Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2925–2930

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PDE4 INHIBITORS: NEW XANTHINE ANALOGUES

John G Montana*, Nicola Cooper, Hazel J Dyke, Lewis Gowers, Joanna P Gregory, Paul G Hellewell[a], Jadwiga Miotla[a] Ken Morris[c], Robert Naylor[b], Bishwa Tuladhar[b] and Julie B H Warneck

Chiroscience Ltd, Cambridge Science Park, Milton Road, Cambridge CB4 4WE, UK

[a]*Department of Pharmacology, National Heart and Lung Institute, Dovehouse Street, London, SW3 6LY, UK*

[b]*Pharmacology Department, University of Bradford, Bradford, BD7 1DP*

[c]*Maybridge Chemical Company Ltd, Trevillett, Tintagel, Cornwall PL34 OHW*

Received 15 June 1998; accepted 10 September 1998

Abstract: Novel xanthine analogues are described which are selective PDE4 inhibitors with improved therapeutic potential over theophylline.

Introduction

Asthma is a chronic, severe, debilitating and often fatal disease whose incidence is increasing, primarily in the Western World. In the UK alone, it is responsible for 2000 deaths per annum among adults and children. Current therapies are based upon inhaled β-agonists[1], which offer only symptomatic relief, and steroids which have associated side effects. There is obviously a requirement for an improved oral anti-inflammatory agent to treat the underlying disease.

Cyclic adenosine monophosphate (cAMP) is converted by some phosphodiesterase enzymes (PDEs) into the inactive acyclic 5'-adenosine monophosphate (5'-AMP). Inhibition of PDE activity thus causes the cellular levels of cAMP to be potentiated, thereby activating the protein kinases responsible for decreasing inflammatory cell activity and airway smooth muscle tone.[2] Seven families of PDEs have been identified to date[3]; PDE4 is cAMP specific and is found in airway smooth muscle, all inflammatory cells and the vascular endothelium and selective inhibitors of PDE4 have shown anti-inflammatory activity in animal models.[4] The anti-inflammatory action stems from the inhibition of cell function and cytokine liberation (e.g. TNF_{α}, IL-2, IL-5, IFN), leading to the inhibition of cell adhesion and proliferation.[1]

Theophylline (1), a dimethylxanthine, has been used to treat asthma for over 60 years. However, its clinical use is limited by adverse reactions on the cardiovascular and central nervous systems as well as its narrow therapeutic index and high inter-individual

*E-mail johnmontana@chiroscience.com Fax +44 1223 420440

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00524-1

variability in absorption, metabolism and clearance[5]. At least some of the beneficial actions of theophylline are associated with its ability to inhibit PDE4[4], but theophylline is both weak and non-selective. Several publications have appeared describing the SAR of theophylline analogues[6]. A more potent and PDE4 selective xanthine should provide an improved agent for the treatment of asthma.

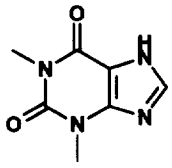
Recent evidence suggests that the PDE4 enzyme possessess two binding sites, a catalytic site and a high affinity site whose nature is not completely understood.[7] However there is increasing support for the hypothesis that binding to this high affinity site (rolipram binding activity, RBA) correlates with the observed side-effects.[8] Thus minimisation of affinity for the high affinity site should provide a compound with an improved therapeutic ratio. Based on this approach, our objective was to identify an orally active PDE4 inhibitor which does not cause nausea/emesis in man whilst maintaining the full spectrum of beneficial biological actions. Theophylline has only weak affinity for the high affinity binding site and the side effects observed with this compound are thought to be associated with activity agaisnt other PDE enzymes and adenosine receptors.

Results

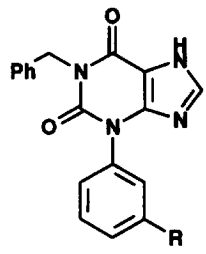
A series of novel xanthines has been prepared and the activities of these compounds are provided in Tables 1, 2 and 3.

Compound	R	PDE4 IC_{50} μM	RBA IC_{50} μM	Ratio PDE4/RBA
theophylline		39%@200	27%@100	
1a	Cl	6.55	0.042	156
1b	CO_2H	110	ND	
1c	CO_2Me	4.08	0.009	453
1d	SOMe	56	1.6	35
1e	NH_2	11	0.32	34
1f	OMe	6.01	0.017	353

Table 1. *In vitro* activity and selectivity of xanthines.
All results are an average of at least 2 determinations, each run in triplicate. ND = not determined

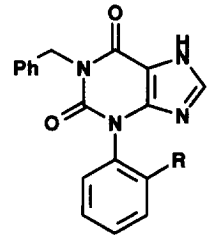


theophylline (1)



(2)

It is evident from the results presented in Table 1 that the nature of R has a dramatic effect on PDE4 activity. Polar groups such as CO_2H and SOMe are not tolerated, halogens and OMe provide compounds with reasonable potency but poor ratio and NH_2 provides a compound with reduced potency. Compound 1f was found to be selective for PDE4 over PDE3 (24% inhibition at 20μM for PDE3). The effect of ortho- substitution was also investigated, and the results for selected compounds are shown in Table 2.



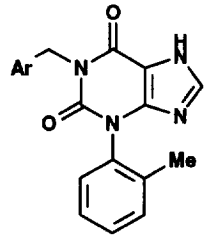
(2)

Compound	R	PDE4 IC_{50} μM	RBA IC_{50} μM	Ratio PDE4/RBA
2a	Me	7.97	0.67	11.9
2b	F	2.4	0.22	10.9
2c	CF_3	20	ND	
2d	SMe	20	ND	
2e	SOMe	100	ND	
2f	^{t}Bu	>20	ND	
2g	H	4.8	0.08	60

Table 2. *In vitro* activity and selectivity of o-substituted xanthines.
All results are an average of at least 2 determinations, each run in triplicate.

Only small lipophilic ortho- substituents are tolerated as can be seen from Table 2. Superior ratios for PDE4 versus high affinity binding were achieved in this series, with

compound (2a) possessing the best ratio seen so far in this type of compound. The ratio PDE4/RBA for compound (2a) is 100 better than the ratio for early compounds such as (1c). Relacement of the aryl portion of the benzyl substituent by heteraromatic groups was also undertaken and results are presented in Table 3.



(3)

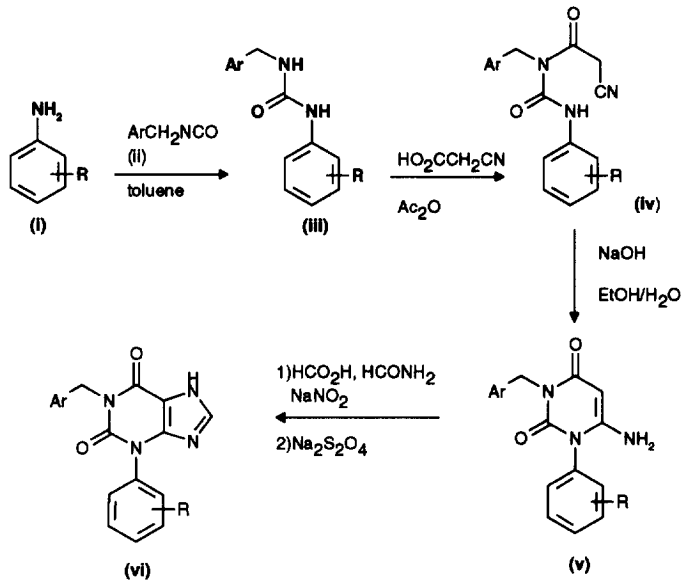
Compound	Ar	PDE4 IC_{50} μM	RBA IC_{50} μM	Ratio PDE4/RBA
3a	Ph	7.97	0.67	11.9
3b	2-furyl	11.95	1.1	10.9
3c	2-thienyl	4.2	1.02	4.1

Table 3. *In vitro* activity and selectivity of xanthines
All results are an average of at least 2 determinations, each run in triplicate.

The thienylmethyl residue provides a useful alternative to benzyl, whereas replacement of benzyl by furfuryl results in a loss of potency. Compound (3c) also demonstrates a good ratio.

Chemistry

The xanthines were prepared using the standard route[9] depicted in Scheme 1. An appropriate aniline (i) was condensed with a suitable isocyanate (ii) to provide a urea (iii) which was acylated with cyanoacetic acid to provide the acylurea (iv). Hydrolysis of the cyano group and *in situ* cyclisation provided the aminouracil[9a, 9b] (v). Nitrosation, reduction of the resultant nitroso intermediate to the corresponding amine and reaction of the resultant diamine with formic acid were all carried out in one pot to provide the desired xanthies[9c] (vi).



Scheme 1. Synthesis of xanthines

In vivo Results

Compound (3c) was selected for *in vivo* evaluation, based on its activity against PDE4 and improved ratio for PDE4 versus high affinity binding. The compound was evaluated in a guinea-pig skin model of eosinophilia[10], a guinea-pig lung model of eosinophilia[11] and a ferret model of emesis[12]. In the skin model, compound (3c) dosed orally demonstrated an excellent inhibition of eosinophilia produced by a range of mediators[13] as shown in Chart 1. In the lung model, compound (3c) demonstrated 56% and 70% inhibition of eosinophilia at oral doses of 10 and 30mg/kg respectively (rolipram 48% at 10mg/kg). No emesis or CNS related side effects were observed when compound (3c) was dosed orally to ferrets at 10mg/kg.

Conclusions

A novel series of xanthines with acceptable PDE4 activity and improved selectivity versus the high affinity site has been identified. A selected compound from this series, compound (3c) has demonstrated excellent *in vivo* activity and a good therapeutic index as determined by efficacious dose versus emetic dose.

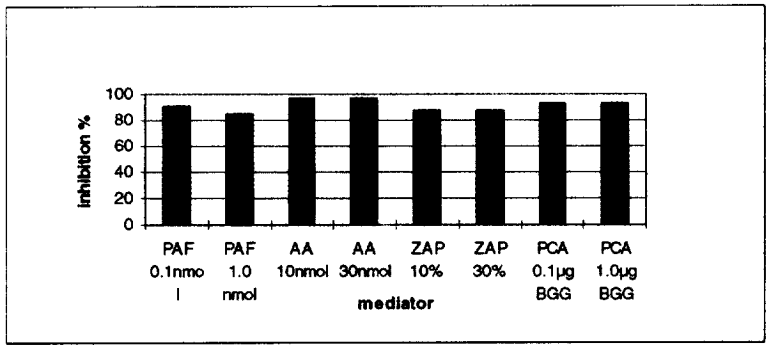


Chart 1. Inhibition of skin eosinophilia by compound (3c) @5mg/kg po

References

1. Giembycz, M. A. *Trends Pharmacol. Sci.* **1996**, *17*, 331-336.
2. Nicholson, C.D.; Shahid, M. *Pulmon. Pharmacol.* **1994**, *7*, 1; Palacios, J.M.; Beleta, J.; Segarra, V. *Il Farmaco* **1995**, *50*, 819.
3. Demoliou-Mason, C.D. *Exp. Opin. Ther. Patents* **1995**, *5*, 417.
4. Teixeira, M.M.; Gristwood, R.W.; Cooper, N.; Hellewell, P.G. *TIPS* **1997**, *18*, 164; Banner, K.H.; Page, C.P. *Clin. Exp. Allergy* **1996**, *26(Supplement 2)*, 2.
5. Perasson, C.G.A.; Andersson, K.E.; Kjellin, G. *Life Sci.* **1986**, *38*, 1057
6. Sakai, R.; Konno, K.; Yamamoto, Y.; Sanae, F.; Takagi, K.; Hasegawa, T.; Iwasaki, N.; Kakiuchi, M.; Kato, H.; Miyamoto, K. *J. Med. Chem.* **1992**, *35*, 4039; Miyamoto, K.; Yamamoto, Y.; Kurita, M.; Sakai, R.; Konno, K.; Sanae, F.; Ohshima, T.; Takagi, K.; Hasegawa, T.; Iwasaki, N.; Kakiuchi, M.; Kato, H. *J. Med. Chem.* **1993**, *36*, 1380; Buckle, R.; Arch, J.; Connolly, B.; Foster, K.; Murray, K.; Readshaw, S.; Smallridge, M.; Smith, D. *J. Med. Chem.* **1994**, *37*, 476.
7. Muller, T.; Engels, P.; Fozard, J.R. *Trends Pharmacol. Sci.* **1996**, *17*, 294; Kelly, J.J.; Barnes, P.J.; Giembycz, M.A. *Biochem.* **1996**, *318*, 425; Jacobitz, S.; McLaughlin, M.M.; Livi, G.P.; Burman, M.; Torphy, T.J. *Mol. Pharmacol.* **1996**, *50*, 891.
8. Duplantier, A.J.; Biggers, M.S.; Chambers, R.J.; Cheng, J.B.; Cooper, K.; Damon, D.B.; Eggler, J.F.; Kraus, K.G.; Marfat, A.; Masamune, H.; Pillar, J.S.; Shirley, J.T.; Umland, J.P.; Watson, J.W. *J. Med. Chem.* **1996**, *39*, 120.

9a. Papesch, V. and Schroder, E.F. *J. Org. Chem.* **1951**, *16*, 1879.

9b. Ohtsuka, Y. *Bull. Chem. Soc. Jap.* **1973**, *46*, 506.

9c. Bredereck, H. and Edenhofer, A. *Chem. Berichte* **1955**, 1306.

10. Teixeira, M.M.; Reynia, S.; Robinson, M.; Shock, A.; Williams, T.J.; Williams, F.M.; Rossi, A.G.; Hellewell, P.G. *Br. J. Pharmacol.* **1994**, *111*, 811; Teixeira, M.M.; Williams, T.J.; Hellewell, P.G.; *Br. J. Pharmacol.* **1993**, *110*, 416.
11. Kallos, P. and Kallos, L. *Int. Arch. Appl. Immunol.* **1984**, *73*, 77; Sanjar, S. Aoki, S. Kristersson, A. Smith, D. and Morley, J.; *Br J Pharmacol.* **1990**, *99*, 679.
12. Costall, B.; Domeney, A. M.; Naylor, R. J; Tattersall, F. D. *Neuropharmacology* **1987**, *26*, 1321-1326.
13. The mediators used as inflammagens in the guinea pig skin eosinophilia model were platelet aggregating factor (PAF), Arachidonic acid (AA) and zymosan-activated plasma (ZAP). Additionally sensitisation to bovine gamma globulin followed by an id challenge of antisera results in a passive cutaneous anaphylactic (PCA) response.