Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3053–3058

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ORALLY ACTIVE INDOLE *N*-OXIDE PDE4 INHIBITORS

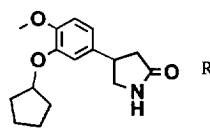
Christopher Hulme,[a,*] Rose Mathew,[a] Kevin Moriarty,[a] Bruce Miller,[a] Mercy Ramanjulu,[a] Paul Cox,[b] John Souness,[b] Ken M. Page,[b] Joanne Uhl,[a] Jeffrey Travis,[a] Richard Labaudiniere,[a] Fu-chih Huang[a] and Stevan W. Djuric[c]

[a]*Rhône-Poulenc Rorer Central Research, 500 Arcola Road, Collegeville, PA 19426, U.S.A.*
[b]*Rhône-Poulenc Rorer Central Research, Dagenham, Essex, United Kingdom*
[c]*Present address: Immunological Diseases Research, Abbott Laboratories, Abbott Park, IL, 60064, U.S.A.*

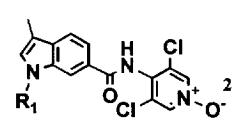
Received 22 July 1998; accepted 9 September 1998

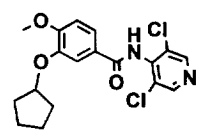
Abstract: This communication describes the synthesis and in vitro and in vivo evaluation of a novel potent series of phosphodiesterase type (IV) (PDE4) inhibitors. Several of the compounds presented possess low nanomolar IC_{50}'s for PDE4 inhibition and excellent in vivo activity for inhibition of TNF-α levels in LPS challenged mice (mouse endotoxemia model). Emesis studies (dog) and efficacy in a SCW arthritis model for the most potent PDE4 inhibitors are presented.

The therapeutic importance of the cytokine tumor necrosis factor (TNF-α) is well-established.[1] The efficacious effects of anti-bodies against TNF-α in the treatment of rheumatoid arthritis have further increased interest in this pivotal cytokine.[2] Several approaches are currently being evaluated for the inhibition of production of this cytokine, as excessive concentrations have been implicated in the pathogenesis of a large number of autoimmune disease states, including asthma, septic shock, and rheumatoid arthritis.[3] Inhibition of the enzyme phosphodiesterase type IV (PDE4), which leads to increased levels of the 2° messenger cyclic-adenosine monophosphate (cAMP) by reducing its PDE4 catalysed hydrolysis to acyclic 5'-AMP, and, consequently a drop in TNF-α production, is one such approach.[4] The PDE4 inhibitor rolipram, **1**, has been the starting point for many approaches for the design of novel PDE4 inhibitors and introduced the now common 3-methoxy-4-cyclopentoxy motif commonly observed in many of these new series.[5]



Rolipram
1



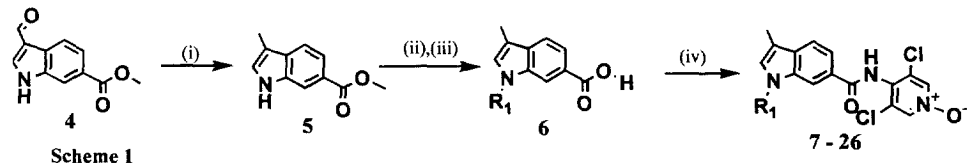


RP 73401
3

This paper presents a novel series of potent indole *N*-oxide PDE4 inhibitors possessing in vivo activity for inhibition of TNF-α release with the general structure, **2**. Encouraging emesis results (dog model) and efficacious effects in the Streptococci cell wall (SCW) arthritis model are also presented. The compounds contain a highly effective indole isostere[6] for the 3-methoxy 4-cyclopentoxy moiety commonly observed in many rolipram-like PDE4 inhibitors and as such represent conformationally constrained analogues of RP73401, **3**, one of the most potent PDE4 inhibitors reported to date.[7] The *N*-oxide derivative of RP73401 reported in the

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00572-1

latter article maintained excellent PDE4 inhibitory activity. The following synthetic scheme was developed to access this class of indole *N*-oxide. Commercially available methyl-3-formylindole-6-carboxylate, **4**, was reduced to the corresponding methyl derivative, **5**, in good yield (70%). The indole, **5**, was alkylated with R_1Cl affording compounds with the general structure **6**. Ester hydrolysis, followed by TBTU [2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate] activation[8] of the newly formed carboxylic acid and coupling with 3,5-dichloroaminopyridine *N*-oxide gave the desired compounds **7** - **26**.

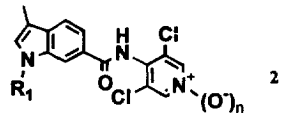


Scheme 1

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) NaH (2.2 equiv), R_1Cl, DMF, 0 °C, 2 h, 70–90%. (iii) LiOH (2 equiv), $MeOH/H_2O$, 3/1, 70 °C, 1 h, 90%. (iv) TBTU (1.1 equiv), N,N-diisopropylethylamine (2 equiv), CH_2Cl_2. Then 4-amino-3,5-dichloropyridine *N*-oxide (14 equiv), $NaAlH_2Et_2$ (6.9 equiv), toluene, 50 °C, 1 h, 40–90%.

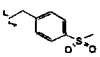
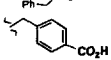
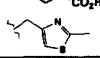
Compounds were evaluated for PDE4 inhibition using the methods of Thompson and Schmeichen.[9] In vivo activity was determined in a mouse endotoxemia assay measuring inhibition of LPS-induced TNF-α production (RP73401 ED_{50} 3.1 mg/kg).[10] Activities of the two standards rolipram, **1** (PDE4 IC_{50} 320 nM and K_i rolipram binding 4.5 nM) and RP73401, **3** (PDE4 IC_{50} 1 nM and K_i rolipram binding 0.4 nM) were determined using these procedures. Emesis studies were performed iv on selected examples in the dog.[11] The pharmacological profiles of a series of indole *N*-oxide analogues of RP73401 are presented in Table 1. Initial PDE4 SAR is clearly analogous to that previously reported for both rolipram and RP73401, namely a preference for bulky lipophilc groups over more polar substitutions. This is exemplified by the lower PDE4 inhibitory activities of **21** (PDE4 IC_{50} 260 nM), **23** (PDE4 IC_{50} 500 nM), and **24** (PDE4 IC_{50} 420 nM). PDE4 activity was slightly improved on moving to the *N*-oxide series as seen by the activities of **8** (PDE4 IC_{50} 100 nM) vs. **9** (PDE4 IC_{50} 30 nM) and **13** (PDE4 IC_{50} 18 nM) vs. **14** (PDE4 IC_{50} 8 nM). Most encouragingly high in vivo activity for the inhibition of TNF-α production was observed for several *N*-oxides. Noteworthy, as they approach the in vivo activity of RP73401, are the cyclohexylmethyl and cyclohexylethylethyl derivatives **9** (ED_{50} 7.1 mpk) and **10** (ED_{50} 7.4 mpk), respectively, with the former showing a dramatic increase in activity over it non-oxide analogue, **8** (ED_{50} 0% at 50 mpk). A commonly observed feature of many rolipram-like PDE4 inhibitors has been an undesirable side-effect profile. Rolipram, **1**, was initially designed as an anti-depressant CNS agent and during clinical evaluation nausea and vomiting was reported.[12] Rolipram was emetic in the dog emesis model at the dose of only 0.03 mg/kg (2/2). Three *N*-oxides **9**, **10**, and **14** exhibiting high in vivo activity for TNF-α release were thus evaluated in this model and showed substantial improvement over both rolipram

and RP73401. Noteworthy is the increased selectivity these compounds possess for the catalytic binding site over the so-called rolipram binding site. The design of compounds possessing such selectivity has been proposed as one solution to address the often seen undesirable side effect profile of many PDE4 inhibitors.[4]



2

Table 1

Compd	n	R_1	PDE4 IC_{50} nM	K_i Rolipram binding nM	ED_{50} (mouse) TNF-α Inhibition	Emesis (dog model) iv
1	-	-	320	4.5	8.2 ± 0.8 mpk	0.03 mg/kg 2/2
3	-	-	1	0.4	3.1 ± 0.5 mpk	0.6 mg/kg 4/4
7	1		19	38	-	-
8	0		100	120	0%@50 mpk	-
9	1		30	86	7.1 mpk	0.8 mg/kg 0/4
10	1		4.1	12	7.4 mpk	1 mg/kg 3/4
11	1		16	67	47%@10 mpk	-
12	1		20	30	8%@10 mpk	-
13	0		18	135	0%@50 mpk	-
14	1		8	60	20 mpk	3 mg/kg 0/4
15	1		24	86	40%@50 mpk	-
16	1		14	67	10 mpk	-
17	1		7	30	34%@50 mpk	-
18	1		46	135	53%@10 mpk	-
19	1		22	51	-	-
20	1		38	116	0%@10 mpk	-
21	1		260	1124	38%@50 mpk	-
22	1		12	71	10%@50 mpk	-
23	1		500	225	40%@50 mpk	-
24	1		420	393	38%@50 mpk	
25	1		1400	3184	-	
26	1		80	64	52%@50 mpk	-

A preliminary investigation into the pharmacokinetic profile of the potent inhibitors **9**[13] and **14**, 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. Impressively, the systemic bioavailability of **9** was 96%. Post iv clearance for both compounds compared to average liver blood flow in the mouse was low, suggesting that a significant first-pass effect was unlikely and that the limiting factors for bioavailability of **14** were likely to be either absorption or metabolism in the GI tract.

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
9	iv	n/a	n/a	3256	3.6	0.3	1.7	n/a
9	oral	439	1.0	3140	2.5	n/a	n/a	**96**
14	iv	n/a	n/a	2016.8	5.1	0.5	0.9	n/a
14	oral	392.7	0.25	668.7	3.7	n/a	n/a	**33**

n/a: not applicable

Compound **9** was evaluated in the Streptococcal cell wall-induced arthritis model in the rat.[15] This animal model is a model of chronic, erosive arthritis and produces a pathology similar to rheumatoid arthritis in man. Twice daily oral administration of **9** over the dose range 3 to 30 mg/kg resulted in dose-dependent inhibition of joint swelling which is an index of antiinflammatory activity (calculated ED_{50} = 23 mg/kg). In this model RP73401 has an ED_{50} of about 20 mg/kg.

Table 3

Treatment	Dose mg/kg (bid), po	Mean % Change Body weight days 0–4	Mean AUC + SEM of daily net joint diameters, days 0–4[A]	%Inhibition vs AUC of Control Joints[B]
Vehicle	10 ml/kg	−5 ± 0.6	4.9 ± 0.5	-
9	3.0	−5 ± 0.7	3.7 ± 0.6	25
9	10.0	−7 ± 0.6	3.3 ± 0.6	33
9	30.0	−13 ± 0.8	1.1 ± 0.4[C]	77

[A]AUC = area under curve, SEM = standard error of the mean. [B]% inhibition versus vehicle with hyper-edematous excluded. [C]$p < 0.05$ vs. vehicle.

In summary, a potent series of indole *N*-oxide PDE4 inhibitors has been reported. The *N*-oxide functionality imparts oral activity for TNF-α inhibition on the indole series with several compounds possessing similar activity to RP73401. Improved emetic and pharmacokinetic profiles were also observed for compound **9** over RP73401. Equally encouragingly, results from a study of the cyclohexylmethyl derivative **9** in a SCW-induced arthritis model were also comparable with RP73401, clearly indicating disease modifying effects.

Acknowledgements: We would like to thank Dr. Sheng-Yuh Tang and Jill Dreibelbis of the mass spectrometry department. Dr. Andy Morley and Stephen Fairway are also thanked.

References and Notes

1. (a) Maini, R. N.; Elliot, M. J.; Brennan, F. M.; Feldmann, M. *Clin. Exp. Immunol.* **1995**, *101*, 207. (b) Beavo, J. A.; Conti, M.; Heaslip, R. J. *Mol. Pharmacol.* **1994**, *46*, 399.

2. Williams, R. D.; Feldmann, M.; Maini, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9783.

3. 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.

4. 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.

5. (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. W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 399. (c) 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. (d) 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. (e) 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. (f) Marivet, M. C.; Bourguignon, J-J.; Lugnier, C.; Mann, A.; Stoclet, J-C.; Wermuth, C-G. *J. Med.Chem.* **1989**, *32*, 1450.

6. Hulme, C.; Moriarty, K.; Miller, B.; Mathew, R.; Ramanjulu, M.; Cox, P.; Souness, J.; Page, K. M.; Uhl, J.; Travis, J. Huang, F-C.; Labaudiniere, R.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1867.

7. (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. (b) 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**.

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

9. Batches of PDE IV 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.

10. 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).

11. Emesis Method: Male and female beagle dogs (8–12 kg) were used. Test compounds were dissolved in DMSO and administered by iv injection (0.3 mL) into a cephalic vein. Animals were monitored for 2 h and the number of emetic episodes was recorded.

12. Lowe, J.A.; Cheng, J. B. *Drugs of the Future* **1992**, *17*, 799.

13. For 1-Cyclohexylmethyl-3-methyl-1*H*-indole-6-carboxylic acid (3,5-dichloro-1-oxido-pyridinium-4-yl)amide, mp 127 °C (amorphous - in vivo results presented with **12** in this form) or 226–228 °C (crystalline - reduced in vivo activity observed in higher melting point form), white solid. For $C_{22}H_{23}Cl_2N_3O_2$ calcd C 61.12, H 5.36, N 9.72. Found C 61.06, H 5.23, N 9.59.

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.

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