Bioorganic & Medicinal Chemistry Letters 19 (2009) 6144–6147

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Optimising metabolic stability in lipophilic chemical space: The identification of a metabolically stable pyrazolopyrimidine CRF-1 receptor antagonist

Duncan C. Miller *, Wolfgang Klute, Andrew Calabrese †, Alan D. Brown

Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 17 July 2009
Revised 3 September 2009
Accepted 4 September 2009
Available online 11 September 2009

Keywords:
Antagonist
Corticotrophin releasing factor 1
Metabolic stability
G-protein coupled receptor

ABSTRACT

Balancing potency and metabolic stability in a target which favours lipophilic ligands is a considerable challenge. Here we describe two strategies employed to achieve this balance in a series of pyrazolopyrimidine CRF antagonists: moderation of lipophilicity, and incorporation of a metabolically stable lipophilic group.

Corticotrophin Releasing Factor (CRF) is a 41 amino acid peptide which is the primary modulator of the HPA (hypothalamic–pituitary–adrenal) axis. CRF is the endogenous ligand for two receptors, CRF-1 and CRF-2, both of which belong to the class B subfamily of G-Protein Coupled Receptors (GPCRs).[1] CRF-1 antagonists have historically been pursued primarily as potential treatments for anxiety and depression.[2] However reports have also implicated CRF in female sexual behaviour.[3] We hypothesised that an antagonist of the CRF-1 receptor may have utility in the treatment of female sexual dysfunction.

Several different classes of small molecule CRF-1 antagonists have been reported in the literature.[4] Representative examples are shown in Figure 1. While there are examples of CRF-1 antagonists which have been studied in the clinic including R121919[5] (**3**), Pexacerfont[6] (**4**) and Emicerfont[7] (**5**), attempts to identify CRF-1 antagonists suitable for clinical development have often been hampered by the apparent intolerance of the small molecule binding site to polar functionality, generally resulting in high lipophilicity for reported small molecule antagonists.[8] This high lipophilicity reduces the chance of achieving a suitable pharmacokinetic profile.[9]

The lead compound for our studies was selected from a review of substrate from previous CRF-1 antagonist programs at Pfizer. Compound **6**[10], a close analogue of the extensively studied pyrrolopyrimidine CRF-1 antagonist Antalarmin,[11] was selected as a promising lead due to its high potency[12] and relatively heteroatom rich, polar pyrazolopyrimidine core (Fig. 2). However this compound was still highly lipophilic with poor in vitro metabolic stability in human liver microsomes.[13]

SAR for CRF-1 potency in chemotypes containing a bicyclic core has been well described in the literature.[4]

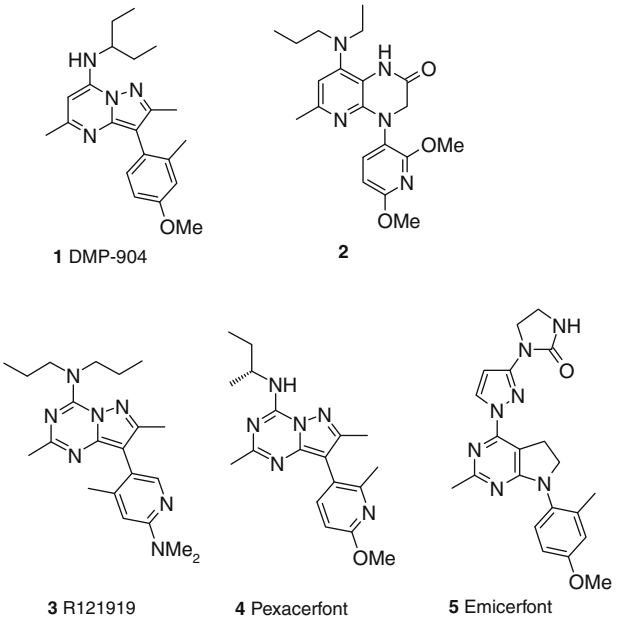
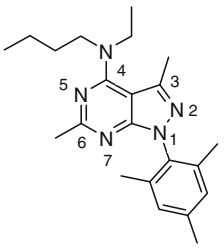


Figure 1. Representative examples of reported CRF-1 antagonists.

* Corresponding author. Tel.: +44 1304 640026.
E-mail address: duncan.miller@pfizer.com (D.C. Miller).
† Celgene, 4550 Towne Centre Court, San Diego, CA 92121, USA.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.016



6 CRF1 Ki 9nM clogP 6.75
HLM Clint >320 l/min/mg

Figure 2. Structure and in vitro functional potency, microsomal stability and clog *P* for Compound **6**.

In an attempt to identify a novel CRF-1 antagonist with good pharmacokinetic parameters we chose to screen all analogues for stability in human and rat liver microsomes in parallel with generating CRF-1 activity data, enabling us to track SAR and physicochemical trends for microsomal stability alongside those for CRF-1 activity.

Our initial design strategy targeted reducing lipophilicity with the aim of improving the metabolic stability of this template through incorporation of more polar substituents at the 1- and 4-positions.

We chose to first explore SAR at the 1-substituent while keeping the 4-substituent constant. For these studies we selected *N*-propyl-*N*-methoxyethylamine as as a less lipophilic replacement of the *N*-ethyl-*N*-butylamine substituent at the 4-position of the pyrazolopyrimidine template as it has previously been reported as retaining good CRF-1 potency when incorporated into this position in several bicyclic CRF-1 antagonist templates[4] (Table 1).

Introduction of a 2-methyl-4-methoxyphenyl substituent into the 1-position of 6,5 bicyclic systems has been widely reported to give good CRF-1 potency.[4] Its introduction into the pyrazolopyrimidine template to give **7** did retain good CRF-1 activity and also encouraging microsomal stability in rat. However stability in human microsomes was poor.[14] Substituting the phenyl ring with other polar substituents (**8–11**) resulted in reduced potency which broadly tracked with the reduction in lipophilicity. Despite this reduction in lipophilicity only **11** showed a significant improvement in both rat and human microsomal stability. Incorporation of substituted pyridines with precedented activity in other 6,5 templates[4,6] either failed to improve microsomal stability (**12–14**) or in the case of **15** improved stability in both rat and human microsomes at the expense of a significant reduction in CRF-1 activity.

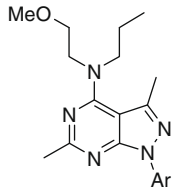
These results suggested that further tuning the lipophilicity of the template through introduction of polarity in the 1-substituent was unlikely to lead to potent compounds with sufficient microsomal stability.

Our attention thus turned to exploring SAR at the 4-position. We elected to explore SAR at this position with the 2-methyl-4-methoxyphenyl substituent in place with the aim of identifying a substituent at the 4-position which would improve human microsomal stability while retaining the encouraging potency and rat stability of compound **7** (Table 2).

Reducing lipophilicity with both tertiary (**16–24**) and secondary amines (**25–30**) uniformly resulted in a significant reduction in potency. Secondary amines were generally more stable than tertiary amines with similar lipophilicity (e.g., **18** vs **27** and **28**).

At this point it appeared that the strategy of reducing lipophilicity through modifications to the 1- and 4-substituents would not deliver a compound with the desired balance of properties. As an alternative strategy two higher lipophilicity analogues, **31** and

Table 1
In vitro functional potency, microsomal stability and clogP for compounds **7–15**



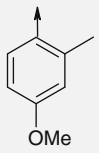
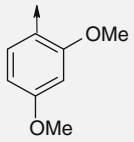
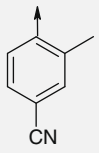
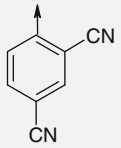
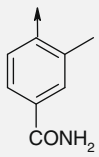
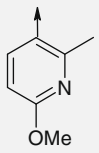
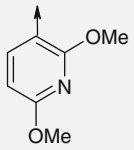
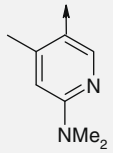
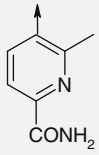
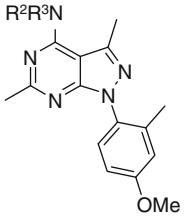
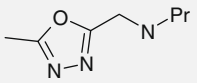
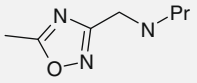
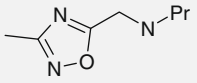
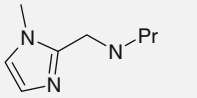
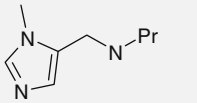
Compd	Ar	K_i (nM)	HLM	RLM	clog *P*
7		13	172	23	4.8
8		74	86	90	4.4
9		101	147	<11	4.1
10		632	151	166	3.1
11		275	17	22	3.5
12		43	117	163	4.1
13		17	218	159	4.0
14		183	160	206	4.1
15		577	38	13	2.8

Table 2
In vitro functional potency, microsomal stability and clog *P* for compounds **7** and **16–32**



Compd	NR^2R^3	K_i (nM)	HLM	RLM	clog *P*
7	N-(*n*Pr)$(CH_2)_2$OMe	13	172	23	4.8
16	N-(Et)$(CH_2)_2$OMe	117	117	104	4.3
17	$N(CH_2CH_2OMe)_2$	193	66	47	3.8
18	N(*n*Pr)(CH_2CH_2OH)	129	80	117	4.1
19	N(*n*Pr)CH_2CONMe_2	1409	18	22	4.2
20		996	34	40	3.3
21		84	51	66	4.2
22		124	29	58	4.2
23		400	96	67	4.4
24		2500	196	85	4.4
25	(*S*)-NHCH(CH_2OMe)Et	135	52	105	4.3
26	(*R*)-NHCH(CH_2OMe)Et	210	37	53	4.3
27	(*S*)-NHCH(*n*Pr)CH_2OH	76	23	41	4.1
28	(*R*)-NHCH(*n*Pr)CH_2OH	574	10	36	4.1
29	(*S*)-NHCH(CH_2OH)Et	620	<7	27	3.6
30	(*R*)-NHCH(CH_2OH)Et	1130	<7	23	3.6
31	$NHCH(Et)_2$	42	64	72	5.4
32	$NHCH(cPr)_2$	35	<12	24	5.2

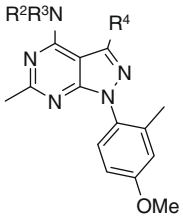
32, were prepared lacking the potentially metabolically vulnerable methoxy and hydroxyl groups present in the earlier secondary amino analogues. These compounds both retained good CRF-1 potency. However despite being effectively isolipophilic they had markedly different microsomal stability profiles with **32** exhibiting much greater microsomal stability in both rat and human systems than **31**. The microsomal stability of **32** was also superior to the more polar analogues prepared in this series.

With the relatively lipophilic *C*,*C*-dicyclopropylmethylamine identified as giving the best balance of CRF-1 potency and microsomal stability at the 4-position our attention now turned to identifying other areas of the template where we might be able to moderate lipophilicity. Analysis of the reported bicyclic CRF-1 antagonists revealed that the methyl at the 6-position is generally required for CRF-1 activity, whereas templates lacking the methyl at the 3-position often retain CRF-1 activity. In an attempt to further reduce the lipophilicity analogues with the 3-methyl deleted were prepared (Table 3).

When the 4-substituent was a tertiary amine deletion of the 3-methyl group resulted in an increase in human microsomal stability, albeit at the expense of a 10-fold reduction in CRF-1 activity (**33** vs **7**).

Intriguingly, when the 4-substituent was a secondary rather than a tertiary amine, the reduction in potency for deletion of the

Table 3
Comparison of pairs of compounds differing only at the 3-position (R^4)



Compd	NR^2R^3	R^4	K_i (nM)	HLM	RLM	clog *P*
7	N(*n*Pr)(CH_2CH_2OMe)	Me	13	172	23	4.8
33	N(*n*Pr)(CH_2CH_2OMe)	H	132	16	387	4.6
25	$NCH(Et)_2$	Me	42	64	72	5.4
34	$NCH(Et)_2$	H	85	15	246	5.1
32	$NHCH(cPr)_2$	Me	35	<12	24	5.2
35	$NHCH(cPr)_2$	H	32	<11	25	5.0

3-methyl group was much less severe (**25** vs **34** and **32** vs **35**). We believe that this observation may be due to the differences in conformational preference between secondary and tertiary amines at this position. Secondary amines can adopt a coplanar conformation with the lone pair of the exocyclic sp^2 nitrogen in conjugation with the pi system of the bicyclic heterocycle (Fig. 3, compound **34**). In this conformation the branched alkyl groups are able to occupy the proposed binding pockets in the CRF-1 receptor above and below the plane of the core. The presence or absence of the 3-methyl group is unlikely to affect this conformation resulting in minimal potency difference. Due to steric clash with the 3-methyl group the preferred conformation for tertiary amines is the non-conjugated form (Fig. 3, compound **7**) which again allows the alkyl groups to occupy the proposed binding pockets. Substitution of the 3-methyl group for a hydrogen atom reduces this steric clash allowing the exocyclic nitrogen to access a low energy coplanar conjugated conformation (Fig. 3, compound **33**).

The use of the C,C-dicyclopropylmethylamine substituent present in our most promising compounds (**32** and **35**) has previously been reported in CRF-1 antagonists.[15] In the reported triazole template this group suffered from significant acid instability. However, in accelerated solution stability studies **35** showed good stability

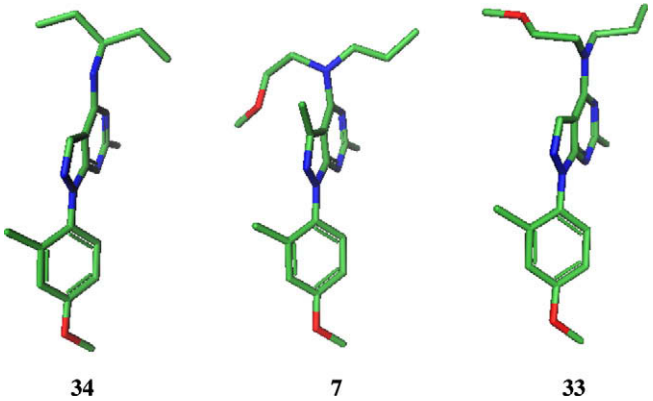
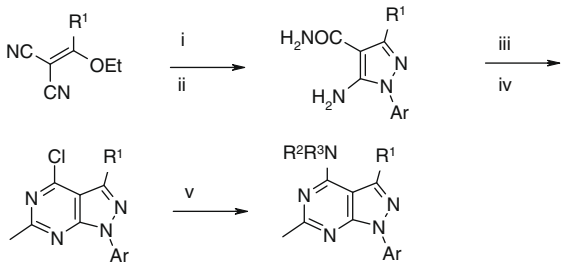


Figure 3. Minimised conformations of **34**, **7** and **33**.

Table 4
In vivo rat pharmacokinetic parameters for compound **35** iv dose 0.1 mg/kg, po dose 5 mg/kg

Compd	Cl (ml/min/kg)	V_d (L/kg)	Fu	$T_{1/2}$ (h)	*F* (%)
35	27	7	0.02	1.9	43



Scheme 1. Reagents: (i) $ArNHNH_2$, MeOH, Et_3N; (ii) NaOH, EtOH, H_2O; (iii) NaOEt, EtOAc, EtOH; (iv) $POCl_3$, dimethylaniline, CH_3CN; (v) *i*$PrNEt_2$, R^2R^3NH, CH_3CN.

across the pH range, with only limited degradation at pH1.2 (51% main band remaining after 14 days at 50 °C). Discussions with colleagues in our pharmaceutical sciences department confirmed that this level of stability should not cause any issues in the development of this compound.

Compound **35** was progressed into a rat pharmacokinetic study to determine how in vitro metabolic stability would translate in vivo (Table 4). Compound **35** exhibited an encouraging pharmacokinetic profile with moderate volume of distribution and clearance suitable for further pre-clinical investigation.

Compounds were conveniently prepared from substituted aryl hydrazines by the method described in Scheme 1. Condensation of the hydrazine with the suitably substituted ethoxymethylidenemalononitrile yielded the cyanopyrazole intermediate, which was hydrolysed to the amide under basic conditions. Cyclisation to the pyrazolopyrimidinone was followed by chlorination to give the chloropyrimidine derivative, which was subsequently displaced with an amine under basic conditions to give target compounds such as **35**.

In summary, introduction of the *C*,*C*-dicyclopropylmethylamine group was instrumental in combining metabolic stability with CRF-1 potency in this pyrazolopyrimidine template. This suggests that in targets which poorly tolerate polar functionality, identifying functional groups which display much higher metabolic stability than would be expected from their lipophilicity can be an effective strategy. Compound **35** has been identified as having an improved balance of potency and in vitro metabolic stability relative to compound **6,** and a pharmacokinetic profile suitable to support further progression of this compound. Further studies will be reported in due course.

Acknowledgements

We acknowledge Denise Harding, Laia Malet and Adam Stennett for compound synthesis, Margaret Jackson, Nick Edmunds, Jo Mulgrew, Graham Baker, David Winpenny, Pauline Carnell and Rachel Russell for biological data and Joanne Phipps for ADME studies.

References and notes

1. Grigoriadis, D. E.; Haddach, M.; Ling, N.; Saunders, J. *Curr. Med. Chem.* **2001**, *1*, 63.
2. Holsboer, F.; Ising, M. *Eur. J. Pharmacol.* **2008**, *583*, 350.
3. (a) Sirinasthsinghji, D. J. S. *Brain Res.* **1985**, *336*, 45; (b) Sirinathsinghji, D. J. S. *Brain Res.* **1986**, *375*, 49; (c) Heinrichs, S. C.; Min, H.; Tamraz, S.; Carmouche, M.; Boehme, S. A.; Vale, W. W. *Psychoneuroendocrinology* **1997**, *22*, 215; (d) Jones, J. E.; Pick, R. R.; Davenport, M. D.; Keene, A. C.; Corp, E. S.; Wade, G. N. *Am. J. Physiol.* **2002**, *283*, R591.
4. (a) Gilligan, P. J.; Baldauf, C.; Cocuzza, A.; Chidester, D.; Zaczek, R.; Fitzgerald, L. W.; McElroy, J.; Smith, M. A.; Shen, H. S. L.; Saye, J. A.; Christ, D.; Trainor, G.; Robertson, D. W.; Hartig, P. *Bioorg. Med. Chem.* **2000**, *8*, 181; (b) Chen, C.; Wilcoxen, K. M.; Huang, C. Q.; McCarthy, J. R.; Chen, T.; Grigoriadis, D. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3669; (c) Dzierba, C. D.; Takvorian, A. D.; Rafalski, M.; Kasireddy-Polam, P.; Wong, H.; Molski, T. F.; Zhang, G.; Li, Y.-W.; Lelas, S.; Peng, Y.; McElroy, J. F.; Zaczek, R. C.; Taub, R. A.; Combs, A. P.; Gilligan, P. J.; Trainor, G. L. *J. Med. Chem.* **2004**, *47*, 5783.
5. Chen, C.; Wilcoxen, K. M.; Huang, C. Q.; Xie, Y. F.; McCarthy, J. R.; Webb, T. R.; Zhu, Y.-F.; Saunders, J.; Liu, X. J.; Chen, T. K.; Bozigian, H.; Grigoriadis, D. E. *J. Med. Chem.* **2004**, *43*, 449.
6. Gilligan, P. J.; Clarke, T.; Liqi, H.; Lelas, S.; Li, Y.-W.; Heman, K.; Fitzgerald, L.; Miller, K.; Zhang, G.; Marshall, A.; Krause, C.; McElroy, J. F.; Ward, K.; Zeller, K.; Wong, H.; Bai, S.; Saye, J.; Grossman, S.; Zaczek, R.; Arneric, S. P.; Hartig, P.; Robertson, D.; Trainor, G. *J. Med. Chem.* **2009**, *52*, 3084.
7. Di Fabio, R.; St-Denis, Y.; Sabbatini, F. M.; Andreotti, D.; Arban, R.; Bernasconi, G.; Braggio, S.; Blaney, F. E.; Capelli, A. M.; Castiglioni, E.; Di Modugno, E.; Donati, D.; Fazzolari, E.; Ratti, E.; Feriani, A.; Contini, S.; Gentile, G.; Ghirlanda, D.; Provera, S.; Marchioro, C.; Roberts, K. L.; Mingardi, A.; Mattioli, M.; Nalin, A.; Pavone, F.; Spada, S.; Trist, D. G.; Worby, A. *J. Med. Chem.* **2008**, *51*, 7370.
8. For recent reviews, see: (a) Tellew, J. E.; Zhiyong, L. *Curr. Top. Med. Chem.* **2008**, *8*, 506; (b) Chen, C. *Curr. Med. Chem.* **2006**, *13*, 1261; (c) Gilligan, P. J.; Li, Y.-W. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 487; (d) Kehne, J.; De Lombaert, S. *Curr. Drug Targets-CNS Neurol. Disord.* **2002**, *1*, 467; (e) Gilligan, P. J.; Robertson, P. J.; Zaczek, R. *J. Med. Chem.* **2000**, *43*, 1641.
9. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Dis.* **2007**, *6*, 881.
10. Gene, M.; Chen, Y. L.; Welch, W. M. Jr. European patent 691128, 1996.
11. Chen, Y. L.; Mansbach, R. L.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. *J. Med. Chem.* **1997**, *40*, 1749.
12. CRF-1 potency is expressed as functional activity measured using CHO cells (Cell Sciences SNB0000377) expressing recombinant human CRF-1 receptor grown in DMEM:F12 (1:1) media containing 10% (V/V) Foetal Bovine Serum (PAA), 400 μg/ml Geneticin (GIBCO-BRL 10131-027) and 1% (V/V) Glutamax in a cell incubator at 37 °C, 5% CO_2 to 70% confluence. FAC hCRF (10 nM) was added with test compound to 10,000 cells/well in Phosphate Buffered Saline containing 500 μM FAC IBMX. The functional response was measured using DiscoveRx HitHunter cAMP II Assay kit (Amersham Biosciences—90-0034-03). Each compound was tested multiple times using a 0.5 log serial dilution dose–response with a top final assay concentration of 20 μM. The % response of the test compound at different test doses were then fitted to a 4-parameter logistic curve to determine the compound IC50. K_i values were determined from the IC50 using the Cheng and Prussoff relationship and the EC_{50} for the agonist dose–response curve which was determined on the same day.
13. Human liver microsome (HLM) and rat liver microsome (RLM) figures are reported as intrinsic clearances (Cl_{int}) with units of μg/ml/mg of protein. This is a measure of oxidative (Phase I) metabolic liability. Unpublished in-house Pfizer experience suggests Cl_{int} figures of <30 are generally have a good chance of translating to moderate to low in vivo clearance, and Cl_{int} figures of >70 are likely to translate to rapid in vivo clearance.
14. The difference between human and rat microsomal stability in this case is surprising. From unpublished in-house experience in these assays rat liver microsomes generally show higher turn-over across chemotypes than human liver microsomes. The high turnover in rat microsomes for this compound suggests a possible atypical species difference in Cyp mediated metabolism for compound **7**.
15. Lowe, R. F.; Nelson, J.; Trunghau, N. D.; Crowe, P. D.; Pahuja, A.; McCarthy, J. R.; Grigoriadis, D. E.; Conlon, P.; Saunders, J.; Chen, C.; Szabo, T.; Ta Kung, C.; Bozigian, H. *J. Med. Chem.* **2005**, *48*, 1540.