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Designing rapid onset selective serotonin re-uptake inhibitors. Part 3: Site-directed metabolism as a strategy to avoid active circulating metabolites: Structure–activity relationships of (thioalkyl)phenoxy benzylamines

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ABSTRACT

A series of thio-alkyl containing diphenylethers were designed and evaluated, as a strategy to competitively direct metabolism away from unwanted amine N-demethylation and deliver a pharmacologically inactive *S*-oxide metabolite. Overall, sulfonamide **20** was found to possess the best balance of target pharmacology, pharmacokinetics and metabolism profile.

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In earlier communications[1] we described our strategy to access selective serotonin re-uptake inhibitors (SSRIs) which demonstrated very rapid elevation of central 5-HT levels, to support rapid-on-set of efficacy, for potential use in acute (*prn*) therapy compatible indications including, male sexual dysfunction. Following identification of clinical agent **1**, Figure 1, we explored a range of simpler, non-chiral expressions, **2**, of our initial lead series. These studies led to the identification of a series of phenoxy benzylamines[2] which possessed excellent SSRI potency and selectivity over other monoamine transporters.[3] In addition, analogues from this series were found to possess lower Vdu than **1**, a potential key feature in securing our clinical objective of delivering rapid systemic exposure[4] of 5-HT.

In particular, **3** was found to possess nano molar potency in the serotonin transporter assay (IC_{50} 5 nM) and good selectivity over inhibition of dopamine and noradrenaline re-uptake (IC_{50} 11,000 nM and 770 nM, respectively). Furthermore, full in vivo profiling of **3** in rat and dog showed the compound to possess excellent pharmacokinetics, supporting predictions to human (T_{max}, 0.5 h, half-life 5 h, and bioavailability ~90%), fully in line with our clinical goals. Unfortunately, in vivo **3** was found to also form the secondary amine as the main metabolite, via N-demethylation (major cytochrome P450 isoform 2D6). This compound was found to be both pharmacologically active (SRI, IC_{50} 50 nM) and possess a relatively long half-life in vivo in rat and dog (10 h). Given that our key goal of was to identify a short half-life compound to support a *prn* profile, the formation of a relatively long-lived and pharmacologically active circulating metabolite, prevented us from moving forward to the clinic with **3**.

While disappointing, the underlying pharmacokinetics of compounds from this series was, however, very attractive. We therefore, set about designing compounds which avoided the formation of an active (secondary amine) metabolite by directing metabolism away from N-demethylation of the amine, to another site in the molecule to give a significantly less active metabolite.

For this strategy to be successful, we reasoned several key objectives must be achieved, including (1) the putative site of metabolism must be pharmacologically tolerated in the (drug) parent (2) any (circulating) metabolite formed must be significantly

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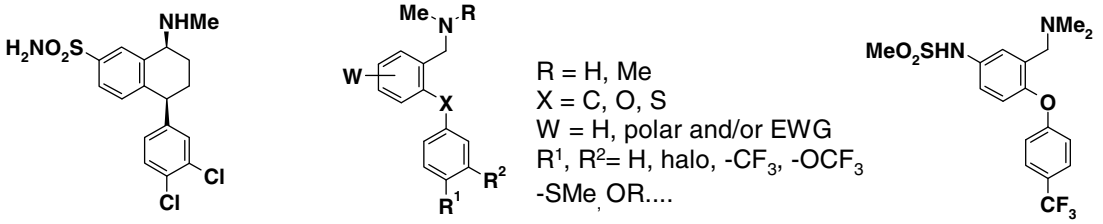
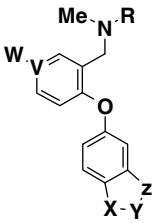


Figure 1. Structures of clinical agent **1**, alternative non-chiral lead series **2** and phenoxy benzylamine **3**.

less active than the parent (>50-fold) to avoid additional pharmacological activity in vivo, and (3) the rate of metabolite formation must be fast enough to successfully compete with amine N-demethylation.

From previous in house experience, we were aware that P450 mediated thio alkyl S-oxidation can be a rapid metabolic process and therefore offered an attractive area for investigation. Our first goal, Table 1, was to establish that high potency and target selectivity, similar to that observed with **3**, could be achieved within this diphenylether template, when containing a thio alkyl moiety.[3c,5] Pleasingly, simple thio methyl analogue **4**, showed good potency in the serotonin transporter assay (IC_{50} 10 nM) and encouraging selectivity over inhibition of dopamine and noradrenaline re-uptake (690- and 60-fold, respectively). Furthermore and key, synthesis and screening of the potential sulfoxide **5** and (less likely) sulfone **6** metabolites, showed these to be >50-fold weaker than **4**, thus building confidence that goals (1) and (2) outlined above, could be achieved. We focused mainly on CYP-mediated metabolism whilst being aware the thio alkyl S-oxidation could equally have been mediated by flavin monoxygenase (FMO).[6] Under our experimental conditions the S-oxidation activity of this enzyme would have been largely indistinguishable from CYP-mediated S-oxidation and so would have added to the SAR complexity around this metabolic route.

Table 1
Human monoamine re-uptake[a] activities and in vitro metabolism data for phenyl ethers **3**–**28**



Compound	R	V	W	X	Y	Z	h-SRI (IC_{50}, nM)	Selectivity over DRI and NRI	Human in vitro metabolism data[d]
3	Me	C	$-NHSO_2Me$	$-CF_3$	—	H	5	2400/170	(N-Demethylation in vivo)
4	Me	C	F	SMe	—	H	10	690/60	NT
5	Me	C	F	–S(O)Me	—	H	600	46/26	NT
6	Me	C	F	$-SO_2Me$	—	H	750	NT	NT
7	Me	C	Me	SMe	—	H	5	820/320	No N-demethylation–Very short half-life
8	Me	C	OMe	SMe	—	H	6	3800/140	Very short half-life
9	Me	N	—	SMe	—	H	2	3600/500	NT
10	Me	N	—	SMe	—	Me	8	3500/220	>20% demethylation
11	Me	N	—	SMe	—	Cl	5	3900/300	ca., 30% demethylation
12	Me	N	—	S	CH_2	CH_2	3	1500/150	25% demethylation
13	Me	C	$-NHSO_2Me$	SMe	—	H	5	711/23	NT
14	H	C	$-NHSO_2Me$	SMe	—	H	12	770/160	—
15	Me	C	$-NHSO_2Me$	SMe	—	F	10	480/70	NT
16	H	C	$-NHSO_2Me$	SMe	—	F	16	290/30	N
17	Me	C	$-NHSO_2Me$	S	CH_2	CH_2	7	93/10	NT
18	Me	C	$-NHSO_2Me$	SMe	—	Me	60	—	NT
19	Me	C	$-NHSO_2Me$	S	$-CH_2CH_2-$	O	15	1000/140	NT
20	Me	C	$-SO_2NH_2$	SMe	—	H	4	630/220	*S*-Oxide
21	Me	C	$-SO_2NH_2$	–S(O)Me	—	H	>1000	c	NT
22	Me	C	$-SO_2NHMe$	SMe	—	H	9	60/25	NT
23	Me	C	$-SO_2NHMe$	–S(O)Me	—	H	>1000	b	NT
24	H	C	$-SO_2NMe(CH_2)_2OH$	SMe	—	H	5	190/1100	NT
25	H	C	$-SO_2NMe(CH_2)_2OH$	–S(O)Me	—	H	>1000	NT	NT
26	Me	C	$-SO_2NH_2$	SMe	—	F	9	620/160	N-Demethylation observed
27	Me	C	$-SO_2NH_2$	SMe	—	Me	5	620/180	*S*-Oxide
28	Me	C	$-SO_2NH_2$	–S(O)Me	—	Me	>10,000	c	NT

[a] See Ref. 1b for description of assay conditions. All assay determination $\geqslant n = 2$.
[b] DRI = 9 μM, NRI = 13 μM.
[c] DRI and NRI >10 μM.
[d] Where N-demethylation was measured as a major metabolic route, S-oxidation was also observed as a competing pathway. No sulfone metabolite was observed.

Potential in vivo metabolism of **4**, via N-demethylation (i) or S-oxidation (ii), using P4502D6 homology modelling[7] is shown in Figure 2. In general, these studies suggested that either orientation (i) or (ii) of **4**, could be accommodated within the cytochrome active site. In the case of N-demethylation (i), a potential π–π interaction between Phe481 and the distal phenyl ring of **4**, could be rationalised to help orientate the molecule towards N-alkylation. Alternatively, modelling for potential S-oxidation (ii), suggested that the presence of a weak H-bond between Asp-301 in the 'I'-helix and the tertiary amine group, could help orientate the molecule to support competing S-oxidation. Furthermore, while ether O-demethylation, and *para*-hydroxylation were metabolic routes often seen with P4502D6,[8] N-demethylation was less common[9] and we felt, therefore, that the presence of an alternative (S-oxidation) route in these substrates would prove competitive in vitro.

With these encouraging data in hand, we next proceeded to investigate the pharmacology and metabolic fates of a series of thio alkyl containing compounds, Table 1. Methyl **7** and methoxy **8** analogues showed good potency (IC_{50} 5 and 6 nM) and selectivity (>100-fold over both dopamine and noradrenaline re-uptake). Encouragingly, while **7** showed no N-dealkylation in human in vitro metabolic studies, both **7** and **8,** however, possessed only very short half-lives in vitro, due, we reasoned, to their relatively highly lipophilicity (log*D* 2.9 (**7**) and 2.7 (**8**)). We next investigated a series of pyridyl analogues[2b] **9**–**12**. These included analogues possessing substitution adjacent to the thio methyl group (**10** and **11**) and cyclising the thio-aklyl group into a ring **12**, as strategies to attempt to modulate the rate of S-oxidation. While these compounds were found to be both potent (IC_{50} 2–10 nM) and selective, they tended to show a significant degree of N-demethylation, in addition to the desired S-oxidation, in vitro. A series of *N*-sulfonamide analogues **13**–**19** were next investigated. Direct thio methyl analogue **13**, of initial lead **3**, showed equivalent potency in the serotonin transporter assay (IC_{50} 5 nM). Unfortunately, this compound was poorly selective over noradrenaline re-uptake inhibition. Disappointingly, this series tended to suffer from either slightly low potency or poor selectivity and was discarded in favour of the more promising reverse sulfonamide analogues.

Primary sulfonamide **20**, for example, was found to be potent (SRI, IC_{50} 4 nM) and selective over both dopamine and noradrenaline re-uptake inhibition (630- and 220-fold, respectively). Excitingly, in vitro, this compound was found to predominantly (>90%) form the sulfoxide (confirmed by HPLC 'spiking' with the sulfoxide, prepared separately) mediated by several P450 isoforms, but predominantly 2D6, with very little N-demethylation observed. Furthermore, the sulfoxide analogue **21** was found to be very weak in the serotonin transporter assay (IC_{50} >1000 nM) with essentially no activity against dopamine or noradrenaline re-uptake. Reassuringly, the presence of the primary sulfonamide did not appear to compromise permeability of this analogue (Caco-2 A–B/B–A 40/41), which subsequently translated to good CNS permeability (*vide infra*). The secondary sulfonamide **22** was also found to be potent (SRI, IC_{50} 9 nM) and the potential sulfoxide metabolite **23** very weak in the serotonin assay. Unfortunately, **22** was found to be poorly selective over dopamine and noradrenaline re-uptake inhibition (60- and 25-fold, respectively). Alkoxy-substituted sulfonamide **24**, was found to show both good potency and selectivity. In addition, the sulfoxide analogue **25** was essentially inactive against the monoamine panel. Unfortunately, the sulfonamide possessed a flawed profile in the Caco-2 assay (A–B/B–A 9/15), raising concerns regarding the potential of this compound to achieve the high CNS exposure desired. The *meta*-fluoro analogue of **20**, **26**, showed similar potency and selectivity, unfortunately, in vitro this compound also underwent a mixture of N-demethylation and S-oxidation. In contrast, the *meta*-methyl analogue, **27**, was found to be potent and selective, with the sulfoxide **28** observed as the major metabolite in human in vitro metabolism studies.

In rat pharmacokinetic studies, Table 2, sulfonamide **20**, was found to possess a rapid T_{max} (0.5 h), low Vdu (74 L/kg) and good CNS penetration, as measured in rat studies (CSF:free 0.9:1), all consistent with our clinical objectives. Furthermore, in both rat and dog pharmacokinetic studies performed using **20**, sulfoxide **21** was found to be the predominant metabolite formed in vivo, possessing a short half-life (<4 h) in both species. In addition to possessing no significant activity against the three monoamine transporters, the highly polar (log*D* < −2) sulfoxide **21** was found to be essentially pharmacologically inactive across a CEREP screening panel of more than 30 receptors, enzymes and ion-channels (IC_{50} >10 μM). Overall, sulfonamide **20** was found to possess the best balance of target pharmacology, pharmacokinetics and metabolite profile. Based on these and other data, sulfonamide **20** was progressed into clinical development.

In summary, a series of thio-alkyl containing diphenylethers were designed and evaluated, as a strategy to competitively direct metabolism away from unwanted amine N-demethylation and deliver a pharmacologically inactive *S*-oxide metabolite. These studies, showed that thio-alkyl substitution, could be tolerated and

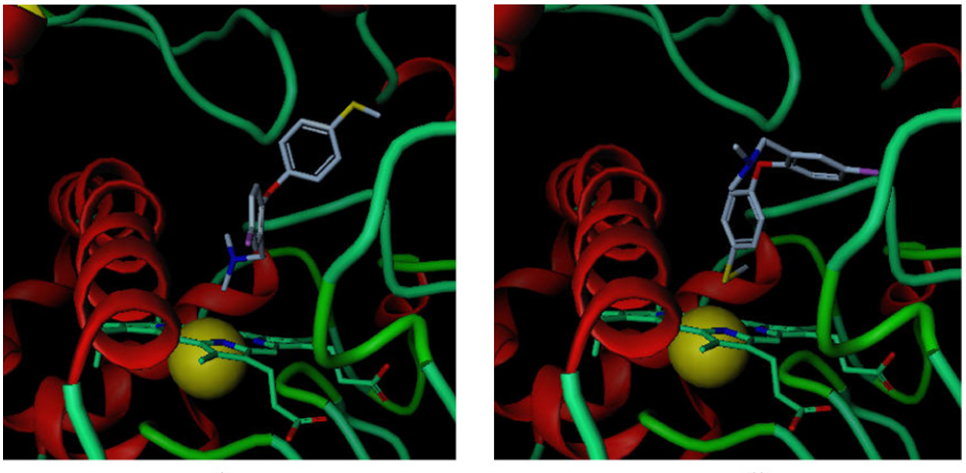
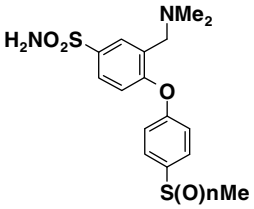


Figure 2. Potential metabolism of **4,** via N-demethylation (i) or S-oxidation (ii), using a CYP4502D6 homology model.

Table 2
Rat pharmacokinetic data on **20**



Compound	*n*	h-SRI (IC_{50}, nM)	Selectivity over DRI and NRI	log*D*	pK_a amine	Caco-2 (A–B/B–A) (%/h)	Rat pharmacokinetics (iv 3 mg/kg (*n* = 5), po 3 mg/kg (*n* = 4)					Rat CNS data[a]
							Blood Cl (ml/min/kg)	Vd (Vdu) (L/kg)	$T_{1/2}$ (h)	*F* (%)	T_{max} (h)	CSF: free (blood)
20	0	4	630/220	1.5	8.4	40/41	19	4 (74)	3	88	0.5	0.9:1
21	1	>1000	—	<−2	8.0	—	—	—	<4	—	—	—

[a] 3 mg/kg iv (1 h post dose).

retain excellent potency against serotonin re-uptake inhibition and also achieve target selectivity over the other monoamine re-uptake transporters. In human in vitro metabolic studies, a range of profiles were observed, all including S-oxidation, demonstrating this route was competitive with N-demethylation. No clear SAR emerged regarding the overall features which influenced the degree of metabolic route selectivity for S-oxidation over N-demethylation in this series. This perhaps reflects a number of factors including potential for relatively broad CYP450 substrate promiscuity, overlapping SAR from multiple CYPs along with potential additional SAR(s) derived from FMO.[5]

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