Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2737–2742

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2-SUBSTITUTED-4-METHOXYBENZIMIDAZOLE-BASED PDE4 INHIBITORS

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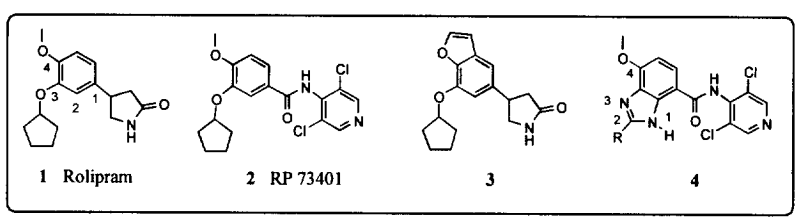
Received 6 July 1998; accepted 19 August 1998

Abstract: A new family of PDE4 inhibitors based on a benzimidazole framework is described. Several of these compounds are orally bioavailable and show efficacy in in vivo models of inflammatory disease.

Cyclic nucleotide phosphodiesterases (PDE1-7) are a family of enzymes that degrade the intracellular secondary messengers cAMP and cGMP[1] thereby limiting a host cell's response to extracellular signals mediated by these carriers. In a number of immunological cell types, elevation of cAMP levels by PDE4 inhibition[2] causes activation of negative feedback mechanisms resulting in suppression of the cell's inflammatory activities.[3] In particular, this type of manipulation brings about a marked reduction in the release of the proinflammatory cytokine TNF-α into the blood.[4] These observations have generated considerable interest in the use of PDE4 inhibitors for the treatment of various immunological disorders, including asthma and rheumatoid arthritis.[5,6]



The discovery of the first PDE4 selective inhibitor, rolipram (**1**), more than two decades ago[7] provided a benchmark for the development of more potent and selective analogues. Initially, the majority of research in this area focused on replacement of the pyrrolidinone of **1** with a wide range of other functionality.[8] In our laboratories, this approach led to the discovery of clinical candidate RP 73401 (**2**).[9] By contrast, modification of the 3,4-dialkoxyphenyl moiety of **1** (or its analogues) has been largely restricted to changes in the nature of the alkoxy substituents. The first example of a more radical alteration of the 4-methoxy-phenyl subunit of **1** was benzofuran **3**.[10a] In this paper, we report the synthesis and biological properties of a series of PDE4 inhibitors

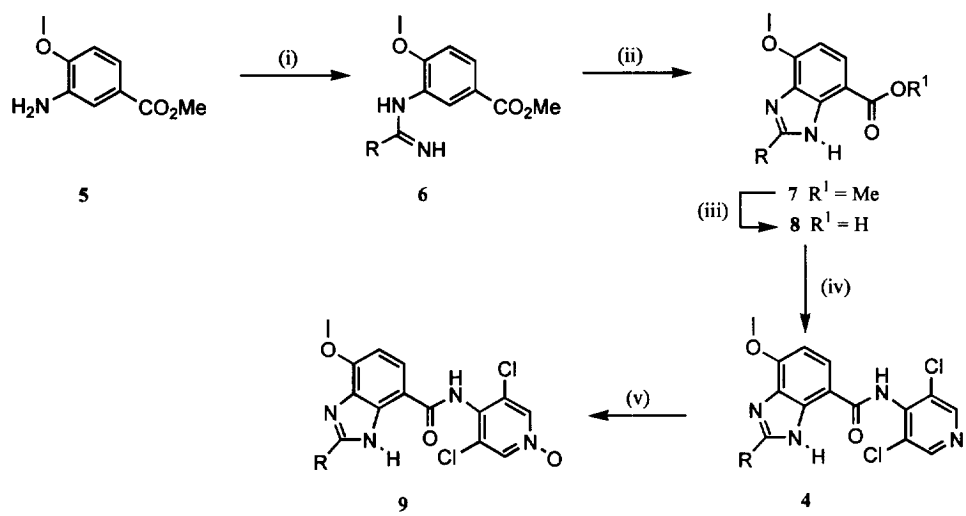
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00497-1

represented by **4**, in which the 3,4-dialkoxyphenyl group of **2** has been replaced with a 2-substituted-7-methoxy-benzimidazole.[10b,c] In implementing this modification, we hoped to retain the necessary recognition elements for enzyme inhibition while (a) reducing the susceptibility of the system to oxidative metabolism[11] (b) increasing water solubility by introduction of an additional heteroatom and (c) providing convenient attachment points (imidazole nitrogens) for the introduction of additional functionality to further probe and define the underlying pharmacophore.

Chemistry

The synthesis of the requisite benzimidazoles is illustrated in Schemes 1 and 2. Fusion of the appropriate nitrile (Scheme 1) with 3-amino-4-methoxy benzoic acid methyl ester **5**, followed by oxidative cyclization of the intermediate amidine **6** furnished the benzimidazole framework **7**.[12] After saponification of the ester group in **7**, the resulting acid **8** was activated (TBTU/DIEA) then coupled to 4-amino-3,5-dichloropyridine, using $NaEt_2AlH_2$,[13] to give the target amide **4**. Where desired, the pyridine nitrogen of **4** was oxidized to the corresponding *N*-oxide **9** using *m*-CPBA.

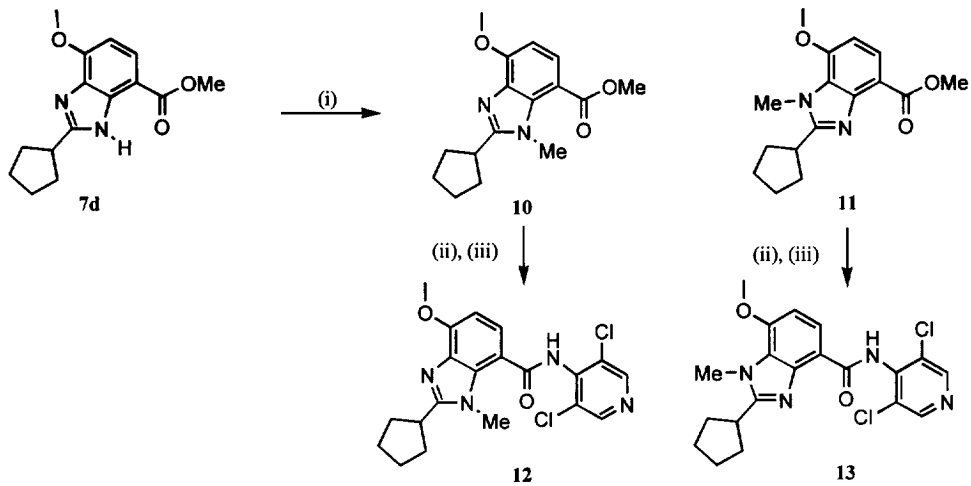
Scheme 1[a]



[a]*Reagents and Conditions*: (i) R-CN/*p*-TSA/Heat (60–90%). (ii) aq NaOCl then $NaHCO_3$/reflux (62-95%). (iii) aq NaOH, 50 °C (90–98%). (iv) TBTU/DIEA, rt then $NaEt_2AlH_2$/4-amino-3,5-dichloropyridine/toluene/THF (20–42% based on **8**). (v) *m*-CPBA, rt.

The *N*-methylated benzimidazoles **12** and **13** (Scheme 2) were prepared by alkylation of **7d** to give **10** and **11** as a 1/7 mixture which was separated by chromatography. Subsequent processing of **10** and **11** individually, as described above, provided the target compounds **12** and **13**. Structural assignment of the isomers was made on the basis of proton-carbon HETCOR analysis.

Scheme 2[a]



[a]*Reagents and Conditions*: (i) NaH/MeI/DMF/4 °C, (89% combined). (ii) aq NaOH, 50 °C (95%). (iii) TBTU/DIEA, rt then $NaEt_2AlH_2$/4-amino-3,5-dichloropyridine/toluene/THF (**12**: 56%; **13**: 43%).

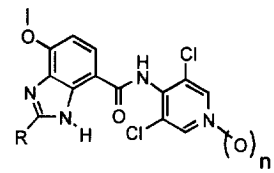
Biological Results

Table 1 summarizes the in vitro activity of a selection of benzimidazoles with respect to both inhibition of isolated guinea-pig macrophage PDE4 (IC_{50})[14] and association with the high affinity rolipram binding site (K_i).[15] In general, inhibition of PDE4 catalytic activity proved to be quite sensitive to side chain modifications at *C*-2 of the benzimidazole ring. The activities (IC_{50}) of **4a–f** clearly establish that substituents at this position play a role in the binding interaction but that a small group, such as a cyclopropyl, provides a sufficient presence to attain high activity. Indeed, increasing the size of the *C*-2 substituent (**4c–4f**) resulted in a reduction in potency. Incorporation of an oxygen atom into the side chain (**4g**) was tolerated providing a useful opportunity for increasing the polarity of these lipophilic compounds (e.g., **4d**, Clog P = 3.5 vs **4g**, Clog P = 1.5). Interestingly, the *N*-oxides **9c,g** were found to be equipotent with the corresponding pyridines **4c,g**. This observation would seem to argue against a direct H-bond between the pyridine ring of **4** and the enzyme and it may be that this interaction is, in fact, mediated by a water bridge. Alkylation of either nitrogen of the benzimidazole ring, as in **12** and **13**, resulted in a significant loss of potency (relative to **4d**). The loss in activity was most severe for **13**, which, although steric effects cannot be ruled out, may well reflect the importance of having an H-bond acceptor at *N*-3 of the benzimidazole.

Kinetic studies have shown that **1** binds to PDE4 in two distinct modes;[16] the so called low affinity site, which is associated with inhibition of catalytic activity and the high affinity site, the physiological significance of which remains unclear.[17] With respect to this discussion, the observation that the benzimidazoles in Table 1

inhibit catalytic activity and bind to the high affinity site, strongly supports the view that these compounds belong to the same pharmacophore family as 1.

Table 1



Compound	R	n	IC_{50} PDE 4 (nM)	K_i High Affinity Binding (nM)
1 (rolipram)			300	5
2 (RP73401)			1	1
4a	CH_3	0	450	712
4b	Cyclopropyl	0	2	1
4c	*Iso*-propyl	0	27	24
4d	Cyclopentyl	0	100	540
4e	$PhCH_2$	0	25	75
4f	$PhCH_2CH_2$	0	23	56
4g	CH_3OCH_2	0	42	25
9c	*Iso*-propyl	1	28	24
9g	CH_3OCH_2	1	45	45
12[a]			540	340
13[a]			4400	10000

[a]See Scheme 2 for structure

Several of the compounds from the benzimidazole series displayed good oral activity. Table 2 shows a selection of animal data for these compounds as measured by (a) inhibition of TNF-α release in mice upon lipopolysaccaride (LPS) challenge[18] (b) inhibition of streptococcal cell wall (SCW) induced arthritis in rats[19] and (c) oral bioavailability in mice.

Table 2

Compound	R[a]	n[a]	TNF-α Release ED_{50} (mg/kg, PO)	SCW-induced Arthritis ED_{50} (mg/kg, PO)	Bioavailability Mouse[b] (F%)
4c	$(CH_3)_2CH$	0	10	-	16
4g	CH_3OCH_2	0	2	10, tid	35
9g	CH_3OCH_2	1	7	5, bid	87

[a]Refers to the structure at the top of Table 1.

[b]Studies were conducted on female Balb/c mice at a dose of 1mg/Kg.

All three compounds were effective in the TNF-α release assay when dosed orally. Two of the compounds (**4g** and **9g**) were sufficiently bioavailable to warrant further evaluation in the SCW arthritis model. Here again, both compounds were effective, although for optimum results, **4g** required dosing three times a day, presumably due to its rapid clearance from plasma (**4g** $T_{1/2}$ = 1.75 h cf **9g** $T_{1/2}$ = 2.5 h).

In conclusion, replacement of the 3,4-dialkoxyphenyl substructure of **2** with a 2-substituted-4-methoxybenzimidazole framework conserves the recognition elements that are required for inhibitory activity against PDE4. Furthermore, appropriate substitution at C-2 of these benzimdazoles results in compounds with good oral bioavailability suggesting that the benzimidazole system may be a useful alternative scaffold to consider when designing new classes of PDE4 inhibitor with improved pharmacokinetic profiles.

Acknowledgements. We would like to thank Dr.Vasant Kumar for the HETCOR spectra and analysis of isomers **12** and **13**. We are also greatful to K.M. Page and his staff for the pharmacokinetic studies on this series of compounds.

References and Notes

1. Beavo, J. A. *Physiol. Rev.* **1995**, *75*, 725.
2. Bourne, H. R.; Lichtenstein, L. M.; Melmon, K. L.; Henry, C. S.; Weinstein, Y.; Shearer, G. M. *Science* **1974**, *184*, 19.
3. Verghese, M. W.; McConnell, R. T.; Strickland, A. B.; Gooding, R. C.; Stimpson, S. A.; Yarnall, D. P.; Taylor, J. D.; Furdon, P. J. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1313.
4. de Brito, F. B.; Souness, J. E.; Warne, P. J. *Emerging Drugs* **1997**, *2*, 249.
5. Marriot, J. B.; Westby, M.; Dalgleish, A. G. *Drug Develop. Today* **1997**, *2*, 273.
6. Palfreyman, M. N. *Drugs of the Future* **1995**, *20*, 793.
7. Schwabe, U.; Miyake, M.; Ohaga, Y.; Daly, J. W. *Mol. Pharmacol.* **1976**, *12*, 900.
8. Reviews: (a) Stafford, J. A.; Feldman, P. L. *Ann. Rep. Med. Chem.*; Academic: New York, 1996; Chapter 8, pp 71–81. (b) Davidsen, S. K.; Summers, J. B. *Exp. Opin. Ther. Patents* **1995**, *5*, 1087. (c) Palfreyman, M. N. and Souness J. E. *Prog. Med. Chem.* **1996**, *33*, 1.
9. Ashton, M. J.; Cook, D. C.; Fenton, G.; Karlsson, J-A.; Palfreyman, M. N.; Raeburn, D.; Ratcliffe, A. J.; Souness, J. E.; Thurairatman, S.; Vicker, N. *J. Med. Chem.* **1994**, *37*, 1696.
10. (a) Stafford, J. A.; Feldman, P. L.; Marron, B. E.; Schoenen, F. J.; Valvano, N. L.; Unwalla, R. J.; Domanico, P. L.; Brawley, E. S.; Leesnitzer, M. A.; Rose, D. A.; Dougherty, R. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1855. (b) For recent patent activity on the replacement of the 3,4-dialkoxyphenyl ring with benzofurans see: Byk Gulden Lomberg: WO 96/03399, 1996 and Chiroscience: WO 97/44337, 1997. (c) For recent reports of other heterocycles as surrogates for the 3,4-dialkoxyphenyl ring see: Hulme, C.; Moriarity, K.; Miller, B.; Mathew, R.; Ramanjulu, M.; Cox, P.; Souness, J.; Page, K. M.; Uhl, J.; Travis, J.; Huang, F-C.; Labaudinere, R.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1867; Chiroscience: WO 97/44036, 1997; Pfizer: WO 97/42174-A1, 1997 and Rhône-Poulenc Rorer: WO 97/48697, 1997.
11. Pharmacokinetic studies on **2** have shown that this compound undergoes extensive first pass metabolism in mice resulting in <5% bioavailability. Page, K. M. unpublished results.
12. Grenda, V. J.; Jones, R. E.: Gal, G.; Sletzinger, M. *J. Org. Chem.* **1965**, *30*, 259.

13. Sim, T. B.; Yoon, N. M. *Synlett.* **1994**, 827.

14. Turner, N. C.; Burns, F. M.; Guermy, T.; Souness, J. E. *Br. J. Pharmacol.* **1993**, *108*, 876. Compounds were routinely tested for PDE4 inhibition only. However, **4d** was evaluated against PDE1-3 (<20% inhibition at 10 μM) and PDE5 (60% at 10 μM vs. Dipyridamole IC_{50} = 0.5 μM under the same conditions)

15. Schmeichen, R.; Schneider, H. H.; Watchtel, H. *Psychopharmacol.* **1990**, *102*, 17.

16. Torphy, T. J.; Stadel, J. M.; Burman, M.; Cieslinski, L. B.; McLaughlin, M. M.; White, J. R.; Livi, G. P. *J. Biol. Chem.* **1992**, *267*, 1798.

17. (a) Barnette, M. S.; Grous, M.; Ciesliski, L. B.; Burman, M.; Christensen, S. B. Torphy, T. J. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1402. (b) Barnette, M.S.; Bartus, O'L. J.; Burman, M.; Christensen, S. B.; Prabhakar, U.S.; Rush, J. A. Trophy, T. J. *Biochem. Pharmacol.* **1996**, *51*, 949.

18. **Murine endotoxemia model**. Compounds were administered orally in a vehicle of 0.24% methylcellulose/0.2% Tween-20 in water to male, Balb/c mice (20–25 g, Harlan–Sprague Dawley, Inc., Indianapolis, IN) 4 h prior to challenge with LPS (*E. coli* 055:B5, Sigma Chemical Co., St. Louis, MO, 30 μg/mouse by intraperitoneal injection). Ninety minutes following LPS challenge, mice were anesthetized with Isoflurane and blood was collected by cardiac puncture. The whole blood was allowed to clot on ice and serum was prepared by centrifugation at 500 × *g* for 10 min. Serum was assayed for TNF-α by ELISA (Genzyme Corp., Cambridge, MA). Dose response curves were generated in duplicate from n = 4–6 mice per dose.

19. Esser, R. E.; Stimpson, S. A.; Cromartie, W. J.; Schwab, J. H. *Arthr. Rheum.* **1985**, *28*, 1402.