Bioorganic & Medicinal Chemistry Letters 22 (2012) 7707–7710

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery and evolution of phenoxypiperidine hydroxyamide dual CCR3/H_1 antagonists. Part II: Optimising in vivo clearance

Mark Furber [d,*], Lilian Alcaraz [a], Christopher Luckhurst [a], Ash Bahl [b], Haydn Beaton [a], Keith Bowers [b], John Collington [c], Rebecca Denton [c], David Donald [a], Elizabeth Kinchin [a], Cathy MacDonald [c], Aaron Rigby [a], Rob Riley [c], Matt Soars [c], Brian Springthorpe [a], Peter Webborn [c]

[a] Medicinal Chemistry, AstraZeneca R&D Charnwood, Loughborough, Leicestershire, LE11 5RH, UK
[b] Discovery Biosciences, AstraZeneca R&D Charnwood, Loughborough, Leicestershire, LE11 5RH, UK
[c] Physical and Metabolic Sciences, AstraZeneca R&D Charnwood, Loughborough, Leicestershire, LE11 5RH, UK
[d] AstraZeneca Respiratory & Inflammation iMED, Pepparedsleden 1, 431 83 Mölndal, Sweden

ARTICLE INFO

Article history:
Available online 10 October 2012

Keywords:
CCR3
H1
Receptor antagonist
Dual inhibitor
Chemokine antagonist
Allergic disease

ABSTRACT

The second part of this communication focuses on the resolution of issues surrounding the series of hydroxyamide phenoxypiperidine CCR3/H_1 dual antagonists described in Part I. This involved further structural exploration directed at reducing metabolism and leading to the identification of compound **60** with a greatly improved in vivo pharmacokinetic profile.

As described in the first part of this communication, dual CCR3/H_1 antagonists were considered as having potential benefit in the treatment of asthma.[1] Further progress in the identification of phenoxypiperidine hydroxy amides as dual CCR3/H_1 antagonists is described in this second part. Although presenting a very promising in vitro profile in terms of potency at both CCR3 and H_1 receptors, low affinity at hERG channel and encouraging in vitro ADME properties, one of the major issues with the lead compound **20** (Fig. 1) identified in Part I, was the poor in vitro to in vivo correlation (ivivc), specifically the high clearance observed in vivo.

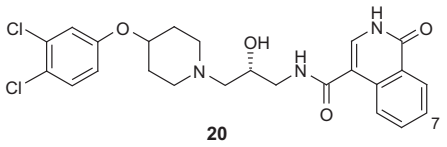
This poor scaling may be connected with the high volumes of distribution associated with these compounds that in turn may be attributed to their lipophilicity. Two principal approaches were investigated to reduce in vivo clearance in rat within the series. The first focused on reducing lipophilicity, with the dichlorophenyl moiety being identified as problematic in this respect. The second focused on identifying and blocking sites of metabolism.

In the first instance, attention was directed toward introducing polarity into the dichlorophenyl portion of the molecule (Table 1). Introduction of a primary amide group into the dichlorophenoxy ring (compound **32**) resulted in a significant drop in measured log D, but only a 10-fold drop in CCR3 affinity (pK_i 7.7).[2] In addition, compound **32** displayed low hERG activity (pIC_{50} <5).[3] Much of this reduction in CCR3 affinity was compensated by a reduction in human plasma protein binding from 98% to 79%; a 10-fold increase in free drug concentration. Furthermore, the compound displayed low in vivo clearance in rat (8.6 μl/min/kg) and a low volume of distribution (V_{ss} 0.5 L/kg) which was more in line with expectations from in vitro rat hepatocyte data (Cl_{int} 2 ml/min/1E^6 cells). Unfortunately, this compound demonstrated poor oral bioavailability in rat (F <5%); likely due to poor intestinal absorption predicted from low calculated permeability, and perhaps not unexpected for a primary carboxamide that possesses a number of additional hydrogen bond donor/acceptor groups. Furthermore, structure–activity proved very tight around this molecule and did not allow modification of the primary carboxamide group. Whilst this molecule itself could not be progressed, it did indicate that with appropriate modification of physicochemical properties a balance of dual CCR3/H_1 potency and suitable PK properties might be achievable.

Table 1 also illustrates the effect of replacing one or both Cl groups with a more polar cyano substituent, with compound **34** affording very similar CCR3 activity (pK_i 8.6) to compound **20** itself. Unfortunately, in vitro stability of this compound in a rat hepatocyte system was disappointing. It should be noted that simple removal of both Cl substituents results in a greater than 2 log drop in CCR3 potency. However, combining the 3-chloro-4-cyano substituted aryl ring system with methanesulfonyl substitution

* Corresponding author. Tel.: +46 31 7761062
E-mail address: mark1.furber@astrazeneca.com (M. Furber).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.09.112



20

CCR3 pKi 8.8, H_1 pKi 8.4, hERG pIC_{50} 5.2
Cl_{int} rat mics 19μL/min/mg; rat heps 4μL/min/10^6 cells;
dog heps 3μL/min/10^6cells; human heps 4μL/min/10^6 cells.

in vivo PK:
rat: Cl 60 mL/min/kg; V_{ss} 12 L/kg; $t_{1/2}$ 2.8 h; F 11%
dog: Cl 18 mL/min/kg; V_{ss} 5.5 L/kg; $t_{1/2}$ 6.0 h; F 40%
logD 3.3; hu ppb 98%; pKa 7.7 (base); 10.9 (acid)

Figure 1. Properties and site of metabolism of compound **20**.

on the isoquinolone ring afforded compound **35** which had a further reduced logD, and was found to be significantly more stable in vitro. Unfortunately, as seen previously, this compound did not scale when progressed to in vivo PK measurements, displaying very high clearance in rat (Cl 70 mL/min/kg).

The second approach adopted to improve in vivo PK properties was to block sites of metabolism in compound **20**. The principal route of metabolism of **20** as determined from in vitro and in vivo rat and in vitro human systems was oxidation in the 7-position of the isoquinolone ring system, with further glucuronidation of the newly introduced hydroxyl group in hepatocytes.

Accordingly, compounds were prepared wherein the isoquinolinone ring system was substituted at or adjacent to the site of metabolism with either a fluorine or a polar group such as methanesulfone or a sulfonamide substituent (Table 2). In some cases this provided the added benefit of lowering logD with the possibility of reducing tissue distribution of the drug.

Within this series of compounds some very high CCR3 and H_1 activities were observed. Compound **49**, in particular, was found to be an extremely potent dual CCR3/H_1 antagonist. Nevertheless, potency was not the main goal in this exercise and compounds **36**, **40**, **43** and **49** were dosed in vivo in rat to ascertain if improved ivivc and lower in vivo clearance could be obtained by this approach. Unfortunately, the outcome was unfavourable; despite lower Cl_{int} values in vitro and lower observed volumes of distribution in vivo (V_{ss} 1.8, 6.4, 4.4, 0.6 L/kg respectively) all four compounds had very high in vivo clearance, approaching rat liver blood flow.

This inability to predict in vivo clearances from in vitro data due to a complete lack of ivivc precluded further progression of this isoquinolinone subseries.

Poor ivivc is not infrequently seen with relatively lipophilic amines and is not always understood. Consequently, a more pragmatic approach was undertaken to concentrate efforts in those subseries whose in vivo clearance was predictable from in vitro measurements, even though this could not be rationalised at the time. This change in direction ultimately provided compounds with lower in vivo clearance. To this aim, we reassessed a number of compounds that that had been synthesised during the course of the project but had not progressed for a range of reasons and elected to revisit compound **53**.

When dosed iv to rat, compound **53** displayed an in vivo clearance of 22 mL/min/kg, a V_{ss} of 10 L/kg, and a $t_{1/2}$ of 6 h. These results were in line with that expected from the in vitro data. When dosed orally in the rat this compound exhibited good plasma exposure and reasonable bioavailability (F 36%). Initially the compound had not been progressed owing to a low hERG margin. In light of the ivivc issues outlined above with other series, close analogues of compound **53** were prepared to explore the possibility of reducing clearance and hERG activity within this series (Table 3).

To overcome the hERG issue with these compounds it was intended to replace the pyrazole ring system in **53** with the pyridone ring present in compound **20** in the hope that the combined features of lower in vivo clearance and low hERG activity could be achieved in one molecule (Table 4). Three compounds were prepared with different aryloxy substituents but all possessing a trifluoromethyl substituted pyridone ring. All three compounds displayed high CCR3 and H_1 potencies, low hERG activity and high in vitro metabolic stability. Investigation of **57** and **58** in vivo showed the compounds scaled from in vitro results and gave good plasma exposure both in rat and in dog. Dosed orally, compound **57** was highly bioavailable in dog (63%) but much less so in rat (<10%). Reasoning that permeability might be an issue for the NH pyridone series, the *N*-methyl pyridone analogue of **57** (compound **60**) was prepared and finally achieved the balance of properties the project sought. Compound **60** retained both CCR3 and H_1 potencies (pK_i 8.4 and 8.3) and low hERG binding efficacy (pIC_{50} 4.9), whilst also exhibiting low clearance and good oral bioavailability in rat (Cl

Table 1
LogD-lowering phenoxy and isoquinolinone substitutions

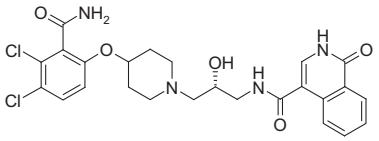
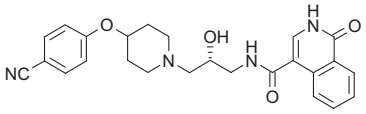
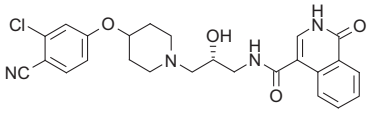
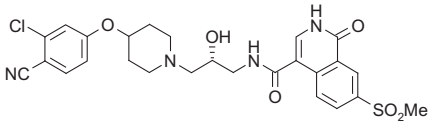
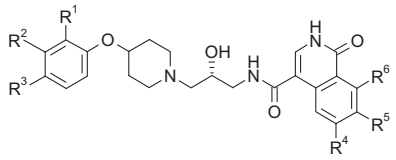
Compound	Structure	logD	CCR3 Binding pK_i[2]	H_1Binding pK_i[2]	Cl_{int} RH[4]	Cl_{int} HLM[4]
32		1.5	7.7	6.6	2	18
33		NT	7.5	6.3	6	18
34		2.6	8.4	6.9	10	22
35		1.4	7.2	6.6	<3	<3

Table 2
Modifications to the substitution of the aryloxy and isoquinolinone ring systems



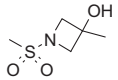
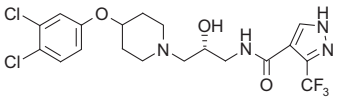
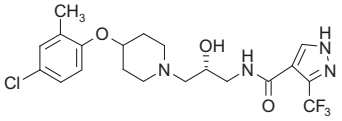
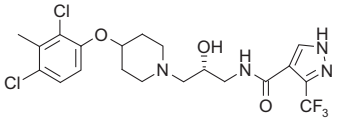
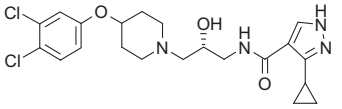
Compound	R^1	R^2	R^3	R^4	R^5	R^6	LogD	CCR3 pK_i[2]	H1 pK_i[2]	Cl_{int} RH[4]	Cl_{int} HLM[4]	hERG binding pIC_{50}[3]
36	H	Cl	Cl	H	SO_2Me	H	2.6	8.1	7.7	3	6	5.2
37	Cl	Me	Cl	H	SO_2Me	H	2.8	8.6	7.3	12	13	<4.5
38	Me	H	Cl	H	SO_2Me	H	2.4	7.8	7.1	7	14	5.7
39	Me	H	Cl	SO_2Me	H	H	NT	8.2	7.2	8	7	<4.5
40	H	Cl	Cl	H	F	H	3.2	8.7	8.1	<3	8	NT
41	Me	H	Cl	H	F	H	NT	8.4	7.9	<3	9	NT
42	H	Cl	Cl	F	H	H	3.9	8.5	8.2	5	8	5.9
43	H	Cl	Cl	H	$SO_2NHCH_2CH_2OH$	H	2.1	8.0	7.3	5	6	5.3
44	H	Cl	Cl	H	SO_2NH_2	H	2.2	7.6	7.7	<3	<3	<5.2
45	Cl	Me	Cl	SO_2Me	H	H	2.8	9.0	7.2	<3	<3	<4.5
46	H	Cl	Cl	H	SO_2NHMe	H	2.8	8.4	7.6	<3	14	5.7
47	H	Cl	Cl	H	SO_2NHc-Pr	H	3.7	8.4	8.3	6	21	6.1
48	H	Cl	Cl	H	SO_2NMe_2	H	3.1	9.1	7.9	10	86	6.0
49	H	Cl	Cl	H		H	3.0	9.5	8.6	7	<3	6.0
50	H	Cl	Cl	H		H	2.5	7.9	7.6	3	18	5.5
51	H	Cl	Cl	H	H	F	3.2	8.7	8.6	<3	6	5.7
52	Me	H	Cl	H	H	F	3.0	9.1	8.6	7	9	<4.5

Table 3
Pyrazole hydroxyamides

Compound	Structure	CCR3/H_1 binding pK_i[2]	hERG pIC_{50} (binding)[3]	PPB (human) %	Cl_{int} RH[4]	Cl_{int} HLM[4]
53		8.2/7.8	5.5–6.5 (variable) (5.5)	98	3	<3
54		8.5/7.6	5.9 (5.5)	96	5	<3
55		9.1/7.4	(5.7)	NT	6	<3
56		8.8/7.9	(5.6)	96	<3	9

9 mL/min/kg, V_{ss}7.3 L/kg, $t_{1/2}$ 9.7 h, F 53%) and dog (Cl 8 mL/min/kg, V_{ss} 6.4 L/kg, $t_{1/2}$ 10.8 h, F 81%).

In summary, issues surrounding the series of hydroxyamide phenoxypiperidines described in Part I had inspired structural explorations culminating in the identification of compound **60**. The enhanced in vivo pharmacokinetic profile of this compound has encouraged further studies in this series and ultimately led to the discovery of a suitable clinical candidate for the project.

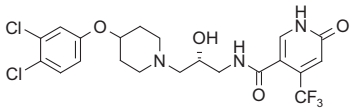
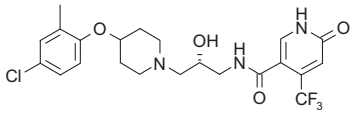
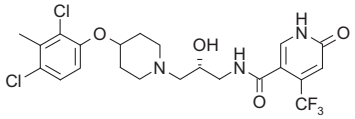
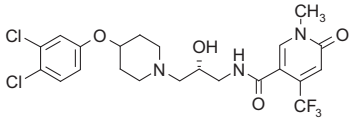
Synthetic chemistry

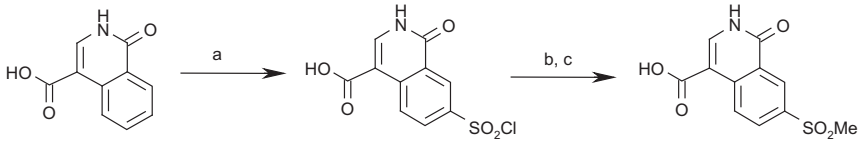
The synthetic routes towards substituted isoquinolinones shown in Tables 1 and 2 are described below (Schemes 1 and 2). The 7-methanesulfonyl-substituted analogue was obtained by chlorosulfonylation of isoquinolinone-4-carboxylic acid followed by reduction and methylation of the resulting sulfonyl chloride (Scheme 1).

The 6-methanesulfonyl, 6-fluoro and 7-fluoro substituted analogues were synthesised as shown in Scheme 2.

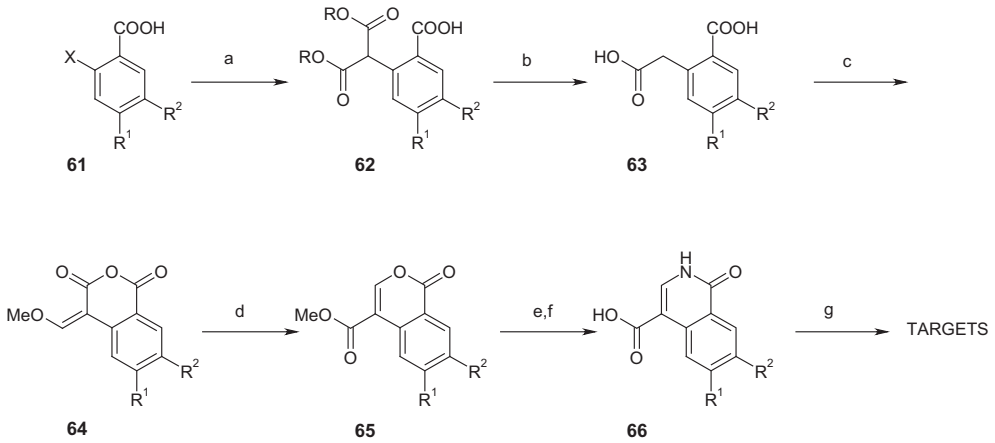
The key step in this sequence was a copper catalysed coupling reaction between an appropriately substituted *ortho*-halo benzoic acid (**61**) and a malonate ester. The coupling conditions were found to be substrate dependent and needed to be optimised individually. The hydrolysis-decarboxylation step was initially performed using concentrated hydrochloric acid and was later replaced by a saponification–decarboxylation procedure under basic conditions,

Table 4
Pyridone hydroxyamides

Compound	Structure	CCR3/H_1 binding pK_i[2]	hERG pIC_{50} (binding)[3]	PPB human (rat)%	Cl_{int} RH[4]	Cl_{int} HLM[4]
57		8.5/8.4	5.0 (5.3)	89(91)	4	<3
58		8.7/8.0	5.0 (5.0)	86	<3	3
59		8.9/8.0	(5.3)	96	3	6
60		8.4/8.3	(4.9)	93(92)	<3	5



Scheme 1. (a) $ClSO_3H$, 100 °C; (b) Na_2SO_3, $NaHCO_3$; (c) MeI, $KHCO_3$.



Scheme 2. (a) For X = Br, R^1 = H, R^2 = F or R^1 = F, R^2 = H: $(MeO_2C)_2CH_2$, NaH, CuBr, 80 °C; for X = Cl, R^1 = SO_2Me, R^2 = H: $(EtO_2C)_2CH_2$, EtOH, 80 °C; (b) conc HCl, 110 °C or NaOH, then HCl; (c) $(MeO)_3$ CH, acetic anhydride, 110 °C; (d) conc H_2SO_4, MeOH; (e) NH_4OAc, AcOH, 80 °C; (f) NaOH; (g) CDI, DMF, then amine.

with shorter reaction times. Treatment of the diacid **63** with trimethylorthoformate yielded the corresponding cyclised anhydride **64** that underwent rearrangement to the lactone **65**. Finally, formation of the desired isoquinolinone acid was achieved by reaction with ammonium acetate followed by hydrolysis of the resulting methyl esters. It is worth noting that the entire sequence could be performed without the need for chromatography since all of the steps proceeded in high yield and the intermediates were isolated as clean solids (>95% purity determined by reverse phase HPLC) at each stage.

Final coupling with the amine **31** described in Part **I** of this communication[1] was carried out under standard coupling conditions, for example, using CDI in DMF.

References and notes

1. Luckhurst, C. A.; Furber, M.; Alcaraz, L.; Bahl, A.; Beaton, H.; Bowers, K.; Collington, J.; Denton, R.; Donald, D.; Kinchin, E.; MacDonald, C.; Rigby, A.; Riley, R.; Soars, M.; Springthorpe, B.; Webborn, P. *Bioorg. Med. Chem. Lett.* **2012**. http://dx.doi.org/10.1016/j.bmcl.2012.09.113.
2. Human CCR3 pK_i was determined in Chinese hamster ovary cells expressing human recombinant CCR3 receptors as described in WO2007102768, example 13. Human H_1 pK_i was determined in competition binding experiments as described in WO2006126948, example 6.
3. hERG pIC_{50} was determined in human embryonic kidney cells via ion flux electrophysiology. hERG binding was performed using the procedure described in WO2005037052.
4. Cl_{int}: HLM = human liver microsomes μL/min/mg protein, RH = rat hepatocytes μL/min/10^6 cells; NT = not tested.