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Orally active PDE4 inhibitors with therapeutic potential☆

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Abstract—Based on the successful results in the clinical trial of Ariflo™, further optimization of the spatial arrangement of the three pharmacophores (carboxylic acid moiety, nitrile moiety and 3-cyclopentyl-4-methoxyphenyl moiety) in the structure of Ariflo **1** was attempted using a bicyclo[3·3·0]octane template instead of a cyclohexane template. As a result, **2a**, **7a** and **7b** were found to be orally active and were predicted to have an improved therapeutic potential based on evaluation by cross-species and same-species comparisons. Structure–activity relationships (SARs) of these compounds are also discussed.

The phosphodiesterases (PDEs)[1] are involved in intracellular degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to form their corresponding 5′-monophosphates, and phosphodiesterase 4 (PDE4)[2] has received considerable attention as a molecular target for the treatment of asthma and other inflammatory diseases.[3,4] Previous efforts in this field have mainly been focused on asthma and chronic obstructive pulmonary disease (COPD).

Many inhibitors have been reported that are under clinical.[5,6] However, the clinical utility of the pioneer compounds has been limited by side effects such as nausea, emesis and increased gastric acid secretion. To obtain efficient PDE4 inhibitors with reduced side effect, two strategies have been tried. The first approach is based on designing compounds with a reduced affinity for the so-called high affinity rolipram-binding site (HPDE4) and high affinity for the catalytic domain (LPDE4).[7,8] Ariflo **1** (Fig. 1) has been reported to be 75-fold more selective than (*R*)-rolipram with respect to LPDE4 activity,[9] and has shown efficacy in human with an improved safety margin. The second strategy is related to the design of PDE4 subtype-selective inhibitors.[10–12] Ariflo **1** is an orally active second-generation PDE4 inhibitor that was also reported to be PDE4D-selective. Recently, the crystal structures of PDE4B[13] and PDE4D[14,15] were determined. These studies would give us structural information about the rolipram binding site and the active site of PDE4 subtypes.

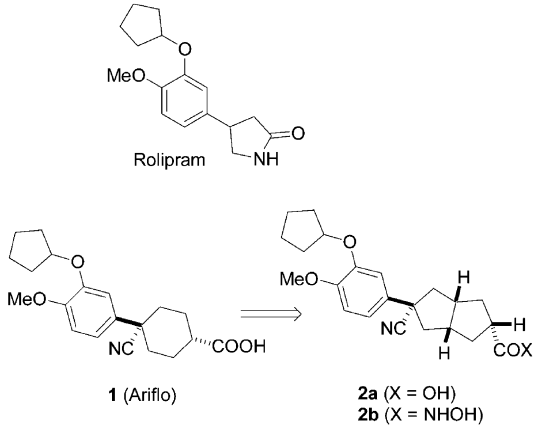

Figure 1. Molecular design of bicyclo[3·3·0]octanes.

The importance of the proper spatial arrangement of the carboxyl moiety relative to the 3-cyclopentyloxy-4-methoxyphenyl and nitrile moieties on the cyclohexane ring is illustrated by comparison of Ariflo **1** (*cis*-isomer) with its *trans*-isomer.[9] More rigid spatial arrangement of these three functional groups within their optimized stereochemistry using another template instead of the cyclohexane ring was predicted to lead to the discovery

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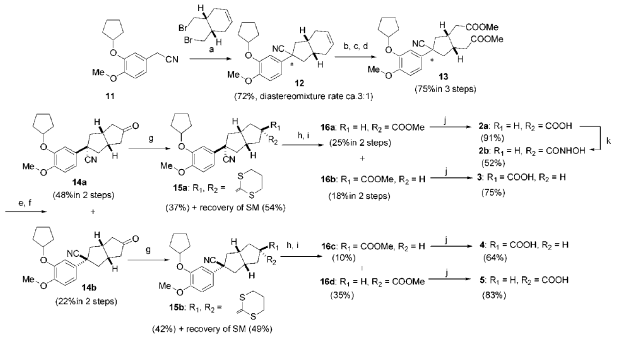


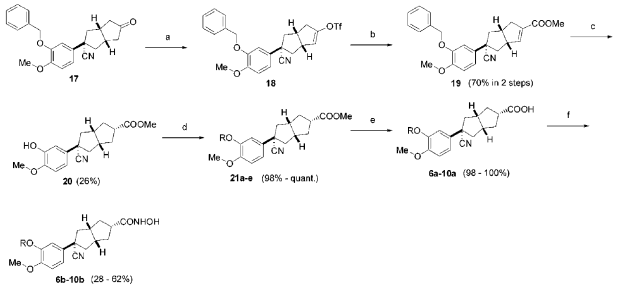
Scheme 1. Synthesis of compounds **2a**, **2b**, **3**, **4** and **5**: (a) NaHMDS, THF, −78°C; (b) O_3, CH_2Cl_2 then PPh_3, −78 °C; (c) $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 2-methyl-2-butene; (d) CH_2N_2, Et_2O, 0°C; (e) NaH, DME, reflux; (f) NaCl, DMSO, H_2O, 165°C; (g) 2-trimethylsilyl-1, 3-dithiane, *n*-BuLi, THF, −78 °C; (h) TFA, H_2O_2aq, CH_3CN, H_2O, then 2N NaOHaq; (i) CH_2N_2, Et_2O; (j) 2N KOHaq, THF, MeOH; (k) $NH_2OC(CH_3)_2OCH_3$, EDC, HOBt, DMF then MeOH, 1N HCl aq.

of a new inhibitor with improved therapeutic potential. Design and synthesis of a series of bicyclo[3·3·0]octane derivatives was carried out with the expectation of obtaining an improved therapeutic potential because the bicyclo[3·3·0]octane template provides more stereochemical diversity (four stereoisomers) and it was expected that derivatives based on the bicyclo[3·3·0]-octane template might show greater potential with respect to PDE4 inhibition, LPDE4 selectivity, and subtype selectivity compared with the corresponding cyclohexane derivatives (two stereoisomers).

As shown in Figure 1, this paper describes the configurational requirements of the above-mentioned three pharmacophores on a new template, the bicyclo[3·3·0]-octane ring, which result in increased beneficial activity and an improved side effect profile.

As outlined in Scheme 1, analogues were prepared from a benzylcyanide **11**[9] by the following synthetic pathway. Dialkylation of **11** with *cis*-4,5-bis(bromomethy)-cyclohex-1-ene provided **12** at a good yield. Ozonolysis of **12**, followed by oxidation and then esterification, gave the diester **13**. Dieckmann condensation of **13**, followed by demethoxycarbonylation, resulted in two separable diastereoisomers **14a** and **14b**, which were converted to ketenedithioacetals **15a** and **15b**, respectively. Deprotection of **15a** gave two separable isomers **16a** and **16b**, which were converted to the carboxylic acid analogues **2a** and **3**, respectively. Condensation of **2a** with *O*-protected hydroxyamine followed by acidic deprotection affored **2b**. According to the procedure described above, **15b** was converted to **16c** and **16d**, which were transformed to **4** and **5**, respectively.

The synthesis of **6**–**10** is outlined in Scheme 2. The enol triflate **18** was prepared from **17**, which was derived from 3-benzyloxy-4-methoxybenzyl cyanide according to the procedure described for the preparation of **14a** from **11**. Palladium-catalyzed insertion of carbon monoxide into **18** in the presence of methanol resulted in **19**, after which catalytic hydrogenation afforded a single



Scheme 2. Synthesis of compounds **6a**–**b**–**10a**–**b**: (a) LiHMDS, $PhNTf_2$, THF, −78 °C rt; (b) $Pd(OAc)_2$, Et_3N, PPh_3, CO, MeOH, DMF; (c) H_2, Pd/C, MeOH; (d) RX, K_2CO_3, DMF or ROH, ADDP, PPh_3, CH_2Cl_2; (e) 1N NaOHaq, MeOH, THF; (f) EDC, HOBt, Et_3N, $NH_2OC(CH_3)_2OCH_3$, DMF then HClaq, MeOH.

isomer **20**. *O*-Alkylation of **20** by the conventional method gave **21a**–**e**, alkaline hydrolysis of which produced **6a**–**10a**, respectively. Condensation of **6a**–**10a** with a protected hydroxylamine, followed by acidic deprotection, led to the corresponding hydroxamic acids **6b**–**10b**, respectively.

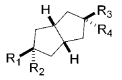
A series of bicyclo[3·3·0]octane derivatives were synthesized and evaluated for their ability to inhibit PDE4 enzyme prepared from U937 cells[16] (a cell line derived from human monocytes). Results are expressed as IC_{50} values, that is, the test compound concentration that gave 50% inhibition relative to the effect of the vehicle. These compounds were also evaluated for their ability to inhibit lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α production in rats.[17] Results are expressed as ID_{50} values, that is, the dose that caused 50% inhibition relative to the effect of the vehicle.

All the four possible isomers **2**–**5** were synthesized and evaluated as demonstrated in Table 1. Compounds **2a**–**b** and **3**, in which the aromatic moiety was located outside the concave bicyclo[3·3·0]octane framework, showed potent inhibitory activity against PDE4. The LPDE4 inhibitory activity of **2a**–**b** was equipotent to that of Ariflo **1**, while that of **3** was 15-fold weaker than that of **1**. Compounds **4** and **5**, with the aromatic moiety located inside the concave framework, exhibited no LPDE4 inhibitory activity at a concentration of 300 nM.

The carboxylic acid group was preferably oriented the *syn*-direction of the nitrile function and located inside the concave molecule, as illustrated by the greater potency of **2a** than **3**. Thus, these results showed that the PDE4 enzyme might recognize the more accesssible aromatic moiety first, while favoring more hindered carboxylic acid and nitrile groups.

Inhibition of TNF-α production in rats was also evaluated using **2a**–**b** and **3**. The in vivo activity of **2a** was 4-fold more potent than that of **1**, while **2b** and **3** were less potent than **1**. Compound **2a** was nearly 10-fold more potent than **3** (based on ID_{50} values) for inhibition of LPS-induced TNF-α production in rats, as predicted from their in vitro SAR.

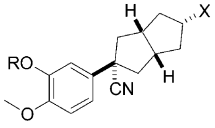
Table 1. Activity profile of bicyclo[3·3·0]octane derivatives

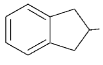


Compd	R_1	R_2	R_3	R_4	Inhibition of LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α[b] ID_{50} (mg/kg, po)
1 (Ariflo)					10	1.7
2a		CN	H	COOH	9.0	0.4
2b		CN	H	CONHOH	9.7	(52%)[c]
3		CN	COOH	H	150	3.8
4	CN		COOH	H	>300	NT[d]
5	CN		H	COOH	>300	NT[d]

[a] Inhibition of PDE4 prepared from U937 cells (a cell line derived from human monocytes). IC_{50} represent a mean of $n=2$.
[b] ID_{50} for inhibition of LPS-induced TNF-α production in rats ($n=7$) 0.5 h after oral dosing of a test compound.
[c] Inhibition% at 3 mg/kg, po.
[d] Not tested.

Table 2. Activity profile of bicyclo[3·3·0]octane derivatives



Compd	R	Inhibition of LPDE4[a] IC_{50} (nM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, po)
2a (X = COOH)		9.0	0.4
2b (X = CONHOH)		9.7	(52%)[c]
6a (X = COOH)	Me—	160	NT[d]
6b (X = CONHOH)		62	(55%)[c]
7a (X = COOH)	Et—	26	2.9
7b (X = CONHOH)		(56%)[b]	1.8
8a (X = COOH)	*i*-Pr—	62	(57%)[c]
8b (X = CONHOH)		12	(47%)[c]
9a (X = COOH)		1.3	1.7
9b (X = CONHOH)		0.85	(17%)[c]
10a (X = COOH)		100	NT[d]
10b (X = CONHOH)		29	3.0

[a] See corresponding footnotes from Table 1.
[b] Inhibition% at 10 nM.
[c] Inhibition% at 3 mg/kg, po.
[d] Not tested.

PDE4 is reported to be inhibited by various hydroxamic acid derivatives.[18,19] This information led us to synthesize a series of hydroxamic acid analogues which were expected to possess strong metal-mediated interactions. Conversion of the carboxylic acid function of **2a** to the hydroxamic acid function of **2b** maintained PDE4 inhibitory activity, but the inhibition of TNF-α production in rats was markedly decreased with **2b** relative to **2a**, probably because of problems such as instability of the hydroxamic acid function and/or poor oral availability.

As illustrated in Table 2, further structural optimization of the cyclopentyl moiety of **2a**, which exhibited the most potent activity among the compounds listed in

Table 3. Biological profile of **1**, **2a**, **7a** and **7b**

Compd	Inhibition of bronchoconstriciton[a] ID_{50} (mg/kg, po)	Inhibition of TNF-α production[c] ID_{50} (mg/kg, po)	Inhibition of gastric emptying[d] ID_{50} (mg/kg, po)	Inhibition of TNF-α production of HWB[f] ID_{50} (μM)
1 (Ariflo)	4.5	1.7	5.7	18
2a	9.6	0.4	(73%)[e]	21
7a	NT[b]	2.9	17	5.8
7b	5.0	1.8	12	0.80

[a] Inhibition of SRS-A-mediated bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs. ($n = 3$–6); OVA challenge 0.15 mg/kg 1 h after oral dosing of a test compound.
[b] Not tested.
[c] See corresponding footnotes from Table 1.
[d] Inhibition of gastric emptying in rats ($n = 5$).
[e] Inhibition% at 10 mg/kg, po.
[f] Inhibition of LPS induced TNF-α production in human whole blood. IC_{50} represent a mean of $n = 3$.

Table 1, was carried out. Replacement of the cyclopentyl moiety of **2a** with a methyl, ethyl and isopropyl groups led to **6a**, **7a**, and **8a**, respectively, which showed reduced LPDE4 inhibitory activity. Replacement of the cyclopentyl moiety of **2a** with an isoindanyl group afforded **9a**, which had 7-fold more potent LPDE4 inhibitory activity, but was nearly 4-fold less potent in the inhibition of LPS-induced TNF-α production presumably because of pharmacodynamic problems. Replacement of the cyclopentyl moiety of **2a** with a cyclopropylmethyl group produced **10a**, which showed 11-fold less potent LPDE4 inhibitory activity. When the corresponding hydroxamic acid analogues **6b**–**10b** were synthesized and evaluated, increased LPDE4 inhibitory activity was shown by all of these compounds. However, the in vivo potency of **9b**, which exhibited the strongest LPDE4 inhibitory activity, was not increased as expected from its in vitro potency and this was also true for the in vivo potency of **8b**.

Further evaluation of compounds **2a**, **7a** and **7b**, which were selected based on their potency in the in vivo TNF-α production assay, was carried out as shown in Table 3. These compounds were evaluated for their abilities to inhibit slow reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction[20,21] (a beneficial effect) and the inhibition of gastric emptying[11] (a side effect) in rats. The results were expressed as ID_{50} values, that is, the dose that caused 50% inhibition relative to the vehicle. These compounds were also evaluated for inhibition of LPS-induced TNF-α production in human whole blood (HWB)[22] to predict their clinical potential. Results were expressed as IC_{50} values, that is, the test compound concentration that caused 50% inhibition relative to the vehicle.

The potency of these compounds against SRS-A-mediated bronchoconstriction in actively sensitized guinea pigs was not always consistent with their inhibition of LPS-induced TNF-α production in rats, probably because of differences in pharmacokinetics related to cross-species comparison. Compound **2a** was 24-fold less potent in guinea pigs than in rats (based on ID_{50} value) and hydroxamic acid analogue **7b** was nearly 3-fold less potent in guinea pigs than in rats.

To assess safety within a single species, inhibition of gastric emptying by **2a**, **7a** and **7b** was evaluated. Compound **2a** caused more than 50% inhibition of gastric emptying at 10 mg/kg, po, while **7a** and **7b** had ID_{50} values of 17 and 12 mg/kg, po, respectively. The dose of **7b** that inhibited gastric emptying was found to be higher than that causing a beneficial effect. Based on its greater potency than that of Ariflo **1** for blocking the LPS-induced TNF-α production in HWB, **7b** was estimated to have an improved therapeutic potential as well as an improved side effect profile.

Based on the hypothesis that more rigid spatial fixing of the three pharmacophores (the carboxylic acid, nitrile, and aromatic moieties) of Ariflo within their optimized stereochemistry using another template could lead to the discovery of a new inhibitor, the design, synthesis, and evaluation of bicyclo[3·3·0]octane derivatives that showed more stereochemical diversity than the cyclohexane derivatives was carried out. Among the compounds tested, **2a**, **7a**, and **7b** potentially had improved therapeutic potential with less side effects based on biological data obtained by both cross-species comparison and same-species comparison. Full details will be reported in due course.

References and notes

1. Essayan, D. M. *J. Allergy Clin. Immunol.* **2001**, *108*, 671.
2. Houslay, M. D. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *69*, 249.
3. Teixeira, M. M.; Gristwood, R. W.; Cooper, N.; Hellewell, P. G. *Trends Pharmacol. Sci.* **1997**, *18*, 164.
4. Burnouf, C.; Pruniaux, M. P. *Curr. Pharm. Des.* **2002**, *8*, 1255.
5. Norman, P. *Exp. Opin. Ther. Patents* **2002**, *12*, 93.
6. Dyke, H. J.; Montana, J. G. *Exp. Opin. Investig. Drugs* **2002**, *11*, 1.
7. Duplantier, A. J.; Biggers, M. S.; Chambers, R. J.; Cheng, J. B.; Cooper, K.; Damon, D. B.; Eggler, J. F.; Kraus, K. G.; Marfat, A.; Masamune, H.; Pillar, J. S.; Shirley, J. T.; Umland, J. P.; Watson, J. W. *J. Med. Chem.* **1996**, *39*, 120.
8. Barnette, M. S.; Grous, M.; Cieslinski, L. B.; Burman, M.; Christensen, S. B.; Torphy, T. J. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1396.

9. Christensen, S. B.; Guider, A.; Forster, C. F.; Gleason, J. G.; Bender, P. E.; Karponski, J. M.; Dewolf, W. E.; Barnette, M. S.; Underwood, D. C.; Griswold, D. E.; Cieslinski, L. B.; Burman, M.; Bochnowicz, S.; Osborn, R. R.; Manning, C. D.; Grous, M.; Hillegas, L. M.; Bartus, J. O.; Ryan, M. D.; Eggleston, D. S.; Haltiwanger, R. C.; Torphy, T. J. *J. Med. Chem.* **1998**, *41*, 821.
10. Muller, T.; Engels, P.; Fozard, J. R. *Trends Pharmacol. Sci.* **1996**, *17*, 294.
11. Andres, J. I.; Alonso, J. M.; Diaz, A.; Fernandez, J.; Iturrino, L.; Martinez, P.; Matesanz, E.; Freyne, E. J.; Deroose, F.; Boeckx, G.; Petit, D.; Diels, G.; Megens, A.; Somers, M.; Wauwe, J. V.; Stoppie, P.; Cools, M.; Clerck, F. D.; Peeters, D.; Chaffoy, D. D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 653.
12. Hersperger, R.; Dawson, J.; Mueller, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 233.
13. Xu, R. X.; Hassell, A. M.; Vanderwall, D.; Lambert, M. H.; Holmes, W. D.; Luther, M. A.; Rocque, W. J.; Milburn, M. V.; Zhao, Y.; Ke, H.; Nolte, R. T. *Science* **2000**, *288*, 1822.
14. Lee, M. E.; Markowitz, J.; Lee, J. O.; Lee, H. *FEBS Lett.* **2002**, *530*, 53.
15. Huai, Q.; Wang, H.; Sun, Y.; Kim, H. Y.; Liu, Y.; Ke, H. *Structure* **2003**, *11*, 865.
16. Torphy, T. J.; Zhou, H.; Cieslinski, L. B. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 1195.
17. Tracey, K. J.; Cerami, A. *Annu. Rev. Med.* **1994**, *45*, 491.
18. Groneberg, R. D.; Burns, C. J.; Morrissette, M. M.; Ullrich, J. W.; Morris, R. L.; Darnbrough, S.; Diuric, S. W.; Condon, S. M.; McGeeham, G. M.; Lahaudiniere, R.; Neuenschwander, K.; Scotese, A. C.; Kline, J. A. *J. Med. Chem.* **1999**, *42*, 541.
19. Kleinman, E. F.; Campbell, E.; Giordano, L. A.; Cohan, V. L.; Jenkinson, T. H.; Cheng, J. B.; Shirley, J. T.; Pettipher, E. R.; Salter, E. D.; Hibbs, T. A.; Dicapua, F. M.; Bordner, J. *J. Med. Chem.* **1998**, *41*, 266.
20. Nakagawa, N.; Obata, T.; Kobayashi, T.; Okada, Y.; Nambu, F.; Terawaki, T.; Aishita, H. *Jpn. J. Pharmacol.* **1992**, *60*, 217.
21. Nakagawa, N.; Obata, T.; Kobayashi, T.; Okada, Y.; Nambu, F.; Terawaki, T.; Furuya, T.; Muryobayashi, K.; Sawada, M.; Aishita, H. *Eur. J. Pharmacol.* **1993**, *235*, 211.
22. Brideau, C.; Staden, C. V.; Sthyler, A.; Rodger, I. W.; Chan, C. C. *Br. J. Pharmacol.* **1999**, *126*, 979.