Bioorganic & Medicinal Chemistry Letters 20 (2010) 137–140

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Addressing species specific metabolism and solubility issues in a quinoline series of oral PDE4 inhibitors

C. Lunniss *,†, C. Eldred ‡, N. Aston, A. Craven, K. Gohil, B. Judkins, S. Keeling, L. Ranshaw, E. Robinson, T. Shipley, N. Trivedi

GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, England, UK

ARTICLE INFO

Article history:
Received 15 October 2009
Revised 4 November 2009
Accepted 5 November 2009
Available online 10 November 2009

Keywords:
PDE4
COPD
Quinoline
Cinnoline
Cynomolgus monkey
PK

ABSTRACT

Species specific conversion of the lead PDE4 inhibitor **1** to the quinolone **3** was identified as the major route of metabolism in the cynomolgus monkey. Modification of the template to give the cinnoline **9** retained potency and selectivity, and greatly improved the pharmacokinetic profile in the cynomolgus monkey compared with **1.** Additional SAR studies aimed at improving the solubility of **9** are also described.

Phosphodiesterase 4 (PDE4) is the predominant isozyme in nearly all immune and inflammatory cells and is a major regulator of cAMP content in airway smooth muscle. Inhibition of PDE4 down regulates cAMP production, mediating bronchodilation and reducing production of inflammatory mediators such as TNF-α. Potential indications for a PDE4 inhibitor as a once daily oral anti-inflammatory agent include chronic obstructive pulmonary disease (COPD), asthma, rhinitis and rheumatoid arthritis.[1,2]

We have previously described identification of the potent quinoline PDE4 inhibitor **1** (PDE4B $pIC_{50} = 9.4$; Roflumilast PDE4B $pIC_{50} = 9.4$).[3] Although this compound has excellent PDE4 potency and selectivity, high in vivo efficacy, and a favourable pharmacokinetic profile in rat and dog,[3] we have identified a species specific metabolic issue with this compound in the cynomolgus monkey. We here disclose details of the metabolic studies with **1**, the pharmacology of the major metabolite, and efforts to circumvent this metabolic issue culminating in the identification of the candidate molecule **9**.

Pharmacokinetic (PK) studies with the oral PDE4 inhibitor **1** identified species differences in PK profile between rat, dog and cynomolgus monkey, with a much shorter half life (0.6 h) in the monkey cf. rat (2.3 h) or dog (2.4 h). Drug metabolism studies were therefore undertaken in rat, dog, monkey and human hepatocytes to identify major metabolites and understand which species might best predict human PK (Fig. 1).

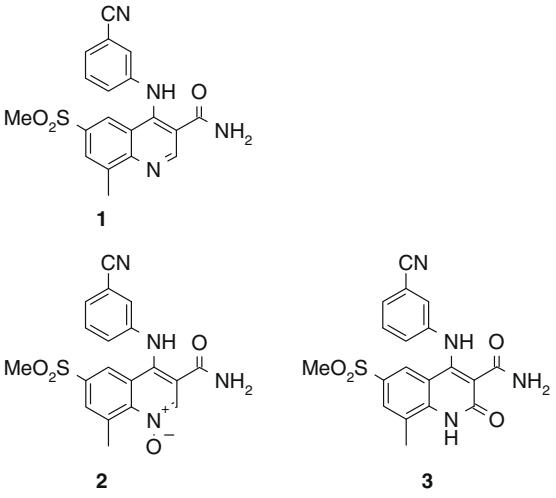


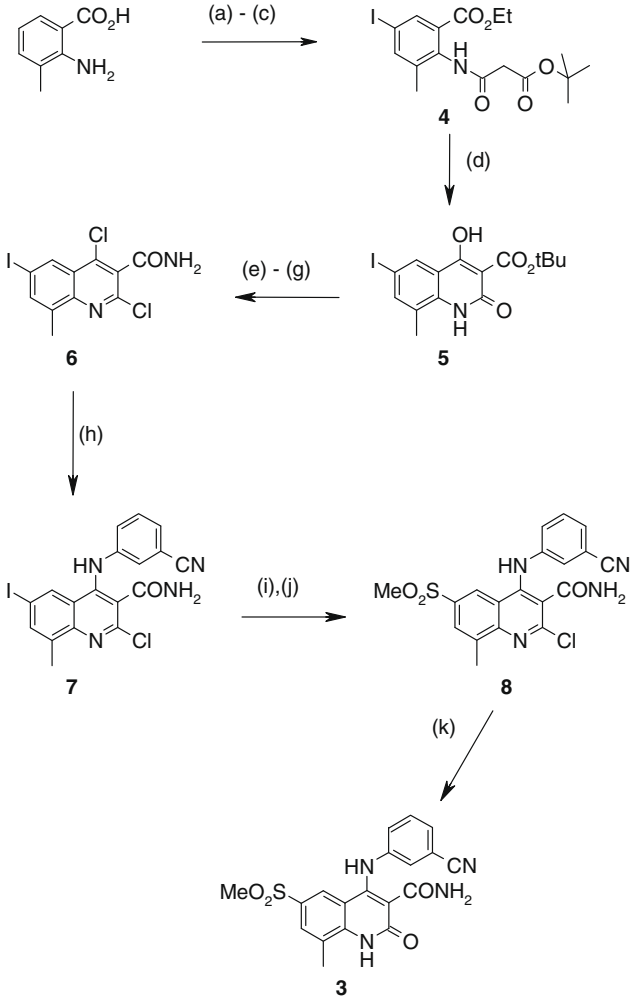
Figure 1. Quinoline **1** and possible metabolites in monkey hepatocytes.

* Corresponding author.
E-mail addresses: c.lunniss@biota.com.au, chris@lunniss.net (C. Lunniss).
† Present address: Biota Holdings Ltd, 585 Blackburn Road, Melbourne, VIC 3185, Australia.
‡ Present address: Colin Eldred Consulting Ltd., Woodridge House, Earlsbrook, Bacton, Stowmarket, Suffolk, IP14 4UA, UK.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.010

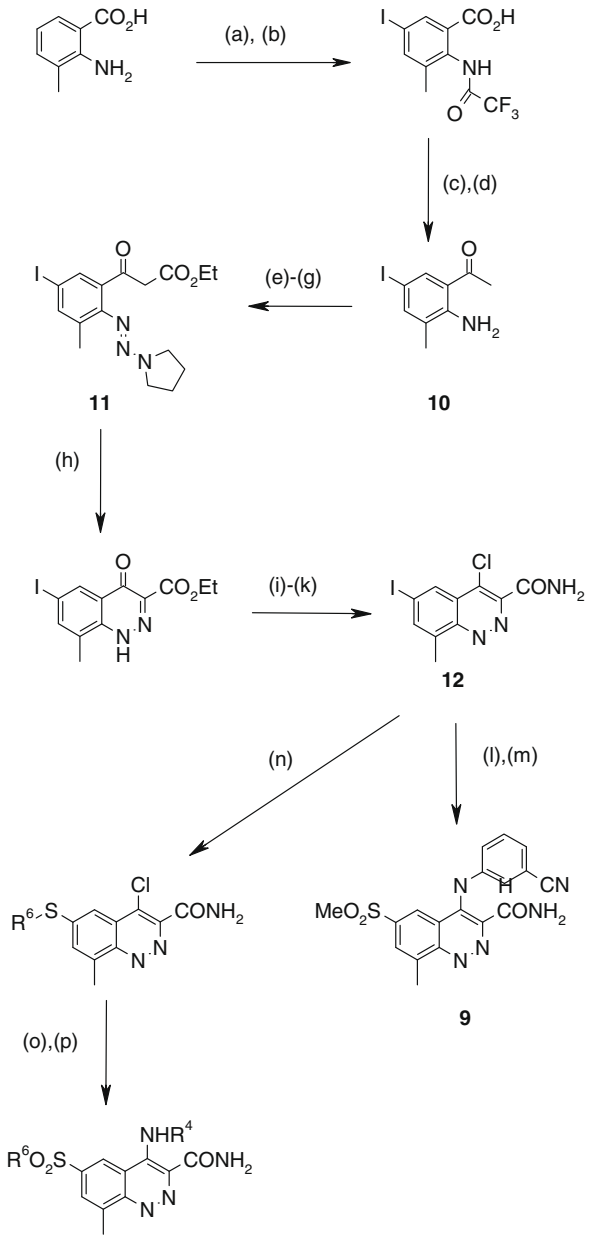
Consistent with the difference in half life, **1** displayed a >20-fold higher rate of metabolism in monkey hepatocytes compared to rat hepatocytes. The major [M+16] metabolite in the monkey was assigned initially as either the *N*-oxide **2** or the 2-quinolone **3**, and is not seen to any significant extent in rat, dog or human hepatocytes. The formation of such a potent active metabolite in the monkey posed potential development issues for **1** since this was the proposed non-rodent species for toxicological evaluation.[4]

The identity of the 2-quinolone **3** as the major metabolite in the monkey was confirmed by synthesis as shown in Scheme 1 (*N*-oxide **2** could not be prepared, despite trying multiple oxidation conditions. The main product isolated was the 2-quinolone possibly owing to spontaneous rearrangement of the *N*-oxide). Preparation of intermediate **4** under standard conditions followed by base catalysed cyclisation gave the hydroxyquinolone intermediate **5**. Acidic hydrolysis of the *t*-butyl ester followed by treatment with phosphoryl chloride gave the corresponding 2,4-dichloroquinolinyl acid chloride; treatment with ammonia gave intermediate **6** without displacement of the aryl chlorine groups. The 4-chloro group was selectively displaced with the sodium salt of 3-aminobenzonitrile to give intermediate **7**. Selective introduction of the methyl sulphone at the quinoline 6-position was achieved by palladium catalysed coupling with the non-nucleophilic (methylthio)tributylstannane reagent,[5] followed by oxidation. Finally, microwave conditions were found most successful for the hydrolysis of the 2-chloroquinoline **8** to give quinolone **3**.



Scheme 1. Synthesis of quinolone metabolite **3**. Reagents and conditions: (a) ICl, HCl, rt, 80%; (b) EtI, Cs_2CO_3, CH_3CN, reflux, 70%; (c) $HO_2CCH_2CO_2C(CH_3)_3$, $(COCl)_2$, $CHCl_3$, DMF (cat), rt, 75%; (d) NaOMe, EtOH, rt, 60%; (e) TFA, rt, 70%; (f) $POCl_3$, 100 °C; (g) NH_4OH/dioxane, 5–10 °C, 60%; (h) 3-aminobenzonitrile, NaH, DMF, 60 °C, 80%; (i) $MeSSnBu_3$, $Pd(PPh_3)_4$, toluene, DMF, 100 °C, 70%; (j) oxone, DMF, rt, 70%; (k) AcOH, H_2O, 180 °C, microwave, 50%.

Since X-ray crystallography studies suggested that hydrogen bonding to the quinoline nitrogen of **1** was a key binding interaction, the potent inhibitory activity of the quinolone **3** (PDE4B pIC_{50} = 9.0) was interpreted as binding of **3** to the enzyme as its 2-hydroxyquinoline tautomer.[8] As the potent metabolite of quinoline **1**, quinolone **3** was further characterised to investigate its potential for development, and showed good rat PK following



Scheme 2. Synthesis of the cinnoline template and compound **9**. Reagents and conditions: (a) ICl, aq HCl, 80%; (b) $(CF_3CO)_2O$, 1,4-dioxane, 70%; (c) MeMgCl (5 equiv), THF, 0 °C, 70%; (d) NaOH, MeOH, 90%; (e) $NaNO_2$, concd HCl, THF, H_2O; (f) pyrrolidine, aq K_2CO_3, 80%; (g) NaH, $CO(OEt)_2$, 80%; (h) CF_3COOH, 75%; (i) NaOH, MeOH, 90%; (j) $POCl_3$; (k) NH_4OH, dioxane, 80%; (l) 3-aminobenzonitrile, MeCN, reflux, 70%; (m) $MeSO_2Na$, CuI, diaminocyclohexane, DMSO, 60%; (n) R^6SSnBu_3, $Pd(PPh_3)_4$, toluene, DMF, 100 °C, typically 70–90%; (o) R^4NH_2, MeCN, reflux (pyridine hydrochloride was added for some aromatic heterocyclic amines), typically 60–80%; (p) oxone, DMF, rt, typically 60–70%.

Table 1
hWB[9] potency and cynomolgus monkey PK of quinolone metabolite **3** cf. quinoline lead **1**

Compound	hWB pIC_{50}[a]	F[b] (%)	Clp[b] (ml/min/kg)	$t_{1/2}$[b] (h)
1	7.9	33	82	0.8
3	7.6	52 (7)	5.9	4.2

[a] Values are means of at least four experiments.
[b] Values are means of three experiments following solution dosing (or suspension dosing using particle size reduced material in parentheses).

Table 2
PDE4B[10] and hWB[9] potency and cynomolgus monkey pharmacokinetics[c] for cinnoline **9**

PDE4B pIC_{50}[a]	hWB pIC_{50}[b]	F%	Clp (ml/min/kg)	$t_{1/2}$ (h)
>9.0	8.6	64 (26)	1.6	7.2

[a] Mean of two determinations.
[b] Mean of >4 determinations.
[c] Values are means of three experiments following solution dosing (or suspension dosing using particle size reduced material in parentheses).

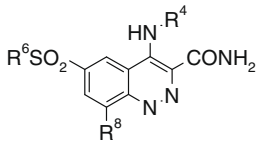
solution dosing. More encouragingly, the monkey PK (again following solution dosing in 10% DMSO, 15% PEG400, 75% water) was much improved over **1**, with good bioavailability and a much longer half life. However poor oral exposure following suspension dosing in 1% methyl cellulose (possibly due to very low aqueous solubility of 1 μg/ml) and poor in vivo efficacy precluded further work with this compound.

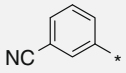
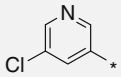
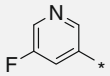
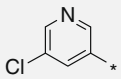
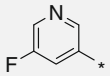
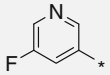
Attention was next turned to modification of the quinoline template to overcome this metabolic issue. It was reasoned that incorporation of a second nitrogen to replace the quinoline with a cinnoline template would prevent 2-quinolone formation, hence the cinnoline analogue **9** was prepared as shown in Scheme 2. The key step of the synthesis was a novel acid catalysed cyclisation of the pyrrolidinyldiazene intermediate **11**,[6] prepared in three steps from the aniline **10** via trapping of the corresponding diazonium salt with pyrrolidine. Ester hydrolysis and chlorination to give the 4-chlorocinnoline 3-acid chloride, followed by ammonia treatment gave the versatile intermediate **12**. The 6-sulphone group was introduced in one step using a copper catalysed coupling with the sodium salt of methanesulphonic acid.[7] Alternatively, in order to facilitate variation of the cinnoline 4-position in the last step, the 4-chloro-6-iodo intermediate **12** could be selectively converted to the 4-chloro-6-alkylthio intermediates using the thioalkyl(tributyl)stannane coupling methodology described in Scheme 1, followed by introduction of the 4-arylamino group and oxidation of sulphide to sulphone as the final step (Table 1).

Compound **9** retained excellent in vitro potency and >100-fold selectivity versus other PDE isozymes (PDE1, 2, 3, 5, 6, 7) and showed a much improved pharmacokinetic profile in the monkey compared with **1** (see Table 2). Despite its low solubility, good oral exposure of **9** could be achieved in both rat and monkey via particle size reduction (wet bead milling). Consequently, cinnoline **9** was selected for further development; its in vivo efficacy will be reported separately.

In order to improve upon the poor solubility of **9**, SAR studies were undertaken with the aim of introducing solubilising modifications onto the cinnoline template (see Table 3). Solubility enhancements of 10-fold could be achieved without loss of potency by replacing phenylamino groups with heteroarylamino groups at this position. In particular, 3-pyridylamino groups

Table 3
SAR studies in the cinnoline series



Compound	R^4	R^6	R^8	PDE4B pIC_{50}[a]	hWB pIC_{50}[a]	Solubility (μg/ml)	Rat F%[a]
9		Me	Me	>9.0	8.6	1	17
13		Me	Me	10.1	8.8	12	50
14		Me	Me	>9.3	8.3	37	34
15		Et	Me	9.5 (n = 1)	8.0	nt[b]	41
16		Me	H	9.1	7.5	7	66
17		^{t}Bu	H	9.3	7.9	1	57
18		Me	F	9.2 (n = 1)	6.8	nt[b]	nt

[a] Values are means of at least two experiments except where indicated in parentheses.
[b] nt = not tested.

(e.g., compounds **13** and **16**) and the 1-ethyl-5-pyrazolylamino (e.g., compound **15**) group gave useful increases in solubility while maintaining good in vitro potency and rat pharmacokinetic profiles.

In addition to modifications at the 4-position, limited variations at the cinnoline 6- and 8-positions were also studied. In line with observations in the related quinoline series,[3] larger alkyl groups were tolerated in the sulphone moiety (compounds **15** and **17**), however this was at the expense of solubility in the case of the *tert*-butyl sulphone **17**. Removal of the 8-methyl group altogether results in ca. 10-fold reduction in potency (cf. compounds **13** and **16**), and 8-fluoro analogues (e.g., **18**) achieve similar potency to their 8-H counterparts. From these SAR studies, compound **13** stood out as being equipotent with the cinnoline lead **9** in vitro and in vivo (data not shown), with an improved pharmacokinetic profile in the rat cf. **9**, and improved solubility.

In conclusion, detailed metabolic studies with the lead quinoline **1** identified the cynomolgus monkey specific quinolone metabolite **3**, which explained the poor pharmacokinetic profile in the monkey and allowed development of a medicinal chemistry strategy to overcome this issue. Synthesis of the cinnoline template led to the discovery of **9** with much improved pharmacokinetics in the monkey, along with a number of more soluble analogues, such as **13**, with promising in vitro and rat pharmacokinetic profiles. More detailed pharmacology of these compounds will be reported elsewhere.

Acknowledgements

The authors would like to thank Don Somers and Maragete Neu for crystallography, Michael Kranz for molecular modeling, Jo Wiseman for the PDE4B enzyme data, and Fiona Lucas and Yemisi Solanke for the hWB data.

References and notes

1. (a) Houslay, M. D.; Schafer, P.; Zhang, K. Y. J. *Drug Discovery Today* **2005**, *10*, 1503; (b) Francisco, J. S.; Hanania, N. A. *Curr. Opin. Pulm. Med.* **2005**, *11*, 129.
2. Hamblin, J. N.; Angell, T. D. R.; Ballantine, S. P.; Cook, C. M.; Cooper, A. W. J.; Dawson, J.; Delves, C. J.; Jones, P. S.; Lindvall, M.; Lucas, F. S.; Mitchell, C. J.; Neu, M. Y.; Ranshaw, L. E.; Solanke, Y. E.; Somers, D. O.; Wiseman, J. O. *Bioorg. Med. Chem. Lett.* **2008**, *14*, 4237.
3. Lunniss, C. J.; Cooper, A. W. J.; Eldred, C. D.; Kranz, M.; Lindvall, M.; Lucas, F. S.; Neu, M.; Preston, A. G. S.; Ranshaw, L. E.; Redgrave, A. J.; Robinson, J. E.; Shipley, T. J.; Solanke, Y. E.; Somers, D. O.; Wiseman, J. O. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1380.
4. Fura, A. *Drug Discovery Today* **2006**, *11*, 133.
5. Dickens, M. J.; Gilday, J. P.; Mowlem, T. J.; Widdowson, D. A. *Tetrahedron* **1991**, *47*, 8621.
6. Dowlatshahi, H. A. *Synth. Commun.* **1985**, *1512*, 1095.
7. Baskin, J. M.; Wang, Z. *Org. Lett.* **2002**, *4*, 4423; Zhu, W.; Ma, D. *J. Org. Chem.* **2005**, *70*, 2696.
8. Frank, J.; Katritzky, A. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1428; Warshel, A.; Papazyan, A. *Curr. Opin. Struct. Biol.* **1998**, *8*, 211.
9. Inhibition of LPS induced TNF-α production in human whole blood was carried out using MSD technology as described in WO2007036733.
10. The ability of compounds to inhibit catalytic activity at PDE4B was determined by Scintillation Proximity Assay (SPA) as described in patent WO2004103998.