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DISCOVERY OF FK453, A NOVEL NON-XANTHINE ADENOSINE A_1 RECEPTOR ANTAGONIST

Atsushi Akahane,* Hirohito Katayama, Takafumi Mitsunaga, Yasuhiro Kita, Takahiro Kusunoki, Takao Terai, Keizo Yoshida, and Youichi Shiokawa

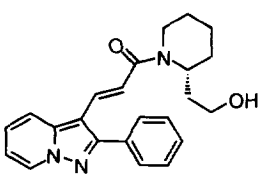
New Drug Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan

Abstract: Novel 2-phenylpyrazolo[1,5-*a*]pyridine-3-acryloylamides were synthesized and evaluated for diuretic activities. FK453 (**1d**), the most potent compound in this series, was found to be a potent and selective adenosine A_1 receptor antagonist, whereas **1e**, the (*S*)-enantiomer of FK453, was a weak and non-selective adenosine antagonist. Copyright © 1996 Elsevier Science Ltd

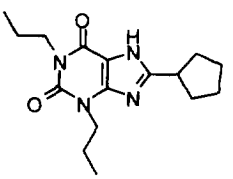
Introduction

Adenosine is a purine nucleoside that is widely distributed throughout the body and is known to modulate a wide variety of physiological functions through interaction with extracellular receptors. The adenosine receptors are classified into three major subtypes, called A_1, A_2 and A_3 receptors.[1-3] Alkyl xanthines such as caffeine and theophylline are known to be prototypic adenosine receptor antagonists. Many alkyl xanthines and non-xanthines have been synthesized and studied as antagonists at A_1 and A_2 receptors.[4-8] Despite the fact that the diuretic action of caffeine and theophylline has been known for many years, the pharmacological basis for this effect has not been fully elucidated, since they are non-selective and weak adenosine antagonists and are also phosphodiesterase inhibitors. Recent reports demonstrated that 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, **2**) and related xanthines, potent and selective adenosine A_1 receptor antagonists, have diuretic, saliuretic, and kaliuretic properties.[8,9]

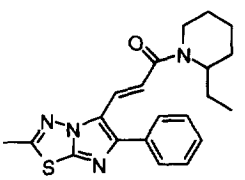
Bay n 1468 (**3**) is a unique diuretic that has uricosuric and renal vasodilating activities, and is structurally unrelated to known xanthine or non-xanthine adenosine antagonists.[10] The pharmacological profiles of Bay n 1468 are different from those of well known thiazides and loop diuretics. However, the mechanism of its action has not been reported. In order to search for more potent diuretics, we selected Bay n 1468 as a lead for a new diuretic and synthesized a new series of pyrazolo[1,5-*a*]pyridines. In this paper, we describe the synthesis, diuretic activities, and the adenosine receptor antagonist activities for FK453 (**1d**) and related analogs.[11-13]



FK 453 (**1d**)



DPCPX (**2**)

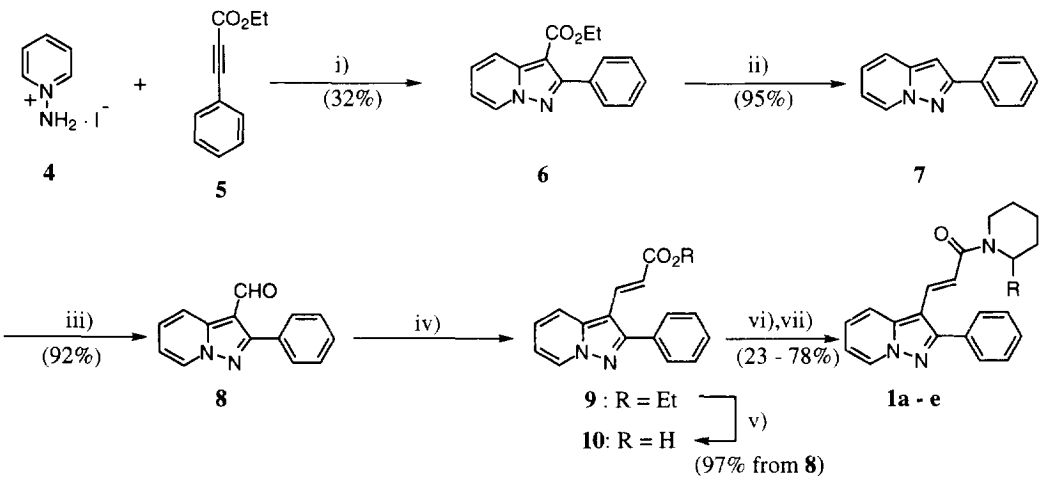


Bay n 1468 (**3**)

Chemistry

Compounds (**1a-e**) in Table 1 and Table 2 were prepared according to Scheme 1. Compound **7**, which was obtained by a modification of the reported method,[14] was converted to aldehyde **8** by Vilsmeyer formylation reaction. The aldehyde **8** was then converted to **10** via Horner-Emmons reaction and hydrolysis of the ester **9**. The carboxylic acid **10** was treated with $SOCl_2$ or isobutyl chloroformate/triethylamine and the resulting acid chloride or mixed anhydride was coupled with piperidine derivatives to give **1a-e**[15]. Chiral 2-ethylpiperidines[16] and 2-hydroxyethylpiperidines[17] were obtained by optical resolution of racemic compounds. Optical purities of **1b-1e** were determined by HPLC to be over 98% ee. It was necessary to perform steps iv) to vii) in the dark, since the *trans* pyrazolo[1,5-*a*]pyridine-3-acrylic acid derivatives were readily isomerized to the corresponding *cis* isomers in solution due to a photochemical *trans-cis* isomerization.[18]

Scheme 1



Reagents: i) KOH/DMF; ii) 47% aq. HBr, Δ; iii) $POCl_3$/DMF; iv) $(EtO)_2POCH_2CO_2Et$, NaH/THF; v) aq. NaOH/MeOH; vi) $SOCl_2/CH_2Cl_2$ or $ClCO_2{}^tBu$, Et_3N/CH_2Cl_2; vii)  , Et_3N/CH_2Cl_2

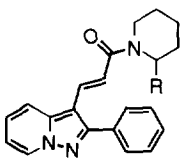
Biological Results and Discussion

The diuretic activities for test compounds (**1a-1e**, **3**) in rats were determined by the previously described method.[12] Test compounds were administered orally (10 mg/kg) and the urine was collected for 6 h. Urine volume and urine sodium excretion were expressed as percent of each control value. The results are shown in Table 1.

In vitro adenosine receptor binding assay and functional assay for **1d**, **1e** and **2** were performed by the previously described method.[13] The results are summarized in Table 2. Adenosine A_1 receptor binding was measured in rat cortical membranes with 1 nM [^{3}H]-cyclohexyladenosine (CHA), and binding to the A_2 receptor was measured in rat striatal membranes using 5 nM [^{3}H]-*N*-ethylcarboxamidoadenosine (NECA) in the presence of 50 nM cyclopentyladenosine (CPA). Adenosine A_1 receptor antagonist activity was evaluated by measuring the inhibition of adenosine induced negative inotropic activities of guinea-pig atria. Adenosine A_2 receptor

antagonist activity was evaluated by measuring the inhibition of adenosine induced relaxation of guinea-pig aorta. Data are shown as IC_{50} values.

Table 1



Compounds	–N R	Diuretic activities in rats[a] UV (% of control)	Na^+ (% of control)
1a	–N	195*	179*
1b	–N (S)	201*	220*
1c	–N (R)	94	91
1d (FK453)	–N (R) OH	272**	280**
1e	–N (S) OH	96	96
3 (Bay n 1468)		242**	278**

[a] dose: 10 mg/kg, p.o.,(n=3); ** P<0.01, * P<0.05: significantly different from each vehicle group (Dunnett's multiple comparison test); UV = urine volume, Na^+ = sodium excretion.

As shown in Table 1, **1a**, **1b**, and **1d** showed diuretic activities at 10 mg/kg p. o.. Among them FK453 (**1d**) was the most potent compound and more potent than Bay n 1468. Interestingly, **1c** and **1e**, enantiomers of **1b** and FK453 respectively, were inactive in this test.

To investigate the mechanism of action of FK453, the following *in vitro* tests were performed. In a receptor binding assay, FK453 showed high affinity (IC_{50} = 17.2 nM) and good selectivity (657 fold) at the adenosine A_1 receptor subtype, whereas **1e**, the (*S*)-enantiomer of FK453, showed markedly low binding affinity and poor selectivity for the receptor binding. Similarly, FK453 had potent antagonist activity (IC_{50} = 0.56 nM) and good selectivity (2017 fold) at the adenosine A_1 receptor subtype, and **1e** had low antagonist activity in the functional assay. Furthermore, FK453 had little effect on adrenergic, muscarinic, histaminergic and serotonergic receptors up to 20 μM, and the phosphodiesterase inhibitory activity of FK453 was very weak (IC_{50} values were 8.0 μM and 14.0 μM for cAMP- and cGMP-phosphodiesterases respectively).[13]

Table 2

Compounds	Adenosine receptor binding[a] IC_{50} (nM) A_1	A_2	A_2/A_1	Adenosine receptor antagonism[b] IC_{50} (nM) A_1	A_2	A_2/A_1
1d (FK453)	17.2	11300	657	0.56	1180	2017
1e	10100	130000	12.9	1180	>100000	> 84.7
2 (DPCPX)	4.7	1130	240	1.31	656	501
3 (Bay n 1468)	n.t.[c]	n.t.[c]	-	(91.0 %)[d]	n.t.[c]	-

[a] 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_2 receptor) (n=3). [b] Inhibition of adenosine induced negative inotropic activity in guinea-pig atria (A_1 receptor) and adenosine induced relaxation in guinea-pig aorta (A_2 receptor) (n=3). [c] n.t.: not tested. [d] inhibition at 10 nM (n=2).

These results demonstrate that FK453 is a highly potent and selective adenosine A_1 receptor antagonist, which causes diuresis.[12] Bay n 1468 is also found to be an adenosine A_1 receptor antagonist.

Conclusions

In summary, FK453 is a novel non-xanthine adenosine receptor antagonist which is highly potent and selective at the adenosine A_1 receptor subtype. The structure of FK453 is different from any known adenosine antagonist. The stereochemistry at the 2-position of the branched piperidine ring of FK453 is very important for adenosine A_1 receptor binding. The diuretic action of FK453 arises from selective adenosine A_1 receptor antagonism.[19] In addition to diuretic activity, FK453 has renal vasodilating activity and renal protective effect, and is useful for the treatment of the diseases such as hypertension and renal failure. [11-13, 20-22]

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