Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1867–1872

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL SERIES OF INDOLE PDE4 INHIBITORS I

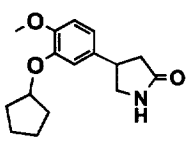
Christopher Hulme,* Kevin Moriarty, Bruce Miller, Rose Mathew, Mercy Ramanjulu, Paul Cox,† John Souness,† Ken M. Page,† Joanne Uhl, Jeffrey Travis, Fu-chih Huang, Richard Labaudiniere, and Stevan W. Djuric

Rhône-Poulenc Rorer Central Research, 500 Arcola Road, Collegeville, PA 19426, U.S.A.
†Rhône-Poulenc Rorer Central Research, Dagenham, Essex, United Kingdom

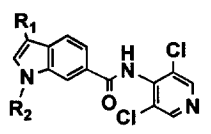
Received 6 April 1998; accepted 26 May 1998

Abstract: This communication describes the synthesis and in vitro evaluation of a novel potent series of phosphodiesterase type (IV) (PDE4) inhibitors. The compounds described contain an indole moiety which replaces the 'rolipram-like' 3-methoxy-4-cyclopentoxy motif. Several of the compounds presented possess low nanomolar IC_{50}'s for PDEIV inhibition. In vivo activities determined from measurement of serum TNF-α levels in LPS challenged mice (mouse endotoxemia model) are also reported.

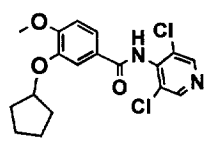
The family of cyclic nucleotide phosphodiesterases (PDE's) regulate levels of the secondary messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) via hydrolysis to their acyclic and inactive analogues 5-adenosine monophosphate (5'-AMP) and 5-guanosine monophosphate (5'-GMP), respectively.[1] Of the seven PDE isoenzymes identified to date,[2] PDE4 is most abundantly found in inflammatory cells such as monocytes and macrophages. The positive effect that increased levels of cAMP has on reducing production of the pro-inflammatory cytokine tumor necrosis factor (TNF-α) and promoting airway smooth muscle relaxation in such proinflammatory cells has generated widespread interest in PDE4 as a potential target for treatment of autoimmune diseases.[3] Many efforts have focused on modification of the archetypal PDE4 inhibitor rolipram, **1**, to access novel series of PDE4 inhibitors with improved pharmacological profiles, including recent reports from this laboratory.[4] In fact, the 3-methoxy-4-cyclopentoxy motif is observed in the majority of PDE4 inhibitors reported to date.[5] This letter reports the synthesis, PDE4 inhibitory activity, and in vivo effects on serum TNF-α levels of a novel series of indoles, with general structure **2**, derived from one of the most potent PDE4 inhibitors reported to date, RP73401, **3**.[6]



Rolipram
1



2

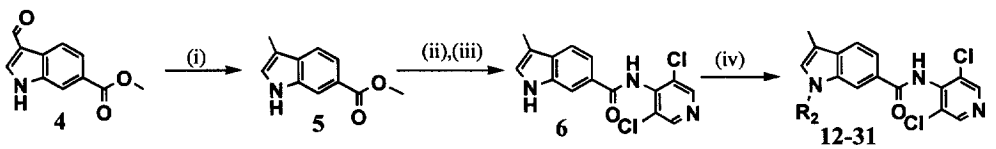


RP 73401
3

The direct replacement of the catechol-like rolipram motif with a 3,4-substituted indole derivative represents one of the first conformationally constrained analogues at these substitution sites to be reported.[7] The following straightforward synthetic scheme was initially developed to probe SAR at R_2, Scheme 1. Based on

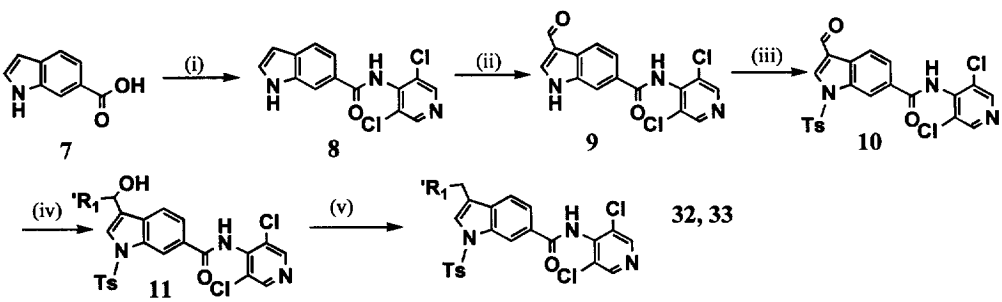
0960-894X/98/$19.00
PII: S0960-894X(98)00324-2

analogous SAR with other rolipram-like inhibitors it was assumed there would be little tolerance for steric bulk at R_1, which is maintained as methyl throughout SAR studies at R_2. Commercially available methyl-3-formylindole-6-carboxylate, **4**, was reduced to the corresponding methyl derivative, **5**, in good yield. Ester hydrolysis, followed by TBTU [2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate] activation[8] of the newly formed carboxylic acid and coupling with 3,5-dichloroaminopyridine gave the intermediate benzamide **6**. SAR studies at R_2 were thus straightforward by facile alkylation/acylation/sulfonylation with the corresponding alkyl/acyl/sulfonyl halides with NaH giving compounds **12–31**.



Scheme 1. *Reagents and conditions*: (i) *p*-toluenesulfonic acid (0.15 equiv), *p*-toluenesulfonylhydrazide (1.2 equiv), sulfolane, DMF, 100 °C. Then $NaCNBH_3$, 100 °C, 2 h, 70%. (ii) LiOH (2 equiv), MeOH:H_2O, 3:1, 70 °C, 1 h, 90%. (iii) TBTU (1.1 equiv), *N,N*-diisopropylethylamine (2 equiv), CH_2Cl_2. Then 4-amino-3,5-dichloropyridine (14 equiv), $NaAlH_2Et_2$ (6.9 equiv), toluene, 50 °C, 1 h, 40 to 90%. (iv) NaH (2.2 equiv), R_2Cl, DMF, 0 °C, 2 h, 70 to 90%.

A brief SAR study of substitution at R_1 was performed via the following general route (Scheme 2). 6-Indole carboxylic acid, **7**, was coupled with 4-amino-3,5-dichloropyrdine to give, **8**. Sequential Vilsmeier oxidation to give **9** and N-sulfonylation produced the desired formyl derivative, **10**. Grignard addition gave the alcohols with general structure **11** and triethylsilane acid catalysed reduction[9] afforded **32** and **33**.



Scheme 2. *Reagents and conditions*: (i) **7**, TBTU (1.1 equiv), *N,N*-diisopropylethylamine (2 equiv), CH_2Cl_2, 2 h, rt. Then 4-amino-3,5-dichloropyridine (10 equiv), $NaAlH_2Et_2$ (5 equiv), toluene, 100 °C, 1 h. Add TBTU activated ester to the latter solution. 100 °C, 2 h, 70%. (ii) $POCl_3$, DMF, 40 °C, 1 h, 30%. (iii) NaH (2.2 equiv), DMF, *p*-toluenesulfonylchloride (1.1 equiv), 0 °C, 1 h, 90%. (iv) R_2MgBr (1.5 equiv), THF, 0 °C, 2 h, 55%. (v) Et_3SiH (2 equiv), BF_3. Et_2O (1 equiv), 25 °C, 3 h, 40%.

Compounds were evaluated for PDE4 inhibition using the methods of Thompson and Schmeichen, respectively.[10] In vivo activity was determined in a mouse endotoxemia assay measuring inhibition of LPS-induced TNF-α production (RP73401 ED_{50} 6–10 mg/kg).[11] Activities of the two standards rolipram (PDE4 IC_{50} 320 nM and K_i rolipram binding 4.5 nM) and RP73401 (PDE4 IC_{50} 1 nM and K_i rolipram binding 0.4 nM) were

determined in-house using these procedures. TNF-α inhibition in intact cells was determined in activated human monocytes.[12] The results are summarized in Table 1.

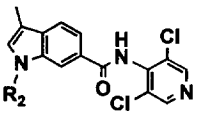
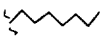
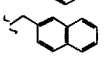
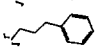
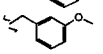
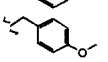
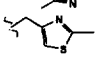
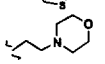
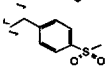
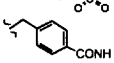
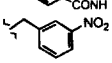


Table 1

Compd	R_1	R_2	PDE4 IC_{50} nM	K_i rolipram binding nM	Whole Cell (HM) IC_{50} nM (RPT240) nM	ED_{50} (mouse) TNF-α Inhib.
12	CH_3		15	374	30%@0.3μM (3)	-
13	CH_3		100	120	200 (8)	0%@50mpk
14	CH_3		3.2	21	2 (0.7)	0%@50mpk
15	CH_3		220	187	12 (0.7)	27%@50mpk
16	CH_3		25	66	11 (3)	22%@50mpk
17	CH_3		18	135	23 (4)	0%@50mpk
18	CH_3		5.2	7	90 (10.5)	0%@50mpk
19	CH_3		70	280	8(0.9)	-
20	CH_3		25	41	168 (3)	17%@50mpk
21	CH_3		46	60	1300 (10)	-
22	CH_3		80	54	-	-
23	CH_3		60	75	-	-
24	CH_3		12	13	60 (3)	20%@30mpk
25	CH_3		187	75	580 (18)	0%@50mpk
26	CH_3		42	24	24.1 (1.5)	0%@50mpk
27	CH_3		380	172	28%@0.3μM (3)	-
28	CH_3		200	300	134 (>1)	0%@50mpk
29	CH_3		300	220	53 (<1)	0%@50mpk
30	CH_3		52	75	34 (2)	16%@50mpk
31	CH_3		45	712	300 (3)	0%@100mpk
32	CH_3CH_2		2900	>3745	-	-
33			>10000	>3745	-	-

The synthetic intermediate **6** (R_2 = H) possessed low PDE4 inhibitory activity (PDE4 IC_{50} 5 μM). Initial efforts therefore concentrated on investigation of lipophilic side chains at R_2. Most encouragingly, excellent PDE4 inhibitory activity was observed for the cyclopentyl analogue, **12**, (PDE4 IC_{50} 15 nM). However, inhibition of TNF-α release (HM) was relatively poor when compared to RP73401 (**12**, 30%@0.3 μM, RP73401 IC_{50} 3 nM). Further compounds **13–24** were thus prepared with differing lipophilic side chains at R_2. Improved PDEIV activities over the cyclopentyl derivative, **12**, were observed for the cyclohexylethyl, **14**, (PDEIV IC_{50} 3.2 nM), the 2-napthyl **18**, (PDE4 IC_{50} 5.2 nM) and *p*-methoxybenzyl, **24** (PDE4 IC_{50} 12 nM) derivatives. Interestingly, a 10-fold drop in both PDE4 activity and whole cell activity was found on changing from 2- to 1-napthyl (**21**, PDE4 IC_{50} 46 nM, WC IC_{50} 1300 nM) substitution. SAR derived from a methoxyl scan of **17**, also indicated a lowering of PDE4 activity when substituting at the 2- and 3-positions with *para*-substitution being preferable (a similar result was observed with an analogous methyl scan but is not reported in this letter). Of additional note is the excellent selectivity of the cyclohexylethyl derivative, **14**, for the catalytic binding site (PDE4 3.2 nM) over the rolipram binding site (K_i 21 nM). Such selective binding is often cited as a potential solution to the often seen emetic side effect of potent PDE4 inhibitors.[13] SAR at R_1 was also briefly investigated maintaining a *p*-toulenesulfonyl group at R_2. In analogous fashion to previous 'rolipram-like' analogues, a large drop in PDE4 activity was observed with increasing steric bulk at this position, exemplified by the ethyl, **32** (PDE4 IC_{50} 2900 nM) and benzyl, **33** (PDE4 IC_{50} >10000 nM) analogues. The highest whole cell activity for TNF-α inhibition was observed for the *N*-cyclohexylethyl, **14** [IC_{50} 2 nM (RP73401 IC_{50} 0.7 nM)] *N*-heptyl, **16** [IC_{50} 11 nM (RP73401 IC_{50} 3 nM)] and *N*-benzyl, **17** [IC_{50} 23 nM (RP 73401 IC_{50} 4 nM)] derivatives. In vivo evaluation of a selection of the compounds in Table 1 revealed poor inhibition of TNF-α relative to RP73401 (ED_{50} 6–10 mg/kg). For example, the *N*-heptyl derivative, **16**, possessing the most potent whole cell activity, only showed 22% inhibition of TNF-α at the high dose of 50 mpk in male Balb/c mice. A preliminary investigation into the pharmacokinetic profile of the potent *p*-methoxybenzyl derivative, **24**, in the female, Balb/c mouse following a single iv and oral dose was therefore undertaken to assess the oral bioavailability of this class of compound.[14] The pharmacokinetic parameters are shown in Table 2.

Table 2

Dose Route	Cp max ($ng.mL^{-1}$)	T max (h)	AUC_{0-x} ($h.ng.mL^{-1}$)	Terminal $t_{1/2}$ (h)	Cl ($L.h^{-1}.kg^{-1}$)	Vdss ($L.kg^{-1}$)	% Bioavailability
iv	n/a	n/a	677.8	2.6	1.48	2.4	n/a
oral	9.9	0.5	25.3	1.7	n/a	n/a	3.7

n/a: not applicable

The systemic bioavailability of this compound was 3.7%. Clearance following a single iv dose was determined to be 1.48 L. $h^{-1}.kg^{-1}$, a figure < 30% that of liver blood flow in the mouse (ca 5.1 L. $h^{-1}.kg^{-1}$). This data suggests that poor absorption, as opposed to a large first pass effect was responsible for the low oral bioavailability of this compound and potentially the low in vivo activity for TNF-α inhibition. A series of

compounds were therefore prepared containing more polar functionality, with an associated lower clogP, by varying R_2. In general, as expected, compounds **25–30**, possessed lower PDE4 activities than their lipophilic counterparts.[1] However, the thiazole, **26**, (PDE4 IC_{50} 42 nM) and the 3-nitrobenzyl derivative, **30** (PDE4 IC_{50} 52 nM) did maintain good potency. Unfortunately, no improvement for in vivo TNF-α release inhibition was observed.

In summary, the synthesis and in vitro evaluation of a novel series of potent PDE4 inhibitors has been reported, demonstrating that the indole skeleton is an effective isostere for the catechol of RP73401. This potency is similarly reflected in several examples assessed for TNF-α release inhibition in the human monocyte screen. PDE4 SAR for the new series paralleled closely that of previous rolipram-like inhibitors, namely little tolerance for steric bulk at R_1 and a preference for large lipophilic groups at R_2. Good whole cell activity, however, did not translate into encouraging TNF-α release in vivo. Further efforts at improving the in vivo potency of this class of compound will be reported in due course.

Acknowledgements We would like to thank Dr Sheng-Yuh Tang and Jill Dreibelbis of the mass spectrometry department.

References and Notes

1. For two reviews see (a) Palfreyman, M. N. *Drugs of the Future* **1995**, *20*, 793. (b) Torphy, T. J.; Livi, G. P.; Christensen, S. B. *DN & P* **1993**, *6*, 203.

2. Beavo, J. A.; Conti, M.; Heaslip, R. J. *Mol. Pharmacol.* **1994**, *46*, 399.

3. (a) Verghese, M. W.; McConnenell, 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. (b) Renz, H.; Gong, J-H.; Schmidt, A.; Nain, M.; Gemsa, D. *J. Immunol.* **1988**, *141*, 2388. (c) Sullivan, P. J.; Bekir, S.; Jaffar, Z.; Page, C. P.; Jeffrey, P. K.; Costello, J. *Lancet* **1994**, *343*, 1006. (d) Raeburn, D.; Underwood, S. L.; Lewis, S. A.; Woodman, V. R. ; Battram, C. H.; Tomkinson, A.; Sharma, S.; Jordan, R.; Souness, J. E.; Webber, S. E.; Karlsson, J-A. *Brit. J. Pharmacol.* **1994**, *113*,1423. (e) Waage, A.; Haltensen, A.; Espink, T. *Lancet* **1987**, *1*, 355. (f) Matsuura, A.; Ashizawa, N.; Asakura, N.; Kumonaka, T.; Aotsuka, T.; Hase, T.; Shimizu, C.; Kurihara, T.; Kobayashi, F. *Biol. Pharm. Bull.* **1994**, *17*, 498. (g) Williams, R. D.; Feldmann, M.; Maini, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9783.

4. (a) Hulme, C.; Poli, G.B.; Huang, F-C.; Souness, J.E.; Djuric, S.W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 175. (b) Hulme, C.; Moriarty, K.; Huang, F-C.; Mason, J.; McGarry, D.; Labaudiniere, R.; Souness, J.; Djuric, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 399.

5. (a) 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. (b) Stafford, J. A.; Veal, J. M.; Feldman, P. L.; Valvano, N. L.; Baer, P. G.; Brackeen, M. F.; Brawley, E. S.; Connolly, K. M.; Domanico, P. L.; Han, B.; Rose, D. A.; Rutkowske, R. D.;Sekut, L.; Stimpson, S. A.; Strickland, A. B.; Verghese, M. W. *J. Med. Chem.* **1995**, *38*, 4972. (c) Masamune, H.; Cheng, J. B.; Cooper, K.; Eggler, J. F.; Marfat, A.; Marshall, S. C.; Shirley, J. T.; Tickner, J. E.; Umland, J. P.;Vazquez, E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1965. (d) Marivet, M. C.; Bourguignon, J-J.; Lugnier, C.; Mann, A.; Stoclet, J-C.; Wermuth, C-G. *J. Med.Chem.* **1989**, *32*, 1450.

6. (a) Ashton, M. J.; Cook, D. C.; Fenton, G.; Karlsson, J-A.; Palfreyman, M. N.; Raeburn, D.; Ratcliff, A. J.; Souness, J. E.; Thurairatnam, S.; Vicker, N. *J. Med. Chem.* **1994**, *37*, 1696.

7. (a) A series of PDE4 inhibitors containing an indazole replacement for the 3-methoxy-4-cyclopentoxy 'rolipram-like' motif has recently been reported : Marfat, A.; WO9742174-A1, **1997**. (b) 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

8. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

9. Orfanopoulos, M.; Smonou, I. *Synth. Comm.* **1988**, *18*, 833.

10. Batches of PDE4 were obtained from guinea-pig macrophages. See Turner, N. C.; Wood, L. J.; Burns, F. M.; Guermy, T.; Souness, J. E. *Br. J. Pharmacol.* **1993**, *108*, 876. PDE4 IC_{50}'s were determined in macrophage homogenates via a two-step radioisotopic method. See Thompson, W. J.; Teraski, W.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucleotide Res.*, **1979**, *10*, 69. K_i values were determined using [^{3}H] rolipram in a guinea pig brain membrane binding assay. See Schmeichen, R.; Schneider, H. H.; Watchtel, H. *Psychopharmacology* **1990**, *102*, 17.

11. For TNF-α production in vivo Male, Balb/c mice (20–25 g, Harlan-Sprague Dawley, Inc., Indianaopolis, IN) were used. For evaluation of test compounds, compounds were administered by oral gavage in a suspension (0.5% methylcellulose/0.2% Tween-80 vehicle) 4 h prior to challenge with lipopolysaccharide (LPS). TNF-α production was elicited by an intraperitoneal injection of LPS (*E. coli* 055:B5, Sigma Chemical Co., St. Louis, MO) at a dose of 30 μg/mouse. Ninety minutes after LPS injection, mice were anesthetized with Isoflurane and blood collected by cardiac puncture. The whole blood was allowed to clot at room temperature and serum was prepared by centrifugation at 500 *g* for 10 min. TNF-α levels in the serum were measured by a mouse TNF-α ELISA (Genzyme Corp., Cambridge, MA).

12. Human peripheral blood monocytes were isolated from heparinized whole blood using Histopaque 1077 (Sigma Chemical Co., St. Louis, MO). The cells were washed and resuspended in tissue culture media (RPMI 1640 with 5% fetal bovine serum, 50 μg/mL gentamicin and nonessential amino acids). Cells were plated in 96-well plates at a density of 2×10^5 cells/well and allowed to adhere for 2 h. Test compounds were added followed by addition of 0.01% *Staphylococcus aureus* (IgGSORB, The Enzyme Center, Malden, MA) to activate the cells. The cells were then incubated overnight and the media was collected and assayed for TNF-α by ELISA (Human TNF-α Duoset, Genzyme Corp., Cambridge, MA).

13. Christensen, S. B.; Guider, A.; Forster, C. J.; Gleason, J. G.; Bender, P. E.; Karpinski, 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.; Ryan, M. D.; Eggleston, D. S.; Haltiwanger, R. C.; Torphy, T. J. *J. Med. Chem.*, **1998**, *41*, 821.

14. IV dosing was performed at 1 mg/kg as a solution in DMSO. Oral dosing was performed at 1 mg/kg as a suspension in 1% aq carboxymethyl cellulose/0.2%Tween 80. A minimum of three animals per time point were used, with animals being sacrificed at the appropriate time after dosing by CO_2 asphyxiation, and blood obtained by cardiac puncture.