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Highly potent and selective chiral inhibitors of PDE5: An illustration of Pfeiffer's rule

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ABSTRACT

A series of potent chiral PDE5 inhibitors are described that are based on the sildenafil architecture but exhibit much greater selectivity over PDE6. Eudismic analysis of the SAR in this series provided a clear illustration of Pfeiffer's rule and indicated that the chiral motif was involved in a highly-stereoselective interaction with PDE5. This PDE5 specificity translated to levels of selectivity over PDE6 that were hitherto unprecedented in the sildenafil scaffold. **UK-371,800** (compound **8**) was identified as a development candidate from this series that married sildenafil-like molecular properties with high selectivity over PDE6. Clinical data confirm that **UK-371,800** has markedly superior human pharmacokinetics to a previously-described higher molecular weight achiral analogue in this template (compound **1**).

The launch of sildenafil (Viagra™)[1] as the first oral treatment for male erectile dysfunction (MED) revolutionised the treatment of this disease.[2] The efficacy of sildenafil (Fig. 1) in MED results from its potent and selective inhibition of the cGMP phosphodiesterase enzyme PDE5. Sexual stimulation leads to the generation of nitric oxide in the *corpus cavernosum*. Nitric oxide activates guanylate cyclase to convert GTP to the second messenger cGMP which, in turn, results in smooth muscle relaxation and the erectile response. cGMP is hydrolysed by PDE5 to inactive GMP. PDE5 inhibitors such as sildenafil thus act to inhibit cGMP breakdown and thereby facilitate penile erection in patients suffering from MED.[3,4]

In addition to treatment of MED, significant research also continues to explore the broader therapeutic utility of PDE5 inhibitors.[4] For example, sildenafil has been shown to exhibit efficacy in pulmonary arterial hypertension, and has been approved (as Revatio™) for treatment of this highly-debilitating condition. The therapeutic value of PDE5 inhibitors has driven ongoing interest in the PDE5 arena. A particular area of focus has been the discovery of new agents with enhanced selectivity for PDE5 over the related enzyme PDE6, compared to sildenafil (Fig. 1).[5]

Initial efforts in this area within our own laboratories focused on further SAR development within our proprietary sildenafil scaffold. **UK-343,664** (compound **1**, Fig. 1) was rapidly identified as a novel sildenafil analogue with markedly improved selectivity for PDE5 over PDE6 and was progressed to clinical development.[6] Unfortunately, **1** exhibited significant non-linearity in its oral pharmacokinetics in humans, with supraproportional increases in exposure with dose (in both C_{max} and AUC).[6] T_{max} was also found to be dose-related, with lower doses showing an apparent slow absorption profile. This non-linear pharmacokinetic behaviour appeared to be related, at least in part, to the finding that **1** was a high affinity substrate for human P-glycoprotein.[6] In contrast, sildenafil itself shows dose-linear pharmacokinetics in humans. Notably, the SAR progression from sildenafil (MWt 474) to **1** (MWt 565) had led us to significantly exceed Lipinski's 'rule-of-5' guidelines for MWt.[7,8] Furthermore, additional SAR analysis in this series indi-

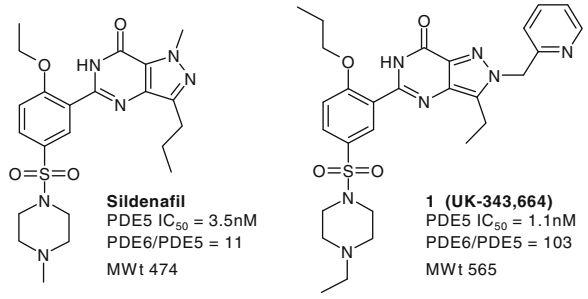

Figure 1. Structures and PDE activity data of sildenafil and **1**.

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cated that the P-glycoprotein issue was not unique to **1** but rather was a common finding for many similarly high MWt analogues. Our efforts thus turned to the challenge of securing very high selectivity for PDE5 over PDE6 in a compound with molecular properties much more akin to sildenafil.

As part of the analysis of analogues of compound **1**, two noteworthy SAR manoeuvres emerged that proved instrumental to this next phase of our programme. First, SAR analysis of the alkoxyarene fragment of **1** showed that significant selectivity enhancements could be secured through introduction of a 2′-methoxyethyloxy pyridyl motif (compound **2**, Fig. 2). With this change in place, we then found that we were able to replace the large *N*-2 pyridylmethyl moiety with a simple methyl group and still maintain good levels of selectivity over PDE6 (compound **3**, Fig. 2). Notably, the discovery of compound **3** (MWt 519) confirmed that it was possible to secure good selectivity for PDE5 over PDE6 in a compound with similar structure and molecular weight to that of sildenafil.

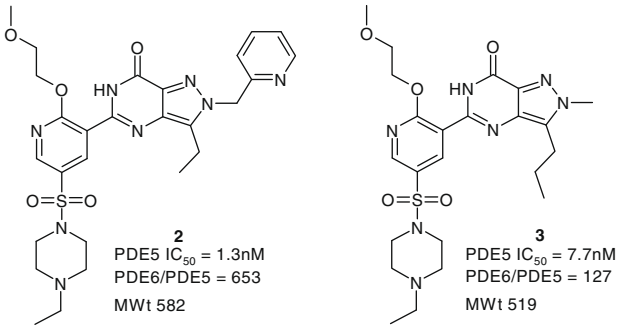


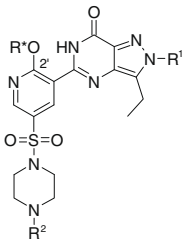
Figure 2. Structures and PDE activity data of key compounds **2** and **3**.

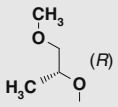
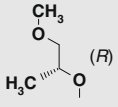
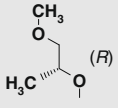
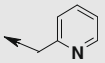
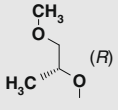
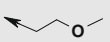
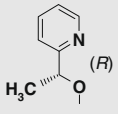
In order to optimise the potency and selectivity of compound **3** further, the project was now faced with a clear challenge: how could this be achieved without again compromising molecular properties? In this context, the introduction of chirality into this series became of interest. Chirality provides a source of molecular complexity that is MWt independent and increasing molecular complexity can drive selectivity.[9] We thus looked to introduce a chiral substituent into this template as a new tactic to differentiate activity *vs* PDE5 and PDE6. The key selectivity SAR observed with the alkoxyarene domain (compounds **2** and **3**), coupled with synthetic expediency, led us to target a small panel of compounds with chiral alkoxy substituents at the 2′-position (Table 1).

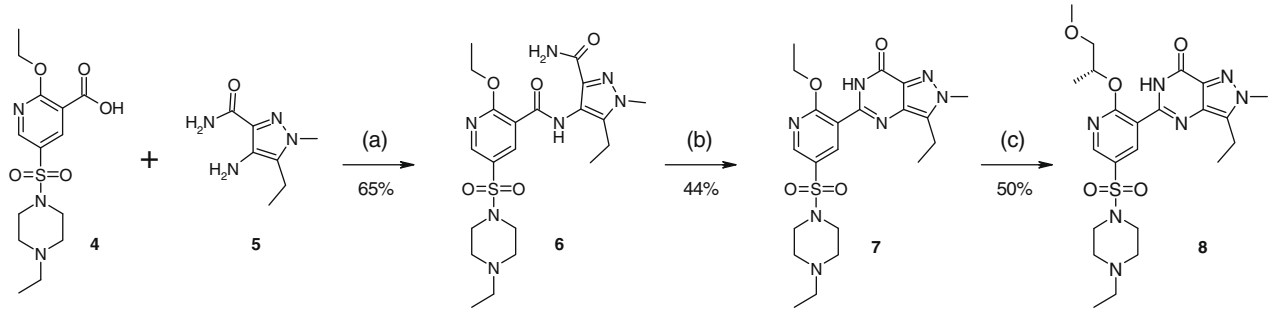
Synthesis of these compounds was straightforward,[10] and took advantage of the ability to introduce the chiral 2′ group *via* a nucleophilic aromatic substitution reaction as the final synthetic step. This general approach is illustrated through the synthesis of target compound **8**, as shown in Scheme 1. Coupling of the known acid **4**[10] and amine **5**[10] led in good yield to amide **6** which underwent base-mediated cyclisation to yield pyrazolopyrimidinone **7**. To prepare the chiral target **8**, compound **7** was simply heated in an excess of the corresponding homochiral alcohol,[11] in the presence of base, to afford **8** as a single enantiomer in good isolated yield.[12,13]

As illustrated in Table 1, introduction of a chiral methyl substituent onto the 2′ methoxyethyloxy group was associated with high potency vs PDE5 in the illustrated (*R*) enantiomer series, but the

Table 1
In vitro inhibition of PDE5 exhibited by chiral compounds **8**–**12** and their corresponding (*S*) enantiomers[14]; selectivity for **8**–**12** over PDE6[14]



Compound	R*O	R^1	R^2	PDE5 IC_{50} (nM)	PDE6 IC_{50} (nM)	Sel PDE6/PDE5	MWt
8		Me	Et	4.2 [(*S*)–**8** = 77]	1978	471	519
9		Me	Pr	5.6 [(*S*)–**9** = 100]	1249	223	533
10			Et	0.62 [(*S*)–**10** = 100]	1539	2482	596
11			Et	0.89 [(*S*)–**11** = 80]	734	825	563
12		Me	Et	3.0 [(*S*)–**12** = 100]	4323	1441	552



Scheme 1. Synthesis of target compound **8**. Reagents and conditions: (a) HOBt, WSCDI, iPr_2NEt, RT; (b) KHMDS, EtOH, 120 °C, sealed vessel, 18 h; (c) KHMDS, xs (*R*)-(−)-1-methoxy-2-propanol, 110 °C, 18 h.

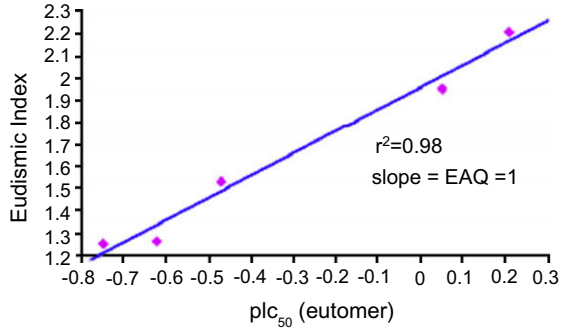


Figure 3. Eudismic analysis of compounds **8**–**12**.

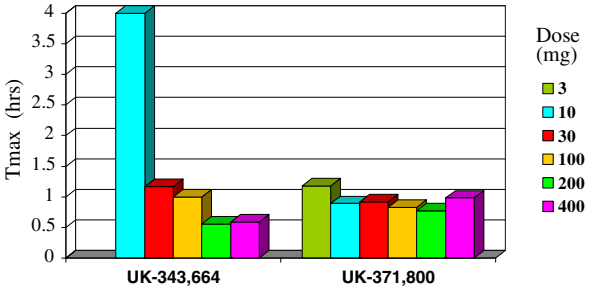


Figure 4. Dose-dependency of T_{max} for **1** (**UK-343,664**) and **8** (**UK-371,800**) in humans.

corresponding (*S*) enantiomer was significantly less active. This highly enantioselective interaction with PDE5 was maintained across the range of analogues. A chiral pyridylmethyl analogue was also explored (compound **12**) and, again, the interaction with PDE5 was highly enantioselective.[13]

Eudismic analysis of the PDE5 inhibition SAR for the chiral compounds in Table 1 provided an interesting illustration of 'Pfeiffer's rule'. In a seminal paper in 1956,[15] Pfeiffer suggested that enantiomeric potency ratios for the interaction of chiral inhibitors with their biomolecular receptors should be directly proportional to the potency of the more active enantiomer. Eudismic analysis is a QSAR formalisation of Pfeiffer's rule.[16,17] In this analysis, the ratio of the distomer (less active enantiomer) and the eutomer (more active enantiomer) is termed the eudismic ratio. The log of the eudismic ratio is termed the Eudismic Index (EI). If Pfeiffer's rule is obeyed, then a plot of EI vs pIC_{50} for the Eutomer should be linear with a slope that is usually between 0.5 and 1.[16,17] This slope is termed the Eudismic Affinity Quotient (EAQ) and a steeper slope (i.e. ~1) is indicative of a highly enantioselective receptor–ligand interaction.

The compounds in Table 1 were subjected to a eudismic analysis and the results of this are shown in Figure 3. The plot of EI and pIC_{50} (eutomer) showed a strong linear correlation ($r^2 = 0.98$) with an EAQ of 1. Further, the high observed EAQ confirmed that the chiral 2′ substituents were involved in an exquisitely stereoselective, highly-complementary interaction within the PDE5 enzyme. This analysis provides a clear illustration of Pfeiffer's rule.

Although PDE5 and PDE6 have high binding site homology, we were intrigued to see if these novel chiral PDE5 inhibitors were able to exploit subtle differences in the PDE5 and PDE6 binding site cavities and deliver greater selectivity. As shown in Table 1, this was indeed found to be the case: all the analogues showed impressive levels of selectivity and some analogues showed selectivities in excess of 1000-fold (which was hitherto unprecedented in the sildenafil scaffold).[18] These data further illustrate the potential of utilising chirality as a tactic to drive selectivity between closely related targets.[19]

Notably, compound **8** (Table 1) proved the most attractive compound for further investigation. This compound delivered on the objective of securing a significant enhancement in PDE5 over PDE6 selectivity relative to sildenafil (471-fold vs 11-fold) in an analogue with similar potency (~4 nM), structure, size (MWt 519 vs MWt 474) and physicochemistry (Log *D* 2.0 vs Log *D* 2.7).[20] Compound **7** exhibited excellent Caco-2 permeability characteristics (15%/h, efflux ratio 1.2), comparable to those of sildenafil (26%/h, efflux ratio ~1). In contrast, **1** (with known non-linear pharmacokinetics), showed only moderate Caco-2 permeability coupled with high efflux (7%/h, efflux ratio 4). These promising in vitro characteristics suggested that compound **8** was not a significant substrate for P-glycoprotein. These data, coupled with supportive preclinical in vivo PK/PD data, led us to progress compound **8** (**UK-371,800**) to clinical evaluation.

In human pharmacokinetic studies, **UK-371,800** exhibited a T_{max} that was independent of dose.[21] This is in stark contrast to **1** which showed significant T_{max} non-linearity (Fig. 4). These data further illustrated that **UK-371,800** was not subject to P-glycoprotein-mediated efflux, thus validating our design strategy to address the non-linearity issues of **1** through securing a smaller compound, more akin to sildenafil and the rule-of-5 guidelines.

In summary, we have described herein a novel series of chiral PDE5 inhibitors that are related to sildenafil but exhibit greatly enhanced levels of selectivity over PDE6. The targeted introduction of chirality proved an effective strategy to secure selectivity enhancements in an 'atom-efficient' manner, thereby avoiding the undesired pharmacokinetic non-linearity observed with a much higher MWt clinical candidate (compound **1**) previously progressed in this template. This series also served to illustrate Pfeiffer's rule and the use of eudismic analysis to establish the specificity of ligand–receptor interactions for chiral compounds. These efforts culminated in the discovery of compound **8** (**UK-371,800**) as a novel chiral PDE5 inhibitor for clinical development.

Acknowledgments

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11. (*R*)-(−)-1-methoxy-2-propanol is commercially available from a variety of suppliers, including Fluka & Acros.
12. *Characterisation data for compound* ***8****:* ^{1}H NMR: δ ($CDCl_3$): 1.04 (3H, t), 1.40 (3H, t), 1.52 (3H, d), 2.42 (2H, q), 2.57 (4H, m), 3.03 (2H, q), 3.15 (4H, m), 3.56 (3H, s), 3.66 (1H, m), 3.77 (1H, m), 4.09 (3H, s), 5.61 (1H, m), 8.62 (1H, s), 10.82 (1H, s). LRMS: m/z 520 $(M+1)^+$. $[\alpha]_D$ +16.6° (*c* 0.10, methanol).
13. Compound **12** was prepared via introduction of the racemic alcohol, followed by chiral resolution. Absolute stereochemistry of **12** is inferred based on potency trends for compounds **8**–**11**. Although this assignment thus has clear caveats, the eudismic analysis is not dependent on absolute configuration. Further, excluding compound **12** from fig. 3 does not impact the linear correlation (r^2 = 0.98).
14. See Ref. 10 for description of PDE5 and PDE6 assays. A detailed protocol from our laboratories for PDE5 and PDE6 assays can also be found in: Allerton, C. M. N.; Barber, C. G.; Beaumont, K. C.; Brown, D. G.; Cole, S. M.; Ellis, D.; Lane, C. A. L.; Maw, G. N.; Mount, N. M.; Rawson, D. J.; Robinson, C. M.; Street, S. D. A.; Summerhill, N. W. *J. Med. Chem.* **2006**, 49, 3581.
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18. Crystallographic studies of compound **8** (**UK-371,800**) in PDE5 have recently been disclosed that indicate a 'flipped' binding mode compared to sildenafil. Homology analysis of this structure with PDE6 provides a structure-based rationale for the enhanced selectivity seen this series, see: Palmer, M. J.; Bell, A. S.; Fox, D. N. A.; Brown, D. G. *Curr. Top. Med. Chem.* **2007**, *7*, 405.
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20. Reported Log *D*s are measured values.
21. **UK-371,800** did exhibit a degree of non-linearity with respect to AUC, but this was only significant at the higher (100–400 mg) doses explored. This AUC non-linearity, which may reflect saturation of CYP3A4 metabolism, was considerably less than that seen for **UK-343,664**.[6]