DOI: 10.1002/cmdc.200600031

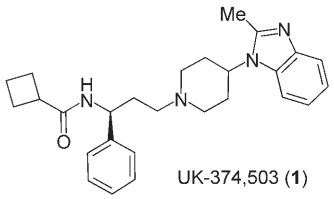
The Discovery of CCR5 Receptor Antagonists for the Treatment of HIV Infection: Hit-to-Lead Studies

Duncan Armour, Marcel J. de Groot, Martin Edwards, Manos Perros, David A. Price,* Blanda L. Stammen, and Anthony Wood[a]

Infection with HIV leads, in the vast majority of cases, to progressive disease and ultimately death. By 2004, just 23 years after AIDS was first recognised, the Joint United Nations Programme on HIV/AIDS estimated that 42 million people worldwide were infected with HIV, with more than 20 million dead since the beginning of the epidemic. Furthermore, rates of infection are once again on the increase in the developed world.[1] Despite the undoubted achievements of highly active antiretroviral therapy (HAART) using cocktails of reverse transcriptase and protease inhibitors, there is still a high unmet medical need for better tolerated, conveniently administered agents to treat HIV and AIDS. New mechanisms of action are particularly attractive to avoid issues of viral resistance.

HIV enters the host cell by fusing the lipid membrane of the virus with the host cell membrane. This fusion is triggered by the interaction of proteins on the surface of the HIV envelope with specific cell surface receptors. One of these is CD4, the main receptor for HIV-1 that binds to gp120, a surface protein on the virus particle. However, CD4 alone is not sufficient to permit HIV fusion and cell entry, an additional coreceptor from the chemokine family of G-protein coupled receptors (GPCRs) is required.[2] The chemokine receptor CCR5 has been demonstrated to be the major coreceptor for the fusion and entry of macrophage tropic (R5-tropic) HIV-1 into cells. R5-tropic strains are prevalent in the early asymptomatic stages of infection. Indeed, the CCR5 monoclonal antibody PRO140 has been demonstrated to potently inhibit a broad range of HIV-1 strains from infecting their target cells.[3] Shifts in tropism do occur during progression, mainly to X4 viruses that use CXCR4 as coreceptor, however, approximately 50% of individuals are infected with strains that maintain their requirement for CCR5. Currently the cause of the switch in tropism in late stage disease is unknown. There is evidence that homozygotes possessing a 32 base pair deletion in the CCR5 coding region are resistant to infection with R5-tropic HIV-1. These homozygotes do not express functional CCR5 receptors on the cell surface. Individuals who are heterozygous for the 32 base pair deletion display significantly longer progression times to the symptomatic stages of infection and evidence is emerging that they respond better to HAART.[4] Moreover, CCR5 deficient individuals are apparently fully immunocompetent, indicating that absence of CCR5 function may not be detrimental and that a CCR5 antagonist should be well tolerated. Currently, some clinical trials involving CCR5 ligands are being halted increasing the focus on this promising mechanism for the treatment of HIV.[5] In particular, aplaviroc was halted due to liver toxicity developed in a Phase IIb clinical trial that was also observed in a subsequent Phase III trial.

Herein, the discovery of hits, design and synthesis, structure–activity relationships (SAR), and biological evaluation of UK-374,503 (**1**) and related compounds are described.



UK-374,503 (**1**)

For the GPCR superfamily, high-throughput screening (HTS) is often considered to be the best route for generating novel, proprietary lead matter for new targets. Using this method many ligands have been identified that act as antagonists or agonists at their target receptors. Frequently these contain structural elements that have been termed "privileged structures" due to the frequency with which they are observed.[6] As yet the origin of this effect remains undetermined, although it has been hypothesised that it may reflect the existence of complementary binding sites within the proteins; may be due to the effective display of functional groups that these templates inherently have due to conformational restriction; or may simply be an artifact of the composition of the compound screening collections that historically have been biased towards compounds from old discovery programmes.[7] Takeda,[8] Merck,[9] and Schering[10] isolated hits from their HTS efforts with known GPCR pharmacophores that have subsequently been developed into potent small molecule CCR5 antagonists. Our CCR5 receptor antagonist programme began independently from, but similar to the approach of the Merck group. HTS was run using inhibition of MIP-1β binding to the human CCR5 receptor which was stably expressed in HEK-293 cells.[11] It was a risk within the project to use the readily available endogenous human ligand MIP-1β as a surrogate for the viral fusion protein, however, the desire to generate lead matter dictated that this risk was acceptable.

This delivered a number of hits from other GPCR programmes such as **2** and **3** (Figure 1). These hits were not considered ideal because of high molecular weight and lipophilicity, polypharmacology, weak binding affinity, and no measurable antiviral activity. Drug discovery programmes have a tendency to increase molecular weight and lipophilicity on the path from lead to development candidate. Thus, the starting point would have to be improved to have any chance of delivering a high quality compound with a good probability of surviving through preclincal and clinical studies.[12] The goal of the

[a] *D. Armour, Dr. M. J. de Groot, Dr. M. Edwards, Dr. M. Perros, Dr. D. A. Price, Dr. B. L. Stammen, Dr. A. Wood*
Pfizer Global Research and Development, Sandwich Laboratories
Ramsgate Road, Sandwich, Kent, CT13 9NJ (UK)
Fax. (+44) 1304-651821
E-mail: david.a.price@pfizer.com

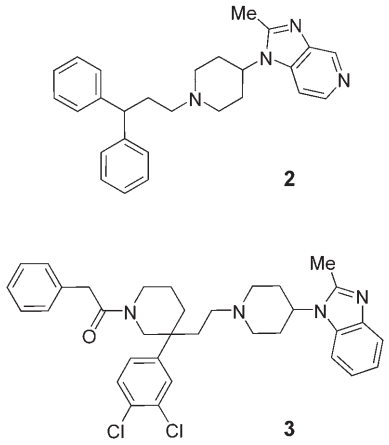


Figure 1. Structures of **2** and **3**. Their inhibitory effect on MIP-1β binding to CCR5-expressing cells was IC_{50} 0.4 μM and 1.1 μM, respectively.

initial hit-to-lead studies was to optimise hits **2** and **3** by combining their most attractive features to produce novel, selective ligands with enhanced ligand efficiency.[13]

The first goal was the replacement of the imidazopyridine moiety in **2**. Imidazopyridines are frequently found to be profound type II cytochrome P450 2D6 inhibitors, as was the case here (CYP 2D6 IC_{50} 40 nM).[14] The inhibition of this enzyme can cause variable drug levels and serious safety concerns when used in combination with other agents, as is routinely the case in HIV treatment regimes. The 3D model of the complex of **2** with cytochrome P450 2D6 indicated that the pyridine nitrogen in **2** was probably ligating directly to the haeme iron of the enzyme, Figure 2.[15] This results in a large increase in the redox potential of P450 and high occupancy of the substrate binding site,[16] leading to a dramatic reduction in turnover rates for the enzyme. This suggested that the carbon analogue **4** would be preferable. The resultant benzimidazole **4** (Figure 3) was a potent inhibitor of MIP-1β binding, albeit without measurable antiviral activity, and a much weaker type I inhibitor of the 2D6 enzyme (CYP 2D6 IC_{50} 710 nM). This was still an issue that required resolution but first an antivirally active analogue had to be found.

The high lipophilicity of **4** was addressed by introducing the amide that featured in hit **3**, to increase the polarity of the template whilst keeping the molecular weight low according to the rule of five.[17] We reasoned that one of the phenyl rings of the diphenylmethyl group restricts the conformational space of the other, an effect known as hydrophobic collapse, and that the introduction of a more polar linker should not interfere with potency.[18] Indeed, the phenyl amide **5** inhibited chemokine binding, albeit slightly more weakly than **4**. Most encouragingly though, moderate levels of antiviral activity could be measured, see Table 1. Subsequently, structure **5** became the focus of the SAR investigations.

Initially, the SAR of different amide substituents was investigated. The compounds were evaluated for their inhibitory effect on MIP-1β binding to CCR5-expressing HEK 293 cells, and for their antiviral activity (AV) against HIV_{BaL} in PM-1 cells.[11] The data for a small number of these are summarised in Table 1.

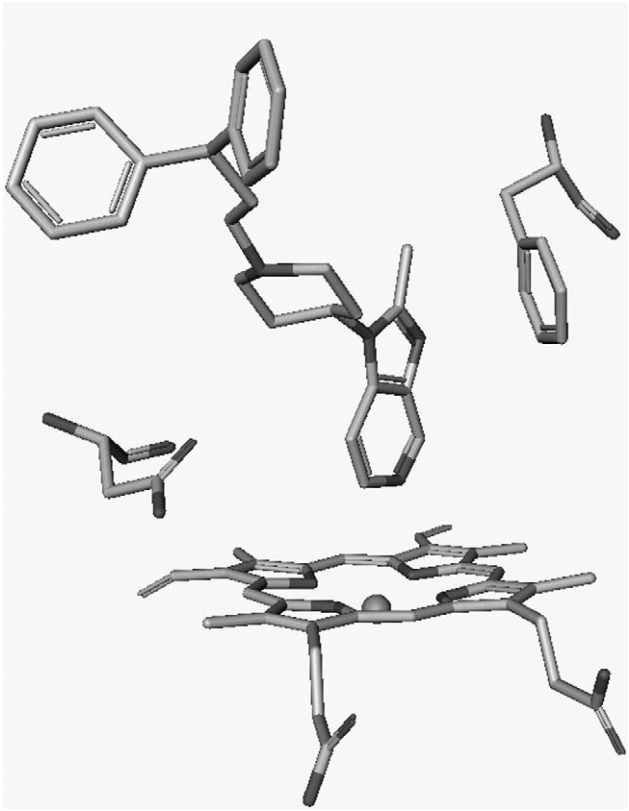


Figure 2. Modelling of the binding mode of **2** to cytochrome P450 2D6 demonstrating potential coordination of the imidazopyridine to the iron.

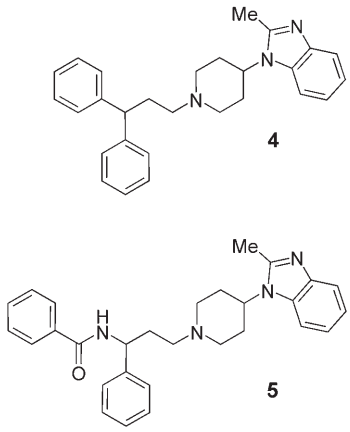
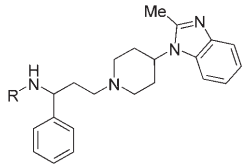
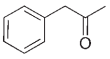


Figure 3. Structures of **4** and **5**. Their inhibitory effect on MIP-1β binding to CCR5-expressing cells was IC_{50} 4 nM and 45 nM, respectively.

Amongst this set of analogues the benzamide **5**, isopropylamide **9**, and cyclobutyl amide **11** were identified as the most active analogues. Compounds with additional polarity (**7** and **10**) showed a sharp decrease in activity suggesting that the amide substituent interacts with a predominantly lipophilic binding site on the CCR5 receptor. More bulky ligands, such as

Table 1. MIP-1β inhibitory activity and antiviral activity of amide analogues.



Compd	R	MIP-1β IC_{50} [nM][a]	AV IC_{50} [nM][b]
5		45	210
6		100	740
7		820	9250
8		270	7110
9		50	700
10		430	>10000
11		40	75

[a] Concentration required to inhibit binding of [^{125}I]MIP-1β by 50%. [b] Concentration required to inhibit replication of HIV_{BaL} into PM-1 cells by 50%. All compounds had negligible cytotoxicity with CC_{30} > 10 μM.

the phenacetyl **6**, had decreased potency. The smallest ligand, acetamide **8**, appeared to be less efficient for binding to CCR5.

Intriguingly, the data for the inhibition of the endogenous chemokine ligand MIP-1β were not predictive of the level of antiviral activity, varying between 5- and more than 100-fold. This discrepancy is not unique to this series and has also been reported by other investigators.[19] More recent work suggests that the binding domains of HIV gp120 and MIP-1α are distinct and separate, although MIP-1α can act as an allosteric inhibitor of gp120 binding.[20,21]

A homochiral synthesis of the two enantiomers unambiguously established that the activity resided with the *S* enantiomer of the benzamide **5** (Figure 4), and the *S* enantiomer of the cyclobutyl amide **1**. Compound **1** was a potent inhibitor of MIP-1β binding, with somewhat improved antiviral activity (Figure 4). This compound had reduced potential as an inhibitor of P450 2D6 (CYP 2D6 IC_{50} 5 μM), however, a clear goal for the project in moving forward was removal of any potential inhibition of metabolising enzymes. Also, when screened for off target pharmacology **1** displayed no significant affinity for other GPCRs at 1 μM. Not surprisingly the most significant off target pharmacology displayed by this series was affinity for the HERG channel. Generation of high selectivity through the use of a high throughput binding assay for the HERG channel will be described in following publications.[22] The *R* enantiomer

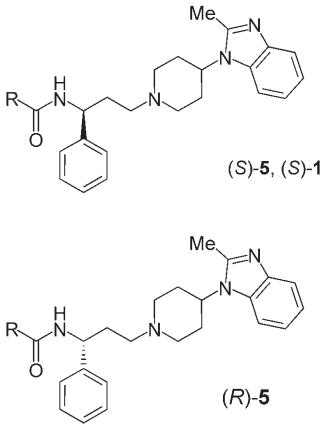


Figure 4. Structures of (*S*)-**5**, (*S*)-**1**, and (*R*)-**5**. Their MIP-1β IC_{50} values were 13 nM, 20 nM, and 580 nM, respectively, and their antiviral activities (AV IC_{50}) were 190 nM, 73 nM, and >10 μM, respectively.

of the benzamide **5** had much reduced affinity and no measurable antiviral activity.

At this stage of the project, **1** was not screened against further HIV isolates as the profile of **1** was still suboptimal with regard to an overall druglike profile. Clearly, as this series developed towards the eventual discovery of maraviroc understanding the potency against a range of HIV isolates was a key part of the screening strategy.

This report describes the discovery of a high quality lead **1** from the HTS hit **2**. Major challenges were introducing antiviral activity while removing Type II P450 interactions, both key goals for generating quality lead matter that eventually lead to the discovery of an orally bioavailable CCR5 antagonist.

Acknowledgements

We would like to acknowledge the technical efforts and assistance of M. Wilkes, A. Warren, N. Puhalo, M. Paradowski, G. L. Smith, R. Halliday, M. Ackland and M. J. de Groot.

Keywords: benzimidazole · CCR5 · HIV · maraviroc · receptors

[1] http://www.unaids.org.
[2] W. S. Blair, P. F. Lin, N. A. Meanwell, O. B. Wallace, *Drug Discovery Today* **2000**, *5*, 183.
[3] A. Trkola, T. J. Ketas, K. A. Nagashima, L. Zhao, T. Cilliers, L. Morris, J. P. Moore, P. J. Maddon, W. C. Olson, *J. Virol.* **2001**, *75*, 579.
[4] N. L. Michael, G. Chang, L. G. Louie, J. R. Mascola, D. Dondero, D. L. Birx, H. W. Sheppard, *Nat. Med.* **1997**, *3*, 338.
[5] Investigational Drugs Database, 873140.
[6] a) B. E. Evans, K. E. Rittle, M. G. Bock, R. M. Dipardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber, P. S. Anderson, R. S. L. Chang, V. J. Lotti, D. J. Cerino, T. B. Chen, P. J. Kling, K. A. Kunkel, J. P. Springer, J. Hirshfield, *J. Med. Chem.* **1988**, *31*, 2235; b) A. A. Patchett, R. P. Nargund, *Annu. Rep. Med. Chem.* **2000**, 35, 289; c) T. Klabunde, G. Hessler, *ChemBioChem* **2002**, *3*, 928.
[7] K. Bondensgaard, M. Ankersen, H. Thogersen, B. S. Hansen, B. S. Wulff, R. P. J. Bywater, *J. Med. Chem.* **2004**, *47*, 888.
[8] M. Shiraishi, Y. Aramaki, M. Seto, H. Imoto, Y. Nishikawa, N. Kanzaki, M. Okamoto, H. Sawada, O. Nishimura, M. Baba, M. Fujino, *J. Med. Chem.* **2000**, *43*, 2049.

[9] C. P. Dorn, P. E. Finke, B. Oates, R. J. Budhu, S. G. Mills, M. MacCoss, L. Malkowitz, M. S. Springer, B. L. Daugherty, S. L. Gould, J. A. DeMartino, S. J. Siciliano, A. Carella, G. Carver, K. Holmes, R. Danzeisen, D. Hazuda, J. Kessler, J. Lineberger, M. Miller, W. A. Schleif, E. A. Emini, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 259.
[10] a) A. Palani, S. Shapiro, J. W. Clader, W. J. Greenlee, K. Cox, J. Strizki, M. Endres, B. M. Baroudy, *J. Med. Chem.* **2001**, *44*, 3339; b) J. R. Tagat, R. W. Steensma, S. W. McCombie, D. V. Nazareno, S. Lin, B. R. Neustadt, K. Cox, S. Xu, L. Wojcik, M. G. Murray, N. Vantuno, B. M. Baroudy, J. M. Strizki, *J. Med. Chem.* **2001**, *44*, 3343 – 3346.
[11] a) C. Combadiere, S. K. Ahuja, H. L. Tiffany, P. M. Murphy, *J. Leukocyte Biol.* **1996**, *60*, 147; b) All biological data is at least $n = 2$ from two separate assays, with $<$ 2-fold difference based on a standard reference compound.
[12] a) M. M. Hahn, A. R. Leach, G. Harper, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 856; b) T. I. Oprea, A. M. Davis, S. J. Teague, P. D. Leeson, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1308.
[13] I. D. Kuntz, K. Chen, K. A. Sharp, P. A. Kollman, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9997.
[14] J. B. Schenkman, S. G. Sligar, D. L. Cinti, *Pharmacol. Ther.* **1981**, *12*, 43.
[15] M. J. de Groot, M. J. Ackland, V. A. Horne, A. A. Alexander, B. C. Jones, *J. Med. Chem.* **1999**, *42*, 1515.
[16] M. Chiba, L. Jin, W. Neway, J. P. Vacca, J. R. Tata, K. Chapman, J. H. Lin, *Drug Metab. Dispos.* **2001**, *29*, 1 – 3.
[17] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
[18] D. H. Rich, *Perspect. Med. Chem.* **1993**, 15.
[19] C. L. Lynch, A. L. Gentry, J. J. Hale, S. G. Mills, M. MacCoss, L. Malkowitz, M. S. Springer, S. Gould, J. A. DeMartino, S. J. Siciliano, M. A. Cascieri, G. Doss, A. Carella, G. Carver, K. Holmes, W. A. Schleif, R. Danzeisen, D. Hazuda, J. Kessler, J. Lineberger, M. Miller, E. A. Emini, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 677.
[20] C. Blanpain, B. J. Doranz, J. Vaklil, J. Rucker, C. Govaerts, S. S. W. Baik, O. Lorthoir, I. Migeotte, F. Libert, F. Baleux, G. Vassart, R. W. Doms, M. Parmentier, *J. Biol. Chem.* **1999**, *274*, 34719.
[21] O. M. Z. Howard, A. K. Shirakawa, J. A. Turpin, A. Maynard, G. J. Tobin, M. Carrington, J. J. Oppenheim, M. Dean, *J. Biol. Chem.* **1999**, *274*, 16228.
[22] J. A. Esté, *Curr. Opin. Invest. Drugs* **2002**, *3*, 383.

Received: February 8, 2006
Revised: March 24, 2006
Published online on June 6, 2006

