Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 693–696

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

RPR203494 a Pyrimidine Analogue of the p38 Inhibitor RPR200765A with an Improved In Vitro Potency

Alan J. Collis, Martyn L. Foster, Frank Halley,* Christopher Maslen, Iain M. McLay, Kenneth M. Page, E. Jane Redford, John E. Souness and Nicola E. Wilsher

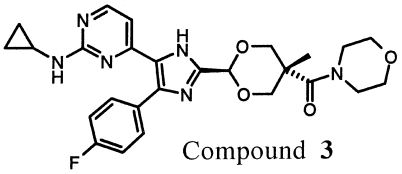
Aventis Pharma, Dagenham Research Centre, Rainham Road South, Dagenham, Essex, RM10 7XS, UK

Received 1 August 2000; accepted 10 January 2001

Abstract—Following the discovery of RPR200765, a series of pyrimidine analogues have been prepared as backups. Amongst them, RPR203494 was identified with a better in vitro profile than RPR200765A. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

p38 is a member of the intracellular family of mitogen activated protein kinase (MAPK), implicated in a phosphorylation cascade leading to the release of IL-1β and TNF-α from leukocytes.[1] IL-1β and TNF-α have been shown to be two important cytokines associated with the initiation and progression of rheumatoid arthritis (RA).[2] A p38 inhibitor would inhibit the release of these cytokines and hence be beneficial in the treatment of RA. Two p38 inhibitors, VX-745 and SB 242235 are currently in clinical phase II and I, respectively, for rheumatoid arthritis and the disease-modifying activity of SB 242235 in an animal model has been the subject of a recent publication.[3] Following the discovery of RPR200765A, a 4-phenyl-5-pyridyl-imidazol-1,3-dioxanyl-morpholinyl methanone[4] a series of 2-aminopyrimidines was prepared. RPR203494, a 2-cyclopropylamino-pyrimidine analogue was found to be more potent than RPR200765A in vitro (see Table 1) and had minimal inhibition of human CYP isozymes:



Compound **3**

RPR203494. $IC_{50} = 9$ nM, $EC_{50} = 60$ nM.

Results and Discussion

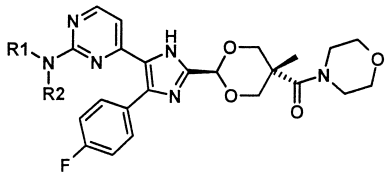
It has been shown recently that the pyridine ring of the pyridinylimidazoles class of p38 inhibitors could be responsible for the inhibition of the cytochrome P450 enzymes and that replacement of the pyridine with a substituted pyrimidine would be beneficial.[5] RPR200765A did indeed show some inhibition of CYP1A2 ($IC_{50} = 4.2\,\mu M$) or CYP2C9 ($IC_{50} = 12\,\mu M$),[4] and the synthesis of pyrimidine analogues was undertaken. The criteria followed for the selection of the amines (R1R2NH) used in the synthesis of the final aminopyrimidines (chemistry section, Scheme 1) was that the final compounds should fulfil the Lipinski rule of 5[6] but with a modified molecular weight of 600. The compounds were also found to have acceptable polar surface area[7] (PSA) with most of the compounds having a PSA < 140 Å^2. All the compounds were screened in a functional assay of lipopolysaccharide (LPS) induced TNF-α release on human monocytes (EC_{50}) and were evaluated in parallel for their in vitro metabolic stability in rat hepatic microsomes. The remaining active and metabolically stable compounds were further evaluated in a combinatorial-pharmacokinetic (combi-PK) study in the rat to establish oral bioavailability. The results are summarised in Table 1. Only the compounds reaching 20% bioavailability were considered for in vivo evaluation, followed by single PK study to confirm absolute bioavailability and to test inhibition of human CYP enzymes. Finally the most interesting compounds were evaluated on p38 kinase (see Table 1 for IC_{50}).

These results showed that di-substitution of the amine was detrimental for activity (entry **2**) and cyclic secondary

*Corresponding author. Tel.: +33-0-15571-3614; fax: +33-0-15571-8014; e-mail: frank.halley@aventis.com

0960-894X/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(01)00034-8

Table 1. Molecular weight, PSA, EC_{50}, in vitro rat metabolism and rat oral bioavailability of the pyrimidine analogues



Compounds	R1	R2	MW	PSA (Å^2)	EC_{50} (nM)	Metabolism (%)	F (%)	IC_{50}[d]
1	Methyl	H	482.5	127	38% (300 nM)	8.1		
2	Methyl	Methyl	496.5	112.8	8% (300 nM)	40.7		
3	Cyclopropyl	H	508.5	127.6	60	43.6	29	9
4	Cyclohexyl	H	550.6	126.4	24	98.6		
5	$-CH_2-(CH_2)_3-CH_2-$		536.6	116.4	26% (300 nM)	99		
6	Carboxymethyl	H	526.5	169.4	>10,000	n/r[a]		
7	Carboxyethyl	H	540.5	169.3	6% (300 nM)	3.5		
8	Aminoethyl	H	511.5	153.7	>10,000	12.0		
9	*N*,*N*-Dimethylaminoethyl	H	539.6	131.7	570	29		
10	*N*,*N*-Dimethylaminopropyl	H	553.6	131.8	27% (300 nM)	18		
11	Hydroxyethyl	H	512.5	149.9	319	18.2		
12	Hydroxypropyl	H	526.6	149.7	57	n/r[a]	2	
13	3-Methoxypropyl	H	540.6	137.9	54	44	15	7
14	H	H	468.5	142	39% (300 nM)	17.6		
15	Allyl	H	508.5	125.3	110	89.1		
16	Cyclopropylmethyl	H	522.6	125.5	69	87.0		
17	Cyanoethyl	H	521.5	151.6	28% (300 nM)	n/r[a]		
18	Propyl	H	510.6	125.3	124	94.5		
19	Benzyl	H	558.6	126	12	68	24	5
20	2-Thienyl-methyl	H	564.6	125.6	70	92		
21	(*R*)-α-Methyl-benzyl	H	572.6	122.1	10	81		
22	(*S*)-α-Methyl-benzyl	H	572.6	122.1	1.5	71	10	
23	2-Pyridyl-methyl	H	559.6	139.4	45	25	25	11
24	4-Methoxybenzyl	H	588.6	137.8	49	23.9	1	
25	4-Fluorobenzyl	H	576.6	125.7	62	44.6	n/d[b]	
26	Phenyl	H	544.6	127.2	54	51	29	16
27	3-Pyridinyl	H	545.6	141.6	231	24.6		
28	4-Pyridinyl	H	545.6	141.7	41	n/r[a]	n/c[c]	
29	3-Methoxyphenyl	H	574.6	139.3	33	54.1	6	

[a]n/r = no result.
[b]n/d = not detected.
[c]n/c = not calculated due to very low level of detection. IC_{50} on p38 kinase.

amines were found to lead to inactive compounds as in the case of piperidine (entry **5**). The introductions of polar groups such as an acid substituent (entries 6 and 7), a primary amine (entry 8) or a tertiary amine (entries 9 and 10) were also found to be detrimental for activity. The potent compounds have nonpolar alkyl or aryl residue, the most potent being the (*S*)-α-methyl-benzyl substituent (entry **22**, $EC_{50} = 1.5$ nM) which is 10 times more potent than (*R*)-isomer **21** or the benzyl **19** as previously found on a related series.[8] Many compounds had high metabolic turnover and were discarded from further consideration although the whole cell activity was high (as in entry **4** for example). The selection criteria for the progression of the compounds were obtained from previous experience, the cut off for metabolism was determined to be 80% for this series and only compounds having an acceptable potency ($EC_{50} < 100$ nM) were considered. Eleven compounds fulfilling these criteria were evaluated in a combi-PK study in the rat (entries **3**, **12**, **13**, **19**, **22**, **23**, **24**, **25**, **26**, **28** and **29**). Four compounds were identified with an oral bioavailability suitable for further investigation (F >20%, entries **3**, **19**, **23** and **26**). The p38 kinase activity measured for the selected compounds showed good correlation with the EC_{50}. The four compounds were screened in vivo in a rat model of SCW induced arthritis, using RPR200765A as a benchmark. Compound **19** was surprisingly found to be almost inactive and compound **23** did not show superiority over RPR200765A. Compound **26** was cytotoxic in several whole cell assays and subsequently discarded. Compound **3** was found to be potent both in the SCW model (Fig. 1) and in the mouse LPS induced TNF-α release assay (Fig. 2).

Compound **3** significantly inhibited the reactivation of ankle swelling at doses from 10 mg/kg/day to 30 mg/kg/day. Compound **3** was then evaluated in a rat PK study as a single compound following administration by the iv and oral routes, the results are shown in Table 2.

The bioavailability of RPR 203494 has been found to be good at 48% but the terminal elimination $T_{1/2}$ following iv and oral administration was only just longer than 1 h.

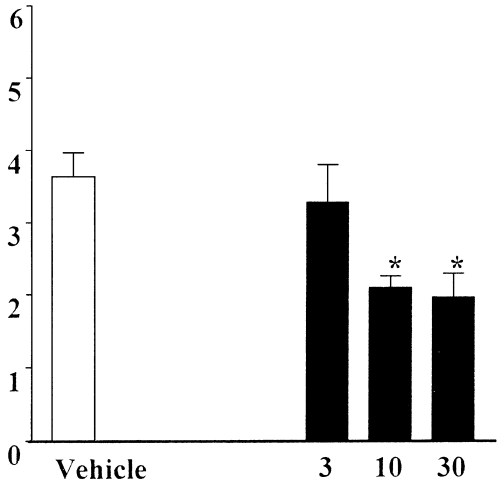


Figure 1. Dose–response relationship for RPR 203494 in the SCW-induced model of arthritis. This figure shows the area under the curve for the change in medial ankle width following iv challenge with SCW and oral administration of compound at 3, 10 and 30 mg/kg.

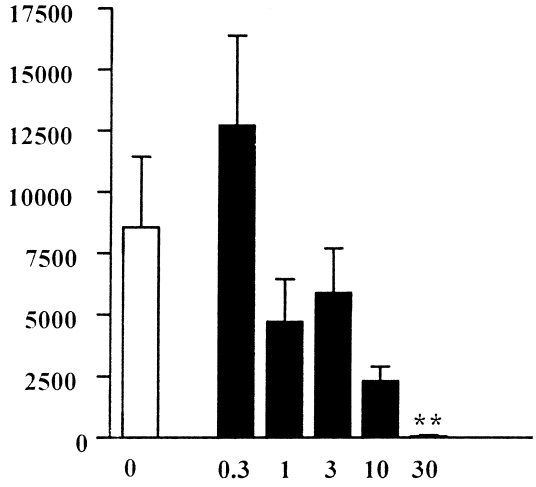


Figure 2. Effect of RPR203494 at 0.3, 1, 3, 10 and 30 mg/kg on LPS (0.1 mg/kg) induced TNF alpha release in male Balb/c mice. Mean ± SEM $*p < 0.05$, $**p < 0.01$, ANOVA with post hoc Dunnett's test compared to vehicle-treated animals.

Table 2. Pharmacokinetic parameters and determination of the absolute oral bioavailability of RPR203494 in the rat

Oral administration at 5 mg/kg				iv administration at 1 mg/kg				
Cp max (ng/mL)	T max (h)	$AUC_{0-\infty}$ (h.ng/mL)	Terminal $T_{1/2}$ (h)	$AUC_{0-\infty}$ (h.ng/mL)	Cl_T (L/h/kg)	Vdss (L/kg)	Terminal $T_{1/2}$ (h)	F%
620	0.25–0.5 h	1388	1.4	580	1.8	1.2	1.2	48

Compound **3** was finally screened using recombinant human CYP enzymes and found to have no inhibitory effect on CYP2D6, CYP1A2 and CYP2C9 up to 50 μM.

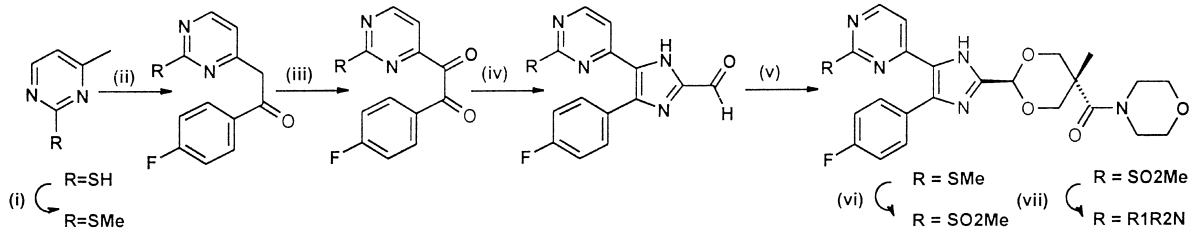
Chemistry

The chemistry was adapted from the synthesis of RPR 200765A,[9] starting from a 2-methythio-4-methylpyrimidine instead of a 4-methylpyridine (Scheme 1).

Biology

Monocyte TNF-α release assay

Adherent human monocytes (100,000 cells/well) were incubated with LPS (10 ng/mL) in the absence and presence of compound for 18 h. Individual experiments were carried out in quadruplicate samples. TNFα was measured by sandwich ELISA and EC_{50} values calculated for the activity of individual compounds. EC_{50} values shown from repeat experiments are means ± SEM ($n = 3$).



Scheme 1. (i) DMFDMA, toluene, reflux (82%); (ii) ethyl 4-fluorobenzoate, HMDSNa, THF, rt (99%); (iii) 48% aq HBr, DMSO, 65 °C (86%); (iv) glyoxaldimethylacetal, NH_4OAc, AcOH, methyl-*tert*-butyl ether, rt (80%); (v) 2,2-dihydroxymorpholinylpropionamide, pTSA, DMF, 80 °C (50%); (vi) mCPBA, DCM, rt (80%); (vii) R_1R_2NH, *N*-methyl pyrrolidinone, 80 °C (20–80%).

Kinase assay

The p38 enzyme assay is carried out at room temperature for 1 h, using 40 ng/well of the mouse enzyme. The substrate, 50 μg/mL ATF-2 is coated onto 96-well plates, the assay is carried out in 25 mM Hepes buffer, pH 7.7 containing 25 mM magnesium chloride, 2 mM dithiothreitol, 1 mM sodium orthovanadate and 100 μM ATP. Phosphorylated ATF-2 is quantitated using phospho-specific ATF-2 primary antibodies. IC_{50} values shown from repeat experiments are means ± SEM ($n = 3$).

Rat SCW model

Lewis rats received an i.a. injection of 10 μg SCW 100P fraction on day 0 followed by an intravenous challenge with 100 μg SCW 100P fraction on day 21. Rats were randomly allocated to receive compound (at 3, 10 or 30 mg/kg/day), on days 20–24 (po, bid.) or vehicle (5 mL/kg/day). Ankle width was measured daily from day 21 to 25. * $p < 0.05$ compare to vehicle, ANOVA with post hoc Dunnett's test.

Mouse TNFα release assay

Compound was administered orally to balb/c mice 30 min prior to LPS (0.1 mg/kg ip) challenge. Serum TNFα levels were determined 90 min after LPS insult. Results represent means ± SEM.

Human CYP inhibition assay[4]

Rapid inhibition assays were undertaken to determine the inhibitory potency (IC_{50}) of the p38 compounds using Gentest recombinant human CYPs. Probe substrates were incubated with the expressed CYPs in the presence or absence of test compound (0.4, 2, 10 and 50 μM).

Conclusion

A rapid investigation of RPR200765A pyrimidine analogues led to the identification of RPR203494 (compound **3**) which was shown to be more potent in vitro ($EC_{50} = 60$ nM, $IC_{50} = 9$ nM on p38 kinase). RPR203494 moreover demonstrated a decreased inhibition of hepatic cytochrome P450 enzymes. Furthermore, RPR203494 was found to have no inhibitory effect on COX1 at 10 μM.

Acknowledgements

The authors thank Adnan Alshaar, Mustafa Bhimani, Brenda Burton and Stuart Parker for technical support.

References

1. Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W.; Strickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Young, P. R. *Nature (London)* **1994**, *372*, 736.
2. Muller-Ladner, U. *Curr. Opin. Rheumatol.* **1996**, *8*, 210.
3. Badger, A. M.; Griswold, D. E.; Kapadia, R.; Blake, S.; Swift, B. A.; Hoffman, S. J.; Stroup, G. B.; Webb, E.; Rieman, D. J.; Gowen, M.; Boehm, J. C.; Adams, J. L.; Lee, J. C. *Arthritis Rheum.* **2000**, *43*, 175.
4. McLay, I. M.; Halley, F.; McKenna, J.; Souness, J. E.; Benning, V.; Birrell, M.; Burton, B.; Belvisi, M.; Collis, A.; Constan, A.; Foster, M. L.; Hele, D.; Jayyosi, Z.; Kelley, M.; Maslen, C.; Miller, G.; Ouldelhkim, M. C.; Page, K.; Phipps, S.; Pollock, K.; Porter, B.; Ratcliffe, A. J.; Redford, E. J.; Webber, S.; Slater, B.; Thybaud, V.; Wilsher, N. *Bioorg. Med. Chem.* **2001**, *9*, 537.
5. Adams, J. L.; Boehm, J. C.; Kassis, S.; Gorycki, P. D.; Webb, E. F.; Hall, R.; Sorenson, M.; Lee, J. C.; Ayrton, A.; Griswold, D. E.; Gallagher, T. F. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3111.
6. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
7. Clark, D. E.; Pickett, S. D. *Drug Discovery Today* **2000**, *5*(2), 49.
8. Liverton, N. J.; Butcher, J. W.; Claiborne, C. F.; Clameron, D. A.; Libby, B. E.; Ngyuen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Danheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neil, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. *J. Med. Chem.* **1999**, *42*, 2180.
9. Cook, D. C.; Lythgoe, D. J.; Thompson, D. M.; Wallace, Paul A.; Walton, J. B. *Org. Process Res. Dev.* Submitted for publication.