Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 739–742

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Anti-HIV-1 Activities and Pharmacokinetics of New Arylpiperazinyl Fluoroquinolones

Toshinori Ohmine,[a] Tetsushi Katsube,[d] Yasunori Tsuzaki,[d] Miho Kazui,[c] Nobuhiro Kobayashi,[c] Tomoaki Komai,[a] Masahiko Hagihara,[d] Takashi Nishigaki,[a] Aikichi Iwamoto,[e] Tomio Kimura,[b] Hiroto Kashiwase[a] and Makoto Yamashita[a,*]

[a]*Biological Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

[b]*Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

[c]*Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

[d]*Pharmaceutical Research Department, Ube Laboratory, Ube Industries, Ltd., 1978-5 Kogushi, Ube City, Yamaguchi 755-0067, Japan*

[e]*Department of Infectious Diseases, Institute of Medical Science, The University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108-8639, Japan*

Received 10 July 2001; accepted 13 December 2001

Abstract—Anti-HIV-1 activities and pharmacokinetics of a series of novel arylpiperazinyl fluoroquinolones are reported. Modification at the C-8 position with a trifluoromethyl group was superior to that with a difluoromethoxy group to achieve higher anti-HIV-1 activity. Two compounds studied exhibited quite high anti-HIV-1 activities ($IC_{50} < 50$ nM) in vitro and high bioavailabilities (BA > 90%) in monkeys. © 2002 Elsevier Science Ltd. All rights reserved.

It has been already more than 10 years since the first anti-HIV chemotherapy was started.[1] Recent progress in combination therapy of several antiretroviral drugs, such as the highly active antiretrovirus therapy (HAART), has achieved long-term control of HIV replication in vivo,[2] resulting in a dramatic reduction in AIDS-related morbidity and mortality.[3] However, there are several problems with HAART, such as a fairly high failure rate to control viremia[4] partly because of resistance to antiretroviral drugs, severe side effects,[5] lack of compliance, drug interaction and high costs.[4b] Therefore, a cheap and safe drug with a new mode of action has long been sought after.

Recently, we reported that several arylpiperazinyl fluoroquinolones exhibited inhibitory activities against HIV-1 replication.[6a,b] The structure–activity relationship study[6b] revealed that the substituent at the C-8 position of arylpiperazinyl fluoroquinolones play an important role in anti-HIV-1 activities. In particular, hydrophobicity of the substituent at this position seems to be one of the key factors for antiviral potency; for instance, the inhibitory activity of a difluoromethoxy analogue was much higher than that of a methoxy analogue.[6b]

Therefore, we synthesized various compounds with a trifluoromethyl group, which has a more hydrophobic property than a difluoromethoxy group, at the C-8 position according to the well-established method,[7] and compared their antiviral activities and cytotoxicities with those of difluoromethoxy analogues (Table 1). While the extent of the effect of substitution varied in each case, all the compounds with a trifluoromethyl group at the C-8 position had higher inhibitory activity against HIV-1 replication than the corresponding difluoromethoxy analogues, as expected. The most potent compound was **3a**, whose IC_{50} was 14 nM.

*Corresponding author. Fax: +81-3-5436-8565; e-mail: myamas@shina.sankyo.co.jp

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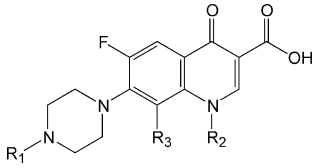
PII: S0960-894X(02)00003-3

Modification with a trifluoromethyl group at the C-8 position also enhanced their cytotoxic properties in parallel (Table 1); consequently, selectivity between anti-HIV-1 activity and cytotoxicity of each compound was similar to that of the corresponding difluoromethoxy analogue.

We next examined the pharmacokinetics of the trifluoromethyl analogues after oral administrations in rats. The results shown in Figure 1 and Table 2 clearly demonstrate that compounds **2a** and **3a** had quite excellent oral bioavailabilities in rats compared to the other six compounds. The peak concentration (C_{max}) in plasma appeared at 1 h after oral administration of compound **2a** and **3a** at a dose of 20 mg/kg, and reached as high as 58.49 and 26.42 μg/mL, respectively. To investigate animal species specificity of bioavailability, we further investigated the pharmacokinetics of some of these trifluoromethyl analogues (compounds **2a**, **3a** and **3c**) in Cynomolgus monkeys (Fig. 2 and Table 3). As shown in Figure 2, mean concentrations of compounds **2a** and **3a** in plasma after oral administrations were fairly high, whereas those of compound **3c** were so low they were almost undetectable. These plasma concentration

Table 1. Comparisons of anti-HIV-1 activities between trifluoromethyl and difluoromethoxy analogues



R_1	R_2	R_3					
		$-CF_3$			$-CHF_2$		
		No.	IC_{50} (μM)	CC_{50} (μM)	No.	IC_{50} (μM)	CC_{50} (μM)
2-Methoxyphenyl	$-CH_3$	**1a**	0.054	0.50	**4a**	0.35	11
2-Methoxyphenyl	$-C_2H_5$	**1b**	0.11	0.75	**4b**	0.22	8.3
2-Methoxyphenyl	Cyclopropyl	**1c**	0.069	0.87	**4c**	0.56	12
2-Pyrimidinyl	$-CH_3$	**2a**	0.049	0.89	**5a**	0.31	13
2-Pyrimidinyl	$-C_2H_5$	**2b**	0.095	1.5	**5b**	0.47	20
2-Pyrimidinyl	Cyclopropyl	**2c**	0.19	5.2	**5c**	3.7	21
2-Pyridyl	$-CH_3$	**3a**	0.014	0.22	**6a**	0.24	9.2
2-Pyridyl	$-C_2H_5$	**3b**	0.026	0.37	**6b**	0.89	4.1
2-Pyridyl	Cyclopropyl	**3c**	0.065	1.4	**6c**	0.49	9.2

Activities of the compounds against HIV-1_{IIIB} replication were based on the inhibition of virus-induced cytopathogenicity in MT-4 cells.[8] Cytotoxicities of the compounds were evaluated in parallel and were based on the viability of mock-infected MT-4 cells as determined by the MTT method.[9]

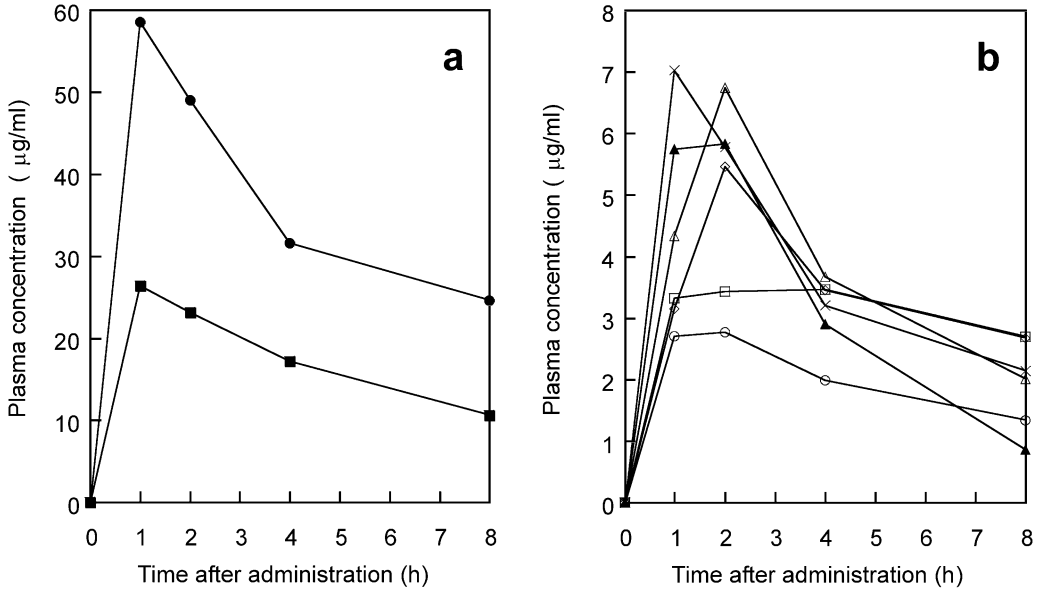


Figure 1. Plasma levels of (a) compounds **2a** (●) and **3a** (■), (b) compounds **1a** (○), **1c** (□), **2b** (◇), **2c** (×), **3b** (△) and **3c** (▲) following oral administrations to Wistar rats. The method and dose of each compound are described in Table 2. Each point represents the mean value of two or three rats.

Table 2. Pharmacokinetic parameters of trifluoromethyl analogues after oral administrations

No.	Dose (mg/kg)	C_{max} (μg/mL)	t_{max} (h)	$AUC_{0-8\ h}$ (μg·h/mL)
1a	10	2.77	2	15.49
1c	20	3.46	4	24.27
2a	20	58.49	1	275.99
2b	20	5.46	2	27.05
2c	20	7.02	1	29.56
3a	20	26.42	1	134.03
3b	20	6.74	2	29.47
3c	20	5.83	2	24.93

All the compounds were suspended in 0.5% carboxymethyl cellulose solution. Wistar rats (SLC Japan, male, 6–9 weeks old) were administered the suspension orally at 10mL suspension/kg, following blood sample collection from the abdominal aorta under anesthesia at a certain time after administration. Plasma samples were extracted with diethyl ether and analyzed by HPLC. The area under the curve ($AUC_{0-8\ h}$) was calculated according to the trapezoidal rule. All the rats were starved for 20 h prior to the administration. The data indicate mean values of three rats.

Table 3. Pharmacokinetic parameters of compounds **2a**, **3a** and **3c** after oral or intravenous administrations to Cynomolgus monkeys

Parameter	Compd **2a**		Compd **3a**		Compd **3c**	
	po	iv	po	iv	po	iv
C_{max} (μg/mL)	1.5±0.5	NA*	1.4±0.5	NA	0.1±0.0	NA
$AUC_{(0-\infty)}$ (μg·h/mL)	18.4±5.5	16.4±4.8	17.2±5.7	18.4±3.3	ND*	3.5±0.6
$MRT_{(0-\infty)}$ (h)	NA	8.2±3.3	NA	9.3±2.1	NA	0.8±0.2
$t_{1/2}$ (h)	8.7±1.9	7.6±3.1	7.5±1.4	8.8±1.9	ND	0.7±0.3
t_{max} (h)	4.0±3.5	NA	2.0±0.0	NA	2.0	NA
CL_{tot} (mL/h/kg)	NA	65.1±21.1	NA	55.7±9.9	NA	293.5±49.6
V_{dss} (mL/kg)	NA	491.0±75.8	NA	509.7±106.6	NA	224.5±23.3
BA (%)	112.2		93.5		4.3	

(Mean±SD, $n=3$). Male Cynomolgus monkeys were administered each compound orally (po) or intravenously (iv) at a dose of 1mg/kg. The compounds were dissolved in polyethylene glycol solution or *N*,*N*-dimethylacetamide/0.1M $NaHCO_3$/saline (1:1:8) at a concentration of 1mg/mL for oral or intravenous administration, respectively. Blood samples were collected at a certain time after administration and were centrifuged to obtain plasma. The protein fraction was removed from the plasma samples by methanol precipitation and the supernatants were analyzed by HPLC. The peak concentration (C_{max}) and the time for peak concentration (t_{max}) are calculated from the experimental data. Other kinetic parameters, such as the area under the plasma concentration–time curve ($AUC_{(0-\infty)}$), the mean residence time [$MRT_{(0-\infty)}$], the elimination half-life ($t_{1/2}$), the total clearance (CL_{tot}), and the distribution volume (V_{dss}), were calculated by fitting a non-compartment model using Win Nonlin software. The absolute bioavailability was calculated as follows: BA (%) = ($AUC_{(0-\infty)}$ in oral administration)/($AUC_{(0-\infty)}$ in intravenous administration)*100. *NA: not applicable; ND: not determined.

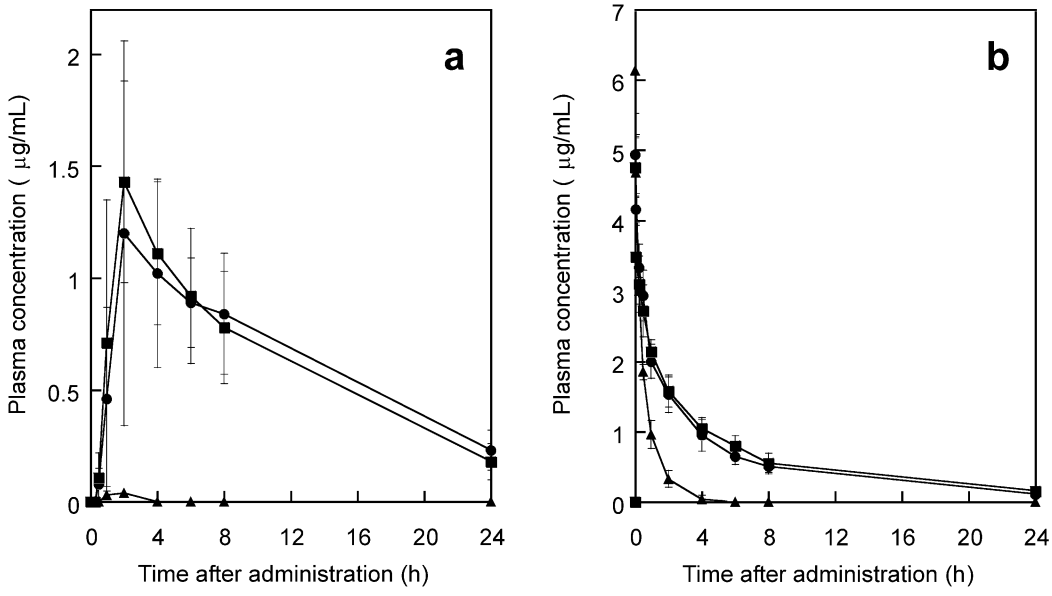


Figure 2. Plasma levels of compounds **2a** (●), **3a** (■) and **3c** (▲) following oral (a) or intravenous (b) administration to Cynomolgus monkeys at a dose of 1mg/kg. The methods are described in the footnotes of Table 3. Each point represents the mean±standard deviation of three monkeys.

profiles in monkeys were similar to those in rats (Table 2 and 3), suggesting that there was no animal species specificity of bioavailability among these compounds. The absolute bioavailabilities of compounds **2a** and **3a** in monkeys were quite high up to 112.2 and 93.5%, respectively (Table 3).

The present study revealed that introduction of a trifluoromethyl group at the C-8 position of arylpiperazinyl fluoroquinolones enhanced their anti-HIV-1 potencies compared to corresponding difluoromethoxy-substituted compounds. The most potent compound in the present study was compound **3a** and its activity was approximately 15-fold higher than compound **4b**, the most active compound in the previous report.[10] At present, the precise mechanism for the enhancement of anti-HIV-1 activity by introduction of a trifluoromethyl group at the C-8 position is unclear. Identification of the target molecule(s) of the drugs would be a clue to clarify this in the future.

Two of the compounds employed in this study, **2a** and **3a**, had quite high bioavailabilities in rats and monkeys (absolute bioavailabilities in monkeys were $>90\%$). These two compounds might be superior candidates for the next generation of anti-HIV-1 chemotherapeutics. In addition, these two compounds inhibit replication of not only HIV-1 replication but also other retroviruses, such as simian immunodeficiency virus and feline immunodeficiency virus (manuscript in preparation). Evaluation of antiviral activities of these compounds in vivo is now in progress using an SIV/Rhesus monkey infection model.

Compound **4b** has been shown to inhibit replication of herpes viruses, including human cytomegalovirus, varicella-zoster virus and herpes simplex virus types 1 and 2, which are important opportunistic pathogens in AIDS patients.[11] Compounds **2a** and **3a** also inhibit human cytomegalovirus replication in vitro and their inhibitory activities are much higher than Compound **4b** (manuscript in preparation). Therefore, the arylpiperazinyl fluoroquinolones reported here have the potential to be unique effective therapeutic agents for AIDS patients. Further investigations, including evaluation of antiviral activity in vivo, elucidation of mode of action and toxicological testing, are now in progress.

Acknowledgements

The authors thank K. Fusegawa and I. Nakayama, Biological Research Laboratories, Sankyo Co., Ltd., for their excellent technical assistance in the animal experiments.

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