Design, Synthesis and Biological Evaluation of a Novel Series of Potent, Orally Active Adenosine A_1 Receptor Antagonists with High Blood–Brain Barrier Permeability

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A novel series of 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines (5—38) were synthesized and evaluated for their *in vitro* adenosine A_1 and A_{2A} receptor binding activities, and *in vitro* metabolism by rat liver in order to search for orally active compounds. Most of the test compounds were potent adenosine A_1 receptor antagonists with high A_1 selectivity and the A_1 affinity and A_1 selectivity of carbonyl derivatives (5—11) was particularly high. In particular, compound 7 was an extremely potent and selective adenosine A_1 antagonist with high A_1 selectivity (Ki=0.026 nM, A_{2A}/A_1=5400). In terms of metabolic stability, 2-oxopropyl (5), 2-hydroxypropyl (12), *N*-methylacetamide (16), 2-(piperidin-1-yl)ethyl (28) and 1-methylpiperidin-4-yl (32, FR194921) were the most stable compounds in this series of analogues. Further *in vivo* evaluation indicated that compounds 5, 13, 17, 28 and 32 were detected in both plasma and brain after oral administration in rats. In particular, 32 displayed good plasma and brain concentrations (dose: 32 mg/kg (n=3); after 30 min, plasma conc.=3390±651 nM, brain conc.=3670±496 nM; after 60 min, plasma conc.=1580±348 nM, brain conc.=2143±434 nM), and a good brain/plasma ratio (1.11±0.060 (30 min), 1.39±0.172 (60 min)). As a result, we could show that 32 is a good candidate for an orally active adenosine A_1 receptor antagonist with high blood–brain barrier permeability and good bioavailability (Ki=6.6 nM, A_{2A}/A_1=820, BA=60.6±4.9% (32 mg/kg)).

Key words adenosine A_1 antagonist; bioavailability; blood–brain barrier permeability; FR194921

In the central nervous system (CNS), adenosine regulates a variety of important physiological functions as a modulator through G-protein coupled extracellular adenosine receptors, which have a seven-transmembrane structure.[1] Adenosine receptors were classified into four subtypes A_1, A_{2A}, A_{2B} and A_3, and these four subtypes of human receptors have been cloned.[2] The A_1 and A_{2A} receptors are high affinity receptors for adenosine, while the A_{2B} and A_3 receptors are low affinity adenosine receptors.[2] With regards to the distribution of adenosine receptors in brain, A_1 receptors are widely present in brain and are especially present in high density in cortex and hippocampus. On the other hand, A_{2A} receptors are present in high density in striatum.[3] On the basis of pharmacological studies in the CNS, it has been suggested that adenosine A_1 receptors play a role in learning and memory.[4] Further investigations on adenosine A_1 receptor antagonists in several animal models have pointed towards novel therapeutic agents[5] for cognitive enhancement,[6] depression[7] and for the treatment of dementia such as senile dementia and Alzheimer's disease.[8] In recent years, several xanthine type adenosine A_1 receptor antagonists have been reported as agents acting in the central nervous system.[5] Apaxifylline (**1**),[9] the active enantiomer of KFM-19, has been developed for the treatment of Alzheimer's disease, senile dementia and cognitive defects, and shows high oral bioavailability (BA) in rats (Chart 1).

We recently reported the discovery of FK838 (**2**)[10] and FR166124 (**3**),[11] potent 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines as adenosine A_1 receptor antagonists with strong diuretic activities after both intravenous (i.v.) and oral (*p.o.*) administration (Chart 1). We had initially hoped that FK838 may be potentially useful for CNS-related diseases, however, from the results of pharmacokinetic studies in rats, it was elucidated that FK838 was not detected in brain after *p.o.* administration,[12] despite the fact that FK838 has good BA.[10] It was reasoned that the poor brain permeability of FK838 was due to the presence of the carboxylic acid group, a feature also present in FR166124. Moreover, a number of FR166124 derivatives were found to be orally active compounds, but were not detected in brain after *p.o.* administration in rats.[12] It was thus necessary to search for novel derivatives with the goal of discovery of compounds having good brain permeability.

Concerning the penetration of small molecule drugs into the CNS, lipophilicity is known to be an important determinant, since lipid-mediated blood–brain barrier transport of lipophilic compounds is an important pathway.[13] Moreover, metabolic stability is also an important factor, since lipophilic drugs are readily metabolized to more polar species, which causes a reduction of lipophilicity.[13a] Furthermore, metabolic stability of drugs is essential, since first path effect by the liver is of fundamental importance for good oral BA. From the results of structure–activity relationships (SAR) study on FR166124 derivatives, various alcohol, ester, nitrile and amides were found to be potent adenosine A_1 receptor antagonists with high A_1 selectivity and these derivatives were more lipophilic compounds than FR166124.[11] Therefore, in order to discover orally active agents with blood–brain barrier permeability, we designed a novel series of 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines, including carbonyl (**5**—**11**), hydroxy (**12**, **13**), amide (**15**—**27**), amine (**28**—**30**) and piperidine derivatives (**32**—**38**) as N2 substituents, in the expectation that such derivatives would have sufficient lipophilicity for blood–brain barrier permeability and would be resistant to metabolism. In this paper, we wish to report the synthesis, adenosine A_1 and A_{2A} binding activities, stability *in vitro* rat liver metabolism, and plasma and brain con-

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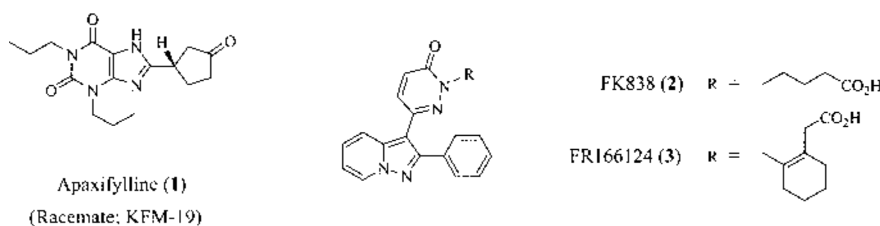
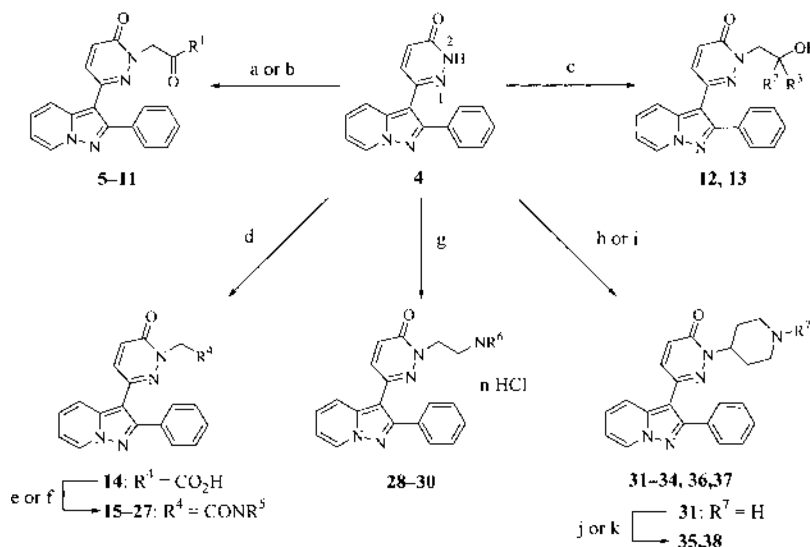


Chart 1. Adenosine A_1 Antagonists



Reagents: (a) chloroacetone, *t*-BuOK, 18-crown-6-ether, DMF; (b) **40** or $BrCH_2COR^1$, NaH, DMF; (c) $CH_2(O)CR^2R^3$, NaOH, $Bn(Et)_3N^+Cl^-$, CH_2Cl_2–H_2O; (d) 1. $BrCH_2CO_2Et$, NaH, DMF, 2. 1 N NaOH, EtOH; (e) amine (R^5NH), EDC·HCl, HOBt, DMF; (f) amine hydrochloride (R^5NH·HCl), EDC, HOBt, DMF; (g) **42** or $Cl(CH_2)_2NR^6$·HCl, NaOH, $Bn(Et)_3N^+Cl^-$, $CHCl_3$–H_2O; (h) 1. 1-Boc-4-hydroxypiperidine, PPh_3, DEAD, THF, 2. 6 N HCl; (i) **44** or R^7OH, PPh_3, DEAD, THF; (j) isopropyl iodide, NaH, DMF; (k) Ac_2O, Pyridine.

Chart 2

centrations in rats after *p.o.* administration of this series and the discovery of **32**, which was found to be a potent, orally active adenosine A_1 receptor antagonist with high blood–brain barrier permeability.

Chemistry Synthetic routes for the novel 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines **5**—**38** prepared in this work are summarized in Chart 2. Starting 6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**4**) was prepared according to the previously described method.[10a,14] Carbonyl derivatives **5**—**11** were prepared from **4** by method A or B. The details of which are as follows: **5** was obtained in 79% yield by alkylation of **4** with chloroacetone using potassium *tert*-butoxide (*tert*-BuOK) as a base (method A). Compounds **6**—**11** were obtained by alkylation of **4** with the appropriate bromoketone using NaH as a base in 16—86% yields (method B). The starting bromoketones were obtained by standard methods. 1-Bromo-3-methyl-2-butanone was prepared according to the reported method[15] and the preparation of bromoketones **40a**—**c** was achieved in a similar manner from the appropriate ketone **39a**—**c** in 78—95%yields (Chart 3). Commercially available 1-bromo-2-butanone and 1-bromopinacolone were used for preparation of **7** and **8**, respectively. Hydroxy derivatives **12** and **13** were prepared by the reaction of **4** with propylene oxide and isobutylene oxide according to the reported method[16] in 71 and 59% yields, respectively (method C).

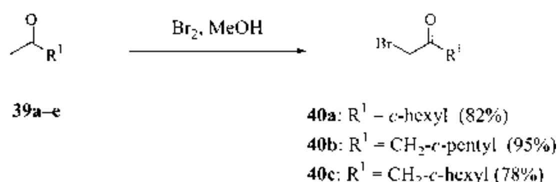


Chart 3

Compound **14** was obtained in 87% yield as a synthetic precursor of amide derivatives by alkylation of **4** with ethyl bromoacetate followed by alkaline hydrolysis of the resulting ester (method D). Preparation of amide derivatives **15**—**27** was accomplished by activation of intermediate **14** as the 1-hydroxybenzotriazole (HOBt) ester followed by treatment with the appropriate amine (method E, 57—96% yields) or amine hydrochloride (method F, 47—96% yields).

Amine derivatives **28** and **29** were prepared by reaction of **4** with 1-(2-chloroethyl)piperidine hydrochloride and 2,6-*cis*-1-(2-chloroethyl)-2,6-dimethylpiperidine (**42**) using NaOH as a base and benzyltriethylammonium chloride as a phase-transfer catalyst[17] in a mixture of $CHCl_3$ and H_2O, followed by treatment with HCl in ethanol (EtOH), in 72 and 93% yields, respectively (method G). Compound **30** was also prepared in 70% yield by the reaction of **4** with 2-(hexamethyl-

eneimino)ethyl chloride according to method G, without HCl treatment (method H). Preparation of **42** was achieved in 62% yield by acylation of 2,6-*cis*-2,6-dimethylpiperidine with ethyl oxalyl chloride, and reduction with lithium aluminum hydride ($LiAlH_4$) in refluxing tetrahydrofuran (THF) followed by treatment with thionyl chloride (Chart 4).

Piperidine derivatives **32**—**34**, **36** and **37** were prepared from **4** with the appropriate 1-substituted-4-hydroxy piperidine using Mitsunobu reaction conditions[18] in 28—71% yields (method J). Commercially available 4-hydroxy-1-methylpiperidine and 1-benzyl-4-hydroxypiperidine were used for preparation **32** and **37**, respectively, and 1-substituted-4-hydroxy piperidines **44a**—**c** were prepared in 83—98% yields from 4-hydroxypiperidine (**43**) by acylation with the appropriate acyl chloride, followed by reduction of the resulting amide (Chart 5). Compound **31** was obtained in 62% yield as a synthetic precursor of **35** and **38** *via* Method I: Mitsunobu reaction of **4** with 1-*tert*-butoxycarbonyl-4-hydroxypiperidine followed by acidic deprotection of the *tert*-butoxycarbonyl group. Preparation of **35** was achieved by alkylation of **31** with 2-iodopropane using NaH as a base (method K). Compound **38** was prepared by acetylation of **31** with acetic anhydride (Ac_2O) in 90% yield (method L).

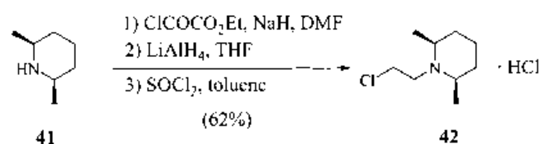


Chart 4

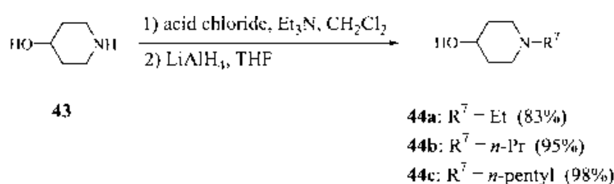


Chart 5

Biology The compounds prepared were evaluated for their *in vitro* adenosine A_1 and A_{2A} receptor binding activities, using receptors cloned from human hippocampus and expressed in Chinese hamster ovary (CHO) cells. Adenosine A_1 and A_{2A} receptor binding was measured using 4.5 nM [^{3}H]-(8-cyclopentyl-1,3-dipropylxanthin ([^{3}H]-DPCPX) and 20 nM [^{3}H]-2-[4-[(2-carboxethyl)phenyl]ethylamino]-5′-*N*-ethylcarbonyladenosine ([^{3}H]-CGS21680), respectively. The *in vitro* A_1 and A_{2A} receptor binding activities are expressed as *Ki* values which were calculated from the IC_{50} values. Selectivity (A_{2A}/A_1) is expressed as the ratio of *Ki* values obtained from the receptor binding assays. Stability to *in vitro* metabolism in rat liver microsomes and cytosol was measured by HPLC analysis, and is presented as percentage of remaining concentration *versus* the initial concentration of test compounds after incubation for 10 min at 37 °C. Stability of compounds **5**—**11** was examined in both microsomes and cytosol, whilst the other compounds were only evaluated in the presence of microsomes.

Selected compounds having potent A_1 affinity, high A_1 selectivity and good metabolic stability were then examined for plasma and brain concentrations *via p.o.* administration. Plasma and brain concentrations, 30 min after *p.o.* administration of test compounds to fasted Sprague-Dawley (SD) male rats were measured by HPLC analysis. The brain/plasma (B/P) ratio is expressed as the ratio of brain and plasma concentrations. Pharmacokinetic parameters of the orally active adenosine A_1 antagonist having the highest brain concentration in rats were also studied.

Results and Discussion

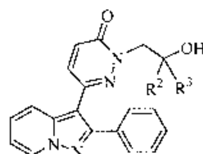
Binding Assays In order to investigate potency as adenosine A_1 antagonists and to search for orally active compounds, we introduced various substituents such as carbonyl (**5**—**11**), hydroxy (**12**, **13**), amide (**15**—**27**), amine (**28**—**30**) and piperidine moiety (**32**—**38**) at the N2 of the pyridazi-

Table 1. Binding Assay and *in Vitro* Metabolism by Rat Liver of Carbonyl Derivatives

Compound	R^1	Method	Yield (%)	clog $P^{a)}$	Adenosine receptor binding[b] *Ki* (nM)		Selectivity[c] A_{2A}/A_1	% of initial concentration[d]	
					A_1	A_{2A}		Microsomes	Cytosol
1				1.33	4.8	530	110	N.T.[e]	N.T.[e]
5	Me	A	79	1.52	0.19	170	900	76.6	1.8
6	Et	B	83	2.04	0.73	650	890	33.2	45.5
7	*i*-Pr	B	80	2.35	0.026	140	5400	8.2	97.5
8	*t*-Bu	B	86	2.75	0.12	340	2800	32.8	98.7
9		B	31	3.55	0.082	87	1100	6.4	90.0
10		B	16	3.61	0.088	120	1400	3.5	98.0
11		B	78	4.17	0.078	54	690	2.6	101.1

a) The clog *P* values were calculated using MacLog *P* from Biobyte Corporation: clog *P* for Macintosh version 2.0.3. *b*) Inhibition of specific binding; A_1: [^{3}H]-DPCPX (4.5 nM), A_{2A}: [^{3}H]-CGS 21680 (20 nM). *c*) Ratio of *Ki* values obtained by receptor binding assay. *d*) Test compounds were incubated for 10 min at 37 °C. Substrate concentration; microsomes: 1 μM, cytosol: 10 μM; Identification limit: 0.05 μg/ml or 0.05 μg/tissue. *e*) N.T.: not tested.

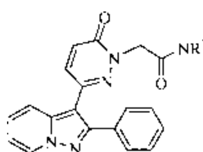
Table 2. Binding Assay and *in Vitro* Metabolism by Rat Liver of Hydroxy Derivatives



Compound	R^2	R^3	Method	Yield (%)	clog *P*[a)]	Adenosine receptor binding[b)] *Ki* (nM)		Selectivity[c)] A_{2A}/A_1	% of initial concentration[d)]
						A_1	A_{2A}		Microsomes
12[e)]	Me	H	C	71	1.73	5.1	250	49	83.2
13	Me	Me	C	59	2.13	0.36	160	440	74.6

a—d) See corresponding footnotes of Table 1. *e*) Racemate.

Table 3. Binding Assay and *in Vitro* Metabolism by Rat Liver of Amide Derivatives



Compound	NR^5	Method	Yield (%)	clog *P*[a)]	Adenosine receptor binding[b)] *Ki* (nM)		Selectivity[c)] A_{2A}/A_1	% of initial concentration[d)]
					A_1	A_{2A}		Microsomes
15	NH_2	F	47	0.54	5.5	460	84	N.T.[e)]
16	NHMe	F	96	0.78	2.5	1900	760	84.0
17	NMe_2	F	85	1.05	18	3800	210	73.0
18	NHEt	F	95	1.30	1.8	1100	610	40.7
19	$NHPr^i$	E	83	1.61	0.29	490	1700	29.8
20	$NHBu^t$	E	86	2.01	0.76	432	570	3.4
21	N(Me)Et	E	57	1.58	4.1	1000	240	33.9
22	NEt_2	E	88	2.11	2.2	920	420	1.5
23	N	E	76	1.69	6.8	2900	430	14.3
24	N OH	F	90	0.66	34	10000 (48)[f)]	>290	N.T.[e)]
25	N	E	86	2.24	1.3	620	480	14.8
26	N O	E	76	1.22	34	10000 (38)[f)]	>290	1.0
27	N S	E	91	1.94	9.1	830	91	1.0

a—e) See corresponding footnotes of Table 1. *f*) (%) inhibition at 10000 nM.

none ring and *in vitro* adenosine A_1 and A_{2A} receptor binding activities were measured (Tables 1—5). As shown in Table 1, all carbonyl derivatives (**5**—**11**) were highly potent adenosine A_1 antagonists with high A_1 selectivity. Amongst them, compound **7** was the most potent adenosine A_1 antagonist (Ki=0.026 nM, A_{2A}/A_1=5400). Introduction of sterically hindered alkyl groups on the side chain increased affinity for the A_1 receptor, but introduction of a cyclohexyl group increased affinity for the A_{2A} receptor (**9**, **11**). Hydroxy derivatives such as tri-substituted **13** showed high A_1 affinity and good A_1 selectivity (Ki=0.36 nM, A_{2A}/A_1=440), but di-substituted analogue **12** had reduced A_1 affinity and A_1 selectivity (Ki=5.1 nM, A_{2A}/A_1=49) (Table 2). Moreover, hydroxy derivatives showed lower A_1 selectivity than carbonyl derivatives and reduction of the carbonyl group to a hydroxy group decreased A_1 affinity, but retained A_{2A} affinity (**5** *versus* **12**). Amide derivatives **15**—**27** also showed high A_1 affinity and good A_1 selectivity (Table 3), **19** (Ki=0.29 nM, A_{2A}/A_1=1700) and **20** (Ki=0.76 nM, A_{2A}/A_1=570) displayed very high A_1 affinity, and **19** showed very high A_1 selectivity. In the series of amide derivatives, mono-substituted amides (**16**, **18**—**20**) showed higher A_1 affinity and A_1 selectivity than di-substituted (**17**, **21**, **22**) and cyclic amide derivatives (**23**—**27**). Table 4 shows the results for amine derivatives **28**—**30**. Compounds **28** (Ki=2.4 nM, A_{2A}/A_1>420) and **30** (Ki=2.6 nM, A_{2A}/A_1=730) showed high A_1 affinity and A_1 selectivity. Introduction of additional methyl groups at the carbon adjacent to the nitrogen in the piperidine ring reduced A_1 affinity (**28** *versus* **29**). As shown in Table 5, piperidine derivatives displayed high A_1 affinity and A_1 selectivity, except for **36** and **37**. Compound **38** showed the highest A_1 affinity (Ki=0.55 nM) amongst them. Introduction of long alkyl (**36**) and aromatic (**37**) moieties to the amino group of the piperidine ring reduced A_1 affinity but retained A_{2A} affinity.

Table 4. Binding Assay and *in Vitro* Metabolism by Rat Liver of Amine Derivatives

Compound	NR^6	*n*	Method	Yield (%)	clog *P*[a)]	Adenosine receptor binding[b)] *Ki* (nM)		Selectivity[c)] A_{2A}/A_1	% of initial concentration[d)]
						A_1	A_{2A}		Microsomes
28		1	G	72	3.58	2.4	1000 (23)[f)]	>420	25.9
29[e)]		1	G	93	4.62	28	10000 (18)[f)]	>360	2.0
30		0	H	70	4.14	2.6	1900	730	7.0

a—d) See corresponding footnotes of Table 1. *e*) Single diastereoisomer. *f*) (%) inhibition at 1000 or 10000 nM.

Table 5. Binding Assay and *in Vitro* Metabolism by Rat Liver of Piperidine Derivatives

Compound	R^7	Method	Yield (%)	clog *P*[a)]	Adenosine receptor binding[b)] *Ki* (nM)		Selectivity[c)] A_{2A}/A_1	% of initial concentration[d)]
					A_1	A_{2A}		Microsomes
32	Me	J	79	2.95	6.6	5400	820	85.1
33	Et	J	28	3.48	4.1	2300	560	75.5
34	*n*-Pr	J	45	4.01	1.2	550	460	58.6
35	*i*-Pr	K	63	3.79	3.1	3500	1100	74.7
36	$(CH_2)_4CH_3$	J	39	5.07	37	1700	46	N.T.[e)]
37	CH_2Ph	J	71	4.94	35	2000	57	N.T.[e)]
38	Ac	L	90	1.94	0.55	150	270	27.6

a—e) See corresponding footnotes of Table 1.

Furthermore, a relationship was found for the analogues prepared here between A_1 affinity and lipophilicity, as expressed by calculated partition coefficient (clog *P*) values. Figure 1 and Eqs. 1—3 show the relationship between adenosine A_1 affinity and the clog *P* value.

Carbonyl derivatives

$$-\log Ki = 0.19(\text{clog}\,P) + 0.41 \quad (1)$$

$n=7$, $r=0.41$, $s=0.44$

Hydroxy and amide derivatives

$$-\log Ki = 0.62(\text{clog}\,P) - 1.48 \quad (2)$$

$n=15$, $r=0.54$, $s=0.56$

Amine and piperidine derivatives

$$-\log Ki = -0.52(\text{clog}\,P) + 1.24 \quad (3)$$

$n=10$, $r=0.76$, $s=0.43$

In the Eqs. 1—3, *n* is the number of compounds, *r* is the correlation coefficient and *s* is the standard error. In both series of the carbonyl derivatives and the hydroxy and amide derivatives having potent A_1 affinity, increasing clog *P* tends to increase A_1 affinity. In contrast, A_1 affinity of amine and piperi-

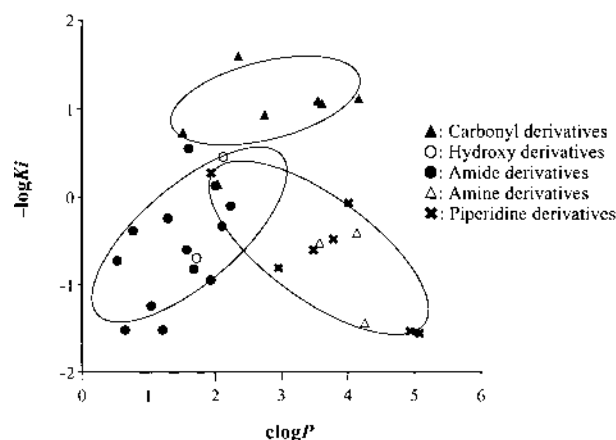


Fig. 1. Relationship between Adenosine A_1 Receptor Affinity and clog *P*

Table 6. Plasma and Brain Concentrations in Rats of Novel 3-(2-Substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo-[1,5-*a*]pyridine Adenosine A_1 Antagonists

Compound	Dose (mg/kg)	After 30 min of *p.o.* administration: Plasma conc.[a] (nM)	Brain conc.[a] (nM)	B/P ratio	After 60 min of *p.o.* administration: Plasma conc.[a] (nM)	Brain conc.[a] (nM)	B/P ratio
1	1.0	4750±371	N.D.[b]		N.T.[c]	N.T.[c]	
1	10	34500±3670	1330±187	0.038±0.002	N.T.[c]	N.T.[c]	
5	10	412±75	415±75[d]	0.478[e]	244±128[d]	73±73[f]	0.497[g]
7	10	N.D.[b]	N.D.[b]		N.D.[b]	N.D.[b]	
8	10	N.D.[b]	N.D.[b]		N.D.[b]	N.D.[b]	
13	10	286±19	172±5	0.605±0.039	205±105[d]	114±55[d]	0.562[h]
17	10	3590±190	445±43	0.124±0.008	1790±161	51±51[f]	0.072[i]
25	10	128±70[d]	N.D.[b]		189±36	N.D.[b]	
28	10	165±14	397±80	2.39±0.347	N.D.[b]	223±89[d]	
32	3.2	389±23	361±5	0.930±0.046	N.T.[c]	N.T.[c]	
32	32	3390±651	3670±496	1.11±0.060	1580±348	2143±434	1.39±0.172

a) Mean±standard error, $n=3$, Identification limit: 0.05 μg/ml or 0.05 μg/tissue. *b*) N.D.: not detected. *c*) N.T.: not tested. *d*) 1/3 N.D. *e*) $n=2$, plasma and brain conc.: No. 1; 558 and 229 nM, No. 2; 383 and 209 nM. *f*) 2/3 N.D. *g*) $n=1$, plasma and brain conc.: 433 and 215 nM. *h*) $n=2$, plasma and brain conc.: No. 1; 267 and 170 nM, No. 2; 347 and 170 nM. *i*) $n=1$, plasma and brain conc.: 2100 and 152 nM.

dine derivatives was decreased due to higher clog*P* values with good correlation. In this series of 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines, it is thus assumed that a suitable clog*P* value for high A_1 affinity ranges from 1.5 to 3.5. Moreover, it is thus concluded that 3-(3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridine is an excellent pharmacophore for the expression of high A_1 affinity.

Metabolism Concerning the oral BA and metabolic stability to maintain lipophilicity, first path effect by the liver is an important factor. Therefore, *in vitro* metabolism by rat liver microsomes as a model of major metabolism was examined, since microsomes contain cytochrome P450s, which display an important role in drug metabolism involving a variety of reactions such as oxidation and reduction.[19] Determination of the metabolites and metabolizing enzymes are important aspects of downstream drug development, however, it is enough at the early stages to measure metabolic stability in terms of percentage of remaining concentration *versus* initial concentration of test compounds. In the case of carbonyl derivatives (**5**—**11**), *in vitro* metabolism by rat liver cytosol was also measured, because it was known that carbonyl groups can be reduced to alcohol groups by metabolism in cytosol. The results are summarized in Tables 1—5. As shown in Table 1, **5** was the most stable in microsomes, but disappeared quickly in cytosol. It was observed that more sterically hindered carbonyl derivatives than **5** had reduced stability in microsomes, but increased stability in cytosol. Both hydroxy derivatives **12** and **13** were more stable in microsomes than the carbonyl derivatives (Table 2). As shown in Tables 3—5, it is apparent that in the series of amide (**15**—**27**), amine (**28**—**30**) and piperidine (**32**—**38**) derivatives, the more sterically hindered the side chain moiety, the more stability in microsomes was reduced, to a similar level as the carbonyl derivatives. Furthermore, it is thus demonstrated that hydroxy and piperidine derivatives have good stability in rat liver microsomes and may be expected to be new compounds with potentially good oral BA and brain permeability.

Plasma and Brain Concentrations, Pharmacokinetics In the next step, we investigated concentrations in plasma and brain in rats dosed by *p.o.* administration with selected compounds. In consideration of the adenosine A_1 affinity, A_1 selectivity and metabolic stability, we selected **5**, **7** and **8** as carbonyl derivatives, **13** as a hydroxy derivative, **17** and **25** as amide derivatives, **28** as an amine derivative and **32** as a piperidine derivative for this study, and the results are summarized in Table 6. In the case of carbonyl derivatives, **5** (clog*P*=1.52) was detected in both plasma and brain after 30 and 60 min of *p.o.* (10 mg/kg) administration (plasma conc.=412±75 nM (30 min); 244±128 nM (60 min), brain conc.=415±75 nM (30 min); 73±73 nM (60 min)), but **7** (clog*P*=2.35) and **8** (clog*P*=2.75) were not detected, even in plasma. Thinking about the metabolic stability of carbonyl derivatives, it was thus suggested that stability in microsomes was a more important factor than that in cytosol for the expression of oral activity. The same result was observed for amide derivatives **17** (clog*P*=1.05) and **25** (clog*P*=2.24). Compound **17**, more stable in microsomes than **25**, had the highest plasma concentration of all tested compounds and good brain concentrations after 30 and 60 min of *p.o.* (10 mg/kg) administration (plasma conc.=3590±190 nM (30 min); 1790±161 nM (60 min), brain conc.=445±43 nM (30 min); 51±51 nM (60 min)). For the amine derivative **28** (clog*P*=3.58) and piperidine derivative **32** (clog*P*=2.95), **28** showed the best B/P ratio after 30 min of *p.o.* (10 mg/kg) administration (plasma conc.=165±14 nM, brain conc.=397±80 nM, B/P=2.39±0.347) and **32** showed the highest brain concentration and a good B/P ratio after 30 and 60 min of *p.o.* (32 mg/kg) administration (plasma conc.=3390±651 nM (30 min); 1580±348 nM (60 min), brain conc.=3670±496 nM (30 min); 2143±434 nM (60 min), B/P=1.11±0.060 (30 min); 1.39±0.172 (60 min)) amongst the tested compounds. Furthermore, **32** showed a good brain concentration and good B/P ratio even at a dose of 3.2 mg/kg (*p.o.*) after 30 min of administration (plasma conc.=389±23 nM, brain conc.=361±5 nM, B/P=0.930±0.046). From the results described above, it was suggested that the presence of the amino moiety on the N2

Table 7. Oral Bioavailability of FR194921 (**32**) of Rats[a]

AUC[b] (μg·h/ml)	C_{max}[c] (μg/ml)	T_{max}[d] (h)	BA (%)
6.91±0.521	2.13±0.091	0.63±0.13	60.6±4.9

a) Dose: 32 mg/kg (*p.o.*, Fasted): Mean±S.E., n=4. *b*) AUC: area under concentration curve. *c*) C_{max}: maximal blood concentration. *d*) T_{max}: time to maximal blood concentration.

substituent is a more important factor than the lipophilicity of test compounds for the expression of high blood–brain barrier permeability after *p.o.* administration.

Finally, concerning the results from the studies of plasma and brain concentrations in rats after oral administration, we selected **32** as the best compound for further pharmacokinetics studies in rats. As shown in Table 7, **32** showed good BA (60.6±4.9%) at a dose of 32 mg/kg (*p.o.*). As a result, **32** was found to be the first 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridine adenosine A_1 antagonist with good oral bioavailability and good blood–brain permeability. Thus, **32** is a good candidate for further pharmacological evaluations.

Conclusion

In summary, we have prepared a novel series of 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines and evaluated them for *in vitro* adenosine A_1 and A_{2A} receptor binding activities, and most were found to be potent adenosine A_1 receptor antagonists with high A_1 selectivity. The SAR study in this series of compounds revealed the following main features: 1) a carbonyl moiety is a good substituent for high A_1 affinity and replacement of the carbonyl group by a hydrophilic moiety such as hydroxy or amide groups decreased both affinity for the adenosine A_1 receptor and A_1 selectivity. 2) In the carbonyl derivatives, A_{2A} affinity was increased by introduction of a cyclohexyl group. 3) Mono-substituted amide derivatives showed higher A_1 affinity and A_1 selectivity than di-substituted and cyclic amide derivatives. 4) In the case of piperidine derivatives, introduction of a lipophilic moiety such as a long chain alkyl or aromatic moiety, reduced A_1 affinity but retained A_{2A} affinity. 5) Lipophilicity is an important factor for expression of high A_1 affinity, with a suitable clog P value being in the range 1.5 to 3.5.

The studies of *in vitro* rat liver metabolism and concentrations in plasma and brain after *p.o.* administration to rats indicated the following features: 1) introduction of a sterically hindered moiety on the N2 substituent reduced the stability in microsomes. 2) An amino group was the best N2 substituent for the expression of blood–brain barrier permeability after *p.o.* administration. It is thus concluded that **32** can possibly lead to a new neuronal therapeutic agent, and is progressed to further pharmacological studies in rats, such as passive avoidance test[4a] and Morris water maze test,[6] the results of which will be reported in due course.

Experimental

Chemistry All melting points (mp) were determined with a Büchi 535 apparatus in open capillaries and are uncorrected. Infrared (IR) spectra were recorded on a Horiba Spectradesk FT-210 or FT-710 spectrometer. ^{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 $CDCl_3$ or dimethylsulfoxide-d_6 (DMSO-d_6). 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 thin layer chromatography plates, followed by visualization with UV light (254, 365 nm) and staining with iodine vapor. Reagents and solvents were used as obtained from commercial suppliers without further purification unless otherwise noted.

Method A. 2-(2-Oxopropyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (5) To a mixture of *tert*-BuOK (5.0 g, 44.5 mmol) and 18-crown-6-ether (740 mg, 2.8 mmol) in *N*,*N*-dimethylformamide (DMF, 80 ml) was added **4** (8.0 g, 27.7 mmol) at 5 °C under a nitrogen atmosphere. After 30 min, to the reaction mixture was added dropwise chloroacetone (3.53 ml, 44.5 mmol), and the reaction was then allowed to warm to ambient temperature and stirred for 1 h. The reaction mixture was partitioned between CH_2Cl_2 and H_2O, and the organic layer was separated, washed with 1 N NaOH and brine, dried over magnesium sulfate ($MgSO_4$), and purified by column chromatography on silica-gel (CH_2Cl_2–methanol (MeOH) 8 : 1 and 4 : 1) and recrystallization from EtOH to give **5** (7.5 g, 79%) as a white solid. mp 190—191 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s), 5.07 (2H, s), 6.80 (1H, d, J=9.7 Hz), 6.93 (1H, td, J=6.9, 1.4 Hz), 7.05 (1H, d, J=9.7 Hz), 7.24—7.30 (1H, m), 7.44—7.49 (3H, m), 7.61—7.66 (2H, m), 7.90 (1H, td, J=8.9, 1.1 Hz), 8.53 (1H, td, J=6.9, 1.0 Hz). IR (KBr) cm^{-1}: 1713, 1664, 1587, 1524, 1493. MS *m/z* 345: (M+H)$^+$. *Anal.* Calcd for $C_{20}H_{16}N_4O_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 69.36; H, 4.63; N, 16.15.

Method B. 2-(2-Oxobutyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (6) To a suspension of NaH (60% dispersion in mineral oil, 200 mg, 5.0 mmol) in DMF (150 ml) was added **4** (1.0 g, 3.5 mmol) at 5 °C under a nitrogen atmosphere. After 30 min, to the reaction mixture was added dropwise 1-bromo-2-butanone (0.46 ml, 4.5 mmol), followed by warming to ambient temperature and stirred for 1 h. The reaction mixture was partitioned between 25% *n*-hexane in ethyl acetate (EtOAc) and H_2O, and the organic layer was separated, washed with 1 N NaOH and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a residual solid which was recrystallized from EtOH to give **6** (1.04 g, 83%) as a white solid. mp 171—172 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7.3 Hz), 2.63 (2H, q, J=7.3 Hz), 5.06 (2H, s), 6.80 (1H, d, J=9.7 Hz), 6.86—6.94 (1H, m), 7.05 (1H, d, J=9.7 Hz), 7.23—7.33 (1H, m), 7.44—7.48 (3H, m), 7.60—7.66 (2H, m), 7.90 (1H, d, J=8.9 Hz), 8.52 (1H, d, J=7.0 Hz). IR (KBr) cm^{-1}: 1728, 1660, 1589, 1529, 1495, 1468. MS *m/z* 359: (M+H)$^+$. *Anal.* Calcd for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63. Found: C, 70.10; H, 5.01; N, 15.37.

Compounds **7**—**11** were prepared following a procedure similar to Method B.

2-(3-Methyl-2-oxobutyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**7**): White solid (80%). mp 171—173 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.13 (6H, d, J=6.8 Hz), 2.78—3.00 (1H, m), 5.19 (2H, s), 6.91 (1H, d, J=9.7 Hz), 7.00—7.15 (2H, m), 7.38—7.65 (6H, m), 7.87 (1H, d, J=8.9 Hz), 8.82 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1726, 1666, 1591, 1527. MS *m/z* 373: (M+H)$^+$. *Anal.* Calcd for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.59; H, 5.41; N, 14.93.

2-(3,3-Dimethyl-2-oxobutyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**8**): White solid (86%). mp 219—220 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (9H, s), 5.22 (2H, s), 6.78 (1H, d, J=9.7 Hz), 6.89 (1H, td, J=6.9, 1.4 Hz), 7.04 (1H, d, J=9.7 Hz), 7.26 (1H, td, J=6.8, 1.1 Hz), 7.41—7.47 (3H, m), 7.59—7.66 (2H, m), 7.87 (1H, d, J=8.9 Hz), 8.51 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1718, 1668, 1652, 1593, 1529, 1491, 1470. MS *m/z* 387: (M+H)$^+$. *Anal.* Calcd for $C_{23}H_{22}N_4O_2$: C, 71.48; H, 5.74; N, 14.50. Found: C, 71.44; H, 5.81; N, 14.48.

2-(2-Cyclohexenyl-2-oxoethyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**9**): White solid (31%). mp 138—139 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.10—2.10 (10H, m), 2.50—2.70 (1H, m), 5.12 (2H, s), 6.78 (1H, d, J=9.7 Hz), 6.84—6.93 (1H, m), 7.03 (1H, d, J=9.7 Hz), 7.21—7.30 (1H, m), 7.43—7.48 (3H, m), 7.61—7.66 (2H, m), 7.87 (1H, d, J=8.9 Hz), 8.51 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1721, 1664, 1633, 1589, 1527, 1498. MS *m/z* 413: (M+H)$^+$. *Anal.* Calcd for $C_{25}H_{24}N_4O_2 \cdot 0.25H_2O$: C, 72.01; H, 5.92; N, 13.44. Found: C, 72.13; H, 5.84; N, 13.48.

2-(3-Cyclopentyl-2-oxobutyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**10**): White solid (16%). mp 150—151 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.11 (10H, m), 2.50—2.70 (1H, m) 5.12 (2H, s), 6.78 (1H, d, J=9.7 Hz), 6.84—6.93 (1H, m), 7.03 (1H, d, J=9.7 Hz), 7.21—7.30 (1H, m), 7.43—7.48 (3H, m), 7.61—7.66 (2H, m), 7.87 (1H, d, J=8.9 Hz), 8.51

(1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1730, 1660, 1635, 1593, 1529, 1497. 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, 72.45; H, 5.84; N, 13.46.

2-(3-Cyclohexyl-2-oxobutyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**11**): White solid (78%). mp 141—142 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 0.90—1.45 (5H, m), 1.65—2.10 (6H, m), 2.46 (2H, d, J=6.9 Hz), 5.04 (2H, s), 6.79 (1H, d, J=9.7 Hz), 6.85—6.93 (1H, m), 7.04 (1H, d, J=9.7 Hz), 7.21—7.30 (1H, m), 7.43—7.49 (3H, m), 7.60—7.66 (2H, m), 7.87 (1H, d, J=8.9 Hz), 8.51 (1H, d, J=7.0 Hz). IR (KBr) cm^{-1}: 1728, 1659, 1633, 1593, 1529, 1497. MS m/z 427: $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{26}N_4O_2 \cdot 0.4H_2O$: C, 72.00; H, 6.23; N, 12.92. Found: C, 72.19; H, 6.20; N, 12.85.

Method C. 2-(2-Hydroxypropyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (12) A mixture of **4** (1.0 g, 3.5 mmol), propylene oxide (1.5 ml, 21.4 mmol), NaH (60% dispersion in mineral oil, 170 mg, 4.2 mmol) and benzyltriethylammonium chloride (80 mg, 0.35 mmol) in a mixture of H_2O (20 ml) and CH_2Cl_2 (20 ml) was stirred for 24 h. The organic layer was separated, washed with H_2O and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a residual solid which was recrystallized from EtOH to give **12** (850 mg, 71%) as a white solid. mp 203—204 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, d, J=6.0 Hz), 3.71 (1H, s), 4.35—4.37 (3H, m), 6.82 (1H, d, J=9.7 Hz), 6.88—6.97 (1H, m), 7.06 (1H, d, J=9.7 Hz), 7.27—7.36 (1H, m), 7.44—7.48 (3H, m), 7.58—7.64 (2H, m), 7.97 (1H, d, J=8.9 Hz), 8.54 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1651, 1579, 1524, 1495, 1466. MS m/z 347: $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.24; H, 5.15; N, 15.85.

Compound **13** was prepared following a procedure similar to Method C.

2-(2-Hydroxy-2-methylpropyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**13**): White solid (59%). mp 199—220 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.18 (6H, s), 4.19 (2H, s), 4.79 (1H, s), 6.88 (1H, d, J=9.6 Hz), 7.03—7.11 (2H, m), 7.35—7.70 (6H, m), 8.12 (1H, d, J=8.9 Hz), 8.81 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1649, 1579, 1522, 1493, 1462. MS m/z 361: $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{20}N_4O_2 \cdot 0.2H_2O$: C, 69.29; H, 5.64; N, 15.39. Found: C, 69.26; H, 5.58; N, 15.39.

Method D. [6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetic Acid (14) To a suspension of NaH (60% dispersion in mineral oil, 2.3 g, 57.5 mmol) in DMF (150 ml) was added **4** (15.0 g, 52 mmol) at 5 °C under a nitrogen atmosphere. After 30 min, to the reaction mixture was added dropwise ethyl bromoacetate (6.3 ml, 57.5 mmol), which was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was partitioned between 25% *n*-hexane in EtOAc and H_2O, and the organic layer was separated, washed with H_2O and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a residual solid, which was triturated with a mixture of EtOAc and *n*-hexane to give ethyl [6-oxo-3-(2-phenyl-pyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetate (94%) as a pale yellow solid. mp 155—157 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7.1 Hz), 4.31 (2H, q, J=7.1 Hz), 5.01 (2H, s), 6.80 (1H, d, J=9.7 Hz), 6.91 (1H, td, J=6.9, 1.4 Hz), 7.04 (1H, d, J=9.7 Hz), 7.24—7.35 (1H, m), 7.44—7.50 (3H, m), 7.60—7.66 (2H, m), 7.96 (1H, td, J=8.9, 1.2 Hz), 8.52 (1H, td, J=6.9, 1.0 Hz). IR (KBr) cm^{-1}: 1745, 1674, 1593, 1525. MS m/z 375: $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96. Found: C, 67.10; H, 4.97; N, 14.59. To a solution of the above ethyl ester in EtOH (400 ml) was added 1 N NaOH (105 ml) and the mixture refluxed for 2 h with stirring. Evaporation of the solvent gave a residue, which was dissolved in H_2O and washed with EtOAc. The aqueous layer was separated, pH was adjusted to pH 1.0 with concentrated HCl. Insoluble material was collected by filtration, washed with H_2O and EtOH, and dried to give **14** (2 steps, 15.6 g, 87%) as a pale yellow solid. mp 247 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 4.91 (2H, s), 6.93 (1H, d, J=9.7 Hz), 7.04—7.14 (2H, m), 7.38—7.66 (6H, m), 7.97 (1H, d, J=8.9 Hz), 8.82 (1H, d, J=6.9 Hz), 13.17 (1H, br s). IR (KBr) cm^{-1}: 1740, 1649, 1574, 1527, 1500, 1466. MS m/z 347. $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{14}N_4O_3 \cdot 0.25H_2O$: C, 65.04; H, 4.16; N, 15.97. Found: C, 65.21; H, 4.06; N, 15.74.

Method E. *N*-Isopropyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (19) To a solution of **14** (1.0 g, 2.89 mmol) in DMF (10 ml) was added successively HOBt (510 mg, 3.77 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl, 723 mg, 3.77 mmol) and isopropylamine (0.32 ml, 3.74 mmol) at ambient temperature under a nitrogen atmosphere and stirred overnight. Evaporation of the solvent gave a residue, which was partitioned between $CHCl_3$ and H_2O. The organic layer was separated, washed with saturated $NaHCO_3$ in H_2O and brine, dried over $MgSO_4$, evaporated, and crystallized from EtOH to give **19** as a white solid (935 mg, 83%). mp 231—232 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (6H, d, J=6.5 Hz), 4.03—4.21 (1H, m), 4.89 (2H, s), 6.20—6.40 (1H, m), 6.80 (1H, d, J=9.7 Hz), 6.92—6.97 (1H, m), 7.06 (1H, d, J=9.7 Hz), 7.27—7.45 (1H, m), 7.45—7.50 (3H, m), 7.59—7.65 (2H, m), 8.10 (1H, d, J=8.9 Hz), 8.53 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1680, 1657, 1585, 1551, 1527. MS m/z 388: $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{21}N_5O_2 \cdot 0.25H_2O$: C, 67.42; H, 5.53; N, 17.87. Found: C, 67.51; H, 5.41; N, 17.86.

Compounds **20**—**23** and **25**—**27** were prepared following a procedure similar to Method E.

N-*tert*-Butyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (**20**): White solid (86%). mp 225—227 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (9H, s), 4.84 (2H, s), 6.16 (1H, br s), 6.79 (1H, d, J=9.7 Hz), 6.87—6.96 (1H, m), 7.05 (1H, d, J=9.7 Hz), 7.26—7.36 (1H, m), 7.44—7.48 (3H, m), 7.60—7.65 (2H, m), 8.09 (1H, d, J=8.9 Hz), 8.52 (1H, d, J=6.9 Hz); IR (KBr) cm^{-1}: 1693, 1662, 1595, 1525. MS m/z 402: $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{23}N_5O_2 \cdot 0.25H_2O$: C, 68.05; H, 5.83; N, 17.25. Found: C, 67.92; H, 5.71; N, 17.21.

N-Ethyl-*N*-methyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (**21**): Pale yellow solid (57%). mp 190—192 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.17, 1.81 (3H (1 : 1), 2×t, J=7.2 Hz), 3.02, 3.11 (3H (1 : 1), 2×s), 3.40—3.57 (2H, m), 5.06, 5.09 (2H (1 : 1), 2×s, J=7.2 Hz), 6.80 (1H, d, J=9.7 Hz), 6.85—6.92 (1H, m), 7.03 (1H, d, J=9.7 Hz), 7.22—7.31 (1H, m), 7.43—7.47 (3H, m), 7.62—7.66 (2H, m), 8.01 (1H, d, J=8.9 Hz), 8.50 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1664, 1593, 1527, 1493. MS m/z 388: $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{21}N_5O_2 \cdot 0.25H_2O$: C, 67.42; H, 5.53; N, 17.87. Found: C, 67.41; H, 5.36; N, 17.89.

N,*N*-Diethyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (**22**): Pale yellow solid (88%). mp 137—138 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, J=7.1 Hz), 1.32 (3H, t, J=7.1 Hz), 3.39—3.54 (4H, m), 5.08 (2H, s), 6.80 (1H, d, J=9.7 Hz), 6.85—6.93 (1H, m), 7.04 (1H, d, J=9.7 Hz), 7.22—7.31 (1H, m), 7.43—7.49 (3H, m), 7.62—7.68 (2H, m), 8.03 (1H, d, J=8.9 Hz), 8.51 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1660, 1589, 1527, 1491. MS m/z 402: $(M+H)^+$; *Anal.* Calcd for $C_{23}H_{23}N_5O_2 \cdot 0.5H_2O$: C, 67.30; H, 5.89; N, 17.06. Found: C, 67.53; H, 5.69; N, 17.14.

2-[2-Oxo-2-(pyrrolidin-1-yl)ethyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**23**): Pale yellow solid (76%). mp 170—171 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.84—2.12 (4H, m), 3.54—3.63 (4H, m), 5.01 (2H, s), 6.80 (1H, d, J=9.7 Hz), 6.90 (1H, t, J=6.9 Hz), 7.03 (1H, d, J=9.7 Hz), 7.19—7.29 (1H, m), 7.43—7.49 (3H, m), 7.62—7.68 (2H, m), 8.05 (1H, d, J=8.9 Hz), 8.52 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1664, 1591, 1527, 1467. MS m/z 400: $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{21}N_5O_2 \cdot 0.25H_2O$: C, 68.59; H, 5.36; N, 17.38. Found: C, 68.33; H, 5.19; N, 17.24.

2-[2-Oxo-2-(piperidin-1-yl)ethyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**25**): Pale yellow solid (86%). mp 205—206 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.40—1.70 (6H, m), 3.40—3.55 (4H, m), 5.07 (2H, s), 6.89 (1H, d, J=9.7 Hz), 7.03—7.12 (2H, m), 7.37—7.53 (4H, m), 7.60—7.66 (2H, m), 7.92 (1H, d, J=8.9 Hz), 8.82 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1660, 1593, 1527, 1497. MS m/z 414: $(M+H)^+$. *Anal.* Calcd for $C_{24}H_{23}N_5O_2$: C, 69.72; H, 5.61; N, 19.64. Found: C, 69.46; H, 5.47; N, 16.19.

2-[2-Oxo-2-(morpholin-4-yl)ethyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**26**): Pale yellow solid (76%). mp 205—206 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 3.58—3.80 (8H, m), 5.09 (2H, s), 6.80 (1H, d, J=9.7 Hz), 6.86—6.95 (1H, m), 7.06 (1H, d, J=9.7 Hz), 7.24—7.34 (1H, m), 7.44—7.49 (3H, m), 7.62—7.67 (2H, m), 8.00 (1H, d, J=8.9 Hz), 8.52 (1H, d, J=7.0 Hz). IR (KBr) cm^{-1}: 1664, 1587, 1529, 1500. MS m/z 416: $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{21}N_5O_3$: C, 66.49; H, 5.09; N, 16.86. Found: C, 66.54; H, 5.23; N, 16.73.

2-[2-Oxo-2-(thiomorpholin-4-yl)ethyl]-6-(2-phenylpyrazolo[1,5-*a*]-pyridin-3-yl)-3(2*H*)-pyridazinone (**27**): Pale yellow solid (91%). mp 207—208 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 2.60—2.80 (4H, m), 3.80—4.00 (4H, m), 5.07 (2H, s), 6.79 (1H, d, J=9.7 Hz), 6.85—6.94 (1H, m), 7.05 (1H, d, J=9.7 Hz), 7.23—7.32 (1H, m), 7.43—7.49 (3H, m), 7.61—7.67 (2H, m), 7.99 (1H, d, J=8.9 Hz), 8.52 (1H, d, J=7.0 Hz). IR (KBr) cm^{-1}: 1664, 1647, 1585, 1527, 1497. MS m/z 432: $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{21}N_5O_2S$: C, 64.02; H, 4.91; N, 16.23. Found: C, 63.97; H, 4.81; N, 16.09.

Method F. *N*,*N*-Dimethyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (17) To a solution of **14** (1.0 g, 2.89 mmol) in DMF (10 ml) was added successively HOBt (510 mg, 3.77 mmol), EDC (0.69 ml, 3.78 mmol) and dimethylamine hydrochloride (307 mg, 3.77 mmol) at ambient temperature under a nitrogen atmosphere and stirred overnight. Evaporation of the solvent gave a residue, which was partitioned between $CHCl_3$ and H_2O. The organic layer was separated, washed with saturated $NaHCO_3$ in H_2O and brine, dried over $MgSO_4$, evapo-

rated, and crystallized from EtOH to give **17** (920 mg, 85%) as a pale yellow solid. mp 189—190 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.90 (3H, s), 3.08 (3H, s), 5.07 (2H, s), 6.89 (1H, d, J=9.7 Hz), 7.04—7.12 (2H, m), 7.37—7.66 (6H, m), 7.93 (1H, d, J=8.9 Hz), 8.82 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1664, 1587, 1525, 1497. MS *m/z* 374: (M+H)$^+$. *Anal.* Calcd for $C_{21}H_{19}N_5O_2 \cdot 0.5H_2O$: C, 65.96; H, 5.27; N, 18.31. Found: C, 66.07; H, 5.27; N, 18.15.

Compounds **15**, **16**, **18** and **24** were prepared following a procedure similar to Method F.

2-[6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (**15**): Pale yellow solid (47%). mp 238—239 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 4.76 (2H, s), 6.89 (1H, d, J=9.7 Hz), 7.07 (1H, d, J=9.7 Hz), 7.04—7.10 (1H, m), 7.35—7.75 (8H, m), 7.97 (1H, d, J=8.9 Hz), 8.82 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1689, 1660, 1633, 1583, 1531, 1518, 1491. MS *m/z* 368: (M+Na)$^+$. *Anal.* Calcd for $C_{19}H_{15}N_5O_2 \cdot 0.4H_2O$: C, 64.73; H, 4.52; N, 19.86. Found: C, 64.83; H, 4.34; N, 19.69.

N-Methyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (**16**): Pale yellow solid (96%). mp 231—232 °C (EtOH). ^{1}H-NMR (CDCl$_3$) δ: 2.78 (3H, d, J=4.9 Hz), 4.93 (2H, s), 6.54 (1H, br s), 6.80 (1H, d, J=9.7 Hz), 6.93 (1H, t, J=6.9 Hz), 7.07 (1H, d, J=9.7 Hz), 7.30—7.38 (1H, m), 7.45—7.49 (3H, m), 7.59—7.63 (2H, m), 8.10 (1H, d, J=8.9 Hz), 8.53 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1670, 1585, 1529. MS *m/z* 360: (M+H)$^+$. *Anal.* Calcd for $C_{20}H_{17}N_5O_2 \cdot 0.25H_2O$: C, 66.01; H, 4.85; N, 19.25. Found: C, 66.02; H, 4.73; N, 19.26.

N-Ethyl-2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1(6*H*)-pyridazin-1-yl]acetamide (**18**): Pale yellow solid (95%). mp 252—254 °C (EtOH). ^{1}H-NMR (CDCl$_3$) δ: 1.17 (3H, t, J=7.3 Hz), 3.28—3.43 (2H, m), 4.92 (2H, s), 6.50 (1H, br s), 6.80 (1H, d, J=9.7 Hz), 6.89—6.97 (1H, m), 7.07 (1H, d, J=9.7 Hz), 7.29—7.38 (1H, m), 7.44—7.49 (3H, m), 7.59—7.65 (2H, m), 8.10 (1H, d, J=8.9 Hz), 8.55 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1684, 1659, 1585, 1556, 1527. MS *m/z* 374: (M+H)$^+$. *Anal.* Calcd for $C_{21}H_{19}N_5O_2 \cdot 0.5H_2O$: C, 65.96; H, 5.27; N, 18.31. Found: C, 65.78; H, 5.02; N, 18.23.

2-[2-((3*R*)-3-Hydroxypyrrolidin-1-yl)-2-oxoethyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**24**): Pale yellow solid (90%). mp 177—178 °C (EtOH). ^{1}H-NMR (CDCl$_3$) δ: 1.95—2.20 (2H, m), 2.79 (1H, br s), 3.50—3.90 (4H, m), 4.45—4.55 (1H, m), 4.94—5.07 (2H, m), 6.79 (1H, d, J=9.7 Hz), 6.88 (1H, t, J=6.9 Hz), 7.04 (1H, d, J=9.7 Hz), 7.20—7.31 (1H, m), 7.41—7.48 (3H, m), 7.61—7.67 (2H, m), 8.02 (1H, d, J=8.9 Hz), 8.50 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1659, 1583, 1527, 1498. MS *m/z* 416: (M+H)$^+$. *Anal.* Calcd for $C_{23}H_{21}N_5O_3 \cdot 0.25H_2O$: C, 65.78; H, 5.16; N, 16.68. Found: C, 65.87; H, 5.05; N, 16.66.

Method G. 6-(2-Phenylpyrazolo[1,5-*a*]pyridin-3-yl)-2-(2-piperidin-1-yl)ethyl-3(2*H*)-pyridazinone Hydrochloride (28) A mixture of **4** (2.0 g, 6.9 mmol), 1-(2-chloroethyl)piperidine hydrochloride (2.7 g, 14.7 mmol), NaH (60% dispersion in mineral oil, 1.12 g, 28 mmol) and benzyltriethylammonium chloride (158 mg, 0.69 mmol) in a mixture of H_2O (50 ml) and $CHCl_3$ (50 ml) was stirred for 24 h. The organic layer was separated, washed with H_2O and brine, dried over $MgSO_4$, and purified by column chromatography on silica-gel (EtOAc and $CHCl_3$–MeOH 30 : 1). Evaporation of the solvent gave a residual solid, which was treated with HCl in EtOH to give **28** as a pale yellow solid (2.18 g, 72%). Recrystallization afford an analytical sample. mp 267—268 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.60 (1H, m), 1.60—1.90 (5H, m), 2.80—3.10 (2H, m), 3.30—3.65 (4H, m), 4.58 (2H, t, J=6.5 Hz), 6.93 (1H, d, J=9.7 Hz), 7.05—7.16 (2H, m), 7.38—7.60 (4H, m), 7.60—7.70 (2H, m), 8.07 (1H, d, J=9.0 Hz), 8.83 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1664, 1630, 1593, 1525, 1493. MS *m/z* 400: (M+H)$^+$. *Anal.* Calcd for $C_{24}H_{25}N_5O \cdot HCl$: C, 66.12; H, 6.01; N, 16.06. Found: C, 66.03; H, 6.06; N, 16.04.

Compound **29** was prepared following a procedure similar to Method G.

2-[2-(2,6-*cis*-2,6-Dimethylpiperidin-1-yl)ethyl]-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone Hydrochloride (29) Pale yellow solid (93%). mp >270 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.30—2.00 (11H, m), 3.30—3.55 (4H, m), 4.40—4.50 (2H, m), 6.97 (1H, d, J=9.7 Hz), 7.09 (1H, d, J=6.9 Hz), 7.23 (1H, d, J=9.7 Hz), 7.40—7.65 (6H, m), 7.99 (1H, d, J=8.9 Hz), 8.84 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1689, 1660, 1633, 1583, 1531, 1518, 1491. MS *m/z* 428: (M+H)$^+$. *Anal.* Calcd for $C_{26}H_{29}N_5O \cdot HCl$: C, 64.79; H, 6.69; N, 14.53. Found: C, 64.74; H, 6.72; N, 14.49.

Method H. 2-(2-Azepan-1-yl)ethyl-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (30) A mixture of **4** (2.0 g, 6.9 mmol), 2-(hexamethyleneimino)ethyl chloride hydrochloride (1.93 g, 9.7 mmol), NaH (60% dispersion in mineral oil, 0.84 g, 21 mmol) and benzyltriethylammonium chloride (158 mg, 0.69 mmol) in a mixture of H_2O (50 ml) and $CHCl_3$ (50 ml) was stirred for 4 h. The organic layer was separated, washed with H_2O and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a residual solid, which was recrystallized from EtOH to give **30** as a pale yellow solid (2.0 g, 70%). mp 132—133 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.50 (8H, br s), 2.63—2.70 (4H, m), 2.88 (2H, t, J=6.7 Hz), 4.22 (2H, t, J=6.7 Hz), 6.86 (1H, d, J=9.6 Hz), 7.00—7.11 (2H, m), 7.39—7.63 (6H, m), 7.95 (1H, d, J=8.9 Hz), 8.81 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1659, 1589, 1529, 1495, 1468. MS *m/z* 414: (M+H)$^+$. *Anal.* Calcd for $C_{25}H_{27}N_5O$: C, 72.61; H, 6.58; N, 16.947. Found: C, 72.47; H, 6.50; N, 16.91.

Method I. 6-(2-Phenylpyrazolo[1,5-*a*]pyridin-3-yl)-2-(piperidin-4-yl)-3(2*H*)-pyridazinone (31) To a solution of **4** (17.9 g, 62.1 mmol), 1-*tert*-butoxycarbonyl-4-hydroxypiperidine (15.0 g, 74.5 mmol) and triphenylphosphine (PPh_3, 24.4 g, 93.0 mmol) in THF (600 ml) was added dropwise diethyl azodicarboxylate (DEAD, 14.7 ml, 93.3 mmol) at −5 to 0 °C under a nitrogen atmosphere. After addition, the reaction mixture was allowed to warm to ambient temperature and stirred overnight. Evaporation of the solvent gave a residue, to which was added 6 N HCl and refluxed for 8 h. The resulting solution was washed with EtOAc, pH of the aqueous layer was adjusted to pH 11.5 with 30% NaOH while keeping the temperature at 5 to 15 °C, followed by extraction three times with $CHCl_3$, dried over $MgSO_4$, and purified by column chromatography on silica-gel ($CHCl_3$–MeOH 40 : 1, 20 : 1 and 10 : 1). Evaporation of the solvent gave a residual solid, which was crystallized from 50% aqueous EtOH (aq. EtOH) to give **31** (14.35 g, 62%) as a pale yellow solid. mp 115—117 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.70—1.85 (4H, m), 2.50—2.70 (2H, m), 3.00—3.10 (2H, m), 4.80—5.00 (1H, m), 6.87 (1H, d, J=9.6 Hz), 7.05—7.13 (2H, m), 7.43—7.63 (6H, m), 7.91 (1H, d, J=8.9 Hz), 8.82 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1657, 1585, 1529, 1495, 1464, 1421. MS *m/z* 372: (M+H)$^+$. *Anal.* Calcd for $C_{22}H_{21}N_5O \cdot 2.25H_2O$: C, 64.21; H, 6.25; N, 17.02. Found: C, 64.26; H, 6.28; N, 16.94.

Method J. 2-(1-Methylpiperidin-4-yl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (32) To a solution of **4** (6.0 g, 20.8 mmol), 1-methyl-4-hydroxypiperidine (3.1 g, 26.9 mmol) and PPh_3 (8.7 g, 33.2 mmol) in THF (120 ml) was added dropwise DEAD (5.3 ml, 33.6 mmol) at −5 to 0 °C under a nitrogen atmosphere and stirred for 4 h. Evaporation of the solvent gave a residue, which was partitioned between EtOAc and 2 N HCl. The aqueous layer was separated and the pH was adjusted to pH 10.7 with 30% NaOH while keeping the temperature at 5 to 15 °C. Insoluble material was collected by filtration, washed with H_2O, dried, dissolved in $CHCl_3$ and purified by column chromatography on silica-gel (EtOAc, $CHCl_3$, $CHCl_3$–MeOH 40 : 1 and 10 : 1). Evaporation of the solvent gave a residual solid, which was crystallized from 50% aq. EtOH to give **32** (6.3 g, 79%) as a pale yellow solid. mp 139—140 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.50—2.10 (6H, m), 2.20 (3H, s), 2.80—3.00 (2H, m), 4.70—4.90 (1H, m), 6.87 (1H, d, J=9.7 Hz), 7.05—7.13 (2H, m), 7.40—7.65 (6H, m), 7.90 (1H, d, J=8.9 Hz), 8.83 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1660, 1589, 1531, 1497, 1466. MS *m/z* 386: (M+H)$^+$. *Anal.* Calcd for $C_{23}H_{23}N_5O \cdot H_2O$: C, 68.47; H, 6.25; N, 17.36. Found: C, 68.71; H, 6.08; N, 17.37.

Compounds **33**, **34**, **36** and **37** were prepared following a procedure similar to Method J.

2-(1-Ethylpiperidin-4-yl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**33**): Pale yellow solid (28%). mp 134—135 °C (50% aq. EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.01 (3H, t, J=7.1 Hz), 1.80—2.15 (6H, m), 2.36 (2H, q, J=7.1 Hz), 2.90—3.10 (2H, m), 4.70—4.90 (1H, m), 6.87 (1H, d, J=9.7 Hz), 7.05—7.15 (2H, m), 7.45—7.65 (6H, m), 7.89 (1H, d, J=8.9 Hz), 8.83 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1660, 1589, 1529, 1493, 1466. MS *m/z* 400: (M+H)$^+$. *Anal.* Calcd for $C_{24}H_{25}N_5O \cdot 0.4H_2O$: C, 70.88; H, 6.39; N, 17.22. Found: C, 71.02; H, 6.35; N, 17.07.

6-(2-Phenylpyrazolo[1,5-*a*]pyridin-3-yl)2-(1-propylpiperidin-4-yl)-3(2*H*)-pyridazinone (**34**): Pale yellow solid (45%). mp 104—106 °C (50% aq. EtOH). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J=7.3 Hz), 1.35—1.55 (2H, m), 1.70—2.35 (8H, m), 2.90—3.05 (2H, m), 4.70—4.90 (1H, m), 6.89 (1H, d, J=9.7 Hz), 7.05—7.30 (2H, m), 7.40—7.60 (6H, m), 7.88 (1H, d, J=8.9 Hz), 8.83 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1657, 1589, 1531, 1468. MS *m/z* 414: (M+H)$^+$. *Anal.* Calcd for $C_{25}H_{27}N_5O \cdot 1.1H_2O$: C, 69.29; H, 6.79; N, 16.16. Found: C, 69.17; H, 6.98; N, 16.27.

2-(1-Pentylpiperidin-4-yl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (**36**): Pale yellow solid (39%). mp 105—106 °C (50% aq. EtOH). ^{1}H-NMR (DMSO-d_6) δ: 0.83—0.91 (3H, m), 1.20—1.45 (6H, m), 1.75—2.15 (6H, m), 2.20—2.35 (2H, m), 2.90—3.05 (2H, m), 4.70—4.90 (1H, m), 6.88 (1H, d, J=9.6 Hz), 7.05—7.15 (2H, m), 7.43—7.51 (4H, m), 7.57—7.62 (2H, m), 7.88 (1H, d, J=8.9 Hz), 8.83 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1660, 1589, 1531, 1497, 1466. MS *m/z* 442: (M+H)$^+$. *Anal.* Calcd for $C_{27}H_{31}N_5O \cdot 0.25H_2O$: C, 72.70; H, 7.12; N, 15.70. Found: C, 72.73; H, 7.10; N, 15.72.

2-(1-Benzylpiperidin-4-yl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-

3(2*H*)-pyridazinone (**37**): Pale yellow solid (71%). mp 184—186 °C (50% aq. EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.70—2.20 (6H, m), 2.93 (2H, br d, J=12.4 Hz), 3.51 (2H, s), 4.70—4.90 (1H, m), 6.88 (1H, d, J=9.6 Hz), 7.00—7.17 (2H, m), 7.20—7.65 (11H, m), 7.88 (1H, d, J=8.8 Hz), 8.84 (1H, d, J=7.0 Hz). IR (KBr) cm^{-1}: 1662, 1589, 1525, 1491, 1460. MS *m/z* 462: (M+H)$^+$. *Anal.* Calcd for $C_{29}H_{27}N_5O \cdot 0.3H_2O$: C, 74.59; H, 5.96; N, 15.00. Found: C, 74.37; H, 6.08; N, 15.36.

Method K. 2-(1-Isopropylpiperidin-4-yl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (35) To a suspension of NaH (60% dispersion in mineral oil, 65 mg, 1.63 mmol) in DMF (20 ml) was added **31** (500 mg, 1.35 mmol) at ambient temperature under a nitrogen atmosphere and stirred for 30 min. To the reaction mixture was added 2-iodopropane (0.55 ml, 5.53 mmol) and stirred for an additional 18 h. To the resulting mixture was added excess triethylamine (Et_3N) and H_2O. Evaporation of the solvent gave a residue, which was partitioned between EtOAc and 1 N HCl. The aqueous layer was separated, adjusted to pH 10 with 4 N NaOH, and extracted with EtOAc. The organic layer was separated, washed with H_2O, dried over $MgSO_4$, evaporated, and crystallized from 50% aq. EtOH to give **35** (350 mg, 63%) as a pale yellow solid. mp 158—159 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.98 (6H, d, J=6.5 Hz), 1.75—2.20 (4H, m), 2.20—2.40 (2H, m), 2.65—2.95 (3H, m), 4.70—4.90 (1H, m), 6.87 (1H, q, J=9.6 Hz), 7.05—7.13 (2H, m), 7.40—7.60 (6H, m), 7.88 (1H, d, J=8.9 Hz), 8.83 (1H, d, J=7.0 Hz). IR (KBr) cm^{-1}: 1660, 1589, 1531, 1468. MS *m/z* 414: (M+H)$^+$. *Anal.* Calcd for $C_{25}H_{27}N_5O \cdot 0.5H_2O$: C, 71.06; H, 6.68; N, 16.57. Found: C, 71.25; H, 6.45; N, 16.78.

Method L. 2-(1-Acetylpiperidin-4-yl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone (38) To a stirred solution of **31** (500 mg, 1.35 mmol) in pyridine (30 ml) was added dropwise Ac_2O (1.28 ml, 13.6 mmol) at ambient temperature under a nitrogen atmosphere and stirred overnight. Evaporation of the solvent gave a residue, which was partitioned between EtOAc and 2 N HCl. The organic layer was separated, washed with saturated NaCl solution in H_2O, dried over $MgSO_4$, evaporated, and crystallized from 50% aq. EtOH to give **38** (500 mg, 90%) as a pale yellow solid. mp 114—117 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.50—2.00 (4H, m), 2.02 (3H, s), 2.60—2.80 (1H, m), 3.15—3.35 (1H, m), 3.90 (1H, br d, J=12.2 Hz), 4.49 (1H, br d, J=13.2 Hz), 5.00—5.20 (1H, m), 6.92 (1H, d, J=9.6 Hz), 7.03—7.12 (1H, m), 7.20 (1H, d, J=9.6 Hz), 7.40—7.60 (6H, m), 7.84 (1H, d, J=8.9 Hz), 8.83 (1H, d, J=6.9 Hz). IR (KBr) cm^{-1}: 1657, 1628, 1585, 1531, 1458. MS *m/z* 414: (M+H)$^+$. *Anal.* Calcd for $C_{24}H_{23}N_5O_2 \cdot 2.5H_2O$: C, 62.87; H, 6.15; N, 15.27. Found: C, 63.13; H, 5.94; N, 15.41.

1-Bromo-2-cyclohexenyl-2-ethanone (40a) To an ice-cooled solution of acetylcyclohexane (**39a**, 5.0 g, 39.6 mmol) in MeOH (30 ml) was added Br_2 (2.0 ml, 39.6 mmol) in a single portion and the reaction temperature was kept below 15 °C until the red color of the solution turned colorless. To the reaction mixture was added H_2O (30 ml) and the mixture stirred overnight at ambient temperature. The resulting solution was extracted three times with 25% *n*-hexane in EtOAc. The extracts were combined, washed twice with 10% K_2CO_3 solution in H_2O, and dried over sodium sulfate. Evaporation of the solvent gave a residue, which was distilled under reduced pressure to give **40a** (6.68 g, 82%) as a pale yellow oil. bp 120—125 °C (18—19 mm.). ^{1}H-NMR (CDCl$_3$) δ: 1.10—1.95 (10H, m), 2.64—2.80 (1H, m), 3.96 (2H, s). MS *m/z* 205, 207: (M+H)$^+$.

Compounds **40b** and **40c** were prepared following a procedure similar to **40a**.

1-Bromo-3-cyclopentyl-2-propanone (**40b**): Pale yellow oil (95%). bp 115—120 °C (18—19 mm.). ^{1}H-NMR (CDCl$_3$) δ: 1.10—1.90 (9H, m), 2.67 (2H, d, J=7.1 Hz), 3.88 (2H, s). MS *m/z* 205, 207: (M+H)$^+$.

1-Bromo-3-cyclohexyl-2-propanone (**40c**): Pale yellow oil (78%). bp 140—145 °C (18 mm.). ^{1}H-NMR (CDCl$_3$) δ: 0.85—1.94 (11H, m), 2.52 (2H, d, J=6.8 Hz), 3.86 (2H, s). MS *m/z* 219, 221: (M+H)$^+$.

2,6-*cis*-1-(2-Chloroethyl)-2,6-dimethylpiperidine Hydrochloride (42) To a suspension of NaH (60% dispersion in mineral oil, 4.4 g, 0.11 mol) in DMF (150 ml) was added dropwise 2,6-*cis*-2,6-dimethylpiperidine (**41**, 13.5 ml, 83 mmol) at 40 °C under a nitrogen atmosphere. After stirring for 1 h, the reaction mixture was allowed to cool to 0 °C, to which was added dropwise ethyl oxalyl chloride (11.2 ml, 0.1 mol) and stirred overnight at ambient temperature. The resulting mixture was partitioned between EtOAc and H_2O, the organic layer was separated, washed with H_2O and saturated NaCl solution in H_2O, and dried over $MgSO_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica-gel (*n*-hexane–EtOAc 9 : 1 4 : 1 and 2 : 1) to give ethyl 2,6-*cis*-2-(2,6-dimethylpiperidin-1-yl)-2-oxoacetate (14.0 g, 66%) as a pale yellow oil. ^{1}H-NMR (CDCl$_3$) δ: 1.22—1.44 (9H, m), 1.50—2.00 (6H, m), 3.75—3.95 (1H, m), 4.20—4.45 (2H, m), 4.60—4.80 (1H, m). MS *m/z* 214: (M+H)$^+$. To a refluxing mixture of $LiAlH_4$ 4.4 g, 0.116 mol) in THF (130 ml) was added dropwise a solution of the above ethyl 2,6-*cis*-2-(2,6-dimethylpiperidin-1-yl)-2-oxoacetate (13.5 g, 63.3 mmol) in THF (35 ml) under a nitrogen atmosphere. After stirring for 2 h, the reaction mixture was allowed to cool to 0 °C and added carefully H_2O (4.4 ml), followed by 4 N NaOH (4.4 ml) and H_2O (13.3 ml) successively. Insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure to give 2,6-*cis*-2,6-dimethyl-1-(2-hydroxyethyl)piperidine (10.0 g, quant.) as a pale yellow oil. ^{1}H-NMR (CDCl$_3$) δ: 1.10 (6H, d, J=6.3 Hz), 1.15—1.72 (6H, m), 2.40—2.60 (2H, m), 2.72 (2H, t, J=6.4 Hz), 2.92 (1H, br s), 3.54 (2H, t, J=6.4 Hz). MS *m/z* 158: (M+H)$^+$. A stirred solution of 2,6-*cis*-2,6-dimethyl-1-(2-hydroxyethyl)piperidine (10.0 g, 63.5 mmol) and thionyl chloride (9.3 ml, 127 mmol) in toluene (100 ml) was stirred for 2.5 h at 80 °C under a nitrogen atmosphere. Evaporation of the solvent gave a residual solid, which was washed with diisopropylether, collected by filtration, and dried under reduced pressure to give **42** (12.8 g, 95%) as a pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ: 1.29—1.38 (6H, m), 1.44—1.90 (6H, m), 3.20—3.60 (4H, m), 3.38—4.05 (2H, m). MS *m/z* 176: (M+H)$^+$.

4-Hydroxy-1-propylpiperidine (44b) To a suspension of 4-hydroxypiperidine (**43**, 5.0 g, 49.4 mmol) and Et_3N (7.6 ml, 54.5 mmol) in CH_2Cl_2 (100 ml) was added dropwise propionyl chloride (4.5 ml, 51 mmol) at −70 to −60 °C under a nitrogen atmosphere. After stirring for 1 h, insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure, to which was added EtOAc, followed by stirring for 30 min. Insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure to give 4-hydroxy-1-propionylpiperidine (7.7 g, 99%) as a yellow oil. ^{1}H-NMR (CDCl$_3$) δ: 1.15 (3H, t, J=7.5 Hz), 1.37—1.59 (2H, m), 1.82—1.96 (2H, m), 2.36 (2H, q, J=7.5 Hz), 3.10—3.30 (2H, m), 3.65—4.20 (3H, m). MS *m/z* 158: (M+H)$^+$. To a suspension of $LiAlH_4$ (1.8 g, 47.4 mmol) in THF (100 ml) was added dropwise a solution of 4-hydroxy-1-propionylpiperidine (5.0 g, 31.8 mmol) in THF (30 ml) at ambient temperature under a nitrogen atmosphere. After stirring for 1 h, the reaction mixture was refluxed for 4 h, and then cooled to 4 °C. To the resulting mixture was added carefully H_2O (1.8 ml), 4 N NaOH (1.8 ml) and H_2O (5.4 ml) successively. Insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure to give **44b** (4.37 g, 96%) as pale yellow oil. ^{1}H-NMR (CDCl$_3$) δ: 0.89 (3H, t, J=7.4 Hz), 1.45—1.69 (4H, m), 1.84—2.19 (5H, m), 2.24—2.33 (2H, m), 2.73—2.84 (2H, m), 3.62—3.76 (1H, m). MS *m/z* 144: (M+H)$^+$.

Compounds **44a** and **44c** were prepared following a procedure similar to **44b.**

1-Ethyl-4-hydroxypiperidine (**44a**): Pale yellow oil (2 steps, 83%). ^{1}H-NMR (DMSO-d_6) δ: 0.96 (3H, t, J=7.2 Hz), 1.24—1.43 (2H, m), 1.60—1.75 (2H, m), 1.85—2.00 (2H, m), 2.26 (2H, q, J=7.2 Hz), 2.60—2.75 (2H, m), 3.33—3.50 (1H, m), 4.52 (1H, d, J=4.2 Hz). MS *m/z* 130: (M+H)$^+$.

4-Hydroxy-1-pentylpiperidine (**44c**): Pale yellow oil (2 steps, 98%). ^{1}H-NMR (CDCl$_3$) δ: 0.89 (3H, t, J=6.4 Hz), 1.20—1.65 (8H, m), 1.85—1.95 (2H, m), 2.00—2.35 (5H, m), 2.72—2.83 (2H, m), 3.50—3.75 (1H, m). MS *m/z* 172: (M+H)$^+$.

Biological Methods. cDNA Cloning and Expression Cells and Membrane Preparation All reagents were biochemical grade. DNA modification enzymes were obtained from Boehringer Mannheim, GIBCO-BRL Takara Shuzo, and TOYOBO. Human hippocampus cDNA library was from Clontech. Adenosine antagonists and agonists were from Research Biochemicals, Inc. Radioligands were from DuPon-NEN. The phage library DNA was subjected to 35 cycles of amplification with each of two oligonucleotide primers for A_1 and A_{2A} receptors cDNA, and each reaction cycle consisted of incubations at 94 °C for 0.5 min, 60 °C for 1 min, and 72 °C for 2 min, with AmpliTaq DNA polymerase (Perkin Elmer). The amplified DNA fragments were subcloned into pUC18, and then isolated clones were sequenced by an Applied Biosystems model 370 DNA sequencer. The expression plasmids were constructed with identified adenosine receptor cDNAs, and the expression cells were established by the Calcium-Phosphate precipitation method. Stable expression cells, derived from CHO, were cultured in minimum essential medium without nucleosides (GIBCO-BRL) containing 10% fetal bovine serum and 100 nM methotrexate, in an atmosphere of 5% CO_2 in air at 37 °C. Transient expression cells, derived from COS 7, were cultured in Dulbecco's modified Eagle's medium. Cells were washed twice with phosphate-buffered saline (PBS) and detached from roller bottle with PBS. Cell suspensions were centrifuged at 1500×g at 4 °C for 10 min and resuspended in 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 2 mM Dithiothreitol, and 1 mM phenylmethanesulfonyl fluoride (buffer A). The cells were allow to stand on ice for 30 min, and then homogenized on ice by ultrasonication. The homogenate was centrifuged at 39000×g at 4 °C for 20 min and washed

three times with buffer A. After centrifugation, the precipitate was suspended in 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$. Protein concentrations were determined by the Bradford method using protein assay kit (Bio Rad). The membrane suspension was stored at −80 °C until assayed.

Receptor Binding Adenosine A_1 and A_{2A} receptor binding were assayed in 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ containing 10 and 20 μg membrane prepared with 3 and 6 U/ml adenosine deaminase, 4.5 nM [^{3}H]-DPCPX (95.3 Ci/mmol; DuPon-NEN.) and 20 nM [^{3}H]-CGS21680 (39.5 Ci/mmol; DuPon-NEN.), and appropriate concentrations of test compounds respectively. Non-specific binding was determined in the presence of 10 μM unlabelled DPCPX and CGS21680. Incubation was carried out at 25 °C for 2 h in a volume of 0.25 ml and terminated by rapid filtration under vacuum through Whatman GF/B filters that had been pretreated with 0.3% polyethyleneimine. The filter was washed with 5×4 ml of ice-cold 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ and the retained radioactivity was quantitated by liquid scintillation counting in 5 ml of Aquasol (National Diagnostics).

In Vitro* Metabolism** Microsomes and cytosol from liver of male SD rats were prepared according to the method described by Sugiura *et al.*[20] Incubation mixture (0.5 ml) contained microsomes (0.5 mg of protein/ml) or cytosol (50 mg of liver/ml), 1 or 10 μM test compounds, 2 mM nicotinamide adenine dinucleotide phosphate, 10 mM glucose-6-phosphate, 5 mM $MgCl_2$, 0.5 units glucose-6-phosphate dehydrogenase and 100 mM phosphate buffer (pH 7.4). After preincubation at 37 °C for 5 min, the reaction was started by addition of test compounds. Incubation was carried out at 37 °C for 10 min and then the reaction was stopped by addition of 0.1 N NaOH (0.5 ml) and $CHCl_3$ (4 ml). The resulting mixture was shaken for 10 min and centrifuged at 1700 ***g for 5 min. The organic layer (3 ml) was separated and evaporated in a stream of nitrogen. The residue was dissolved in methanol (100 μl) and analyzed by HPLC.

Quantitation in Plasma and Brain, Pharmacokinetics SD male rats (7—9 weeks) fasted for 24 h were administered with test compound in 0.5% methyl cellulose. After 30 or 60 min, rats were anesthetized with ether to allow blood sampling from the abdominal aorta. Where indicated, rats were then perfused with 0.9% saline *via* an aortic catheter before the cerebrum was removed. The heparinized plasma and cerebrum were stored at −20 °C until measurement of the concentration by HPLC. The heparinized plasma (0.1 ml) or 20% cerebrum homogenate (0.5 ml) was mixed with methanol (50 μl) and then to the mixture was added 0.1 N NaOH (0.5 ml) and $CHCl_3$ (4 ml). The resulting mixture was shaken for 10 min and centrifuged at 1700 ***g*** for 5 min. The organic layer (3 ml) was separated and evaporated in a stream of nitrogen. The residue was dissolved in 150 μl of HPLC mobile phase and analyzed by HPLC. Quantitation was achieved using a standard curve which was made by adding standard solutions of test compounds to control plasma or brain homogenate instead of methanol. The lower limit of quantitation was 0.05 μg/ml for plasma and 0.05 μg/g tissue for brain.

HPLC Analysis The HPLC system consisted of a Waters LC Module 1 and a Millennium 2010J chromatography manager. All samples were resolved using a CAPCELL PAC C_{18} UG120 column (4.6×150 mm, Shiseido) maintained at 30 °C. The column was eluted at 1 ml/min with various mixtures of 20 mM phosphate buffer (pH 6.5) and acetonitrile. The column eluent was monitored at 250 nm.

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