Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2137–2140

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

7-Methoxybenzofuran-4-carboxamides as PDE 4 Inhibitors: A Potential Treatment for Asthma

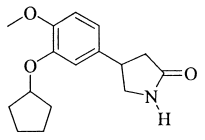
George Buckley,[a] Nicola Cooper,[a] Hazel J. Dyke,[a] Fiona Galleway,[a] Lewis Gowers,[a] Joanna C. Gregory,[a] Duncan R. Hannah,[a] Alan F. Haughan,[a] Paul G. Hellewell,[b] Hannah J. Kendall,[a] Christopher Lowe,[a] Robert Maxey,[a] John G. Montana,[a,*] Robert Naylor,[c] C. Louise Picken,[a] Karen A. Runcie,[a] Verity Sabin,[a] Bishwa R. Tuladhar[b] and Julie B. H. Warneck[a]

[a]*Celltech-Chiroscience Ltd, Granta Park, Abington, Cambridge CB1 6GS, UK*
[b]*Department of Pharmacology, National Heart and Lung Institute, Dovehouse St, London SW3 6LY, UK*
[c]*Department of Pharmacology, University of Bradford, Bradford, BD7 1DP, UK*

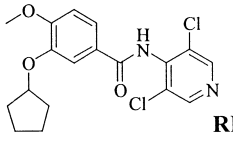
Received 17 April 2000; revised 12 July 2000; accepted 18 July 2000

Abstract—The synthesis and pharmacological profile of a novel series of 7-methoxybenzofuran-4-carboxamides is described. Some of these compounds were found to be potent inhibitors of phosphodiesterase type 4 (PDE4).

Posphodiesterase type 4 (PDE4) is a cAMP-specific phosphodiesterase present in inflammatory cells and airway smooth muscle. It catalyses deactivation of cAMP by hydrolysis of the phosphodiester bond. The elevated levels of cAMP which result from inhibition of PDE4 cause activation of the protein kinases responsible for decreasing inflammatory cell activity and airway smooth muscle tone, leading to supression of inflammatory cell functions and relaxation of airway smooth muscle.[1] These effects have prompted the investigation of PDE 4 inhibitors as a potential treatment for asthma.[2] The first selective PDE4 inhibitor to be identified was rolipram,[3] which also caused side effects of nausea and emesis. It has been suggested that in addition to binding to the catalytic site on the enzyme, rolipram also binds to a high-affinity site (known as the rolipram binding site)[4] and it is believed that binding to the high affinity site correlates with the emetic side effects.[5] Modifications to the structure of rolipram have been carried out to identify compounds with the PDE4 inhibiting properties of rolipram but without the side effects, giving leads such as **RP 73401**, in which the pyrrolidinone moiety is replaced with 3,5-dichloropyridyl-4-carboxamide.[6]



Rolipram



RP 73401

Our objective was to identify PDE4 inhibitors with good selectivity for the catalytic site over the rolipram binding site. Since inhibition of PDE3 may result in cardiotoxicity, selectivity for PDE4 over the PDE3 isozyme is also important.[7]

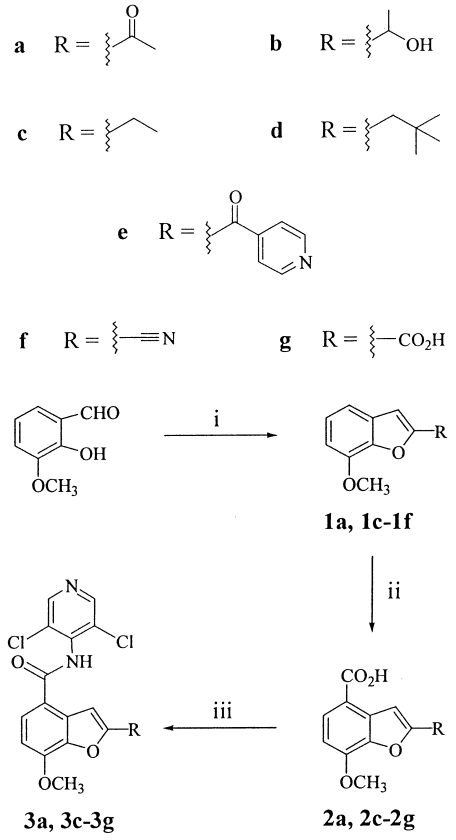
We have prepared a series of novel PDE4 inhibitors in which the 3,4-dialkoxyphenyl unit of **RP 73401** is replaced with a 7-methoxybenzofuran.[8] A variety of substituents have been incorporated at the 2-position of the benzofuran to investigate their effect on potency and selectivity.

Following the procedure of René and Reyer,[9] *o*-vanillin was reacted with the appropriate α-halo compounds in the presence of ethanolic potassium hydroxide to give the 2-substituted 7-methoxybenzofurans **1a**, **1e** and **1f**.

*Corresponding author. Tel.: +44-1223-896513; e-mail: johnmontana@chiroscience.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00426-1

1c and **1d** were obtained by Wolff–Kishner reduction of the corresponding carbonyl compounds. 7-Methoxybenzofuran-2-carboxylic acid **1g** was commercially available.[10] Introduction of a carboxylic acid at the 4-position of the 2-substituted benzofurans **1a** and **1c**–**1g** was carried out either by bromination followed by carbonylation, or by formylation followed by oxidation. The resulting acids **2a** and **2c**–**2g** were converted to acid chlorides and then coupled with the anion of 4-amino-3,5-dichloropyridine (preformed using sodium hydride in DMF) to give **3a** and **3c**–**3g** (Scheme 1). **3a** was reduced to the alcohol **3b** using sodium borohydride.



Scheme 1. Reagents: (i) XCH_2R, KOH, EtOH; (ii) Br_2, MeOH, then CO, $PdCl_2(PPh_3)_2$, Et_3N, H_2O, THF or $TiCl_4$, Cl_2CHOCH_3, then $NaClO_2$, $NaHPO_4$, ^tBuOH; (iii) $C_2Cl_2O_2$, DMF, CH_2Cl_2 then 4-amino-3,5-dichloropyridine, NaH, DMF.

Table 1. 2-Substituted benzofuran-4-carboxamides[a]

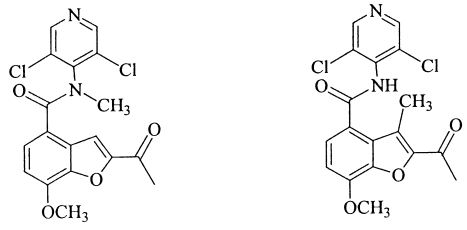
	PDE4 IC_{50}[11]	RBA IC_{50}[12]	PDE4:RBA[b]	PDE3
Rolipram	3.5	0.02	175	27%
3a	0.0016	0.0434	0.037	32%
3b	0.0086	0.0188	0.46	23%
3c	0.027	0.023	1.17	46%
3d	0.017	0.075	0.23	34%
3e	0.00241	0.0081	0.30	7%
3f	0.0068	0.0065	1.05	31%
3g	0.77	1.65	0.47	38%

[a]Values are shown as IC_{50} (μM) or percent inhibition at 20 μM and are the means of at least two experiments. RBA = rolipram binding assay.
[b]PDE4 was obtained from human U937 cells, rolipram binding protein was obtained from rat brain tissues, and PDE3 was obtained from human platelets.

The 7-methoxybenzofuran-4-carboxamides **3a**–**3g** were screened in in vitro assays (Table 1).

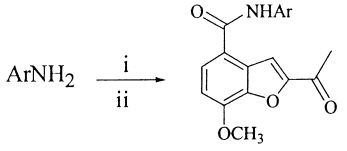
The 2-acetylbenzofuran **3a** was found to be the best compound in the series **3a**–**3g**. It was a potent inhibitor of PDE4, showed excellent selectivity for the catalytic site over the rolipram binding site, and was inactive against PDE3. The alcohol **3b** was also a potent inhibitor of PDE4, but its ratio of binding to the catalyic site compared with binding to the rolipram site was not as good as that of the ketone **3a**. The ethyl compound **3c** was 10-fold less potent than the ketone **3a**, illustrating the need for a hydrogen-bonding group at the 2-position for optimal activity. Replacing the cyclopentyl group in rolipram with bulky lipophilic substituents has been reported to improve the PDE4:RBA ratio,[13] but in this series the 2,2-dimethylpropyl compound **3d** was found to be 10-fold less selective than the 2-acetyl compound **3a**. The pyridylcarbonyl compound **3e** and the nitrile **3f** were both very potent inhibitors of PDE4, but their PDE4:RBA ratios were not satisfactory. The carboxylic acid **3g** had little PDE4 inhibitory activity.

Having identified the 2-acetyl-7-methoxybenzofuran **3a** as a potent selective inhibitor of PDE4, we went on to investigate the effect of methylating the amide nitrogen and the 3-position of the benzofuran ring on potency and selectivity. The *N*-methyl and 3-methyl analogues of **3a** (**4** and **5** respectively) were prepared and screened in in vitro assays. They showed only modest PDE4 inhibitory activity (Table 2).



4 **5**

We also investigated the effect of replacing the 3,5-dichloropyridyl moiety with other substituted 6-membered aromatic rings. A variety of aromatic amines were coupled



Scheme 2. Reagents: (i) NaH, DMF; (ii) 2-acetyl-7-methoxybenzofuran.

Table 2. Methylated 2-acetylbenzofuran-4-carboxamides[a]

	PDE4 IC_{50}[11]	RBA IC_{50}[12]
4	0.33	0.15
5	0.32	0.16

[a]Values are shown as IC_{50} (μM) and are the means of at least two experiments. RBA = rolipram binding assay.

Table 3. *N*-Heterocyclic 2-acetylbenzofuran-4-carboxamides[a]

	Ar	PDE4 IC_{50}[11]	RBA IC_{50}[12]	PDE4:RBA
3a	3,5-Dichloropyrid-4-yl	0.0016	0.0434	0.037
6a	3-Chloropyrid-4-yl	0.0063	0.032	0.197
6b	Pyrid-4-yl	0.12	0.25	0.48
6c	2-Chloropyrid-3-yl	0.875	0.199	4.40
6d	3-Methylpyrid-2-yl	0.452	1.85	0.24
6e	5-Chloropyrimidin-4-yl	0.164	0.0596	2.75
6f	2-Chlorophenyl	0.054	0.248	0.21
6g	2,6-Dichloro-4-cyanophenyl	0.0851	0.228	0.37

[a]Values are shown as IC_{50} (μM) and are the means of at least two experiments. RBA = rolipram binding assay.

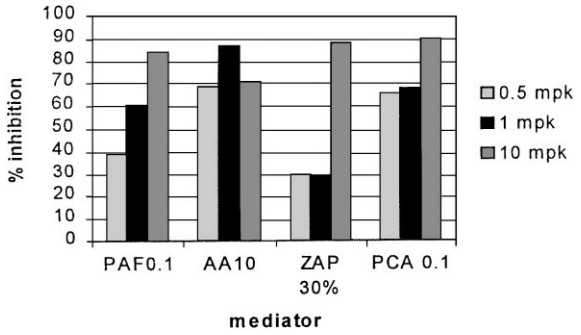


Figure 1. Inhibition of eosinophilia in the guinea pig by oral dosing of **3a**. Values are shown are the means of two experiments.

with 2-acetyl-7-methoxybenzofuran 4-carboxylic acid (Scheme 2) to give the compounds **6a**–**6h**, which were screened in in vitro assays as described above (Table 3).

The monochloropyridyl compound **6a** was almost as potent as the dichloro analogue **3a**, but its ratio was inferior. The pyridyl compound **6b** was much less active, indicating that for good activity against PDE4 it was essential to have at least one substituent *ortho* to the amide bond. Moving the pyridine nitrogen around the ring as in **6c** and **6d** was also detrimental. Location of the pyridine nitrogen *meta* to the amide bond (**6c**) resulted in selectivity for the rolipram binding site over the catalytic site, as did incorporation of a second nitrogen into the ring as in the pyrimidine **6e**. Removing the pyridine nitrogen (**6f**) caused a 10-fold drop in activity. A nitrile substituent *para* to the amide bond was tolerated, but the 4-cyano-2,6-dichlorophenyl compound **6g** was neither as active nor as selective as the corresponding dichloropyridyl compound **3a**.

On the basis of its high potency against PDE4 and good selectivity for the catalytic site over the rolipram binding site, compound **3a** was selected for evaluation in a guinea pig skin eosinophilia model.[14] It was administered po at 0.5, 1 and 10 mpk, and showed good activity across a range of mediators[15] as shown in Figure 1.

3a was also assessed for emetic and CNS side effects in a ferret emesis model.[16] Neither emesis nor CNS effects were observed when **3a** was dosed orally at 10 mpk indicating that there is a significant difference between the efficacious and emetic doses for this compound.

In summary, we have identified a series of novel benzofuran based PDE4 inhibitors. The 2-acetyl compound **3a** showed good oral activity in a functional model of inflammation at doses which showed none of the CNS and emetic side effects associated with the prototypical PDE4 inhibitor, rolipram. Work is now in progress to optimise this lead.

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