Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2167–2172

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Muscarinic M_3 Receptor Antagonists with (2*R*)-2-[(1*R*)-3,3-Difluorocyclopentyl]-2-hydroxyphenylacetamide Structures. Part 2

Yoshio Ogino, Norikazu Ohtake,* Kensuke Kobayashi, Toshifumi Kimura, Toru Fujikawa, Takuro Hasegawa, Kazuhito Noguchi and Toshiaki Mase

Banyu Tsukuba Research Institute in collaboration with Merck Research Laboratories, Okubo-3, Tsukuba 300-2611, Ibaraki, Japan

Received 3 March 2003; accepted 2 April 2003

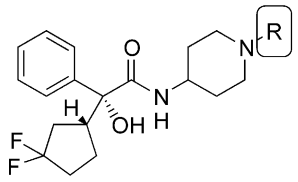
Abstract—Optimization of the amine part of our original muscarinic M_3 receptor antagonist **1** was performed to identify M_3 receptor antagonists that are superior to **1**. Compounds carrying a variety of diamine moieties without hydrophobic substituent on the nitrogen atom were screened against the binding affinity for the M_3 receptor and the selectivity for M_3 over the M_1 and M_2 receptors. This process led to a 4-aminopiperidinamide (**2l**) with a K_i value of 5.1 nM and with a selectivity of the M_3 receptor that was 46-fold greater than that of the M_2 receptor. Further derivatization of **2l** by inserting a spacer group or by incorporating alkyl group(s) into the amine part resulted in the identification of an 4-(aminoethyl)piperidinamide **2l**–**b** with a K_i value of 3.7 nM for the M_3 receptor and a selectivity for the M_3 receptor that was 170-fold greater than that of the M_2 receptor.

Introduction

Pharmaceutical research into therapeutic agents that are selective for muscarinic receptor subtypes has focused on exploration of orally-active, muscarinic M_3 receptor-selective antagonists for the treatment of respiratory disorders such as chronic obstructive pulmonary disease (COPD) and urinary tract disorders such as urinary incontinence (UI). Some muscarinic M_3 receptor antagonists have been identified and are under clinical development.[1–6] Among them, an inhaled M_3 antagonist, tiotropium, has been developed for the treatment of COPD. Tiotropium was non-selective toward the muscarinic receptor subtypes in the binding assay, however, it showed kinetic selectivity toward the M_3 receptor over the M_2 receptor (dissociation from the M_2 receptor is faster than from the M_3 receptor).[7] Based on a hypothesis that M_2-sparing M_3 receptor antagonists in the binding basis would have clinical benefit over non-selective muscarinic antagonists or kinetically selective antagonists, we focused our search on this type of M_3 receptor antagonists. As a result, we identified an 1-(6-aminopyridyn-2-ylmethyl)piperidinamide (**1**) that possesses high binding affinity ($K_i = 2.8$ nM) for the M_3 receptor and has selectivity for the M_3 receptor that is 190-fold greater than that of the M_2 receptor in the class of (2*R*)-2-[(1*R*)-3,3-difluorocyclopentyl]-2-hydroxy-2-phenylacetamides. The in vitro metabolism study of **1** indicated that this compound was easily metabolized to an *N*-dealkylated one (**2a**) in rats. The binding data of **2a** ($K_i = 320$ nM for the M_3 receptor, $M_2/M_3 = 47$-fold) suggested that the piperidinyl side chain was essential for the compound's potency for the M_3 receptor and for its selectivity for the M_3 receptor over the M_2 receptor. We considered that new antagonists lacking the aminopyridylmethyl moiety would be more metabolically stable and would lead to more potent and longer-acting M_3 antagonists. Therefore, we focused on optimization of the amine part of **1**. As a result of extensive derivatization, we have found a 4-(2-aminoethyl)piperidinamide (**2l**–**b**) that showed better in vitro metabolic stability in the rat, dog, and human hepatic microsomes, that had potent binding affinity for the M_3 receptor, and that had a higher selectivity (170-fold) for the M_3 receptor than for the M_2 receptor. In this paper, we describe the synthesis and structure–activity relationships (SARs)

*Corresponding author. Tel.: +81-298-77-2221; fax: +81-298-77-2029; e-mail: ootakenr@banyu.co.uk

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00350-0



1 R = 6-aminopyridyn-2-ylmethyl

2 R = H

Figure 1.

of (2*R*)-2-[(1*R*)-3,3-difluorocyclopentyl]-2-hydroxy-2-phenylacetamides that contained a variety of diamine moieties (Fig. 1).

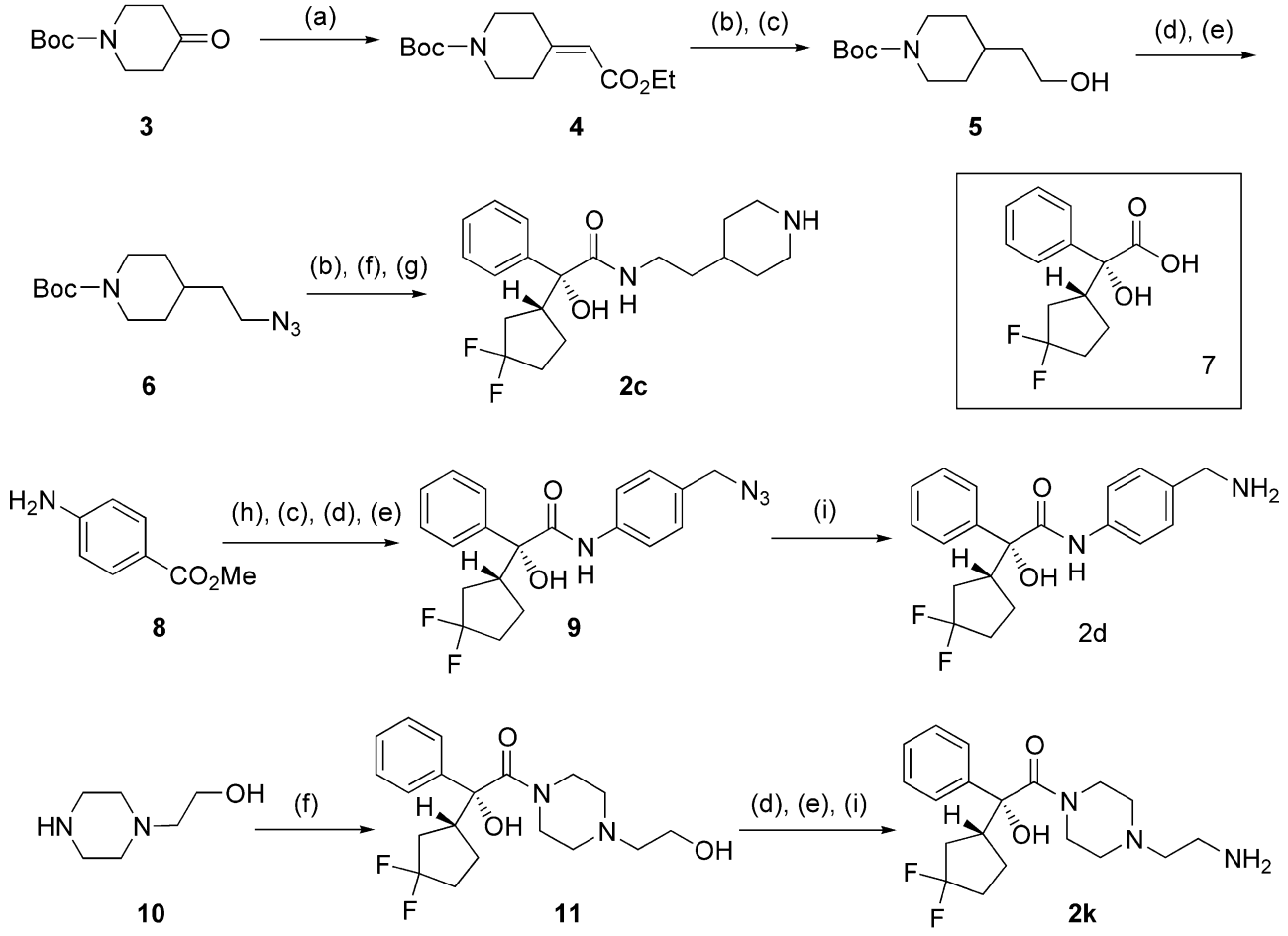
Chemistry

Synthetic methods of representative compounds (**2c**, **2d**, **2k**, **2l–c**, **2l–g** and **2l–i**) are summarized in Schemes 1 and 2. First, the synthesis of compound **2c** was started with the preparation of its amine part. Treatment of *N*-Boc-piperidone **3** with Horner–Emmonds reagent gave **4**, in which the double bond was hydrogenated and subsequently the ester moiety was reduced with LAH to produce an alcohol **5** in a 85% yield. The primary alcohol moiety of **5** was converted to an azide **6** via substitution of the corresponding mesylate with NaN_3. Coupling of **6** with a 2-(3,3-difluorocyclopentyl)-2-hydroxy-2-phenyl acetic acid **7** under a standard condition (WSC, HOBT) followed by hydrogenation of the azide group produced **2c** in 21% yield.

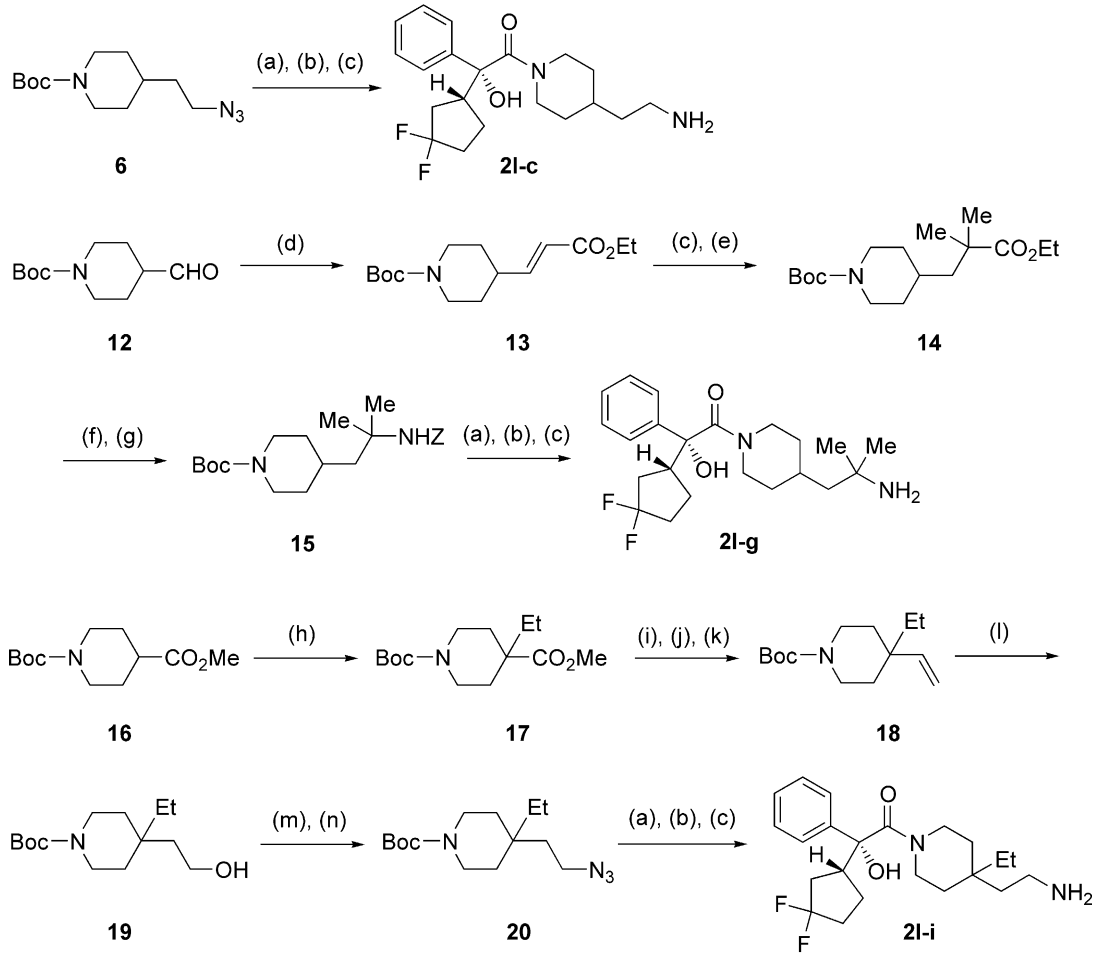
In order to prepare compound **2d**, methyl *p*-aminobenzoate was coupled with the corresponding acid chloride of **7** in a 51% yield. The ester moiety of the coupling product was reduced with LAH to yield an alcohol, which was converted to an azide **9** in a 57% yield in a manner similar to that described above. The azide group was reduced with PPh_3 in THF–H_2O yielded **2d** in a quantitative yield.

Compound **2k** was synthesized from *N*-(2-hydroxyethyl)pyperazine **10**. After the coupling of **10** with **7** (47% yield), the primary alcohol of the resulting compound **11** was converted to the amine in a similar manner to that described for the preparation of **2d** to produce **2k** in 87% yield.

Scheme 2 shows the synthesis of the representative piperidinamides (**2l–c**, **2l–g**, **2l–i**). The Boc group of the



Scheme 1. Synthesis of the representative piperidinamides (**2c**, **2d**, and **2k**). Reagents and conditions: (a) 60% NaH, $(EtO)_2CH_2CO_2Et$, THF, 0 °C; (b) 10% Pd/C, H_2, MeOH, rt; (c) LAH, THF, rt; (d) MsCl, NEt_3, THF, 0 °C; (e) NaN_3, DMF, rt; (f) **7**, WSC, HOBt, DMF, rt; (g) TFA, rt; (h) $SOCl_2$, DMA, −10 °C; (i) PPh_3, THF–H_2O, rt.



Scheme 2. Synthesis of the representative piperidinamides (**2l–c**, **2l–g**, and **2l–i**). Reagents and conditions: (a) TFA, $CHCl_3$, rt; (b) **7**, WSC, HOBt, $CHCl_3$, rt; (c) 10% Pd/C, H_2, MeOH, rt; (d) 60% NaH, $(EtO)_2CH_2CO_2Et$, THF, 0 °C; (e) LDA, MeI, THF, −78 °C; (f) NaOH, MeOH, rt; (g) $(PhO)_2PON_3$, BnOH, THF, 80 °C; (h) LDA, EtBr, THF, −78 °C; (i) LAH, THF, rt; (j) SO_3Py, DMSO, NEt_3, rt; (k) PPh_3CH_3Br, *n*-BuLi, THF, −78 °C; (l) BH_3, THF, 0 °C, then 30% H_2O_2; (m) MsCl, NEt_3, THF, 0 °C; (n) NaN_3, DMF, rt.

piperidines (**6**, **15**, **20**) with a protected primary amine or an azide group (a precursor of an amine) was deprotected by treatment with TFA to produce the corresponding 1-(non-protected)-piperidines, which were coupled with the acid **7** to yield in 50–70% yield. Finally, the benzyloxycarbonyl group or the azide of the coupling products were reduced by a catalytic hydrogenation to produce the target compounds in a good yield.

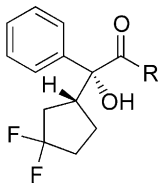
Results and Discussion

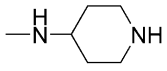
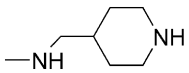
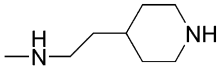
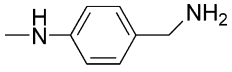
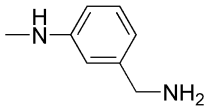
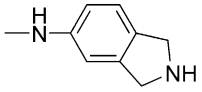
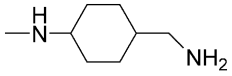
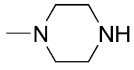
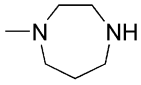
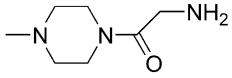
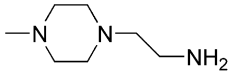
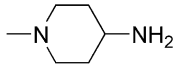
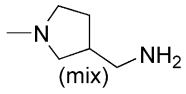
Compounds were tested in initial screens for binding affinity (K_i values) against human muscarinic receptor subtypes (hM_1, hM_2 and hM_3) in transfected CHO cells.[8,9] Subsequently, selected compounds were examined for their in vitro metabolic stability in rat, dog, and human hepatic microsomes.[6]

As described above, the 4-piperidinamide **2a** lost much of its binding affinity ($K_i = 320$ nM) for the M_3 receptor (Table 1). In contrast, the 4-aminomethylpiperidinamide **2b** showed a 18-fold improvement in the binding affinity ($K_i = 17$ nM) for the M_3 receptor as compared with that of **2a**. However, an 4-aminoethylpiperidine **2c** showed only a 4-fold improvement ($K_i = 89$ nM) in the M_3 binding affinity. These compounds retained the selectivity for M_3 over the M_2 receptor to some extent, which was probably due to the 2-(3,3-difluorocyclopentyl)-2-hydroxyphenylacetic acid moiety.[6]

Compounds with a substituted aniline (**2d**, **2e**, **2f**) or a cyclohexylamine (**2g**) showed low binding affinity (Table 2). Based on our previous finding that an amide hydrogen in the 2-cyclobutyl-2-hydroxyphenylacetamide enhanced the compound's binding affinity for the M_3 receptor and increased the compound's selectivity for the M_3 receptor over that for the M_2 receptor,[8] both piperazinamide **2h** and homopiprazinamide **2i** were predicted to show low M_3 binding affinity and M_3 selectivity over the M_2 receptor subtype. However, **2i** showed moderate M_3 affinity ($K_i = 17$ nM) and M_3 selectivity (66-fold) over the M_2 receptor. Furthermore, a 4-aminoethylpiperazinamide **2k** showed a nano-molar order M_3 binding affinity ($K_i = 9.6$ nM) with 85-fold M_3 selectivity over the M_2 receptor. We assumed that the primary amine moiety of **2k** played a key role in enhancing both the M_3 affinity and selectivity, while the tertiary amine moiety of the piperazine group did not

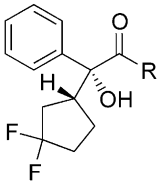
Table 1. The binding affinity of the compounds (**2a**–**2m**) to the muscarinic receptor subtypes

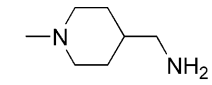
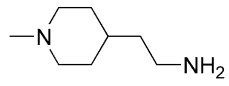
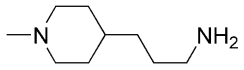
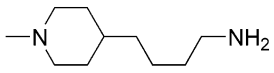
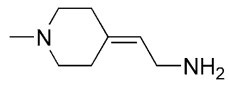
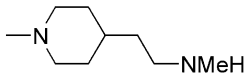
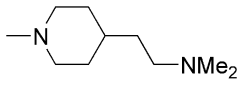
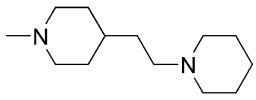
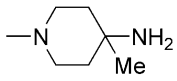
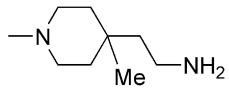
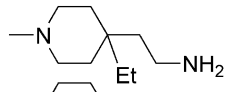
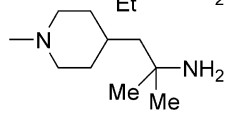


Compd	R	Binding affinity[a] (K_i, nM)			Selectivity
		M_3	M_1	M_2	M_2/M_3
2a		320	310	15,000	47
2b		17	23	2200	130
2c		89	140	3900	43
2d		650	740	5300	8
2e		93	210	2900	31
2f		290	630	15,000	51
2g		100	55	6800	68
2h		50	30	1200	24
2i		17	14	1100	66
2j		16	21	300	18
2k		9.6	11	820	85
2l		5.1	3.0	240	46
2m		2.9	2.1	130	44

[a]Values are the mean of two or more independent assays.

Table 2. The binding affinity of the compounds (**2l-a**–**1l-l**) to the muscarinic receptor subtypes



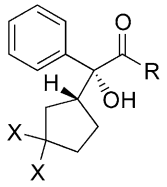
Compd	R	Binding affinity[a] (K_i, nM)			Selectivity
		M_3	M_1	M_2	M_2/M_3
2l-a		11	11	760	69
2l-b		3.7	6.6	630	170
2l-c		47	92	2800	45
2l-d		76	140	4200	56
2l-e		1.2	1.3	55	46
2l-f		120	140	5400	45
2l-g		930	760	15,000	16
2l-h		2400	1900	36,000	15
2l-i		8.3	4.7	640	77
2l-j		3.3	1.5	210	65
2l-k		7.0	1.0	300	43
2l-l		26	37	1000	39

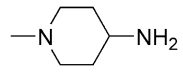
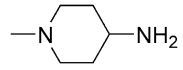
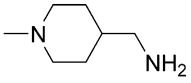
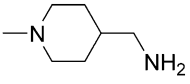
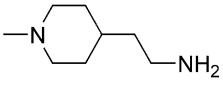
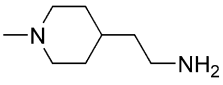
[a]Values are the mean of two or more independent assays.

contribute to either the binding affinity or the selectivity. Based on this assumption, we prepared a 4-aminopiperidinamide **2l** and assessed its binding affinity. As expected, **2l** showed a potent M_3 binding affinity ($K_i = 5.1$ nM) and moderate M_3 selectivity ($M_2/M_3 = 46$-fold). A pyrolidinamide **2m** exhibited higher M_3 binding affinity than **2l** did; however, its selectivity was comparable. Therefore, further derivatization on the amine part of the piperidinamide **2l** was prioritized.

With respect to optimization of M_3 affinity and selectivity, a methylene (**2l**–**a**), an ethylene (**2l**–**b**), a propylene (**2l**–**c**), or a butylene (**2l**–**d**) spacer was inserted between the piperidine and primary amine moiety of **2l**. Compound **2l**–**b** with an ethylene spacer showed the most potent M_3 binding affinity ($K_i = 3.7$ nM) and

Table 3. The binding affinity to the muscarinic receptor subtypes and metabolic stability of the representative compounds



Compd	X	R	Binding affinity[a] (K_i nM)			Selectivity	Metabolic stability %[b] (Rat, dog, human)
			M_3	M_1	M_2	M_2/M_3	
23	H		1.4	0.98	11	7.9	40, 89, 92
2l	F		5.1	3.0	240	46	100, 88, 86
24	H		1.7	1.8	28	16	72, 100, 91
2l-a	F		11	11	760	69	98, 100, 100
25	H		1.1	2.1	41	36	77, 84, 86
2l-b	F		3.7	6.6	630	170	100, 100, 82
1			2.8	1.5	530	190	46, 84, 80

[a]Values are in the mean of two or more independent assays.
[b]% remaining after 30-min incubation in each hepatic microsomes.

selectivity ($M_2/M_3 = 170$-fold), while compound **2l–d** ($K_i = 76$ nM) with a butylene spacer showed an approximately 15-fold reduction in the M_3 binding affinity as compared to **2l**. Replacement of the ethylene spacer with an ethylidene one (**2l–e**) resulted in an increase in M_3 binding affinity ($K_i = 1.2$ nM), while the M_3 selectivity of **2l–d** significantly decreased ($M_2/M_3 = 46$-fold). It is interesting to note that compound **2l–b** showed high M_3 binding affinity and selectivity since the piperidine nitrogen of the amine part was acylated with the acid **7**. In contrast, compound **2c**, in which the primary amine moiety of the same amine part was connected with the acid **7**, resulted in a large reduction in M_3 binding affinity and selectivity in comparison with that of **2l–b**.

The effects of the substituents on the nitrogen of the primary amine moiety in **2l–b** on the M_3 affinity and selectivity were investigated. Replacement of the *N*-methylamono (**2l–f**), *N*,*N*-dimethylamino (**2l–g**) or pentamethylene imine (**2l–h**) group with the primary amine of **2l–b** significantly reduced the M_3 binding affinity and M_3 selectivity over the M_2 subtype. In particular, M_3 binding affinity decreased in **2l–h**, which suggests that the bulkiness around the primary amine moiety affects the binding interaction between the antagonist molecule and M_3 receptor subtype. Also, incorporation of alkyl group(s) into the α-carbon (**2l–l**) or the γ-carbon (**2l–j** and **2l–k**) to the primary amine group in **2l–b** resulted in a decrease in M_3 selectivity over the M_2 receptor.

The effects of the 3,3-difluorocyclopentyl moiety of the representative compounds (**2l**, **2l–a**, and **2l–b**) on the M_3 binding affinity and selectivity, and in vitro metabolic stability were examined (Table 3). Incorporation of the 3,3-difluorocyclopentyl group resulted in a 3- to 6-fold decrease in the M_3 binding affinity, but a 4- to 6-fold improvement in the selectivity for the M_3 receptor over that of the M_2 receptor. In particular, the 3,3-difluorocyclopentyl moiety contributed to an enhancement in the in vitro metabolic stability especially against rat hepatic microsomes.

With respect to the pharmacokinetic profiles of **2l–b** in dogs (Table 4), the oral bioavailablity (F) of **2l–b** was

Table 4. Pharmacokinetic parameters of **2l–b** after iv (0.1 mg/kg) and po (0.3 mg/kg) administration to dogs

iv		po	
AUC (ng h/mL)	150	AUC (ng h/mL)	440
CL_{tot} (mL/min/kg)	13	F (%)	98
V_{dss} (mL/kg)	9400	C_{max} (ng/mL)	43
		$t_{1/2}$ (h)	6.9

approximately 100%. The excellent in vitro metabolic stability of **2l**–**b** would reflect its high oral bioavailability. The total clearance of **2l**–**b** (Cl_{tot} = 13 mL/min/kg) in dogs was slow, which resulted in a long plasma half life ($T_{1/2}$ = 15 h).

Conclusion

Optimization of the (2*R*)-2-[(1*R*)-3,3-difluorocyclopentyl]-2-hydroxy-2-phenylacetamides by focusing on the amine part of the molecule led to 4-aminopiperadinamide **2l**, which has potent binding affinity for the M_3 receptor (K_i = 5.1 nM) and moderate selectivity for M_3 over the M_2 receptor (M_2/M_3 = 46-fold). Further optimization of the primary amine part of **2l** resulted in the identification of **2l**–**b** with an 4-(2-aminoethyl)piperidine group (K_i = 3.7 nM, M_2/M_3 = 170-fold). As expected, this compound showed improved metabolic stability in the rat and dog in vitro hepatic microsome assays because the major metabolic site of **1** was deleted. In addition, this compound showed good oral bioavailability in dogs. These results suggested that a heteroaromatic side chain of the piperidine moiety in **1** was not essential to maintain the high M_3 binding affinity and selectivity over the M_2 receptor and that deletion of this side chain contributed the metabolic stability of the molecules as well.

Acknowledgements

We are grateful to Kimberley Marcopul and Clinical Literature Information Center (CLIC), Merck & Co., Ltd. for their critical reading of this manuscript.

References and Notes

1. Doods, H. N. *Drug News Perspect*. **1992**, 345.
2. Wang, P.; Luthin, G. R.; Ruggieri, M. R. *J. Pharmacol. Exp. Ther*. **1995**, *273*, 959.
3. Alabaster, V. A. *Life Sci*. **1997**, *60*, 1053.
4. Takeuchi, M.; Naito, M.; Morihira, K.; Ikeda, M.; Isomura, Y. *Jpn. Kokai Tokkyo Koho* **1995**, JP 07258250; *Chem. Abstr*. 175858.
5. Miyachi, H.; Kiyota, H.; Segawa, M. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2163.
6. Mitsuya, M.; Kobayashi, K.; Kawakami, K.; Satoh, A.; Ogino, Y.; Kakikawa, T.; Ohtake, N.; Kimura, T.; Hirose, H.; Sato, N.; Numazawa, T.; Hasegawa, T.; Noguchi, K.; Mase, T. *J. Med. Chem*. **2000**, *43*, 5017.
7. Disse, B.; Speck, G. A.; Rominger, K. L.; Witek, T. J., Jr.; Hammer, R. *Life Sci*. **1999**, *64*, 457.
8. Mitsuya, M.; Mase, T.; Tsuchiya, Y.; Kawakami, K.; Hattori, H.; Kobayashi, K.; Ogino, Y.; Fujikawa, T.; Satoh, A.; Kimura, T.; Noguchi, K.; Ohtake, N.; Tomimoto, K. *Bioorg. Med. Chem*. **1999**, *7*, 2555.
9. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol*. **1973**, *22*, 3099.