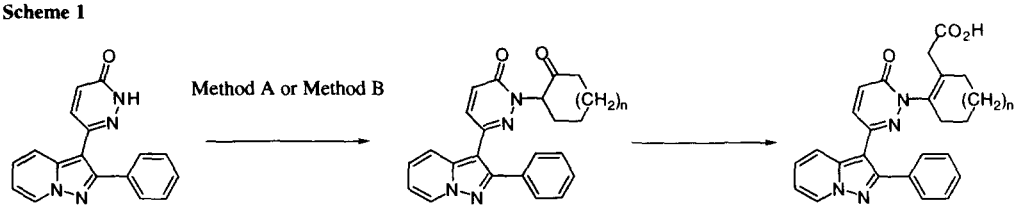
Figure 2

Chemistry

Starting 3-(3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridine (**4**) was prepared according to the reported method.[14,19,20] Cycloalkenyl acetic acid derivatives **3**[21], **8** and **9** in Table 1, were prepared by the methods shown in Scheme 1. Compounds **5** (n = 0) and **6** (n = 1) were synthesized from **4** *via* method A: reaction with 2-chlorocycloalkanone and Horner–Emmons reaction followed by alkaline hydrolysis. Compound

7 (n = 2) was synthesized from **4** *via* method B: reaction with cycloheptheneoxide, oxidation of the resulting alcohol and Horner–Emmons reaction followed by acidic deprotection of the *tert*-butyl ester. The low yields in these reactions were due to the following reasons: 1) starting **4** remained in the alkylation reaction of **4** and competing *O*-alkylation was observed in the case with 2-chlorocyclohexanone.[22] 2) Varying quantities of the *exo*-(*E*)-double bond isomers were also obtained in the Horner–Emmons reaction.

Scheme 1

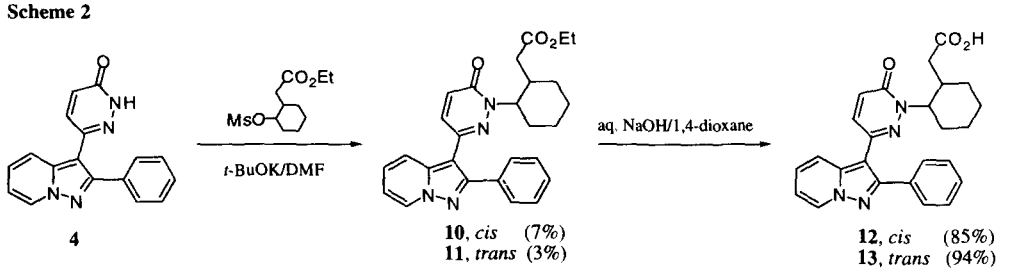


Method A: i) 2-chlorocycloalkanone, NaH/DMF, ii) $(EtO)_2P(O)CH_2CO_2Et$, NaH/toluene, iii) aq. NaOH/1,4-dioxane or MeOH

Method B: i)  , NaH/DMF, ii) PDC, CH_2Cl_2, iii) $(EtO)_2P(O)CH_2CO_2Bu^t$, NaH/toluene, iv) TFA/CH_2Cl_2

Racemic cycloalkyl acetic acid derivatives **12** and **13** (Table 1) were synthesized from **4** *via* reaction with the corresponding mesylate and separation of isomers **10**[23] and **11**[24] using silica-gel chromatography followed by alkaline hydrolysis as shown in Scheme 2. Initially, direct catalytic hydrogenation of **3** was attempted for the synthesis of cycloalkyl acetic acid derivatives, but **12** or **13** could not be obtained.[25] Hence, we selected the alternative method described above in spite of the low isolated yields.

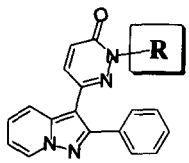
Scheme 2

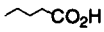
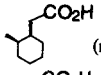
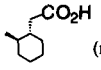


Biological Results

Adenosine receptor binding assay was performed by the previously described method,[12] and the results are summarized in Table 1 in comparison with DPCPX (xanthine type adenosine A_1 receptor antagonist),[26] FK453 and FK838. As shown in Table 1, the best compound as a selective adenosine A_1 receptor antagonist was **3** (FR166124) displaying high affinity (IC_{50} = 15 nM) and high selectivity (410-fold). Compounds **3**, **8**, **9** and **12** were more potent and selective than FK838. Whilst for **13** the affinity for the adenosine A_1 receptor was the most potent (IC_{50} = 3.6 nM) amongst the prepared compounds, the selectivity was low (28-fold). Additionally, **3** showed higher selectivity than **12** and **13**. This fact suggested that in this series the internal double bond of the cycloalkenyl acetic acid moiety played an important role in determining the selectivity for the adenosine A_1 receptor.

Table 1



Compound[a]	R	Adenosine receptor binding[b] IC_{50} (nM)		selectivity[c]
		A_1	A_{2A}	A_{2A} / A_1
DPCPX[d]		4.7	1100	230
1 (FK453)		17	11000	650
2 (FK838)		120	5900	49
3 (FR166124)		15	6200	410
8		11	840	76
9		16	5500	340
12	 (racemic)	34	5500	160
13	 (racemic)	3.6	100	28

[a] All test compounds were dissolved in DMSO. [b]Inhibition of [^{3}H]-CHA specific binding to rat cortical membranes (A_1 receptor) and [^{3}H]-NECA specific binding to rat striatal membranes (A_{2A} receptor) (n = 3). [c] Ratio of IC_{50} values obtained by receptor binding assay. [d] DPCPX: 8-Cyclopentyl-1,3-dipropylxanthine.

Water Solubility

An additional goal of our study was the discovery of a potent adenosine A_1 receptor antagonist with high water solubility. The water solubility of the sodium salt of FR166124[27] was measured[28] and the result is shown in Table 2 in comparison with that of FK838.[14] As a result, we found that FR166124 had more than 20-fold higher water solubility than FK838.

Table 2

FK838-Na	10 mg/mL
FR166124-Na	> 200 mg/mL[a]

[a] Upper limit was not determined.

Discussion

FR166124 was designed based on the hypothesis that the high selectivity for the adenosine A_1 receptor of FK453 was due to the presence of the (2*R*)-2-(2-hydroxyethyl)piperidine ring of the acryloylamide as a conformationally limiting factor. In order to prove this hypothesis, the X-ray crystal structure of FR166124 was investigated (Figure 3). It was found that the pyridazinone ring and the cyclohexenyl acetic acid group were oriented approximately perpendicular due to the steric hindrance between them. Superposition of FR166124 (**3**) and FK453 (**1**), in consideration of the orientation of the cyclohexenyl acetic acid group and the (2*R*)-2-(2-hydroxyethyl)piperidine ring, based on X-ray crystallographic analysis are shown in Figure 4. As a result, it was found that the structure of FR166124 resembled closely that of FK453. It was thus clear that the pyridazinone

cyclohexenyl acetic acid group was an excellent substituent to induce binding and to mimic the conformation of FK453.

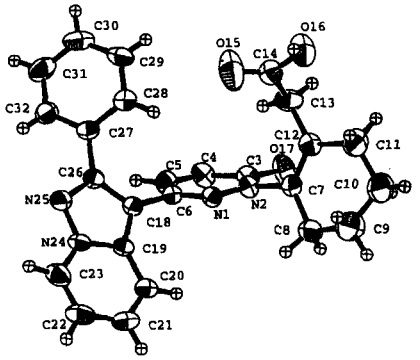


Figure 3. Ortep plot of FR166124 (**3**).
Selected bond angles (°): C(18)-C(6)-N(1)=117.3(5), C(6)-N(1)-N(2)=117.3(4), N(1)-N(2)-C(7)=113.1(4), N(1)-N(2)-C(3)=126.0(5), N(2)-C(7)-C(12)=118.4(5), N(2)-C(7)-C(8)=114.7(5), C(7)-C(12)-C(13)=123.6(6), C(7)-C(12)-C(11)=119.2(5).

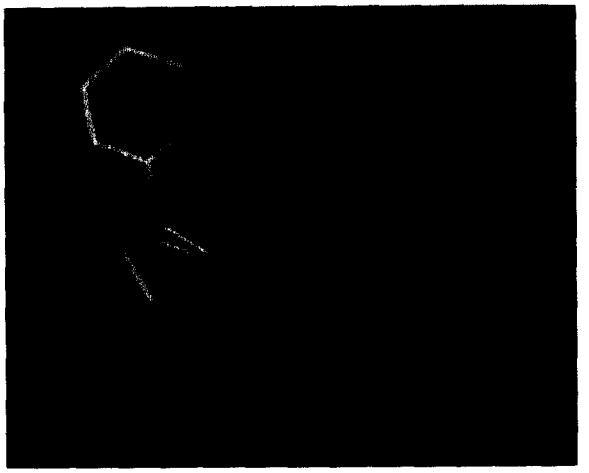


Figure 4. Superposition of **1** (pink) and **3** (blue).

Conclusion

FR166124 is a novel pyrazolo[1,5-*a*]pyridine adenosine A_1 receptor antagonist which is highly potent and selective to the adenosine A_1 receptor subtype, and has good solubility in water as the sodium salt. Further evaluation of diuretic activity, vasodilating activity and renal protective effect are now underway and will be reported in due course.

Acknowledgments: The authors would like to acknowledge Dr. Hirokazu Tanaka and Dr. Hisashi Takasugi for continuing interest and encouragement, and Dr. David Barrett for helpful discussions and for critical evaluation of this manuscript.

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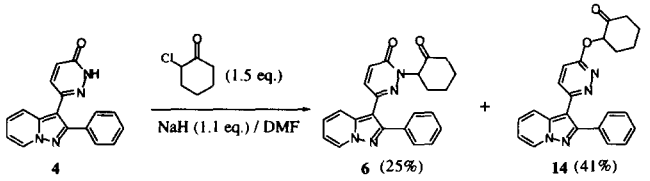
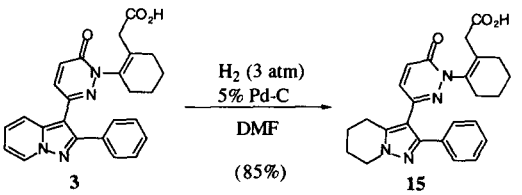
21. *Selected data for FR166124* (**3**): mp 218-219 °C (aq. EtOH); FT IR (KBr) 1722, 1639, 1579, 1531, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70-2.00 (m, 4H), 2.20-2.70 (m, 4H), 2.91 (d, 1H, *J* = 14.0 Hz), 3.18 (d, 1H, *J* = 14.0 Hz), 6.89-7.00 (m, 2H), 7.17 (d, 1H, *J* = 9.6 Hz), 7.30-7.61 (m, 6H), 7.94 (d, 1H, *J* = 9.0 Hz), 8.55 (d, 1H, *J* = 6.9 Hz), 12.16 (s, 1H); (+) APCI MS *m/z* 427 $(M+H)^+$; Anal. Calcd for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14. Found: C, 70.29; H, 5.21; N, 13.05.
22. In the reaction of **4** with 2-chlorocyclohexanone, **6** and **14** were obtained in 25% and 41% yields respectively.



23. *Selected data for* **10**: mp 151-152 °C (EtOAc-IPE); IR (Nujol) 1715, 1640, 1625, 1590, 1525 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10 (t, 3H, *J* = 7.1 Hz), 1.41-2.55 (m, 10H), 2.95-3.03 (m, 1H), 3.83-4.13 (m, 2H), 5.15 (dt, 1H, *J* = 12.0, 4.0 Hz), 6.75 (d, 1H, *J* = 9.6 Hz), 6.91 (dt, 1H, *J* = 6.9, 1.3Hz), 7.00 (d, 1H, *J* = 9.6 Hz), 7.32 (ddd, 1H, *J* = 9.0, 6.9, 1.3 Hz), 7.43-7.48 (m, 3H), 7.57-7.62 (m, 2H), 7.94 (d, 1H, *J* = 9.0Hz), 8.53 (d, 1H, *J* = 6.9Hz); (+) APCI MS *m/z* 457 $(M+H)^+$; Anal. Calcd for $C_{27}H_{28}N_4O_3 \cdot 0.2H_2O$: C, 70.47; H, 6.16; N, 12.18. Found: C, 70.36; H, 6.22; N, 12.07.
24. *Selected data for* **11**: mp 172-173 °C (EtOAc); IR (Nujol) 1720, 1650, 1625, 1580, 1520 cm^{-1}; ^1H NMR($CDCl_3$) δ 1.18 (t, 3H, *J* = 7.1 Hz), 1.32-2.35 (m, 10H), 2.47-2.70 (m, 1H), 3.98-4.10 (m, 2H), 4.91 (dt, 1H, *J* = 10.0, 4.1 Hz), 6.72 (d, 1H, *J* = 9.7 Hz), 6.92 (dt, 1H, *J* = 6.9, 1.4Hz), 6.95 (d, 1H, *J* = 9.7 Hz), 7.36 (dd, 1H, *J* = 8.9, 6.9 Hz), 7.43-7.47 (m, 3H), 7.60-7.65 (m, 2H), 8.08 (d, 1H, *J* = 8.9Hz), 8.53 (d, 1H, *J* = 6.9Hz); (+) APCI MS *m/z* 457 $(M+H)^+$; Anal. Calcd for $C_{27}H_{28}N_4O_3 \cdot 0.2H_2O$: C, 70.47; H, 6.16; N, 12.18. Found: C, 70.36; H, 6.07; N, 11.93.
25. Catalytic hydrogenation of FR166124 (**3**) gave **15** as the sole product in 85% yield. *Selected data for* **15**: mp 128-131 °C (aq. EtOH); FT IR (KBr) 1722, 1657, 1587, 1504 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70-2.60 (m, 11H), 2.80-3.16 (m, 5H), 4.15-4.40 (m, 2H), 6.89 (d, 1H, *J* = 9.6 Hz), 7.07 (d, 1H, *J* = 9.6 Hz), 7.35-7.50 (m, 5H); (+) APCI MS *m/z* 431 $(M+H)^+$; Anal. Calcd for $C_{25}H_{26}N_4O_3 \cdot H_2O$: C, 66.95; H, 6.29; N, 12.49. Found: C, 66.98; H, 6.32; N, 12.54.



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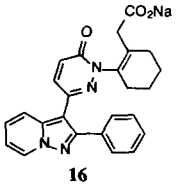
27. *Preparation of the sodium salt of FR166124* (**16**). **3** was dissolved in equimolar amounts of 0.1 N aqueous sodium hydroxide solution. After stirring for 1 hour at ambient temperature, the solution was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized from a mixture of acetone and water (10 : 1) to give **16** as yellow crystals. *Selected data for* **16**: mp 180-182 °C (aq. acetone); FT IR (KBr) 3423, 1653, 1583, 1531 cm^{-1}; ^1H NMR (D_2O) δ 1.60-2.40 (m, 8H), 2.60-2.81 (m, 2H), 6.83 (d, 1H, *J* = 9.6 Hz), 6.94-7.01 (m, 1H), 7.02 (d, 1H, *J* = 9.6 Hz), 7.30-7.50 (m, 6H), 7.66 (d, 1H, *J* = 8.9 Hz), 8.34 (d, 1H, *J* = 6.9 Hz); (+) FAB MS *m/z* 427.1 $(M+H-Na)^+$, 449.1 $(M+H)^+$; Anal. Calcd for $C_{25}H_{21}N_4O_3Na \cdot 2.2H_2O$: C, 61.52; H, 5.25; N, 11.48. Found: C, 61.42; H, 5.24; N, 11.39.



28. The sodium salt of FR166124 (**16**) was added to water and agitated at ambient temperature until insoluble **16** remained.