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Overcoming HERG affinity in the discovery of the CCR5 antagonist maraviroc

David A. Price,* Duncan Armour, Marcel de Groot, Derek Leishman, Carolyn Napier, Manos Perros, Blanda L. Stammen and Anthony Wood

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

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Abstract—The discovery of maraviroc **17** is described with particular reference to the generation of high selectivity over affinity for the HERG potassium channel. This was achieved through the use of a high throughput binding assay for the HERG channel that is known to show an excellent correlation with functional effects.

Drug induced QT prolongation has now been reported for both antiarrhythmic and non-cardiovascular related drugs and has led to the removal of some compounds from the market. The majority of drugs recognized to cause acquired long QT syndrome do so by blocking a human cardiac potassium channel known as the human ether-a-go-go related gene (HERG) potassium channel.[1] The function of HERG channels is to conduct the rapidly activating delayed rectifier potassium current (IKr) which has a key role in the control of cardiac rhythm.[2] This paper describes the challenges faced during the discovery of the CCR5 antagonist maraviroc **17** for the treatment of HIV infection and in particular it focuses on the importance of generating appropriate selectivity over affinity for the HERG ion channel. This selectivity is particularly important in the HIV arena as drugs to treat HIV are rarely given in isolation, rather in combination with other agents to prevent emergence of viral resistance. Many of the agents that a CCR5 antagonist could be co-administered with have known interactions with cytochrome P450 enzymes that may affect circulating levels of other compounds in the treatment regime. This, combined with our desire to maintain a free plasma concentration of the CCR5 antagonist above the antiviral IC_{90}, drives the need for a large safety window. Thus, achieving high selectivity with respect to HERG affinity and so IKr blockade was a key objective for the project from the outset. Since completing this work other scientists active in the CCR5 arena have also reported issues around HERG affinity and QT prolongation.[3,4]

HERG inhibition is commonly tested using whole cell voltage clamp technique, however, this is a technically challenging, low throughput technique. Within the CCR5 project to rapidly generate the required HERG SAR we used a predictive, high throughput, preclinical ligand binding screen using the tritiated class III antiarrhythmic agent dofetilide and the HERG channel stably expressed in HEK-293 cells. Although, binding assays do not provide functional information on drug effects, we have shown that [^{3}H]-dofetilide binding can be effectively used as a discovery screening assay for QT prolongation and this assay enabled the project to design out unwanted ion channel pharmacology.[5]

Our lead compound **1** was a potent ligand for the CCR5 receptor as measured by its ability to displace the endogenous radiolabelled protein MIP-1β from the receptor stably expressed in HEK-293 cells. Compound **1** was also a potent ligand for the HERG channel displaying 80% inhibition of the binding of tritiated dofetilide when screened at 300 nM. The alternative isomer **2** was prepared in which the piperidine ring of the tropane is forced into a pseudo-boat conformation to minimise 1,3-diaxial strain between the tropane bridge and the benzimidazole. This compound is essentially equipotent to **1** in affinity for the CCR5 receptor and for the HERG channel, Table 1.

Keywords: Maraviroc; Tropanes; Triazoles; Ion channel; Herg.
* Corresponding author. Tel.: +44 1034 644690; e-mail: david.a.price@pfizer.com

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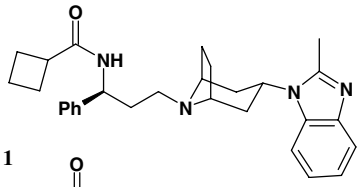
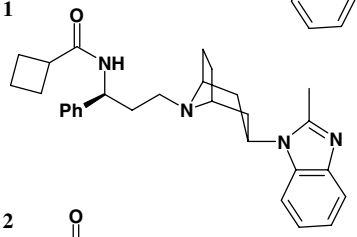
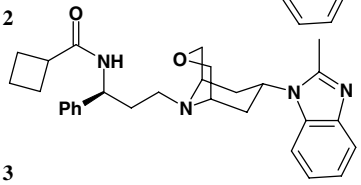
Table 1. MIP-1β inhibitory activity and HERG channel activity for compounds **1**, **2** and **3**

Compounds	MIP-1β IC_{50}[a] (nM)	HERG channel inhibition[b]
 **1**	2	80% @ 300 nM
 **2**	6	99% @ 1 μM
 **3**	39	70% @ 300 nM

[a] The concentration required to inhibit binding of [^{125}I]MIP-1β by 50%.
[b] Percentage inhibition of [^{3}H]dofetilide binding to HERG stably expressed on HEK-293 cells.

We proposed that this unwanted HERG affinity could be removed by moderating the basicity/steric environment of the central nitrogen or altering the orientation and/or substitution patterns of the aromatic rings in the compounds. An alternative strategy of reducing the intrinsic lipophilicity of the compounds was followed in the desire to improve other parameters such as stability to oxidative metabolism and improving the biopharmaceutical properties in general as well as to remove unwanted pharmacology.

To probe the importance of the basicity of the nitrogen, the oxogranatane (**3**) was prepared where the oxygen bridgehead has a profound effect on the basicity of the nitrogen without causing a dramatic change in overall structure. Compound **1** has a measured pKa of 7.8, while introduction of the oxygen in **3** reduces the pKa to 6.0, essentially **3** at physiological pH 7.4 is less than 3% protonated. Interestingly, within this series such a dramatic reduction in basicity has had negligible effect on the affinity of the compound for the HERG channel and attention turned to the alternative approach of altering the aromatic rings' orientation and substitution pattern. It is interesting to note that a recent publication around modification of the dofetilide structure **4** suggests that the basic centre of dofetilide itself is not essential to effects on the HERG channel.[6] The *N*-acetyl compound **5** is still essentially equipotent with dofetilide **4** suggesting that it possesses a high affinity for the HERG channel even within a non basic expression.

Attention turned to the second strategy of modifying the aromatic rings present in **1**. Progress in the solution of several ion channel structures has increased our understanding of the possible molecular interactions of molecules with ion channels.[7] The crystal structure of the HERG channel itself has yet to be solved but closely related potassium channels such as the bacterial KcsA have been.[8] This information, coupled with homology models based upon the KcsA channel, site-directed mutagenesis studies on the HERG channel and analysis of structure activity relationships of known ligands, has allowed research groups to build realistic models of the HERG channel to predict how compounds might bind.[9,10] There are increasing numbers of PDB entries using both NMR and crystallography techniques of channels captured in both the open and closed forms. Docking of compound **1** into this model of the HERG channel suggests that the phenyl ring of the benzimidazole overlaps perfectly with a lipophilic binding area represented in light grey, Figure 1. Modification of the stereoelectronics of this heterocyclic motif was a priority to disrupt this overlap. The cyclobutyl group of the amide also overlaps neatly with a lipophilic binding area suggesting that introduction of polar groups onto the amide substituent could decrease HERG affinity. These polar motifs would be poorly accommodated by the hydrophobic amino acid residues in the channel (Table 2).

These docking experiments suggested that looking for alternative expressions for the benzimidazole would be a priority to reduce affinity for the HERG channel. Attempts to introduce polar substituents onto the phenyl ring of the benzimidazole were synthetically challenging and also were increasing molecular weight in a series that was already >450. It was therefore decided that complete replacement of the benzimidazole rather than modification was the best strategy, Table 3. The initial targets were prepared in the piperidine template rather

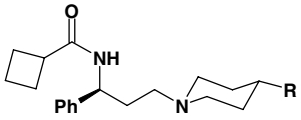
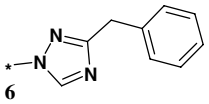
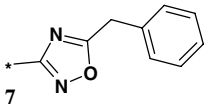
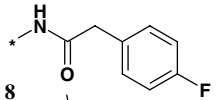
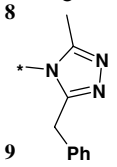
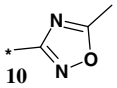
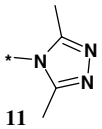
Figure 1. Dock of compound **1** into model of the HERG channel. HERG pharmacophore is superimposed on the channel model, lipophilic binding areas are shown as light grey rings and the basic centre is a blue ball. For clarity the side chains in green are for only 2 of the 4 protein units that make up the tetrameric channel.

than tropanes as the synthetic chemistry was dramatically simplified.

Disappointingly, from the initial set of analogues **6–8** prepared it was apparent that regardless of the expression used, whether triazole, oxadiazole or amide, a pendant phenyl ring appeared to be optimal for binding to the HERG channel as well as CCR5. In compound **9** using the alternative regioisomeric triazole enabled the phenyl ring to have a dramatically different vector and it was clear that the CCR5 receptor did not tolerate this substitution pattern. With this information in hand a more radical approach was taken to completely remove the phenyl ring and just have simple methyl substitution on the heterocycle, generating compounds **10** and **11**.

Compound **11** was judged to have the most useful profile for a number of reasons, it was synthetically simple to transfer into the tropane series, we could independently vary two positions on the triazole moiety for opti-

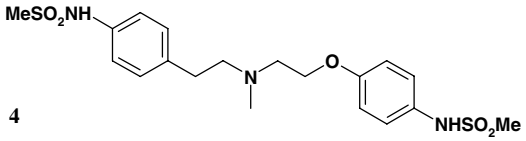
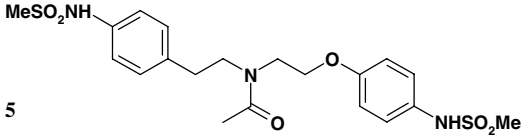
Table 3. MIP-1β inhibitory activity and HERG channel activity for compounds

Compound	MIP-1β IC_{50}[a] (nM)	HERG channel inhibition[b]
		
 **6**	5	44% @ 100 nM
 **7**	20	50% @ 100 nM
 **8**	0.7	30% @ 100 nM
 **9**	250	Not tested
 **10**	70	Not tested
 **11**	50	5% @ 100 nM

[a] The concentration required to inhibit binding of [^{125}I]MIP-1β by 50%.

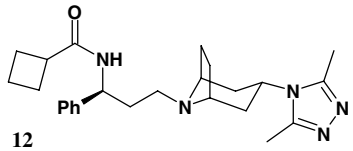
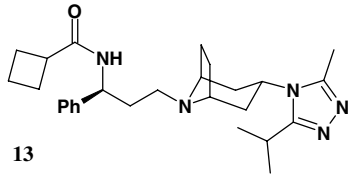
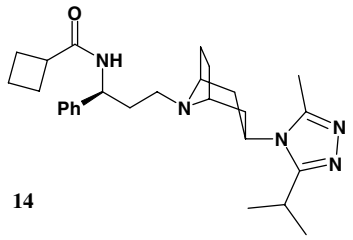
[b] Percentage inhibition of [^{3}H]dofetilide binding to HERG stably expressed on HEK-293 cells.

Table 2. HERG channel activity for compounds **3** and **4**

Compound	HERG channel inhibition[a] (μM)
 **4**	0.011
 **5**	0.021

[a] The effective concentration required to delay the effective refractory period (ERP) 10 ms.

Table 4. Antiviral activity and HERG channel activity for compounds

Compound	Antiviral IC_{90}[a] (nM)	HERG channel inhibition[b]
 **12**	13	10% @ 100 nM
 **13**	8	14% @ 100 nM 30% @ 300 nM
 **14**	101	Not tested

[a] The concentration required to inhibit replication of HIV_{BaL} in PM-1 cells by 90%.

[b] Percentage inhibition of [^{3}H]dofetilide binding to HERG stably expressed on HEK-293 cells.

mal profile and when screened at 100 nM there seemed to be no significant affinity for the HERG channel.[11]

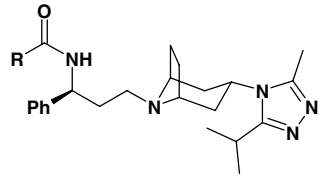
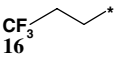
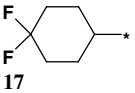
Transferring our learnings into the tropane series gave compound **12** and optimisation of the alkyl substitution pattern on the triazole for antiviral effects gave **13**. When the alternative tropane isomer **14** was screened, there was a significant decrease in antiviral potency observed and no further analogues were prepared in that isomeric series (Table 4).

We were delighted to find that compound **13** had good levels of antiviral potency, however, it was disappointing that as we had increased the lipophilicity in the series affinity for the HERG channel had also started to return. Comparing compounds **2** and **13** does show that the triazole series had a superior profile to the benzimidazoles regarding HERG pharmacology and this prompted us to focus our efforts into the triazole series. Compound **13** was also successfully progressed through a whole cell voltage clamp experiment to confirm that the high throughput screening assay we were relying on was predictive of functional effects. With this confirmation we felt confident in the data we were generating and the value of the binding assay to enable successful decision making.

From our model of the HERG channel described earlier we now turned our attention to modifying the cyclobutyl amide and thus further reduce any affinity for the channel. This work was rapidly performed as the amide coupling could be undertaken in the final step enabling parallel chemistry techniques to be used. The design criteria for selection of acid monomers were strict in terms of physiochemistry of final products (log *D* range 1.5–2.3) so that compounds prepared would have the best chance of possessing good pharmacokinetic and biopharmaceutical properties. This log *D* range was decided upon from analysis of previous compounds prepared within the project as being most appropriate. In terms of the structural features of the monomers used as diverse a range as possible was selected to maximise learnings from the screening results, Table 5.

Ring homologation of **13** (log *D* 1.6) to compound **15** (log *D* 2.1) immediately gave an indication that an increase in antiviral activity was possible with a small decrease in affinity for the HERG channel. This reduction is presumably due to some deleterious steric clash with residues in the ion channel. Compound **16** (log *D* 1.8) also showed a small decrease in affinity for the HERG channel compared to the lead **13** and this suggested that there could be a further reduction in affinity for HERG by fluorination of the amide substituent.[12] Combining the learnings from **15** and **16** leads to the design and synthesis of the 4,4-difluorocyclohexyl group of maraviroc **17** (log *D* 2.1) which showed no binding to the HERG channel when screened at 300 nM. The level of antiviral potency displayed was outstanding with a nanomolar IC_{90}. Maraviroc has also been tested at 1000 nM and showed no significant binding to the HERG channel at the higher concentration. Within the triazole series the 4,4-difluorocyclohexyl group is unique in its antiviral profile and lack of affinity for the HERG channel. The 4,4-difluorocyclohexyl group is clearly not tolerated within the ion channel due to the steric demands of the cyclohexyl group and also the dipole generated by the difluoro moiety.

Table 5. Antiviral activity and HERG channel activity for compounds

Compound	Antiviral IC_{90}[a] (nM)	HERG channel inhibition[b]
		
 **13**	8	30% @ 300 nM
 **15**	2	18% @ 300 nM
 **16**	14	14% @ 300 nM
 **17**	2	0% @ 300 nM

[a] The concentration required to inhibit replication of HIV_{BaL} in PM-1 cells by 90%.

[b] Percentage inhibition of [^{3}H]dofetilide binding to HERG stably expressed on HEK-293 cells.

In conclusion, this paper describes the discovery of maraviroc focusing on the use of a high throughput binding assay to generate high selectivity over affinity for the HERG ion channel. We discovered specific structure–activity relationships that enabled us to moderate HERG affinity in a lipophilicity independent manner and from work in the benzimidazole series it would also appear that HERG affinity is independent of the pK_a of the basic centre.

References and notes

1. Fermini, B.; Fossa, A. A. *Nat. Rev. Drug Disc.* **2003**, *2*, 447.
2. Keating, M. T.; Sanguinetti, M. C. *Cell* **2001**, *104*, 569.
3. Esté, J. A. *Curr. Opin. Invest. Drugs* **2002**, *3*, 383.
4. Shu, M.; Loebach, J. L.; Parker, K. A.; Mills, S. G.; Chapman, K. T.; Shen, D. S.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Di Salvo, J.; Lyons, K.; Pivnichny, J. V.; Kwei, G. Y.; Carella, A.; Carver, G.; Holmes, K.; Schleif, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Linebrger, J.; Miller, M. D.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 952.
5. Greengrass, P. M.; Stewart, M.; Wood, C. M.; Patent Application WO 03021271.
6. Liu, H.; Ji, M.; Luo, X.; Shen, J.; Huang, X.; Hua, W.; Jiang, H.; Chen, K. *J. Med. Chem.* **2002**, *45*, 2953.
7. Domene, C.; Haider, S.; Sansom, M. S. P. *Curr. Opin. Drug Disc. Dev.* **2003**, *6*, 619.
8. Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C. *Proc. Nat. Acad. Sci.* **2000**, *97*, 12329.
9. Cavalli, A.; Poluzzi, E.; De Ponti, F.; Recanatini, M. *J. Med. Chem.* **2002**, *45*, 3844.
10. Perry, M.; de Groot, M. J.; Helliwell, R.; Leishman, D.; Tristani-Firouzi, M.; Sanguinetti, M. C.; Mitcheson, J. *Mol. Pharmacol.* **2004**, *66*, 240.
11. Price, D. A.; Gayton, S.; Selby, M. D.; Ahman, J.; Haycock-Lewandowski, S. *Synlett* **2005**, *7*, 1133.
12. Price, D. A.; Gayton, S.; Selby, M. D.; Ahman, J.; Haycock-Lewandowski, S.; Stammen, B. L.; Warren, A. *Tetrahedron Lett.* **2005**, *46*, 5005.