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Novel Adenosine A_1 Receptor Antagonists. Synthesis and Structure–Activity Relationships of a Novel Series of 3-(2-Cyclohexenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines

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Abstract—A novel series of 3-(2-cyclohexenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines was synthesized and evaluated for in vitro adenosine A_1 and A_{2A} receptor binding activities. Most of the cyclohexenyl derivatives (**7a**–**e**, **8a**–**s**) were found to be potent adenosine A_1 receptor antagonists. In a series of analogues of FR166124 (**3a**), alcohol **7c**, nitrile **7e** and amide derivatives (**7d**, **8c**, **8r**) were found to be more potent A_1 antagonists with higher A_{2A}/A_1 selectivity than FR166124. Amongst them, **8r** showed considerable water solubility (33.3 mg/mL), but lower than that of the sodium salt of FR166124 (> 200 mg/mL). Additionally, FR166124 had strong diuretic activity by both p.o. and iv administration in rats (minimum effective dose = 0.1 and 0.032 mg/kg, respectively).

Introduction

Adenosine plays a variety of important physiological roles through extracellular adenosine receptors which are classified into four subtypes called A_1, A_{2A}, A_{2B} and A_3.[1–4] With regards to possible therapeutic applications of adenosine antagonists, effects on the cardiovascular system, kidney and central nervous system have been studied, and extensive pharmacological studies have suggested that selective antagonists of the A_1 receptor may lead to novel therapeutic agents for the treatment of certain kidney and central nervous system diseases.[2,5–10] The low water solubility of the known adenosine A_1 receptor antagonists[6] has made it especially difficult to develop therapeutic agents, however, several xanthine derivatives are known to be water-soluble A_1 receptor antagonists.[11,12] We recently reported the discovery of FK453 (**1**)[13–17] and FK838 (**2**),[18–21] two potent 3-substituted 2-phenylpyrazolo[1,5-*a*]pyridine adenosine A_1 receptor antagonists with strong diuretic activities (Fig. 1). Moreover, we have also reported the discovery of FR166124 (**3a**, Fig. 1), a more potent derivative with higher A_{2A}/A_1 selectivity that has very high water solubility as the sodium salt (> 200 mg/mL).[22]

The design process leading to the discovery of FR166124 involved introduction of various cyclic acid groups to the *N*2′ of the pyridazinone ring, in place of the butyric acid group of FK838, as substituents to induce rigidity and mimic the postulated conformation of FK453.[22] A cyclohexenyl acetic acid group was found to be especially effective. In order to discover even more potent and A_{2A}/A_1 selective analogues, we then designed a series of FR166124 derivatives in order to further elucidate the importance of the cyclohexenyl acetic acid moiety. In this paper, we wish to report the synthesis and structure–activity relationships (SAR) of a novel series of 3-(2-cyclohexenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines, including

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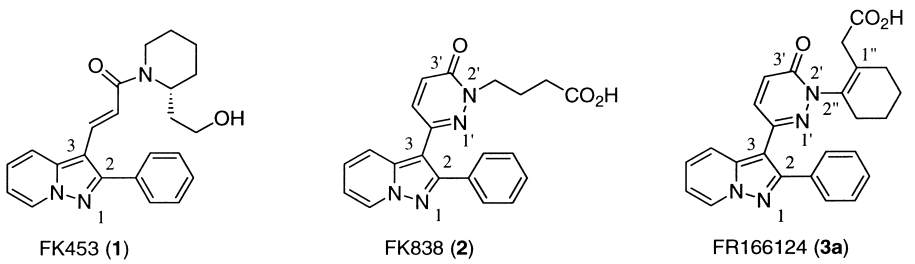
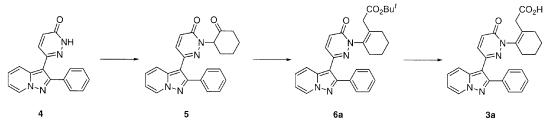


Figure 1. 3-Substituted-2-phenylpyrazolo[1,5-*a*]pyridine adenosine A_1 receptor antagonists.

alcohol **7c**, nitrile **7e**, amide derivatives (**7d,8a–8s**), and a series of olefin isomers of FR166124.

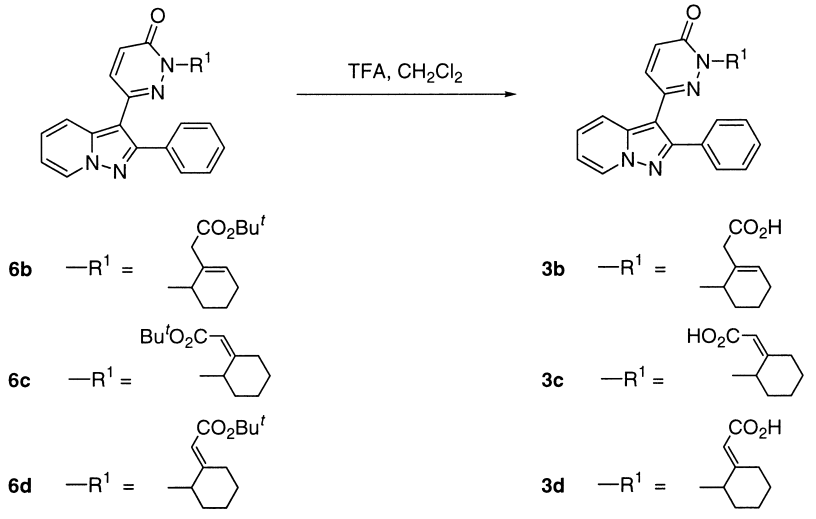
Chemistry

Synthetic routes for the novel 3-(2-cyclohexenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines **3**, **7** and **8** prepared in this work are shown in Schemes 1–3. The olefin isomers (**3b–d**) of **3a** were prepared by acidic deprotection of the corresponding *tert*-butyl esters **6b–d** which were obtained in 65–76% yields by the previously described methods (eq. 1).[23]

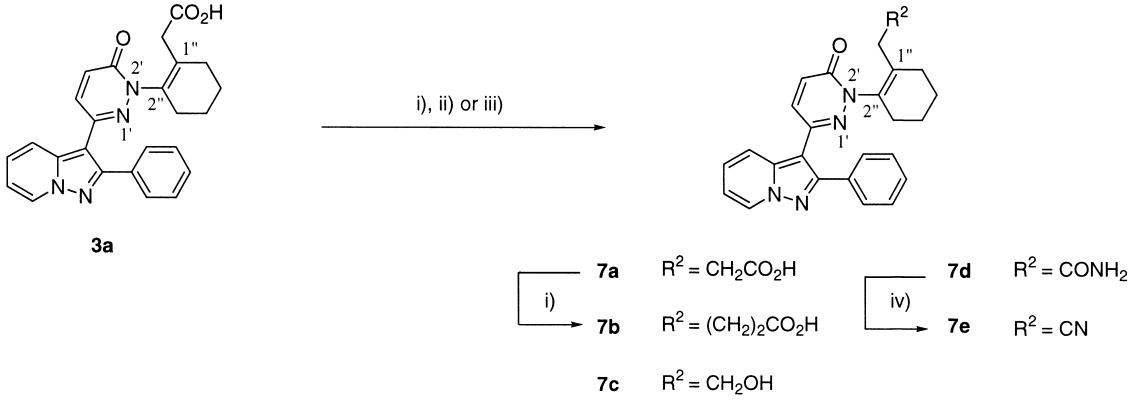


(1)

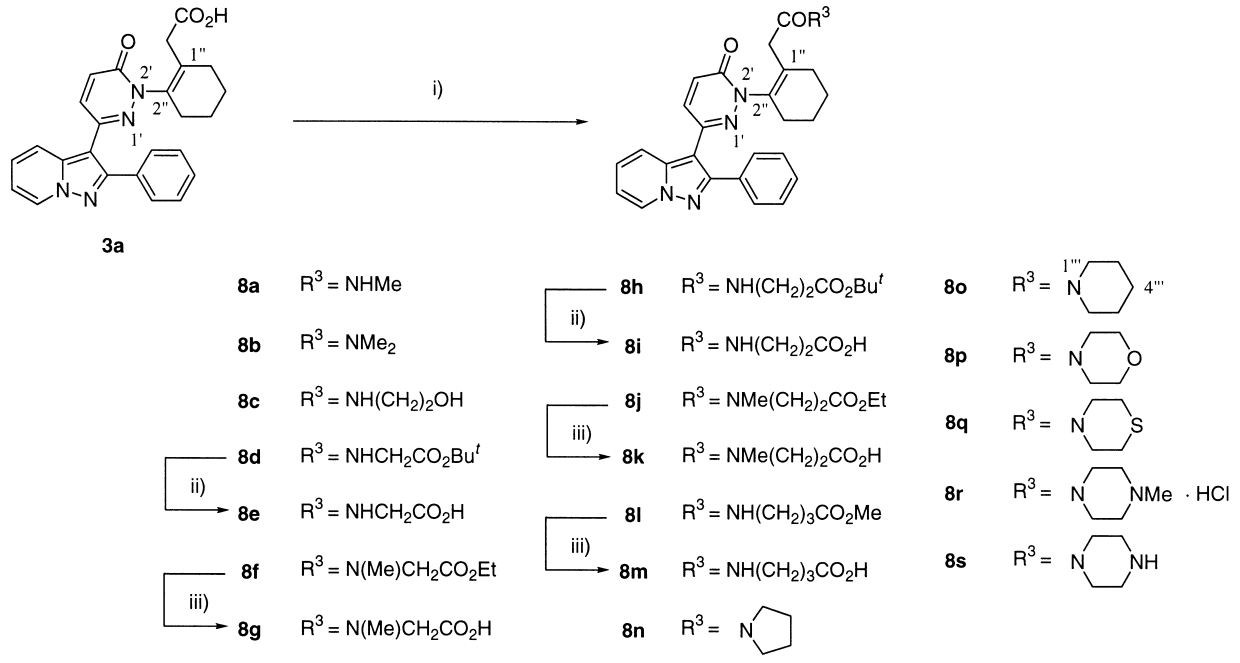
One carbon homologated compound **7a** was prepared from **3a** in 31% yield by Arndt–Eistert reaction using trimethylsilyldiazomethane and benzylalcohol followed



Scheme 1. Synthesis of olefin isomers of FR166124 (**3a**).



Scheme 2. Synthesis of *N*2′-cyclohexenyl analogues. Reagents and conditions: (i) 1. $SOCl_2/CH_2Cl_2$; 2. trimethylsilyldiazomethane/THF–CH_3CN; 3. 2,4,6-trimethylpyridine, $PhCH_2OH$; 4. 1 N NaOH aq, THF–H_2O (**7a, 7b**). (ii) BH_3·THF, THF (**7c**). (iii) 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), HOBt, NH_4Cl/THF–CH_2Cl_2 (**7d**). (iv) TFAA, pyridine, CH_2Cl_2 (**7e**).



Scheme 3. Synthesis of amide derivatives of FR166124 (**3a**). Reagents and conditions: (i) R^3H·HCl, EDC, HOBt/THF–CH_2Cl_2 (**8a, b, f, j, l**); R^3H, EDC·HCl, HOBt/THF–CH_2Cl_2 (**8c, p, q**) or DMF (**8n, o**); $(COCl)_2$/DMF–CH_2Cl_2, then R^3H·HCl, Et_3N/CH_2Cl_2 (**8d, h**); R^3H, EDC·HCl, HOBt, Et_3N/DMF, then 4 N HCl/EtOAc (**8r**); 1-triphenylmethylpiperazine, EDC·HCl, HOBt/THF–CH_2Cl_2, then HCO_2H, c-HCl (**8s**) (ii) TFA/CH_2Cl_2 (**8e, i**), (iii) 1 N NaOH/1,4-dioxane-H_2O (**8g, k, m**).

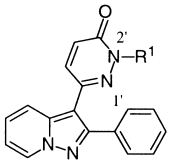
by alkaline hydrolysis. Two carbon homologated compound **7b** was prepared from **7a** in 80% yield by repetition of the same procedure. Alcohol **7c** was prepared in 70% yield by reduction of **3a** using BH_3·THF complex. The preparation of amide derivatives **7d**, **8a–c** and **8n–q** was accomplished by activation of **3a** as the HOBt (HOBt) ester, followed by treatment with an appropriate amine or amine hydrochloride in 40–92% yields. Compound **7d** was easily converted to **7e** by dehydration using trifluoroacetic anhydride (TFAA) in 62% yield. Compounds **8e** and **8i** were prepared by reaction of the acid chloride generated from **3a** with $(COCl)_2$ and the appropriate amino ester, followed by acidic deprotection of the resulting *tert*-butyl esters **8d** and **8h** using trifluoroacetic acid (TFA), in 55 and 43% yields, respectively. Compounds **8g**, **8k** and **8m** were prepared in 37–60% yields by reaction of the HOBt ester of **3a** with the appropriate amino ester, followed by alkaline hydrolysis of the resulting esters **8f**, **8j** and **8l**, respectively. Compound **8r** was prepared in 59% yield by reaction of the HOBt ester of **3a** with 1-methylpiperazine followed by treatment with hydrogen chloride in EtOAc. Compound **8s** was prepared in 76% yield by reaction of the HOBt ester of **6a** with 1-triphenylmethylpiperazine[24] followed by deprotection of the triphenylmethyl group with a mixture of concd HCl in HCO_2H.

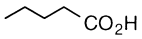
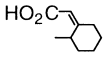
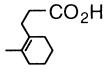
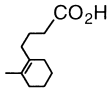
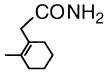
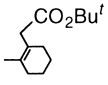
Results and Discussion

The prepared compounds were evaluated for in vitro adenosine A_1 and A_{2A} receptor binding activities according to the reported method.[16] Adenosine A_1 receptor binding was measured in rat cortical membranes using 1 nM [^{3}H]-cyclohexyladenosine (CHA). Adenosine A_{2A} receptor binding was measured in rat striatal membranes using 5 nM [^{3}H]-*N*-ethylcarboxamideadenosine (NECA) in the presence of 50 nM cyclopentyladenosine (CPA) to eliminate the adenosine A_1 component. The in vitro A_1 and A_{2A} receptor binding activities are expressed as the nanomolar concentration of a compound required for 50% inhibition (IC_{50}) of specific binding of [^{3}H]-CHA and [^{3}H]-NECA, respectively. Selectivity (A_{2A}/A_1) is expressed as the ratio of IC_{50} values obtained by receptor binding assay. Table 1 shows the results of binding assay and the selectivity of these new *N*2′-cyclohexenyl analogues, in comparison with DPCPX (xanthine type adenosine A_1 receptor antagonist),[25] FK453 and FK838. The results of binding and selectivity for the new amide derivatives of FR166124 are shown in Table 2.

In order to investigate the potency and selectivity for adenosine A_1 receptors of this type of compounds, we initially synthesized and evaluated the in vitro activity of *N*2′-cyclohexenyl analogues **3** and **7**, which are olefin isomers and related compounds, derived from FR166124 (Table 1). With regards to the stereochemistry of the double bond, olefin isomers **3b** and **3d** displayed 2-fold lower affinity for the adenosine A_1 receptor (IC_{50} = 33 and 32 nM, respectively), and the A_{2A}/A_1 selectivity was also 2-fold lower (230 and 210-fold, respectively) than that of FR166124. The affinity of isomer **3c** was 5-fold less potent (IC_{50} = 72 nM) and 3-fold less selective (130-fold) than FR166124. Thus, it was elucidated that the position of the double bond of the cyclohexenyl group and the orientation of the carboxylic acid group were critical for A_1 affinity and

Table 1. Binding assay and selectivity of *N*2′-cyclohexenyl analogues

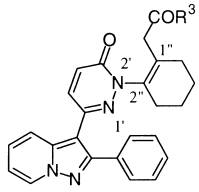


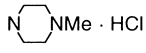
Compound[a]	$-R^1$	Adenosine receptor binding[b] IC_{50} (nM) A_1	A_{2A}	Selectivity[c] A_{2A}/A_1
DPCPX[d]		4.7	1100	230
FK453 (**1**)		17	11000	650
FK838 (**2**)		120	5900	49
3a (FR166124)		15	6200	410
3b[e]		33	7500	230
3c[e]		72	9300	130
3d[e]		32	6600	210
7a		28	7600	270
7b		30	3900	130
7c		2.1	2500	1200
7d		1.8	740	410
7e		6.4	3300	520
6a		8.6	1700	200

[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.
[e]Racemic compounds.

especially for A_{2A}/A_1 selectivity. The one-carbon homologated compound **7a** was 2-fold less potent ($IC_{50}=28$ nM) and 2-fold less selective (270-fold) than FR166124. The two-carbon homologated compound **7b** was 2-fold less potent ($IC_{50}=30$ nM) and 3-fold less selective (130-fold) than FR166124. It is thus clear that FR166124 is the best compound in the series of *N*2′-cyclohexenylcarboxylic acids (**3a**–**d**, **7a**, **7b**). *tert*-Butyl ester **6a**, which is the synthetic precursor of FR166124,[23] showed high A_1 affinity ($IC_{50}=8.6$ nM), but the A_{2A}/A_1 selectivity was low (200-fold). On the other hand, alcohol **7c**, amide **7d** and nitrile **7e** showed very high A_1 affinity and very high A_{2A}/A_1 selectivity. In particular, **7c** was 7-fold more potent ($IC_{50}=2.1$ nM) and 3-fold more selective (1200-fold) than FR166124; **7d** was 8-fold more potent ($IC_{50}=1.8$ nM) than FR166124. From the results obtained in this series, we thus selected **7d** as a new lead compound since a variety of derivatives can be synthesized by chemical modification of the amide moiety, despite the fact that **7d** also displayed 8-fold more potent adenosine A_{2A} receptor binding ($IC_{50}=740$ nM) than FR166124.

Table 2. Binding assay and selectivity of amide derivatives of FR166124



Compound[a]	R^3	Adenosine receptor binding[b] IC_{50} (nM) A_1	A_{2A}	Selectivity[c] A_{2A}/A_1
8a	NHMe	6.8	1900	280
8b	NMe_2	1.7	530	310
8c	$NH(CH_2)_2OH$	2.0	890	450
8e	$NHCH_2CO_2H$	16	7600	480
8g	$N(Me)CH_2CO_2H$	59	14000	240
8i	$NH(CH_2)_2\ CO_2H$	9.2	1900	210
8k	$NMe(CH_2)_2\ CO_2H$	30	1300	43
8m	$NH(CH_2)_3\ CO_2H$	12	1400	120
8n		3.1	640	210
8o		9.2	1600	170
8p		5.0	1200	240
8q		8.7	1800	210
8r		7.9	4200	530
8s		21	6100	290

[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.

In order to optimize the amide substituent, a variety of derivatives were examined and the results are collected in Table 2. Although introduction of two methyl groups or a hydroxyethyl group to the amide nitrogen of **7d** retained A_1 affinity and A_{2A}/A_1 selectivity (**8b**, **8c**), introduction of only one methyl group reduced potency and A_{2A}/A_1 selectivity (**8a**). Introduction of a carboxyl

group into the side chain of the amide group reduced A_1 affinity (**8e**, **8g**, **8i**, **8k**, **8m**) and A_{2A}/A_1 selectivity (**8g**, **8i**, **8k**, **8m**). Moreover, when an additional methyl group was introduced to the amide nitrogen of **8e** and **8i**, the A_1 affinity and A_{2A}/A_1 selectivity were reduced (**8e** versus **8g**, **8i** versus **8k**). Amides derived from cyclic amines (**8n**–**s**) had lower A_1 affinity than **7d**, however **8r** derived from *N*-methylpiperazine showed high A_{2A}/A_1 selectivity (530-fold) compared to **7d** (410-fold). Despite the fact that replacement of the C4‴ of piperidine (**8o**) by heteroatoms such as oxygen (**8p**), sulfur (**8q**), methylamino (**8r**) and amino (**8s**) had little effect on A_1 receptor binding, the A_{2A}/A_1 selectivity of **8r** was higher than that of **7d**.

As a result of these investigations, alcohol **7c**, nitrile **7e** and amide derivatives (**7d**, **8c**, **8r**) were found to be more potent adenosine A_1 receptor antagonists with higher A_{2A}/A_1 selectivity than FR166124. In vitro adenosine A_1 receptor affinity of the cyclohexenyl derivatives (**7**, **8**) decreased in the order: CH_2CONMe_2 (**8b**), CH_2CONH_2 (**7d**), $CH_2CONH(CH_2)_2OH$ (**8c**), $(CH_2)_2OH$ (**7c**) < $CH_2CON(\text{-}C_4H_8\text{-})$ (**8n**) > CH_2CN (**7e**), $CH_2CONHMe$ (**8a**) > $CH_2CON(\text{-}C_2H_4\text{-X-}C_2H_4\text{-})$, (**8o**–**s**, e.g. X = O, S, NH), $CH_2CONH(CH_2)_nCO_2H$ (**8e**, **8i**, **8m**, $n = 1$–3) > $(CH_2)_nCO_2H$ (**7a**, **7b**, $n = 1$, 2) > $CH_2CON(Me)(CH_2)_nCO_2H$ (**8g**, **8k**, $n = 1$, 2). Concerning the receptor binding of FR166124 derivatives, introduction of a more hydrophobic moiety than a carboxylic acid group (e.g. **5a**, **7c**–**e**, **8b**, **8c**, **8n**) at the C1″ of the cyclohexene ring increased both A_1 and A_{2A} affinity. Introduction of hydrophilic groups such as carboxylic acid or amino moieties at the amide portion decreased A_{2A} affinity (e.g. **8g**, **8s**). In the case of amide derivatives of FR166124, it is inferred that hydrophobicity is an important factor for increasing A_1 affinity and A_{2A}/A_1 selectivity rather than steric factors.

In terms of solubility, **8e**, **8g**, **8i**, **8k**, **8m** and **8r** can be expected to have high water solubility, since they possess a carboxylic acid moiety, which can be converted to the sodium salt, or amino groups, obtained as a hydrochloride salt, in the amide moiety. In spite of these features, sodium salts of carboxylates (**8e**, **8g**, **8i**, **8k**, **8m**) were so hygroscopic that suitable solids for measurement of solubility could not be obtained. After consideration of A_1 affinity, A_{2A}/A_1 selectivity and chemical properties, the water solubilities of **7c**, **7d**, **7e**, **8c** and **8r** were measured by adding the test compound to water and agitating at ambient temperature until insoluble material remained (Table 3). Although, **7c** is a highly potent adenosine A_1 receptor antagonist with high A_{2A}/A_1 selectivity, solubility in water was very low (6.4 μg/mL). Compound **8r** showed good water solubility (33.3 mg/mL) but lower than that of the sodium salt of FR166124 (> 200 mg/mL).

Whilst a number of these analogues of FR166124 were found to be more potent adenosine A_1 receptor antagonists with high A_{2A}/A_1 selectivity than FR166124, after considering water solubility and synthetic cost, FR166124 was selected as the best compound for further evaluation. To investigate the potential as a therapeutic agent, diuretic activity was determined according to the reported method.[15] Table 4 shows the minimum effective dose of FR166124 in rats by p.o. and iv administration. It was found that FR166124 and FK838 had equal diuretic activity under the conditions of p.o. administration, but in the case of iv administration, FR166124 was three fold more potent than FK838. More potent adenosine A_1 receptor antagonists with high A_{2A}/A_1 selectivity derived from FK838 have stronger diuretic activity. It was thus concluded that FR166124 can possibly lead to a new therapeutic agent, and was progressed to further pharmacological study, the results of which will be reported in due course.

Table 3. Water solubility of selected compounds

Compound	Solubility
1 (FK453)	11.9 μg/mL
2 (FK838)[a]	10 mg/mL
3a (FR166124)[a]	> 200 mg/mL[b]
7c	6.4 μg/mL
7d	25.6 μg/mL
7e	2.8 μg/mL
8c	185.6 μg/mL
8r	33.3 mg/mL

[a]Water solubility was measured as sodium salt.
[b]Upper limit was not determined.

Table 4. Diuretic activity of FR166124 in rats[a]

	Minimum effective dose[b] (mg/kg)	
Compound	p.o.	iv
2 (FK838)	0.1	0.1
3a (FR166124)	0.1	0.032

[a]Test compounds were administered ($n = 3$) and the urine was collected for 3 h (iv) and 6 h (p.o.).
[b]Statistical evaluation was performed by Dunnett's multiple comparison test ($P < 0.05$ were considered significant).

Conclusion

In summary, we have prepared a novel series of 3-(2-cyclohexenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines and evaluated their in vitro adenosine A_1 and A_{2A} receptor binding activities, leading to alcohol **7c**, nitrile **7e** and amide derivatives (**7d**, **8c**, **8r**), which were found to be more potent adenosine A_1 receptor antagonists with higher A_{2A}/A_1 selectivity than FR166124. Amongst them, **7c**, **7e** and **8r** were found to be highly potent and highly A_{2A}/A_1 selective adenosine A_1 receptor antagonists. Furthermore, **8r** has high water solubility, but less than that of the sodium salt of FR166124. The SAR study in this series of compounds revealed the following main features. (1) The position of the double bond of the cyclohexenyl group and the chain length of the carboxylic acid group were critical for high A_1 affinity and high A_{2A}/A_1 selectivity. (2) Replacement of the carboxylic acid group of FR166124 by hydrophobic moieties such as hydroxy, nitrile and carbamoyl groups increased A_1 affinity and A_{2A}/A_1 selectivity. (3) Certain amide derivatives of FR166124 with an additional amino group on the

amide moiety have high water solubility. (4) 3-(2-Cyclohexenyl-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines were potent adenosine A_1 receptor antagonists with high A_{2A}/A_1 selectivity. (5) Diuretic activity in rats of FR166124 (iv) was more potent than FK838.

Experimental

General procedures

All melting points (mp) were determined with a Büchi 535 apparatus in open capillaries and are uncorrected. Infrared spectra were recorded on a Horiba Spectradesk FT-210 spectrometer (FT-IR) or Hitachi 260-10 spectrometer (IR) as KBr disks, neat or in solution as indicated. ^{1}H NMR spectra were measured with a Bruker AC200P (200 MHz). Chemical shifts are given in parts per million (ppm) using tetramethylsilane as the internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. All *J* values are given in Hz. Mass (MS) spectra were measured on a Hitachi model M-1000H mass spectrometer using APCI for ionization. Elemental analyses were carried out on a Perkin–Elmer 2400 CHN elemental analyzer. Column chromatography was performed with the indicated solvents using Merck silica gel 60 (70–230 mesh). Monitoring of reactions was carried out using Merck 60 F_{254} silica gel, glass-supported TLC plates, followed by visualization with UV light (254 and 365 nm) and staining with iodine vapor. Reagents and solvents were used as obtained from commercial suppliers without further purification.

6-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenylacetic acid (3b). To a solution of *tert*-butyl 6-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]-pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenylacetate **6b** (15 g, 31 mmol) in CH_2Cl_2 (30 mL) was added dropwise TFA (24 mL, 310 mmol) at 5 °C under a nitrogen atmosphere. After the addition, the reaction mixture was allowed to warm to ambient temperature and stirred for 2 h. Evaporation of the solvent gave a residue which was azeotroped twice with toluene (100 mL). The resultant residue was partitioned between EtOAc and 1 N aqueous NaOH solution. The aqueous layer was separated, its pH was adjusted to 3.3 with 2 N aqueous HCl and extracted with CH_2Cl_2, which was then washed with water, dried over $MgSO_4$ and evaporated. The resultant solid was recrystallized from 70% aqueous EtOH to give **3b** (9.96 g, 75%) as a white solid. Mp 164.0–166.0°C; FT-IR (KBr) 1720, 1641, 1571, 1529 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.50–2.00 (m, 6H), 2.77 (ABq, 2H, *J* = 15.9, 15.8 Hz), 5.67 (br-s, 1H), 5.97 (s, 1H), 6.86 (d, 1H, *J* = 9.6 Hz), 7.03–7.11 (m, 2H), 7.38–7.63 (m, 6H), 7.93 (d, 1H, *J* = 8.9 Hz), 8.81 (d, 1H, *J* = 6.9 Hz), 12.13 (br-s, 1H); 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, 70.58; H, 5.27; N, 13.51.

Preparation of **3c** and **3d** was carried out by a method similar to that described for **3b** from the corresponding *tert*-butyl esters **6c** and **6d**, respectively.

2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-(*Z*)-cyclohexylidene-1-acetic acid (3c). White solid (65%); mp 256 °C (dec.) (aq EtOH); FT-IR (KBr) 1714, 1651, 1585, 1527, 1495 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.41–1.70 (m, 2H), 1.80–2.60 (m, 6H), 5.85–6.19 (m, 2H), 6.93 (t, 2H, *J* = 6.2 Hz), 7.17–7.34 (m, 2H), 7.43–7.46 (m, 3H), 7.55–7.56 (m, 2H), 7.80 (d, 1H, *J* = 8.8 Hz), 8.56 (d, 1H, *J* = 6.9 Hz); 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, 70.25; H, 5.26; N, 12.86.

6-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-(*E*)-cyclohexylidene-1-acetic acid (3d). White solid (76%); mp 250–253 °C (aq EtOH); FT-IR (KBr) 1714, 1651, 1595, 1527 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.20–2.20 (m, 7H), 3.80–4.00 (m, 1H), 4.93 (s, 1H), 5.40–5.11 (m, 1H), 6.98 (d, 1H, *J* = 9.7 Hz), 7.04–7.11 (m, 1H), 7.21 (d, 1H, J = 9.7 Hz), 7.40-7.63 (m, 6H), 7.85 (d, 1H, *J* = 8.9 Hz), 8.84 (d, 1H, *J* = 6.9 Hz), 12.21 (br-s, 1H); 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, 70.18; H, 5.02; N, 13.51.

3-[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]propionic acid (7a). To a suspension of **3a** (0.2 g, 0.47 mmol) in CH_2Cl_2 (4 mL) was added $SOCl_2$ (0.046 mL, 0.61 mmol) at ambient temperature under a nitrogen atmosphere. After stirring for 2.5 h, the reaction mixture was evaporated under reduced pressure. The resultant residue was dissolved in a mixture of THF (2 mL) and CH_3CN (2 mL) and to this mixture was added a solution of 10% trimethylsilyldiazomethane in *n*-hexane (2.2 g, 1.5 mmol) at 0 °C under a nitrogen atmosphere, then the mixture was stirred for an additional 3 h. Evaporation of volatiles gave a residue which was treated with benzyl alcohol (1.0 mL) and 2,4,6-trimethylpyridine (1.0 mL), then the mixture was stirred at 180–185 °C for 7 min under a nitrogen atmosphere. The reaction mixture was partitioned between EtOAc and 10% aqueous citric acid solution, and the organic layer was separated, washed in turn with water and brine, dried over $MgSO_4$, evaporated, and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH 100:1 elution) to give benzyl 3-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]propionate (87.0 mg, 35%) as a solid. Mp 111–113 °C (Et_2O); IR (Nujol) 1735, 1710, 1670, 1630, 1595, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.65–2.00 (m, 4H), 2.10–2.60 (m, 8H), 5.02 (s, 2H), 6.76 (d, 1H, *J* = 9.7 Hz), 6.88 (dt, 1H, *J* = 1.4, 6.9 Hz), 7.00 (d, 1H, *J* = 9.7 Hz), 7.20–7.30 (m, 6H), 7.43–7.47 (m, 3H), 7.60–7.66 (m, 2H), 7.89 (d, 1H, *J* = 8.9 Hz), 8.50 (d, 1H, *J* = 6.9 Hz); MS *m*/*z* 531 $(M+H)^+$. A mixture of the above benzyl ester (74 mg, 0.14 mmol), 1 N aqueous NaOH solution (0.28 mL) and THF (4 mL) was refluxed with stirring for 5 h. Evaporation of the solvent gave a residue which was partitioned between EtOAc and water. The aqueous layer was separated, acidified with 1 N aqueous HCl and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a residual solid which was recrystallized from aqueous EtOH to give **7a** as a white solid (54.0 mg,

88%). Mp 184–185 °C; IR (Nujol) 1695, 1655, 1625, 1585, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.55–1.81 (m, 4H), 2.00–2.35 (m, 8H), 6.91 (d, 1H, J = 9.7 Hz), 7.07 (dt, 1H, J = 1.3, 6.9 Hz), 7.14 (d, 1H, J = 9.7 Hz), 7.20–7.70 (m, 6H), 7.79 (d, 1H, J = 8.9 Hz), 8.82 (d, 1H, J = 6.9 Hz), 12.06 (s, 1H); MS m/z 441 $(M+H)^+$. Anal. calcd for $C_{26}H_{24}N_4O_3$: C, 70.89; H, 5.49; N, 12.72. Found: C, 70.57; H, 5.60; N, 12.42.

4-[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]butyric acid (7b). **7b** was prepared according to a method similar to that described for **7a**, using **7a** as the starting material instead of **3a**. White solid (2 steps, 80%); mp 145–147 °C (EtOAc–*n*-hexane); IR (Nujol) 1700, 1655, 1625, 1585, 1510 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.20–1.90 (m, 8H), 2.00–2.50 (m, 6H), 6.90 (d, 1H, J = 9.7 Hz), 7.07 (t, 1H, J = 6.9 Hz), 7.13 (d, 1H, J = 9.7 Hz), 7.38–7.65 (m, 6H), 7.80 (d, 1H, J = 8.8 Hz), 8.82 (d, 1H, J = 6.9 Hz), 11.92 (s, 1H); MS m/z 455 $(M+H)^+$. Anal. calcd for $C_{27}H_{26}N_4O_3$: C, 71.35; H, 5.77; N, 12.33. Found: C, 70.93; H, 5.76; N, 12.23.

2-[2-(2-Hydroxyethyl)-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (7c). To a solution of **3a** (2.07 g, 4.86 mmol) in THF (40 mL) was added dropwise a solution of BH_3·THF complex in THF (1 M solution, 9.72 mL, 9.72 mmol) at −10 °C over a period of 10 min under a nitrogen atmosphere. The reaction mixture was allowed to warm to ambient temperature and stirred for an additional 5.5 h, then cooled to 10 °C and treated with 1 N aqueous HCl. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed in turn with 1 N aqueous NaOH solution, water and brine, dried over $MgSO_4$, evaporated, and purified by column chromatography on silica gel (toluene:MeOH 10:1 elution) to give **7c** (1.41 g, 70%) as a white solid. Recrystallization afforded an analytical sample; mp 198–199 °C (aq EtOH); IR (Nujol) 3375, 1650, 1630, 1585, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.66–1.97 (m, 4H), 2.01–2.24 (m, 2H), 2.33–2.47 (m, 4H), 3.23 (t, 1H, J = 5.4 Hz), 3.63–3.83 (m, 2H), 6.85 (t, 1H, J = 9.7 Hz), 6.92 (t, 1H, J = 6.9 Hz), 7.08 (d, 1H, J = 9.7 Hz), 7.32 (dd, 1H, J = 8.9, 6.9 Hz), 7.45–7.50 (m, 3H), 7.60–7.65 (m, 2H), 7.91 (d, 1H, J = 8.9 Hz), 8.53 (d, 1H, J = 6.9 Hz); MS m/z 413 $(M+H)^+$. Anal. calcd for $C_{25}H_{24}N_4O_2$: C, 72.79; H, 5.86; N, 13.58. Found: C, 72.52; H, 5.97; N, 13.56.

2-[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetamide (7d). To a solution of **3a** (432 mg, 1 mmol) in a mixture of CH_2Cl_2 (20 mL) and THF (10 mL) was added successively HOBt (162 mg, 1.2 mmol), EDC (0.28 mL, 1.5 mmol) and NH_4Cl (65 mg, 1.2 mmol) at ambient temperature under a nitrogen atmosphere and stirred overnight. The reaction mixture was partitioned between EtOAc and water, and the organic layer was separated, washed in turn with water, saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, evaporated, and purified by column chromatography on silica gel (EtOAc elution) to give **7d** (185 mg, 43%) as a beige solid. Recrystallization afforded an analytical sample. Mp 204–205 °C (EtOAc); FT-IR (KBr) 1672, 1659, 1589, 1527 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.85 (m, 4H), 2.05–2.40 (m, 4H), 2.71 (ABq, 2H, J = 14.7, 20.7 Hz), 6.96 (d, 1H, J = 9.7 Hz), 7.04–7.18 (m, 2H), 7.38–7.70 (m, 6H), 7.92 (d, 1H, J = 8.9 Hz), 8.82 (d, 1H, J = 6.9 Hz); MS m/z 426 $(M+H)^+$. Anal. calcd for $C_{25}H_{23}N_5O_2$·0.5H_2O: C, 69.11; H, 5.57; N, 16.12. Found: C, 69.49; H, 5.51; N, 16.18.

Using the same procedure **8a**, **8b**, **8f**, **8j** and **8l** were also prepared as described for **7d** from the appropriate amine hydrochloride.

***N*-Methyl-2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetamide (8a).** Amorphous powder (43%). FT-IR (KBr) 1659, 1587, 1529 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.80–2.00 (m, 4H), 2.15–2.50 (m, 4H), 2.60–2.70 (m, 1H), 2.74 (d, 3H, J = 4.7 Hz), 3.12 (d, 1H, J = 14.8 Hz), 6.84 (t, 1H, J = 9.6 Hz), 6.90–7.00 (m, 1H), 7.11 (d, 1H, J = 9.6 Hz), 7.29–7.65 (m, 6H), 7.86 (d, 1H, J = 9.0 Hz), 8.54 (d, 1H, J = 6.9 Hz); MS m/z 440 $(M+H)^+$. Anal. calcd for $C_{26}H_{25}N_5O_2$·0.5H_2O: C, 69.63; H, 5.84; N, 15.61. Found: C, 69.80; H, 5.83; N, 15.89.

***N*,*N*-Dimethyl-2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetamide (8b).** White solid (40%); mp 178–179 °C (EtOH); FT-IR (KBr) 1666, 1639, 1591, 1527 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.85 (m, 4H), 2.05–2.40 (m, 4H), 2.69 (s, 3H), 2.80 (s, 3H), 2.93 (s, 2H), 6.89 (d, 1H, J = 9.7 Hz), 7.07–7.11 (m, 2H), 7.37–7.66 (m, 6H), 7.90 (d, 1H, J = 8.9 Hz), 8.81 (d, 1H, J = 6.9 Hz); MS m/z 454 $(M+H)^+$. Anal. calcd for $C_{27}H_{27}N_5O_2$·0.5H_2O: C, 70.11; H, 6.10; N, 15.14. Found: C, 70.33; H, 5.97; N, 15.20.

Ethyl [methyl-[2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetyl]amino]-acetate (8f). Yellow solid (80%); mp 56–60 °C (EtOAc–*n*-hexane); FT-IR (KBr) 1743, 1670, 1639, 1591, 1527 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.96–1.67 (m, 3H), 1.60–1.85 (m, 4H), 2.05–2.40 (m, 4H), 2.70–3.00 (m, 5H), 3.90–4.10 (m, 4H), 6.80–7.13 (m, 3H), 7.35–7.63 (m, 6H), 7.89 (d, 1H, J = 8.9 Hz), 8.81 (d, 1H, J = 6.9 Hz); MS m/z 526 $(M+H)^+$. Anal. calcd for $C_{30}H_{31}N_5O_4$: C, 68.55; H, 5.94; N, 13.32. Found: C, 68.32; H, 6.06; N, 12.86.

Ethyl 3-[methyl-[2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]-pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetyl]-amino]propionate (8j). Amorphous foam (75%). FT-IR (KBr) 1736, 1666, 1645, 1593, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95–1.16 (m, 3H), 1.55–1.80 (m, 4H), 2.10–2.50 (m, 6H), 2.65–3.20 (m, 7H), 3.80–4.05 (m, 2H), 6.88 (d, 1H, J = 9.7 Hz), 7.00–7.12 (m, 2H), 7.30–7.65 (m, 6H), 7.90 (d, 1H, J = 8.9 Hz), 8.81 (d, 1H, J = 6.9 Hz); MS m/z 540 $(M+H)^+$.

Methyl 4-[2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetylamino]-butyrate (8l). Amorphous foam (61%). FT-IR (KBr) 1736, 1668, 1589, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ

1.45–1.85 (m, 6H), 2.16–2.35 (m, 6H), 2.65–3.10 (m, 4H), 3.53 (s, 3H), 6.95 (d, 1H, $J=9.7$ Hz), 7.03–7.12 (m, 1H), 7.15 (d, 1H, $J=9.7$ Hz), 7.35–7.67 (m, 7H), 7.91 (d, 1H, $J=8.9$ Hz), 8.82 (d, 1H, $J=6.9$ Hz); MS m/z 526 $(M+H)^+$.

[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetonitrile (7e). To a stirred mixture of **7d** (2.15 g, 5 mmol) and pyridine (2.1 mL, 25 mmol) in CH_2Cl_2 (25 mL) was added dropwise TFAA (1.1 mL, 7.5 mmol) at 0 °C, followed by warming to ambient temperature and stirring an additional 3 h under a nitrogen atmosphere. The reaction mixture was partitioned between EtOAc and water, and the organic layer separated, washed in turn with water, saturated aqueous $NaHCO_3$ solution, 1 N aqueous HCl, brine, saturated aqueous $NaHCO_3$ solution, water and brine, dried over $MgSO_4$, evaporated, and purified by column chromatography on silica gel (CH_2Cl_2:EtOAc 100:3 and 100:5 elution) to give **7e** (1.28 g, 62%) as a beige solid. Recrystallization afforded an analytical sample; mp 163–165 °C (EtOH); FT-IR (KBr) 2251, 1734, 1670, 1633, 1595, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.85 (m, 4H), 2.05–2.40 (m, 4H), 3.26 (s, 2H), 6.94 (d, 1H, $J=9.7$ Hz), 7.04–7.17 (m, 2H), 7.38–7.69 (m, 6H), 7.87 (d, 1H, $J=8.9$ Hz), 8.83 (d, 1H, $J=6.9$ Hz); MS m/z 408 $(M+H)^+$. Anal. calcd for $C_{25}H_{21}N_5O{\cdot}0.5H_2O$: C, 72.10; H, 5.32; N, 16.82. Found: C, 72.03; H, 5.13; N, 16.54.

***N*-(2-Hydroxyethyl)-2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetamide (8c).** To a solution of **3a** (800 mg, 1.88 mmol) in a mixture of CH_2Cl_2 (10 mL) and THF (10 mL) was added successively HOBt (305 mg, 2.26 mmol), EDC·HCl (541 mg, 2.82 mmol) and 2-aminoethanol (0.14 mL, 2.26 mmol) at ambient temperature under a nitrogen atmosphere and stirred for 3 h. The reaction mixture was partitioned between EtOAc and water, and organic layer was separated, washed in turn with 1 N HCl, water, saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, evaporated, and purified by column chromatography on silica gel (EtOAc elution) to give **8c** (500 mg, 57%) as a solid. Recrystallization afforded an analytical sample; mp 204–205 °C (EtOAc); FT-IR (KBr) 3315, 1657, 1585, 1529 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.75–2.00 (m, 4H), 2.10–2.50 (m, 4H), 2.60–2.80 (m, 1H), 3.10–3.30 (m, 2H), 3.50–3.90 (m, 4H), 6.83 (d, 1H, $J=9.7$ Hz), 6.90–7.00 (m, 1H), 7.14 (d, 1H, $J=9.7$ Hz), 7.30–7.40 (m, 1H), 7.45–7.70 (m, 6H), 7.87 (d, 1H, $J=8.9$ Hz), 8.54 (d, 1H, $J=6.9$ Hz); MS m/z 470 $(M+H)^+$. Anal. calcd for $C_{27}H_{27}N_5O_3{\cdot}H_2O$: C, 66.51; H, 6.00; N, 14.36. Found: C, 66.19; H, 5.82; N, 14.18.

Using a similar procedure **8n–q** were also prepared as described for **8c**, from the appropriate amine.

2-[2-[2-Oxo-2-(pyrrolidin-1-yl)ethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (8n). White solid (75%); mp 178–179 °C (EtOAc); IR (Nujol) 1660, 1635, 1585, 1525 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51–2.76 (m, 12H), 2.86–3.55 (m, 6H), 6.77 (d, 1H, $J=9.7$ Hz), 6.92 (t, 1H, $J=6.9$ Hz), 7.03 (d, 1H, $J=9.7$ Hz), 7.36 (dd, 1H, $J=8.9$, 6.9 Hz), 7.45–7.48 (m, 3H), 7.60–7.65 (m, 2H), 8.11 (d, 1H, $J=8.9$ Hz), 8.53 (d, 1H, $J=6.9$ Hz); MS m/z 480 $(M+H)^+$. Anal. calcd for $C_{29}H_{29}N_5O_2$: C, 72.63; H, 6.10; N, 14.60. Found: C, 72.61; H, 6.19; N, 14.50.

2-[2-[2-Oxo-2-(piperidin-1-yl)ethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (8o). White solid (76%); mp 184–185 °C (EtOAc–Et_2O); IR (Nujol) 1660, 1635, 1585, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28–1.60 (m, 6H), 1.70–1.97 (m, 4H), 2.20–2.58 (m, 4H), 3.09 (s, 2H), 3.20–3.43 (m, 4H), 6.78 (d, 1H, $J=9.7$ Hz), 6.93 (dd, 1H, $J=8.9$, 6.7 Hz), 7.03 (d, 1H, $J=9.7$ Hz), 7.35 (dd, 1H, $J=8.9$, 6.7 Hz), 7.46–7.49 (m, 3H), 7.60–7.63 (m, 2H), 8.05 (d, 1H, $J=8.9$ Hz), 8.52 (d, 1H, $J=6.7$ Hz); MS m/z 494 $(M+H)^+$. Anal. calcd for $C_{30}H_{31}N_5O_2{\cdot}0.5H_2O$: C, 71.69; H, 6.42; N, 13.96. Found: C, 71.71; H, 6.31; N, 13.76.

2-[2-[2-(Morpholin-4-yl)-2-oxoethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (8p). White solid (89%); mp 202–204 °C (EtOAc–Et_2O); IR (Nujol) 1655, 1590, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70–2.00 (m, 4H), 2.10–2.60 (m, 4H), 3.10 (s, 2H), 3.30–3.70 (m, 8H), 6.76 (d, 1H, $J=9.8$ Hz), 6.92 (t, 1H, $J=6.9$ Hz), 7.05 (d, 1H, $J=9.8$ Hz), 7.34 (t, 1H, $J=6.9$ Hz), 7.45–7.49 (m, 3H), 7.59–7.63 (m, 2H), 7.99 (d, 1H, $J=9.0$ Hz), 8.52 (d, 1H, $J=6.9$ Hz); MS m/z 496 $(M+H)^+$. Anal. calcd for $C_{29}H_{29}N_5O_3$: C, 70.29; H, 5.90; N, 14.13. Found: C, 70.19; H, 5.84; N, 13.72.

2-[2-[2-Oxo-2-(thiomorpholin-4-yl)ethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (8q). White solid (92%); mp 181–183 °C (EtOAc); FT-IR (KBr) 1660, 1587, 1529 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60–2.00 (m, 4H), 2.10–2.70 (m, 8H), 3.10 (m, 2H), 3.60–4.00 (m, 4H), 6.78 (d, 1H, $J=9.7$ Hz), 6.94 (t, 1H, $J=6.9$ Hz), 7.05 (d, 1H, $J=9.7$ Hz), 7.26–8.05 (m, 6H), 8.00 (d, 1H, $J=9.0$ Hz), 8.55 (d, 1H, $J=6.9$ Hz); MS m/z 512 $(M+H)^+$. Anal. calcd for $C_{29}H_{29}N_5O_2S$: C, 68.08; H, 5.71; N, 13.69. Found: C, 67.96; H, 5.66; N, 13.42.

***tert*-Butyl [2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetylamino]acetate (8d).** To a solution of **3a** (222 mg, 0.52 mmol) and DMF (1 drop) in CH_2Cl_2 (4 mL) was added dropwise $(COCl)_2$ (99 mg, 0.78 mmol) at 5 °C under a nitrogen atmosphere. After stirring for 20 min at the same temperature, the reaction mixture was allowed to warm to ambient temperature, stirred for an additional 2 h and concentrated under reduced pressure to give a residue. To an ice-cooled mixture of glycine *tert*-butyl ester hydrochloride (95.8 mg, 0.57 mmol) and Et_3N (158 mg, 1.56 mmol) in CH_2Cl_2 (5 mL) was added a solution of the above residue in CH_2Cl_2 (2 mL). After the addition, the reaction mixture was allowed to warm to ambient temperature and stirred for an additional 5.5 h. The reaction mixture was washed in turn with 1 N aqueous HCl, saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, evaporated, and purified by column chromatography on silica gel (CH_2Cl_2:EtOAc 10:1 elution) to give **8d** (152.9 mg, 55%) as a foam. IR (CH_2Cl_2) 3280, 1735, 1650, 1585, 1525 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.75–1.92 (m, 4H), 2.11–2.52 (m,

4H), 2.78 (d, 1H, $J=14.7$ Hz), 3.16 (d, 1H, $J=14.7$ Hz), 3.74 (dd, 1H, $J=17.6$, 5.5 Hz), 3.91 (dd, 1H, $J=17.6$, 6.0 Hz, 6.84 (d, 1H, $J=9.7$ Hz), 6.93 (td, 1H, $J=6.9$, 1.4 Hz), 7.10 (d, 1H, $J=9.7$ Hz), 7.32 (ddd, 1H, $J=8.9$, 6.9, 1.1 Hz), 7.46–7.51 (m, 3H), 7.60–7.68 (m, 3H), 7.88 (d, 1H, $J=8.9$ Hz), 8.53 (d, 1H, $J=6.9$ Hz); MS m/z 540 $(M+H)^+$.

***tert*-Butyl 3-[2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetylamino]-propionate (8h). 8h** was obtained from **3a** by the same procedure described for **8d** using *tert*-butyl 3-aminopropionate instead of *tert*-butyl 2-aminoacetate. Foam (43%). IR (CH_2Cl_2) 3280, 1720, 1655, 1585, 1525 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.75–1.96 (m, 4H), 2.15–2.30 (m, 2H), 2.43 (t, 4H, $J=6.9$ Hz), 2.71 (d, 1H, $J=14.7$ Hz), 3.09 (d, 1H, $J=14.7$ Hz), 3.35–3.49 (m, 2H), 6.82 (d, 1H, $J=9.7$ Hz), 6.93 (td, 1H, $J=7.0$, 1.3 Hz), 7.09 (d, 1H, $J=9.7$ Hz), 7.32 (dd, 1H, $J=8.9$, 7.0 Hz), 7.46–7.49 (m, 4H), 7.60–7.65 (m, 2H), 7.87 (d, 1H, $J=8.9$ Hz), 8.53 (d, 1H, $J=7.0$ Hz); MS m/z 554 $(M+H)^+$.

2-[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetylamino]acetic acid (8e). To a solution of **8d** (141 mg, 0.262 mmol) in CH_2Cl_2 (0.28 mL) was added dropwise TFA (299 mg, 2.62 mmol) at 5 °C under a nitrogen atmosphere. After the addition, the reaction mixture was allowed to warm to ambient temperature and stirred for 14 h. Evaporation of the solvent gave a residue which was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was acidified with 1 N aqueous HCl, extracted with CH_2Cl_2. The extracts were combined, washed with brine, dried over $MgSO_4$, and evaporated to give **8e** (106.0 mg, 83%) as a pale yellow solid. Recrystallization afforded an analytical sample; mp 190–192°C (EtOAc); IR (Nujol) 3280, 1727, 1675, 1650, 1585, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.72–2.63 (m, 8H), 2.79 (d, 1H, $J=14.9$ Hz), 3.15 (d, 1H, $J=14.9$ Hz), 3.96 (t, 2H, $J=5.7$ Hz), 6.92 (td, 1H, $J=7.0$, 1.3 Hz), 6.94 (d, 1H, $J=9.7$ Hz), 7.11 (d, 1H, $J=9.7$ Hz), 7.35 (dd, 1H, $J=8.9$, 7.0 Hz), 7.45–7.50 (m,3H), 7.57–7.63 (m, 2H), 7.72 (t, 1H, $J=5.8$ Hz), 7.88 (d, 1H, $J=8.9$ Hz), 8.55 (d, 1H, $J=7.0$ Hz); MS m/z 484 $(M+H)^+$. Anal. calcd for $C_{27}H_{25}N_5O_4$: C, 67.07; H, 5.21; N, 14.48. Found: C, 66.80; H, 5.38; N, 14.10.

3-[2-[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetylamino]propionic acid (8i). 8i was obtained from **8h** by the same procedure described for **8e**. Pale yellow solid (86%). Mp 183–184 °C (EtOAc); IR (Nujol) 3275, 1720, 1670, 1650, 1580, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.69–2.01 (m, 4H), 2.09–2.48 (m, 4H), 2.54 (t, 2H, $J=6.5$ Hz), 2.74 (d, 1H, $J=15.0$ Hz), 3.09 (d, 1H, $J=15.0$ Hz), 3.41–3.52 (m, 2H), 6.89 (d, 1H, $J=9.7$ Hz), 6.93 (td, 1H, $J=7.0$, 1.3 Hz), 7.11 (d, 1H, $J=9.7$ Hz), 7.34 (dd, 1H, $J=8.9$, 7.0 Hz), 7.45–7.50 (m, 3H), 7.57–7.64 (m, 3H), 7.88 (d, 1H, $J=8.9$ Hz), 8.55 (d, 1H, $J=7.0$ Hz); MS m/z 498 $(M+H)^+$. Anal. calcd for $C_{28}H_{27}N_5O_4$: C, 67.59; H, 5.47; N, 14.08. Found: C, 67.25; H, 5.57; N, 13.77.

[Methyl-[2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetyl]amino]acetic acid (8g). To a solution of **8f** (710 mg, 1.35 mmol) in a mixture of 1,4-dioxane (7 mL) and water (4 mL) was added 1 N aqueous NaOH solution (3.4 mL, 3.38 mmol) at ambient temperature and the mixture was stirred for 2 h. The reaction mixture was partitioned between EtOAc and water, and the aqueous layer was separated. The pH was adjusted to 1.5 with 6 N aqueous HCl, extracted with EtOAc, washed in turn with water and brine, dried over $MgSO_4$, evaporated, and purified by column chromatography on silica gel (CH_2Cl_2:MeOH 20:1, 10:1 and 6:1 elution) to give **8g** (300 mg, 46%) as a white solid. Recrystallization afforded an analytical sample; mp 135–138 °C (aq EtOH); FT-IR (KBr) 1730, 1657, 1585, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.85 (m, 4H), 2.05–2.40 (m, 4H), 2.70–3.00 (m, 5H), 3.80–3.90 (m, 2H), 6.83–7.12 (m, 3H), 7.35–7.65 (m, 6H), 7.85–7.96 (m, 1H), 8.81 (d, 1H, $J=6.9$ Hz); MS m/z 498 $(M+H)^+$. Anal. calcd for $C_{28}H_{27}N_5O_4 \cdot 0.25H_2O$: C, 69.99; H, 5.52; N, 13.95. Found: C, 69.98; H, 5.85; N, 14.06.

Using a similar procedure **8k** and **8m** were prepared as described for **8g**.

3-[Methyl-[2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetyl]amino]-propionic acid (8k). White solid (80%); mp 138–140 °C (aq EtOH); FT-IR (KBr) 1720, 1660, 1633, 1587, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.60–1.85 (m, 4H), 2.05–2.40 (m, 6H), 2.60–3.20 (m, 7H), 6.82–6.91 (m, 1H), 7.00–7.11 (m, 2H), 7.40–7.65 (m, 6H), 7.85–7.95 (m, 1H), 8.80 (d, 1H, $J=6.8$ Hz); MS m/z 512 $(M+H)^+$. Anal. calcd for $C_{29}H_{29}N_5O_4$: C, 68.09; H, 5.71; N, 13.69. Found: C, 67.87; H, 5.67; N, 13.60.

4-[2-[2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazinyl]-1-cyclohexenyl]acetylamino]butyric acid (8m). White solid (80%); mp 206–207 °C (EtOH); FT-IR (KBr) 1726, 1659, 1585, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43–1.85 (m, 6H), 2.09–2.35 (m, 6H), 2.65–3.10 (m, 4H), 6.95 (d, 1H, $J=9.7$ Hz), 7.04–7.11 (m, 1H), 7.15 (d, 1H, $J=9.7$ Hz), 7.35–7.70 (m, 7H), 7.91 (d, 1H, $J=8.8$ Hz), 8.82 (d, 1H, $J=6.8$ Hz); MS m/z 512 $(M+H)^+$. Anal. calcd for $C_{29}H_{29}N_5O_4$: C, 68.09; H, 5.71; N, 13.69. Found: C, 67.90; H, 5.78; N, 13.69.

2-[2-[2-(4-Methylpiperazin-1-yl)-2-oxoethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone hydrochloride (8r). A mixture of **3a** (500 mg, 1.2 mmol), 1-methylpiperazine (0.14 mL, 1.3 mmol), Et_3N (0.2 mL, 1.4 mmol), HOBt (180 mg, 1.3 mmol) and EDC·HCl (250 mg, 1.3 mmol) in DMF (10 mL) was stirred for 16 h at ambient temperature under a nitrogen atmosphere. The reaction mixture was partitioned between EtOAc and water, and the organic layer was separated, washed with water, dried over $MgSO_4$ and evaporated to give a residue. This residue was treated with 4 N HCl in EtOAc and the resultant solid was collected by filtration to give **8r** (380 mg, 59%) as a beige solid. Recrystallization afforded an analytical sample; mp 188–191 °C (H_2O); IR (Nujol) 2675, 2600, 1650, 1630, 1580, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ

1.55–190 (m, 3H), 2.00–2.50 (m, 3H), 2.60–3.60 (m, 10H), 2.66 (s, 3H), 3.87 (s, 1H), 4.34 (s, 1H), 6.90 (d, 1H, $J=9.7$ Hz), 7.05–7.13 (m, 1H), 7.10 (d, 1H, $J=9.7$ Hz), 7.40–7.60 (m, 4H), 7.61–7.70 (m, 2H), 7.89 (d, 1H, $J=8.8$ Hz), 8.83 (d, 1H, $J=6.9$ Hz), 11.01 (s, 1H); MS m/z 509 $(M+H)^+$. Anal. calcd for $C_{30}H_{33}N_6O_2Cl\cdot 2.2H_2O$: C, 61.63; H, 6.44; N, 14.37. Found: C, 61.41; H, 6.61; N, 14.21.

2-[2-[2-Oxo-2-(piperazin-1-yl)ethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (8s). To a solution of **3a** (3.0 g, 7.03 mmol) in a mixture of CH_2Cl_2 (30 mL) and THF (30 mL) was added successively HOBt (1.14 g, 8.44 mmol), EDC·HCl (2.02 g, 10.55 mmol) and 1-triphenylmethylpiperazine (2.77 g, 8.44 mmol) at ambient temperature under a nitrogen atmosphere and stirred for 1.5 h. The reaction mixture was partitioned between EtOAc and water, and the organic layer was separated, washed in turn with water, saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to give 2-[2-[2-oxo-2-(4-triphenylmethylpiperazin-1-yl)ethyl]-1-cyclohexenyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (500 mg, quant.) as a solid: mp 208–209 °C (EtOAc); FT-IR (KBr) 1662, 1637, 1587, 1529 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70–2.00 (m, 4H), 2.20–2.60 (m, 4H), 2.70–2.80 (m, 4H), 3.10 (s, 2H), 3.30–3.70 (m, 4H), 6.77 (d, 1H, $J=9.7$ Hz), 6.89–6.96 (m, 1H), 7.04 (d, 1H, $J=9.7$ Hz), 7.30–7.40 (m, 1H), 7.45–7.70 (m, 5H), 8.00 (d, 1H, $J=8.9$ Hz), 8.52 (d, 1H, $J=6.9$ Hz); MS m/z 495 $(M+H)^+$. Anal. calcd for $C_{29}H_{30}N_6O_2\cdot 0.5H_2O$: C, 69.17; H, 6.20; N, 16.69. Found: C, 69.41; H, 6.35; N, 16.41. To a solution of the above compound in HCO_2H (10 mL) was added concd HCl (1.43 mL) at ambient temperature and the mixture was stirred for 2 h. The reaction mixture was partitioned between EtOAc and water, and the aqueous layer was separated and washed with EtOAc. The pH of the solution was adjusted to 12 with 4 N aqueous NaOH solution and then extracted twice with CH_2Cl_2. The extracts were combined, washed with brine, dried over $MgSO_4$, evaporated and purified by column chromatography on silica gel (CH_2Cl_2:MeOH 20:1 and 10:1 elution) to give **8s** (2.65 g, 76%) as beige solid. Recrystallization afforded an analytical sample; mp 208–209 °C (EtOAc); FT-IR (KBr) 1662, 1637, 1587, 1529 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70–2.00 (m, 4H), 2.20–2.60 (m, 4H), 2.70–2.80 (m, 4H), 3.10 (s, 2H), 3.30–3.70 (m, 4H), 6.77 (d, 1H, $J=9.7$ Hz), 6.89–6.96 (m, 1H), 7.04 (d, 1H, $J=9.7$ Hz), 7.30–7.40 (m, 1H), 7.45–7.70 (m, 5H), 8.00 (d, 1H, $J=8.9$ Hz), 8.52 (d, 1H, $J=6.9$ Hz); MS m/z 495 $(M+H)^+$. Anal. calcd for $C_{29}H_{30}N_6O_2\cdot 0.5H_2O$: C, 69.17; H, 6.20; N, 16.69. Found: C, 69.41; H, 6.35; N, 16.41.

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References

1. *Adenosine and Adenosine Receptors*, Williams, M., Ed.; The Humana Press: Clifton, New Jersey; 1990; pp 1–15.
2. Williams, M. *Med. Res. Rev.* **1989**, *9*, 219.
3. Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, K.; Jacobson, K. A.; Leff, P.; Williams, M. *Pharmacol. Rev.* **1994**, *46*, 143.
4. Dalziei, H. H.; Westfall, D. P. *Pharmacol. Rev.* **1994**, *46*, 449.
5. Poulsen, S.-A.; Quinn, R. J. *Bioorg. Med. Chem.* **1998**, *6*, 619.
6. For a recent review of adenosine A_1 receptor antagonists, see Müller, C. E. *Exp. Opin. Ther. Patents* **1997**, *7*, 419.
7. Müller, C. E.; Stein, B. *Curr. Pharm. Des.* **1996**, *2*, 501.
8. Jacobson, K. A.; Trivedi, B. K.; Churchill, P. C.; Williams, M. *Biochem. Pharmacol.* **1991**, *41*, 1399.
9. Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407.
10. Suzuki, F. *Drug News Perspect* **1992**, *5*, 587.
11. Suzuki, F.; Shimada, J.; Nonaka, H.; Ishii, A.; Shiozaki, S.; Ichikawa, S.; Ono, E. *J. Med. Chem.* **1992**, *35*, 3578.
12. Ceccarelli, S.; Altobelli, M.; D'Alessandro, A.; Paesano, A. *Res. Commun. Mol. Pathol. Pharmacol.* **1995**, *87*, 101.
13. Akahane, A.; Katayama, H.; Mitsunaga, T.; Kita, Y.; Kusunoki, T.; Terai, T.; Yoshida, K.; Shiokawa, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2059.
14. Terai, T.; Kita, Y.; Kusunoki, T.; Ando, T.; Shimazaki, T.; Deguchi, Y.; Akahane, A.; Shiokawa, Y.; Yoshida, K. *Eur. J. Pharmacol.* **1990**, *183*, 1057.
15. Terai, T.; Kita, Y.; Kusunoki, T.; Ando, T.; Nagatomi, I.; Horiai, H.; Akahane, A.; Shiokawa, Y.; Yoshida, K. *Drug Dev. Res.* **1995**, *36*, 25.
16. Terai, T.; Kita, Y.; Kusunoki, T.; Shimazaki, T.; Ando, T.; Horiai, H.; Akahane, A.; Shiokawa, Y.; Yoshida, K. *Eur. J. Pharmacol.* **1995**, *279*, 217.
17. Terai, T.; Kusunoki, T.; Kita, Y.; Akahane, A.; Shiokawa, Y.; Kohno, Y.; Horiai, H.; Uehara, Y.; Yoshida, K. *Cardiovascular Drug Rev.* **1997**, *15*, 44.
18. Akahane, A.; Katayama, H.; Mitsunaga, T.; Kato, T.; Kinoshita, T.; Kita, Y.; Kusunoki, T.; Terai, T.; Yoshida, K.; Shiokawa, Y. *J. Med. Chem.* **1999**, *42*, 779.
19. Kusunoki, T.; Kita, Y.; Akahane, A.; Shiokawa, Y.; Kohno, Y.; Horiai, H.; Sendoh, H.; Yoshida, K.; Tanaka, H. *Can. J. Physiol. Pharmacol.* **1994**, *72*(Suppl. 1), 505.
20. Horiai, H.; Yoshida, K.; Minoura, H.; Takeda, M.; Nakano, K.; Hanaoka, K.; Kusunoki, T.; Terai, T.; Ohtsuka, M.; Shimomura, K. *Can. J. Physiol. Pharmacol.* **1994**, *72*(Suppl. 1) 505.
21. Takeda, M.; Kohno, Y.; Y.; Esumi, K.; Horai, H.; Ohtsuka, M.; Shimomura, K.; Imai, M. *Jpn J. Pharmacol.* **1994**, *64* (Suppl 1), 176P.
22. Kuroda, S.; Akahane, A.; Itani, H.; Nishimura, S.; Durkin, K.; Kinoshita, T.; Tenda, Y.; Sakane, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1979.
23. Kuroda, S.; Akahane, A.; Itani, H.; Nishimura, S.; Durkin, K.; Kinoshita, T.; Nakanishi, I.; Sakane, K. *Tetrahedron* **1999**, *55*, 10351.
24. Maccoss, M.; Wagner, A. F.; Tolman, R. L. European Patent 330263. *Chem. Abstr.* **1989**, *112*, 55917.
25. Bruns, R. F.; Fergus, J. H. *J. Pharm. Pharmacol.* **1989**, *41*, 590.