Bioorganic & Medicinal Chemistry Letters 19 (2009) 1084–1088

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The design and discovery of novel amide CCR5 antagonists

David C. Pryde *, Martin Corless, David R. Fenwick, Helen J. Mason, Blanda C. Stammen, Peter T. Stephenson, David Ellis, David Bachelor, David Gordon, Christopher G. Barber, Anthony Wood, Donald S. Middleton, David C. Blakemore, Gemma C. Parsons, Rachel Eastwood, Michelle Y. Platts, Keith Statham, Kerry A. Paradowski, Catherine Burt, Wolfgang Klute

Department of Chemistry, Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 17 November 2008
Revised 6 January 2009
Accepted 6 January 2009
Available online 10 January 2009

Keywords:
CCR5
Antagonist
Chemokine
HIV
AIDS
Antiviral

ABSTRACT

The synthesis of a range of novel amine-containing structures and their primary potency as inhibitors of HIV-1 fusion via blocking of the CCR5 receptor is described. The development of the medicinal chemistry strategy and SAR's which led to the identification of the piperidine amide compounds **33** and **36** as excellent leads for further evaluation is described, along with key physicochemical data which highlighted their lead potential.

Chemokines are a large family of pro-inflammatory peptides which exert their effects through a range of at least 17 G-protein coupled receptors (GPCR's) leading to downstream chemotaxis, angiogenesis, cell differentiation and other biological activities.[1] Recently, antagonism of the CCR5 receptor has been targeted as a potential treatment for HIV infection, based on the finding that CCR5 antibodies can block infection of macrophages by M-tropic HIV.[2] Further, CCR5Δ32 homozygotes are highly resistant to R5-tropic HIV infection, while heterozygotes do become infected, but progress more slowly to AIDS and respond better to treatment. It has become apparent that CCR5 is the coreceptor for the most commonly transmitted HIV-1 strains which predominate during the early stages of infection and remain the dominant form in >50% of late stage HIV-1 infected patients.[3] Blockade of the CCR5 receptor appears not to be associated with any mechanism-related side effects, making this a highly compelling target for drug discovery.[4]

Over the past several years, there has been intensive research interest in small molecule antagonists of the CCR5 receptor resulting in the disclosure of several distinct chemical series.[5] This has led to the first launched CCR5 antagonist, maraviroc (**1**),[6] and a range of other agents at various stages of development (Fig. 1). As the work described within this paper was being carried out, literature examples of CCR5 antagonists such as E-913 (**2**),[7] SCH-C (**3**),[8] and TAK-779[9] (**4**) were all being reported to share potent antiviral activity within a range of chemotypes. Despite the diversity of structures represented in Fig. 1, a common feature of all of these series is the presence of a basic nitrogen atom which is believed to 'anchor' the ligand to E283 within the transmembrane region of the CCR5 receptor,[10] and a proximal lipophilic group, which likely lies in close contact with a tyrosine residue, Y108.

Structural overlays of all 4 structures are at best imperfect, and it is clear that the structures can occupy distinct, but partially overlapping regions of the CCR5 receptor. Literature reports suggest that the TAK series, exemplified by TAK-779 binds in a cavity formed by transmembrane helices 1, 2, 3 and 7 near the extracellular surface of CCR5.[11] A similar binding cavity has been reported for SCH-C based on an alanine scan method.[12] This site only partially overlaps with a putative binding site, proximal to the extracellular side of helices 2, 3, 6 and 7, of a distinct series of compounds reported by the Merck group, based on a homology model constructed from the crystal structure of bovine rhodopsin.[13] The E913 series of compounds is reported to additionally make contacts with the second extracellular loop.[14] Finally, site directed mutagenesis studies have supported the maraviroc series binding in a distinct region of the receptor to SCH-C.[10] These data demonstrate that a range of structurally distinct chemotypes can be accommodated by the CCR5 receptor and retain potent antagonism.

* Corresponding author. Tel.: +44 0 1304 64.
E-mail address: David.Pryde@pfizer.com (D.C. Pryde).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.01.012

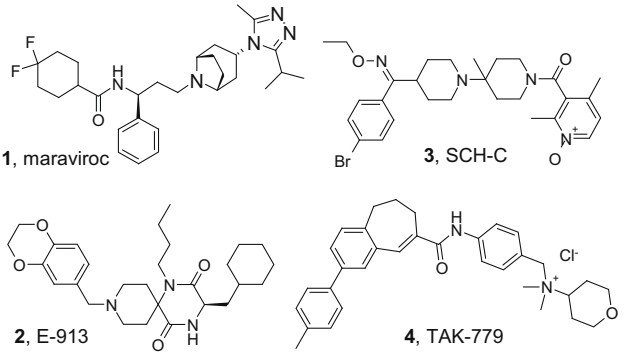
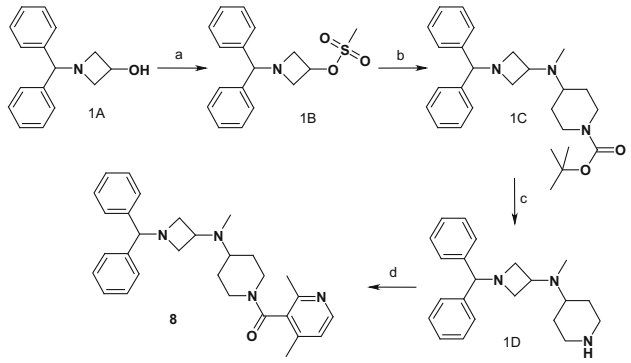


Figure 1. Literature examples of potent CCR5 antagonists taken from the time of the work described within this paper.



Scheme 1. Reagents and conditions: (a) MsCl, Et_3N, DCM, 0 °C, 100%. (b) 1-BOC-4-methylaminopiperidine, Na_2CO_3, MeCN, rt, 53%. (c) TFA, DCM, rt, 100%. (d) 2,4-dimethyl-nicotinic acid, WSCDI, HOBt, Hunigs base, DMAP, DMF, rt, 76%.

Following our extensive investigations around piperidine and tropane amide series of CCR5 antagonists, we were seeking a complementary series of novel structures with high antiviral potency, good metabolic stability and high oral absorption, primarily to provide agents with an orthogonal structure and potential resistance profile to that of maraviroc. One arm of these investigations involved the syntheses of a number of variant heterocyclic core templates based around the piperidine amide function as represented in SCH-C,[15] but incorporating maraviroc-like amide functionality which had been shown to impart favourable properties on the maraviroc series.[6b] Inspiration for the heterocyclic cores was taken from the extensive literature which exists around other peptidic GPCR antagonists,[16] with structural templates built around a central basic core. For synthetic expedience, it was decided to target only right hand side amides which would introduce both synthetic flexibility and the potential for late stage parallel chemistry into our syntheses. This basic strategy is shown in Figure 2.

For example, the targeted core structures featured mono- and bicyclic amine groups, with linking groups of variant length, functionality and number of rotatable bonds to cast our net as wide as possible. Examples of these groups are shown in the below tables.

The synthesis of these compounds was straightforward and largely derivative. The central linker group was constructed by a combination of reductive amination or alkylation chemistry, from which the final compounds were synthesised by simple amide formation. A typical synthetic scheme is shown in Scheme 1 below for the azetidine **8**.

All other targets represented in the data tables below were accessed using analogous chemistry, starting from amine/carbonyl combinations. In the cases where the amine component was not commercially or easily available, the synthesis of that component is detailed in the Supplementary Information section.

Table 1 shows the initial examples made in this investigation, all based on a left hand side benzhydryl group and a 2,6-dimethyl pyridyl amide on the right hand side.[17] The linking groups examined included monocycles and bicycles, contained bridging heterocycles and contained both monobasic or dibasic amines where the basic group is either part of a ring or acyclic. Initial targets covered a relatively wide range of calculated pK_a values (calculated pK_as of approximately 6–9), although it should be stressed that very few of these calculated values were confirmed by measurement. The activity measurements in Table 1 reflect the inhibition of cell–cell fusion between HIV-1 gp160-expressing CHO cells and CD4/CCR5-expressing HeLa-P4 cells which has been shown to offer an excellent correlation with anti-HIV activity.[6,18] Also detailed in Table 1 are *c*Log*P* values for these initial targets. While relatively high, our strategy relied on simple chemistry to rapidly identify suitable core templates, which we could then return to and optimise physicochemical properties. Monocyclic linkers such as those contained in compounds **5**–**7** showed very little activity, although the incorporation of a piperidine ring in the centre of a linking group in **16** gave a 56 nM inhibitor of cellular fusion. The azetidine **8** showed very encouraging 24 nM fusion IC_{50}, and the isomeric analogue **15** showed a similarly encouraging 102 nM.

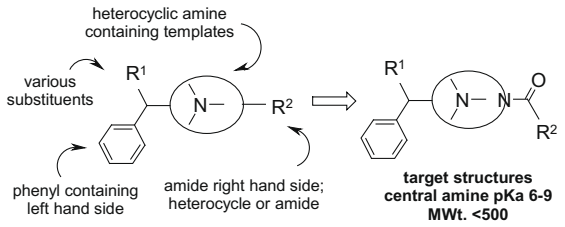
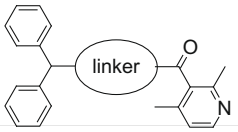


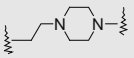
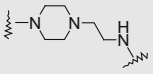
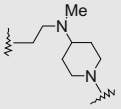
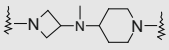
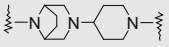
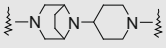
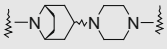
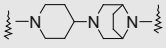
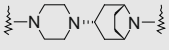
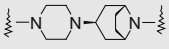
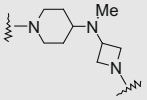
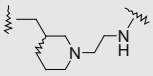
Figure 2. Design strategy for a novel series of CCR5 antagonists.

Structures in which a piperazine, tropane or azatropane formed bicyclic core templates such as those in **11**–**14** largely ablated activity, although isomeric analogues **9** and **10** had significantly improved activity, and highlighted the need to hit upon just the right template and functional substituents to ensure CCR5 potency.

Having established several lead compounds within new templates which possessed encouraging activity, we set about reducing the relatively high Log*P* of these leads primarily through the incorporation of an amide group in the left hand side portion of the molecules, initially via small alkyl or cycloalkyl amides which we knew had precedented antiviral potency in the maraviroc series. These structures are shown in Table 2 and incorporate both pyridyl and phenyl right hand side amides. The monocyclic structure of **16** was reproduced in the amide **17**, but this resulted in significant loss of potency. The azetidine structure of **8** was incorporated into the amides **21** and **22**, albeit with the addition of an extra methylene group to avoid the introduction of an unstable aminal functional group, and again this resulted in a loss of fusion potency. Building upon the finding that some bicyclic structures in Table 1 were potent, we set about incorporating several bicyclic core templates into the basic target structures using combinations of azetidine, pyrrolidine and piperidine rings. This was largely an unsuccessful exercise viz **19**–**20**, **23**–**25** and **27**–**29**, until the *bis*-piperidine **30** was made, and showed to have very encouraging potency and moderate lipophilicity (IC_{50} 145 nM, *c*Log*P* 3.5). These results showed the importance of suitably orienting the left hand side functionality to achieve good primary potency, and that presumably very few of our test structures achieved a correct orientation. The only other compound in Table 2 which had an IC_{50} below 3 μM in the fusion assay was the azetidine analogue **26** albeit some 20 fold weaker.

Table 1
Initial examples of linking groups in a benzhydryl–pyridyl amide series



Compound #	Linker	gp160 fusion IC_{50}, nM[a]	cpka[b] (cLogP)
5		0%@10 μM	6.5 (5.0)
6		15%@100 nM	6.1 (4.4)
7		13%@100 nM	8.7 (4.3)
8		24	8.0 (4.4)
9		313	7.8 (4.4)
10		41	8.9 (4.4)
11[d]		25%@10 μM	9.4 (4.5)
12		12%@100 nM	7.7 (4.5)
13		21%@10 μM	7.3 (4.4)
14		6%@100 nM	7.3 (4.4)
15		102 nM	8.2 (4.9)
16[c]		56	8.5 (5.9)

[a] The concentration of test compound required to inhibit the cell–cell fusion of HeLa-P4 cells expressing recombinant human CCR5 and CD4 at the cell surface and encoding an HIV-1 long terminal repeat regulated B-Gal reporter gene with CHO cells expressing cell surface Tat and HIV-1 gp160 by 50%. IC_{50} determinations were the mean of at least two replicates.[18]
[b] Calculated using the ACD Labs pKa prediction software.
[c] Racemic mixture.
[d] Mixture of diastereoisomers.

Our efforts now focussed entirely around the lead structure **30**, summarised in Table 3. These designs kept the rightmost piperidine ring invariant (either as a simple piperidine or a tropane equivalent), and sought to alter the remainder of the structure. The most successful design feature identified was a benzyl amino group appended to the amide N atom, which provided all of the most active compounds, and importantly simplified any synthesis and stereochemistry issues significantly. Compounds **32**, **33**, **34**, **36** and **38** all possessed very similar structures and shared modest to excellent activity. A 3-linked piperidine **35** was significantly poorer than its 4-linked equivalent, as was a left hand ring tropane in **31** and it was notable that rigidifying the central region of the *bis*-piperidine system in **37** gave a further potency enhancement.

Table 2
Left-hand side amide analogues of compounds from Table 1

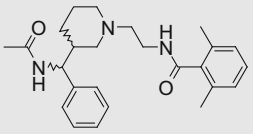
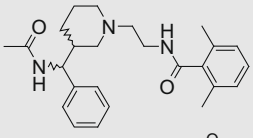
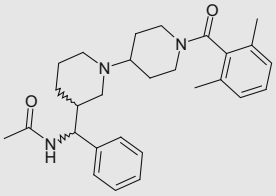
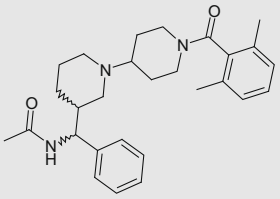
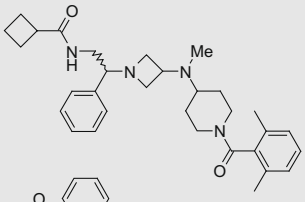
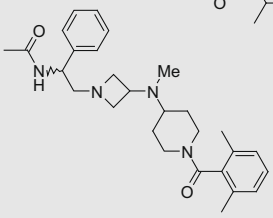
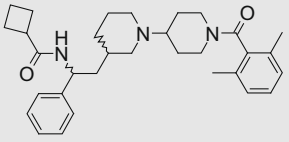
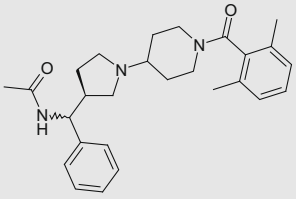
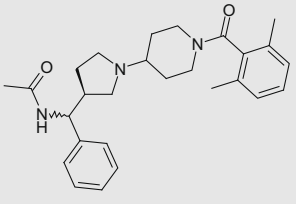
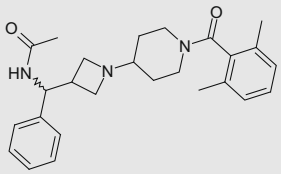
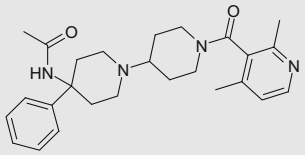
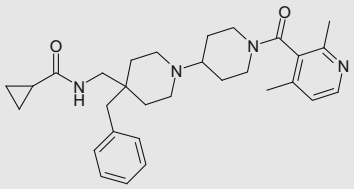
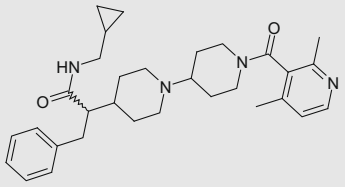
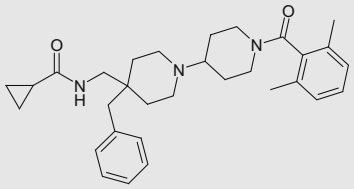
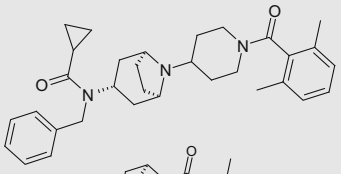
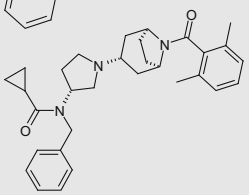
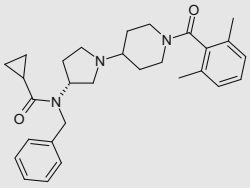
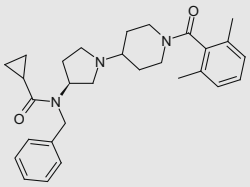
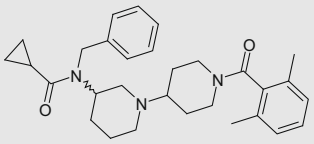
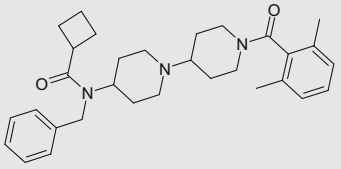
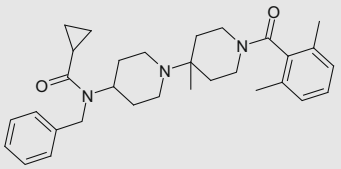
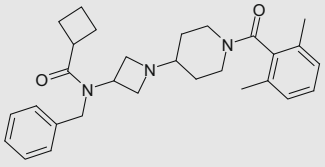
Compound	Structure[a]	gp160 fusion IC_{50}, nM[b]	cLogP
17[c]		20% @10 μM	2.7
18[c]		12% @10 μM	2.7
19[c]		28% @10 μM	2.6
20[c]		0% @10 μM	2.6
21		20% @10 μM	4.2
22		50% @10 μM	3.5
23[d]		20% @10 μM	4.2
24[c]		18% @1 μM	2.1
25[c]		0% @ 10 μM	2.1

Table 2 (*continued*)

Compound	Structure[a]	gp160 fusion IC_{50}, nM[b]	cLogP
26		2490	1.5
27		20% @10 μM	1.1
28		18% @1 μM	1.9
29		20% @10 μM	2.7
30		145	3.5

[a] Compounds **17**–**23**, **26** and **29** were all tested as racemates.
[b] The concentration of test compound required to inhibit the cell–cell fusion of HeLa-P4 cells expressing recombinant human CCR5 and CD4 at the cell surface and encoding an HIV-1 long terminal repeat regulated B-Gal reporter gene with CHO cells expressing cell surface Tat and HIV-1 gp160 by 50%. IC_{50} determinations were the mean of at least two replicates.[18]
[c] Single, unassigned diastereoisomer.
[d] Mixture of diastereoisomers.

Table 3
Analogues designed around compound **30**

Compound	Structure	gp160 fusionIC_{50}, nM[a]	cLogP
31		10%@1 μM	3.3
32		1	3.9
33		1	3.4
34		1230	3.4
35[b]		19%@10 μM	4.2
36		3	3.3
37		0.5	3.5
38		5800	4.6

[a] The concentration of test compound required to inhibit the cell–cell fusion of HeLa-P4 cells expressing recombinant human CCR5 and CD4 at the cell surface and encoding an HIV-1 long terminal repeat regulated B-Gal reporter gene with CHO cells expressing cell surface Tat and HIV-1 gp160 by 50%. IC_{50} determinations were the mean of at least two replicates.[18]
[b] Compound **35** was tested as a racemate.

It is interesting to note the difference in potency between the very similar structures represented by 3-linked piperidine **35**, 4-linked piperidine **36**, pyrrolidine **33** and the related architecture of **25** which may have been difficult to fully elucidate had we not set out with a flexible design approach to synthesising a wide range of targets.

The result of these investigations was the identification of several highly potent and promising novel agents. Physicochemical and in vitro ADME data was obtained on two compounds, **33** and **36**, along with confirmation of competitive antagonism by **33** of the CCR5 receptor using a MIP-1β displacement binding assay (Table 4).

Both compounds were moderately lipophilic, which partly explained their very poor metabolic stability in hepatic microsomes. Examination of the route by which compound **33** was metabolised[20] revealed that a facile oxidation of the benzylic methylene of the *N*-benzyl group was responsible for the very short microsomal half-life. This was mirrored in the predominant metabolic route of compound **36**.

Table 4
Selected properties of compounds **33** and **36**

Compound	33	36
MWt.	460	487
[a]LogD	2.6	3.5
[b]HLM Clint μL/min/mg	195	205
[c]BaL IC_{90}, nM	2	40
[d]MIP-1β IC_{50}, nM	8	ND
[e]hERG IC_{50}, nM	9800	3410
Caco-2 AB/BA	23/26	ND
TPSA[19]	44	44

[a] Partition coefficient measured in 1-octano/aqueous buffer at pH 7.4

[b] Determined using pooled liver microsomes in phosphate buffer at pH7.4 and 1 μM substrate concentration, with sampling made at the 30min timepoint.

[c] The concentration at which replication of the R5 HIV-1 viral strain Ba-L is 90% inhibited by test compounds in the PM-1 cell line as measured by p24 antigen output.

[d] The concentration of test compound required to inhibit binding of [^{125}I]-labelled MIP-1β to CCR5 stably expressed on MIP34.10 cells by 50%.

[e] The concentration of test compound required to inhibit binding of [^{3}H]dofetilide to HERG stably expressed on HEK293 cells by 50%.

Nonetheless, the series represented by compounds **33** and **36** offered the potential for high permeability, relatively weak binding to the hERG ion channel,[21] and highly promising levels of antiviral activity. They therefore represented excellent starting points for a lead development programme with the aims of further driving down antiviral potency and increasing metabolic stability, the results of which will be disclosed elsewhere.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.012.

References and notes

1. Viola, A.; Luster, A. D. *Ann. Rev. Pharm. Toxicol.* **2008**, *48*, 171.
2. Cocchi, F.; DeVico, A. L.; Garzino-Demo, A.; Arya, S. K.; Gallo, R. C.; Lusso, P. *Science* **1995**, *270*, 1811.
3. Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C. M.; Saragosti, S.; Lapoumeroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. *Nature* **1996**, *382*, 722.
4. (a) Dorr, P.; Perros, M. *Ex. Op. Dr. Discov.* **2008**, *3*, 1345; (b) Perros, M. *Adv. Antiviral Drug Design* **2007**, *5*, 185.
5. Barber, C. G. *Curr. Opin. Invest. Drugs* **2004**, *5*, 851.
6. (a) Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.; Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. *Antimicrob. Agents Chemother.* **2005**, *49*, 4721; (b) Wood, A.; Armour, D. *Progress Med. Chem.* **2005**, *43*, 239.
7. Maeda, K.; Yoshimura, K.; Shibayama, S.; Habashita, H.; Tada, H.; Sagawa, K.; Miyakawa, T.; Aoki, M.; Fukushima, D.; Mitsuya, H. *J. Biol. Chem.* **2001**, *276*, 35194.
8. Strizki, J. M.; Xu, S.; Wagner, N. E.; Wojcik, L.; Liu, J.; Hou, Y.; Endres, M.; Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Tagat, J. R.; McCombie, S.; Cox, K.; Fawzi, A. B.; Chou, C.-Chu.; Pugliese-Sivo, C.; Davies, L.; Moreno, M. E.; Ho, D. D.; Trkola, A.; Stoddart, C. A.; Moore, J. P.; Reyes, G. R.; Baroudy, B. M. *PNAS* **2001**, *98*, 12718.
9. Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. *PNAS* **1999**, *96*, 5698.
10. Dorr, P. K.; Todd, K.; Irvine, R.; Robas, N.; Thomas, A;, Fidock, M.; Sultan, H.; Mills, J.; Perrucio, F.; Burt, K.; Rickett, G.; Perkins, H.; Griffin, P.; Macartney, M.; Hamilton, D.; Westby, M.; Perros, M. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2005, Washington DC, USA. b. Kondru, R.; Zhang, J.; Ji, C.; Mirzadegan, T.; Rotstein, D.; Sankuratri, S.; Dioszegi, *M. Mol. Pharm.*, **2008**, 73(3), 789.
11. Dragic, T.; Trkola, A.; Thompson, D. A.; Cormier, E. G.; Kajumo, F. A.; Maxwell, E.; Lin, S. W.; Ying, W.; Smith, S. O.; Sakmar, T. P.; Moore, J. P. *PNAS* **2000**, *97*, 5639.
12. Tsamis, F.; Gavrilov, S.; Kajumo, F.; Seibert, C.; Kuhmann; Shawn; Ketas, T.; Trkola, A.; Palani, A.; Clader, J. W.; Tagat, J. R.; McCombie, S.; Baroudy, B.; Moore, J. P.; Sakmar, T. P.; Dragic, T. *J. Virol.* **2003**, *77*, 5201.
13. Castonguay, L. A.; Weng, Y.; Adolfsen, W.; Di Salvo, J.; Kilburn, R.; Caldwell, C. G.; Daugherty, B. L.; Finke, P. E.; Hale, J. J.; Lynch, C. L.; Mills, S. G.; MacCoss, M.; Springer, M. S.; DeMartino, J. A. *Biochemistry* **2003**, *42*, 1544.
14. Maeda, K.; Das, D.; Ogata-Aoki, H.; Nakata, H.; Miyakawa, T.; Tojo, Y.; Norman, R.; Takaoka, Y.; Ding, J.; Arnold, G. F.; Arnold, E.; Mitsuya, H. *J. Biol. Chem.* **2006**, *281*, 12688.
15. For a description of SAR within this series, see: Palani, A.; Shapiro, S.; Josien, H.; Bara, T.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J. M.; Baroudy, B. M. *J. Med. Chem.* **2002**, *45*, 3143.
16. For a recent review, see: Blakeney, J. S.; Reid, R. C.; Le, G. T.; Fairlie, D. P. *Chem. Rev.* **2007**, *107*, 2960.
17. For a discussion of rotameric properties of this structural class, see: Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Blythin, D.; Cox, K.; Wagner, N. E.; Strizki, J.; Baroudy, B. M.; Dan, N. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 705.
18. Bradley, J.; Gill, J.; Bertelli, F.; Letafat, S.; Corbau, R.; Hayter, P.; Harrison, P.; Tee, A.; Keighley, W.; Perros, M.; Ciaramella, G.; Sewing, A.; Williams, C. *J. Biomol. Screen.* **2004**, *9*, 516.
19. For comparison, maraviroc has a TPSA of 63.
20. Carried out by incubation with human liver microsomes, and analysis of the supernatant by mass spectrometry.
21. For a discussion of working around hERG issues in the maraviroc series, see: Price, D. A.; Armour, D.; De Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4633.