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An in situ oxidation strategy towards overcoming hERG affinity

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ABSTRACT

In an effort to overcome hERG affinity with a lead compound, several S-oxide and N-oxide analogues were synthesised with a much improved hERG profile but low in vivo absorption. This led to the implementation of an in situ oxidation strategy wherein a sulfide was dosed orally and systemic levels of the corresponding sulfoxide and sulfone were monitored. SAR and pharmacokinetic data to support this as a possible strategy are presented, although ultimately the approach was shown not to be suitable due to very low levels of active circulating metabolites.

The advent of Highly Active Anti-RetroViral Therapy (HAART) has significantly changed the face of managing HIV infection,[1] and its success in suppressing viral replication and reducing HIV associated deaths is due to the combined use of agents which target up to three different viral targets.[2] Nonetheless, the emergence of resistance to some of the components of HAART, and the toxicity of some of the currently available drugs has necessitated the continuance of the search for new effective antiviral agents, and in particular of new mechanistic classes directed to both virus and host targets with superior efficacy and/or safety profiles.[3]

One such target is CCR5, a host chemokine receptor expressed on white blood cells, which functions as a co-receptor for the HIV-1 virus.[4] Homozygotes which lack the CCR5 receptor have demonstrated very high resistance to infection, and blocking of this receptor with antagonists has been shown to produce a potent antiviral response in patients.[5] Previous publications from Pfizer have detailed the discovery programme in which the high throughput screening lead **1** was optimised through to maraviroc **2** (see Fig. 1), which was launched several years ago as the first-in-class CCR5 antagonist for the treatment of HIV.[6]

During this programme, an extensive medicinal chemistry effort initially identified the tetrahydropyran derivative **3** as a promising lead compound on the basis of high antiviral activity, but suffered from high metabolic instability and off-target affinity for the hERG channel.[7]

Whilst the main direction of the programme took us from **1** to **2**,[6] we wish to describe in this Letter a parallel medicinal chemistry effort in which attempts to improve the pharmacokinetic and cardiac safety properties of **3** were made by specifically targeting polar, particularly oxidised, analogues of the tetrahydropyran ring. This effort was initiated by the observation that charged analogues

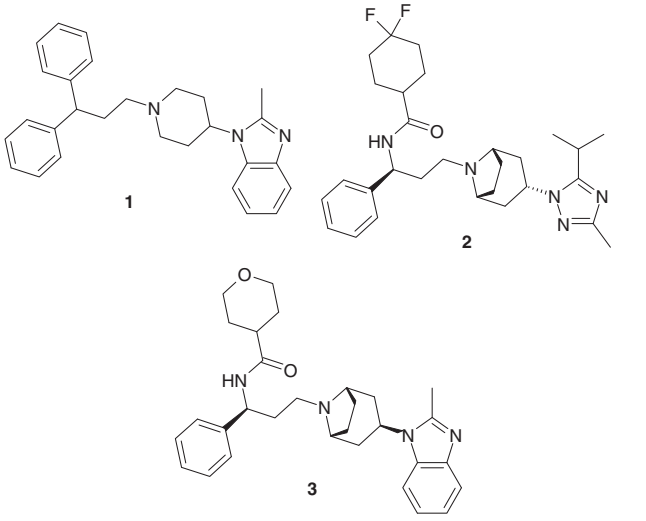

Figure 1. A high throughput screening hit (**1**) for the Pfizer medicinal chemistry programme which led to maraviroc (**2**) and an intermediate lead compound (**3**).

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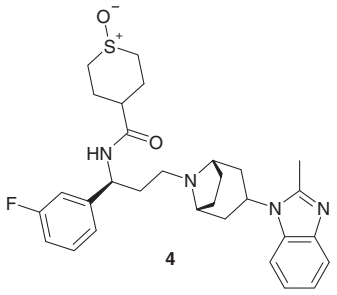


Figure 2. Profile of a lead sulfoxide analogue **4**. *Abbreviations*: RRCK, A sub clone of MDCK cells known to express low levels of *p*-glycoprotein used to assess membrane permeability; HLM, human liver microsomes; hERG, human ether-a-go-go related gene; Cl, clearance; *F*, oral bioavailability; V_d, volume of distribution; Caco-2, human epithelial cell line derived from a colon carcinoma; HPLC, high pressure liquid chromatography. Measured partition coefficients were generated at pH7.4 in mixtures of 1-octanol and 0.1 M sodium phosphate buffer using the shake-flask method.

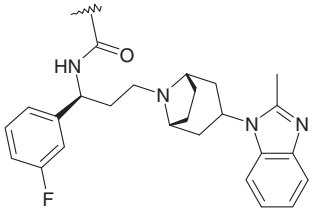
of **3** such as the tetrahydrothiopyran S-oxide **4** displayed excellent cell-based activity[8] and selectivity over the hERG channel (Fig. 2).

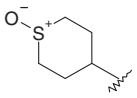
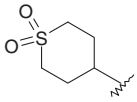
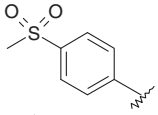
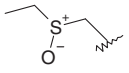
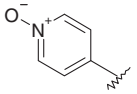
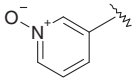
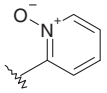
Compound **4** was taken into an in vivo rat pharmacokinetic study, but suffered from high clearance and very low oral bioavailability. Several analogues of this sulfoxide group, for example, the related sulfone and the analogous pyridine N-oxides were then synthesised with the purpose of identifying an analogue with improved in vivo pharmacokinetics, while retaining the favourable potency and hERG window of **4**.

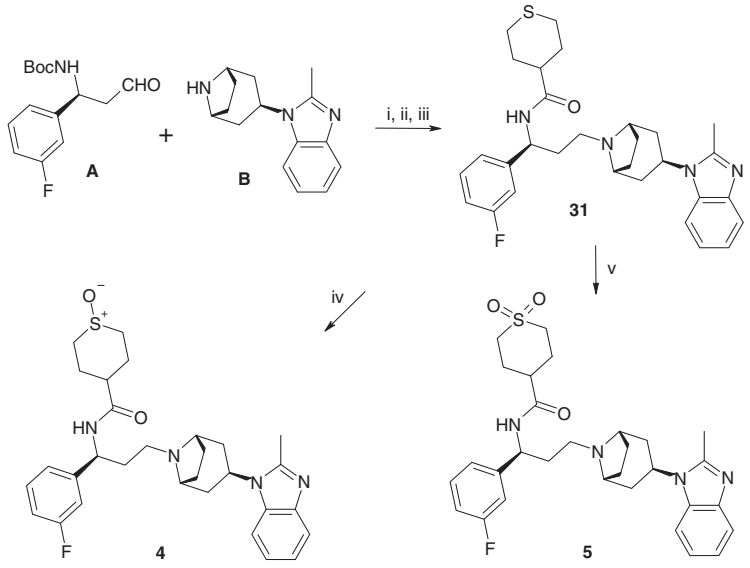
The chemistry to prepare the analogues described in this paper is entirely analogous to that described previously for the maraviroc programme. S- and N-oxidations were preferably carried out at a late stage on the corresponding sulfide or pyridine derivatives. In the case of the sulfoxides, diastereoisomers were separated by reverse phase HPLC. Starting materials for the oxidations were constructed from the appropriate aldehyde **A** and amine **B** which were prepared according to the published methods.[9,10] An example of a complete synthetic procedure is detailed in Scheme 1 below.

Simple oxidised analogues were targeted first and initially assessed for potency in a cell–cell fusion assay[8] as shown in Table 1. The sulfoxide **4** and sulfone **5** derivatives showed equivalent activity. The alternative aromatic sulfone **6** was some 200-fold less active, and the aliphatic sulfoxide **7** was more than 500 times less potent than **4**. The isomeric pyridine N-oxides **8**–**10** were all significantly less active than the starting point **4**, with the *o*-isomer **10** significantly weaker than the *m* and *p* isomers **8** and **9**.

Table 1
Oxygenated amide substituent SAR based on the lead **4**

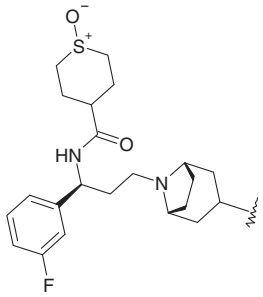


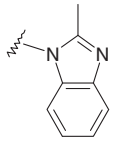
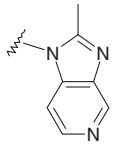
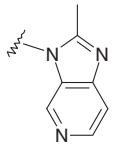
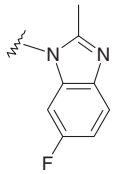
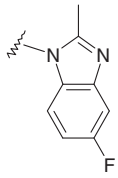
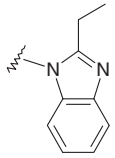
Compound	Structure	*c* log *P*	gp160 fusion inhibition (IC_{50}, nM)
4		1.8	0.2
5		1.7	0.2
6		4.2	40
7		3.2	>100
8		3.1	4
9		3.1	5
10		3.1	>100



Scheme 1. Reagents and conditions: (i) $NaBH(OAc)_3$, AcOH, DCM (80%); (ii) 4 M HCl in dioxane (95%); (iii) EDCI, HOBT, tetrahydro-2*H*-thiopyran-4-carboxylic acid, DCM (65%); (iv) NaIO4, 4:1 MeOH/water (40%); (v) H_2O_2 (30 wt % in water) (77%).

Table 2
Heterocyclic tropane SAR based on the lead **4**



Compound	Structure	*c* log *P*	gp160 fusion inhibition (IC_{50}, nM)
4		1.8	0.2
11		0.7	4
12		0.7	1
13		2.0	0.01
14		2.0	0.4
15		2.4	0.05

This initial tranche of compounds showed that good levels of potency were attainable with a variety of amide substituents, and excluded the aliphatic sulfoxide group in **7** and the *o*-pyridine N-oxide in **10** from further consideration. Also apparent was that the tetrahydrothiopyran template for either a sulfoxide or sulfone was especially favoured for potency. Retaining the sulfoxide group in this template, our attention turned to the benzimidazole group, and a range of benzimidazole derivatives were synthesised and investigated as detailed in Table 2.

These data showed that relatively small variations in the nature of the substituent heterocycle could lead to significant differences in cell-based potency. For example, inserting a nitrogen atom into the 6-position of the benzimidazole ring in **4** gave the imidazopyridine **12**, some fivefold less potent than the starting compound, whereas insertion of a fluorine atom in the same position in **13** gave a 20-fold increase in potency. Interestingly the same functionality of compounds **12** and **13** applied to the 5-position (viz. **11** and **14**) gave subtly different results, while the ethyl-substituted benzimidazole **15** gave improved potency, albeit in a more lipophilic structure. Overall, all benzimidazole substituents in Table 2 retained good levels of potency, and at this stage did not rule out any analogues, but rather prompted us to investigate combinations of the functionality described within Tables 1 and 2 to identify optimal compounds. In this phase of the investigation, we also profiled each compound for its affinity for the hERG channel as shown in Table 3. In general, the 6-fluoro group exemplified in **13**, **17**, **22** and **29** supported higher fusion potency than the other tropane substituents. Also in general, the phenyl sulfone, phenyl sulfoxide and pyridine N-oxide groupings in **6**, **8**, **9** and **20**–**30** all carried varying degrees of hERG affinity, with the *p*-pyridine N-oxide group of compounds **8**, **26**, **27** and **28** showing the greatest affinity amongst this set of compounds. These data focussed attention on both the tetrahydrothiopyran S-oxide and S,S-dioxide analogues of compounds **4**, **5** and **12**–**19**. These compounds were all 1 nM or better inhibitors of cell-cell fusion with no measurable inhibition of the hERG channel at 10 μM concentration. To assess if any of these compounds offered a more appealing profile than **4**, in vitro microsomal stability and permeability was evaluated for this subset of compounds, as shown in Table 4.

From Tables 3 and 4, compound **17** had excellent potency and hERG selectivity and subsequently found to possess reasonable HLM stability (Fig. 3). While the Caco-2 and RRCK measured permeability was not ideal and arguably not significantly different to that of the starting compound **4**, it was decided to take **17** into an in vivo study to obtain in vivo confirmation of its absorption profile. The rat pharmacokinetics of **17** was assessed and **17** was found to have similarly high Cl and low oral bioavailability to **4**. A portal vein/systemic crossover study in rat confirmed that the absorption of **17** was very low, approximately 3%.

The in vitro data for both **4** and **17** was therefore, while encouraging, not borne out by sufficient in vivo absorption in rat. At this point, we returned to the starting point of this programme of work, namely compound **3**, and considered alternative ways in which we could access the favourable permeability properties of a compound like **3** (Caco-2 AB/BA 23/42, RRCK 9) with the potency and hERG selectivity profile of **4**. This prompted us to look at the lower oxidation analogue of **4**, namely the sulfide **31**.

Our thinking was that the lipophilic, membrane-permeable sulfide **31**, albeit hERG-liable, could be dosed and following rapid and complete absorption, would then undergo rapid first-pass hepatic oxidation to a much more selective, metabolically robust derivative which would retain antiviral activity. This strategy is summarised in Figure 4.

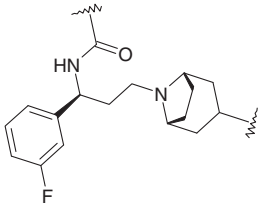
Compound **31** was indeed confirmed as possessing high in vitro metabolic instability and high permeability, and as suspected, **31** also had significant hERG affinity. The in vitro microsomal oxidation of **31** was also confirmed as resulting in the generation of both **4** and **5** as primary metabolites.

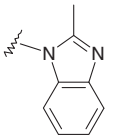
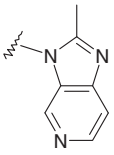
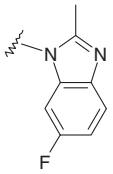
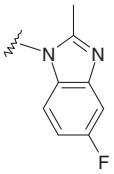
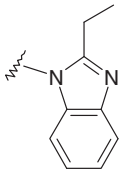
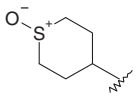
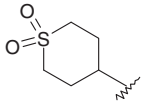
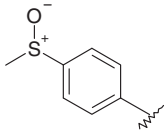
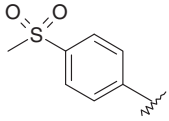
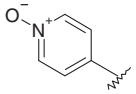
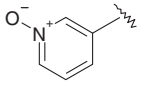
It therefore appeared that the in situ oxidation strategy could be well-tested using **31** as a prodrug starting point.

Compound **31** was dosed to the rat, and plasma samples analysed for the presence of parent compound **31**, and for active circulating metabolites **4** and **5**. Data is shown in Table 5 and clearly shows that **31** is rapidly metabolised in vivo, with minimal plasma exposure of the parent compound detected.

Following oral administration of **31** to the rat, and taking into account plasma protein binding, the sulfoxides **4** exhibited a free AUC approximately 15-fold higher than parent in favour of diastereoisomer **4A**. However although the sulfoxides were observed at concentrations above parent based on a metabolite clearance of

Table 3
Composite SAR around lead compound **4** through combination of amide and heterocyclic substituents



Compound gp160 (IC_{50}, nM) [^{3}H]-dof binding (IC_{50}, nM)					
	**4** *0.2* >10,000	**12** *1* >10,000	**13** *0.01* >10,000	**14** *0.4* >10,000	**15** *0.05* >10,000
	**5** *0.2* >10,000	16 *0.2* >10,000	17 *0.01* >10,000	18 *0.03* >10,000	**19** *0.3* >10,000
	**20** *40* NT	21 *2* >10,000	**22** *1* 8000	**23** *36* NT	NS
	**6** *40* 855	**24** *1* 4330	NS	**25** *8* 1180	NS
	**8** *4* 261	**26** *4* 1480	NS	**27** *0.1* 327	**28** *24* 663
	**9** *5* 5920	NS	**29** *0.5* >10,000	**30** *1* 5240	NS

NS = not synthesised.

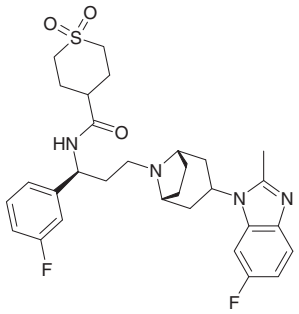
Table 4
Microsomal stability, permeability and selected physicochemical data for compounds **4**, **5** and **12**-**19**

Compound	*c* log *P* (log *D*)	HLM Clint[d] (μL/min/mg)	Caco-2[e] AB/BA ($\times 10^{-6}$ cm/s)	RRCK[f] ($\times 10^{-6}$ cm/s)
4A[a]	1.8 (2.3)	36	2/20	1
4B[b]	1.8 (2.3)	111	ND	1
5	1.7 (2.8)	23	<1/20	4
12[c]	0.7 (1.7)	50	2/2	ND
13[c]	2.0 (ND)	ND	2/18	ND
14[c]	2.0 (2.7)	93	ND	2
15[c]	2.4 (2.8)	89	ND	2
16	0.6 (1.9)	<8	ND	1
17	1.9 (2.9)	10	5/31	2
18	1.9 (3.0)	14	3/21	2
19	2.2 (3.7)	25	ND	3

[a] Diasteroisomer **1**, unassigned absolute configuration.
[b] Diasteroisomer **2**, unassigned absolute configuration.
[c] Mixture of diasteroisomers.
[d] Human liver microsomal stability (0.8 mg/mL protein, 1 μM substrate concentration).
[e] Flux (5 μM, pH7.4).
[f] Permeability as measured in a Pfizer specific cell line (2 μM, pH 7.4).

approximately liver blood flow in the rat (70 mL/min/kg) oral exposure indicated a bioavailability of <10%.

It was unclear why was the exposure of the oxidised metabolites was so low, given that the parent was highly permeable, and highly susceptible to oxidation to give these metabolites. The reasons for this observation are not fully understood but potential explanations include gut wall metabolism of **31** to the sulfoxide(s)



17
gp160 IC_{50} 0.01 nM
log *D* 2.9
Caco-2 AB/BA 5/31 x10^{-6} cm/s
HLM 10 μL/min/mg
RRCK 2 x10^{-6} cm/s
hERG IC_{50} >10 μM
Rat PK (1mg/kg IV, 2mg/kg PO)
Estimated Cl ~85 mL/min/kg
Rat absorption
~3 %

Figure 3. Sulfone analogue **17** of the lead compound **4**.

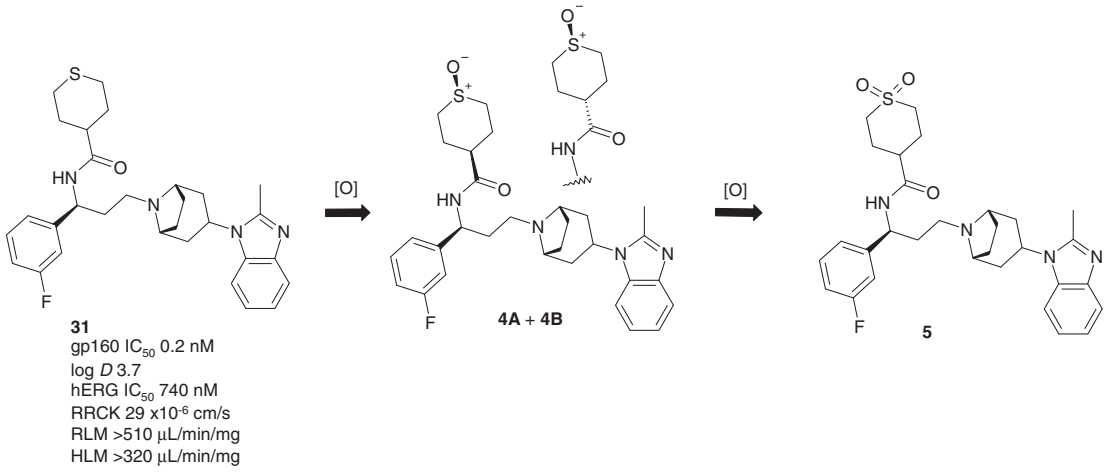


Figure 4. Oxidation strategy starting with the sulfide **31**.

Table 5
Rat plasma pharmacokinetics of **31**

Compound	**31**				
Dose (route)	1 mg/kg (iv)	3 mg/kg (po)			
		31	**4A**	**4B**	**5**
Clearance (mL/min/kg)	60		—	—	—
Cl_u (mL/min/kg)	2431	—	—	—	—
V_{dss} (L/kg)	2.0	—	—	—	—
V_{du} (L/kg)	81	—	—	—	—
Terminal $T_{1/2}$ (h)	0.8	—	—	—	—
Bioavailability	—	1%	—	—	—
Absorption	—	9%	—	—	—
AUC (ng h/mL)	270	10	11	2	<1
Free AUC (ng h/mL)	6.7	0.25	1.7	0.3	<0.1

followed by subsequent metabolism and/or uptake by the liver for excretion. The precise fate of **31** was not explored further. Overall the data shown above was sufficient to indicate the use of **31** as a potential oxidisable precursor to be non-viable due to the limited formation and systemic exposure of the active metabolite.

Through the course of this work, we were intrigued by the possibility that S- and N-oxide derivatives could offer an excellent compromise between on the one hand high cell-based activity and a wide hERG selectivity window and acceptable oral absorption on the other. We were able to identify several compounds which were likely to come close to being optimal within the series, but confirmed that oral bioavailability in the rat for two compounds was low, as a result of very low absorption. We then investigated a prodrug strategy in which we sought to dose a highly permeable, metabolically labile sulfide and monitor for active oxidised metabolites in the systemic circulation. This approach was also compromised by low exposure of both parent and active metabolites, and prompted the project to investigate alternative options.[11–13]

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