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Identification of an Orally Bioavailable, Potent, and Selective Inhibitor of GlyT1

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ABSTRACT Amalgamation of the structure–activity relationship of two series of GlyT1 inhibitors developed at Merck led to the discovery of a clinical candidate, compound **16** (DCCCyB), which demonstrated excellent in vivo occupancy of GlyT1 transporters in rhesus monkey as determined by displacement of a PET tracer ligand.

KEYWORDS Inhibitor, GlyT1, structure–activity relationship, DCCCyB, PET tracer ligand

16 (DCCCyB)

The hypofunction of *N*-methyl-D-aspartate (NMDA) receptors has been implicated in the pathophysiology of schizophrenia, with evidence coming from both preclinical models[1–3] and the limited clinical data available.[4–10] The latter includes the reversible psychosis induced in nonschizophrenics by NMDA antagonists such as phencyclidine (PCP), and the clinical efficacy observed when antipsychotic medication was supplemented with the obligatory NMDA coagonist glycine or with sarcosine, a weak endogenous inhibitor of type 1 glycine uptake transporters (GlyT1). Most importantly, this adjunctive therapy has been shown to give significant improvements in the negative and cognitive symptoms of stable schizophrenics, for which significant unmet medical need remains due to the lack of efficacy of conventional antipsychotics against these symptoms.[6] In the brain, glycine levels are thought to be maintained tonically at submaximal concentrations in the synapse by GlyT1.[11] This suggests that the pharmacological manipulation of synaptic glycine concentration using a GlyT1 inhibitor may be a viable method of potentiating NMDA receptor function in vivo, hence ameliorating the negative and cognitive symptoms of schizophrenia. To test this hypothesis, there has been an industry wide effort to identify potent and selective GlyT1 inhibitors.[12–15]

Recent communications from our laboratory have disclosed two related series of hGlyT1 inhibitors, typified by the piperidine sulfonamide **1**[16] and cyclohexyl sulfone **2**,[17] which demonstrate excellent selectivity over the related GlyT2 and TauT transporters (Figure 1). Early issues for the piperidine series were poor oral bioavailability in rat and dog due to high plasma clearance, although this was resolved in the 2,4-dichlorophenyl analogue **1**.

However, compound **1** was shown to have unacceptably high covalent binding in vivo to both rat liver and plasma proteins after oral dosing at 20 mg/kg (6 h postdose: liver, 153 pmol equiv/mg protein; plasma, 214 pmol equiv/mg protein). Metabolite identification experiments using radiolabeled piperidine sulfonamide analogues, or trapping experiments with radiolabeled cyanide, led us to hypothesize that the observed covalent binding was due to oxidation of the piperidine ring leading to the formation of a reactive species. High levels of covalent binding have been linked to increased incidence of idiosyncratic toxicities of compounds in the clinic;[18] therefore, further chemical optimization was undertaken in the sulfone series. The required cyclohexyl sulfone derivatives were accessed as delineated in Scheme 1.[19] The *cis* and *trans* isomers of the key alcohol intermediate **A** were separated chromatographically and assigned based upon nuclear Overhauser effect NMR experiments.

Where the required thiol was readily available, formation of the mesylate of **A** followed by displacement gave the thioether. Alternatively, the thioether was accessed via Mitsunobu chemistry with thioacetate followed by a one-pot deprotection–alkylation protocol. Oxidation of the thioether with oxone gave the required final compound.

The inhibition at hGlyT1 transporters and microsomal turnover in rat and human microsomes for a selection of heterocyclic sulfone compounds is given in Table 1.

Received Date: May 11, 2010
Accepted Date: June 21, 2010
Published on Web Date: June 25, 2010

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hGlyT1 IC_{50} 26 nM
hGlyT1 IC_{50} 5.4 nM

Figure 1. Structures of hGlyT1 inhibitors.

Scheme 1[a]

[a] Reagents and conditions: (a) KHMDS, THF and then cyclopropylmethyl bromide. (b) $LiAlH_4$, Et_2O. (c) 2,4-Dichlorobenzoyl chloride, Hunigs base, DCM. (d) HCl(aq), THF. (e) $NaBH_4$, EtOH. (f) Chromatographic separation of isomers. (g) MsCl, pyr and then RSNa. (h) PPh_3, $N_2(CO_2i\text{Pr})_2$, thioacetate. (i) LiOH, THF/H_2O and then RBr. (j) Oxone, acetone/water.

Table 1. hGlyT1 Potency and Human and Rat Liver Microsomal Turnover of Selected Heterocyclic Sulfone Analogues

Cpd	R	amide / sulfone	hGlyT1 IC_{50} [a] (nM)	LM % Turnover[b] H	LM % Turnover[b] R
2	1-methyl-1,2,3-triazolyl	*Cis*	5.4	28	10
3	1-methyl-1,2,3-triazolyl	*Trans*	1.9	28	23
4	NH-triazolyl	*Cis*	3000	-	-
5	NH-triazolyl	*Trans*	3000	-	-
6	methylisoxazolyl	*Cis*	7.3	40	41
7	methylisoxazolyl	*Trans*	8.6	35	51
8	methylthiadiazolyl	*Trans*	3333	-	-
9	methylpyridazinyl	*Trans*	29	35	40

[a] IC_{50} values are averages of at least two measurements. The hGlyT1$_a$ isoform was used for the assay. [b] Turnover of GlyT1 compounds (1 μM) in rat and human liver microsomes. All incubations were carried out at 37 °C for 15 min. Protein concentration = 0.5 mg/mL; cosolvent = 0.99% MeCN + 0.01% DMSO. For compounds in this series, LM % turnover ≤40 was considered acceptable for further compound progression.

Compound **2** exhibited excellent oral bioavailability in the rat and occupied GlyT1 transporters in vivo, as adjudged by our previously reported in vivo binding assay in the rat[20] using a proprietary GlyT1 radiolabel, with an Occ_{50} of 3.4 mg/kg.[17] Analysis of the plasma and brain drug levels required to achieve Occ_{50} (1.2 and 0.2 μM, respectively) revealed a low brain to plasma ratio of 0.16. A similarly low brain to plasma ratio of 0.1 was determined from a 10 mg/kg oral dose of compound **3**. A subsequent study in mdrla +/+ and −/− mice determined the ratio between the brain:blood ratios of the −/− and +/+ animals to be 8.7, suggesting compound **3** to be a P-gp substrate.

Compound **2** did not inhibit common Cyp isoforms (2D6, 2C9, and 3A4: IC_{50} > 10 μM); however, the NH-triazole analogues **4** and **5**, potential metabolites of compounds **2** and **3**, respectively, proved to be extremely potent inhibitors of Cyp 2C9 (compound **5** Cyp 2C9: IC_{50} = 10 nM). The potent Cyp inhibition, in combination with the high plasma Occ_{50} due to the P-gp issue, precluded the further development of triazole analogues **2** and **3**. Heterocyclic sulfone analogues in which the pendant alkyl group was linked through carbon exhibited either increased microsomal turnover (**6** and **7**) or reduced potency at hGlyT1 (**8** and **9**).

Investigation of simple alkyl sulfone derivatives related to compound **1** (Table 2) established that the structure−activity relationship (SAR) was reminiscent of the previously described 4-pyridyl piperidine series[16] with a significant reduction in potency observed in the series propyl **10**, ethyl **11**, and methyl **12**. In the alkyl sulfone series, a more stringent requirement for the *cis* relationship between the sulfone and the amide was observed than in the heterocyclic series, with compounds **10** and **11** demonstrating >10-fold greater potency relative to **13** and **14**. Although the cyclobutylmethyl compound **15** demonstrated a loss in potency relative to propyl analogue **10**, the cyclopropylmethyl compound **16** (DCCCyB) retained potency but with improved microsomal stability. Compound **16** demonstrated an acceptable level of in vivo covalent binding (<25 pmol equiv/mg after a 20 mg/kg oral dose) in the rat and was selected for further profiling.

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DOI: 10.1021/ml1001085 | *ACS Med. Chem. Lett.* 2010, *1*, 350–354

ACS Medicinal Chemistry Letters

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Table 2. hGlyT1 Potency and Human and Rat Liver Microsomal Turnover of Selected Alkyl Sulfone Analogues

Cpd	R	amide / sulfone	hGlyT1 IC_{50} [a] (nM)	LM % Turnover[b] H	LM % Turnover[b] R
10	Pr	*Cis*	36.9	55	53
11	Et	*Cis*	132.4	-	-
12	Me	*Cis*	3333	-	-
13	Pr	*Trans*	1627	-	-
14	Et	*Trans*	1323	-	-
15	cyclobutylmethyl	Cis	629	-	-
16	cyclopropylmethyl	Cis	29	35	40

[a] IC_{50} values are averages of at least two measurements. The $hGlyT1_a$ isoform was used for the assay. [b] Turnover of GlyT1 compounds (1 μM) in rat and human liver microsomes. All incubations were carried out at 37 °C for 15 min. Protein concentration = 0.5 mg/mL; cosolvent = 0.99% MeCN + 0.01% DMSO. For compounds in this series, LM % turnover ≤40 was considered acceptable for further compound progression.

Table 3. Pharmacokinetic Parameters of Compound **16** in Preclinical Species

		rat	rhesus	dog
dose (iv and po)	mg/kg	1.0	1.0	1.0
Cl	mL/min/kg	36	24	4.9
Vd(ss)	L/kg	4.1	2.3	3.1
$T_{1/2}$	h	2.4	1.5	10
F	%	65	2	48
C_{max}	μM	0.14	0.04	1.39
T_{max}	h	0.8	2.7	1.0

The pharmacokinetic parameters of compound **16** in preclinical species are given in Table 3. Clearance is low in dogs and moderate in rats and rhesus monkey, and this combined with moderate Vd(ss) in all species gave acceptable half-life values. Oral bioavailability of 65 and 48% in rat and dog, respectively, was obtained using the 0.5% methocel suspension dosing vehicle.

Compound **16** was not a substrate for human or mouse P-gp, had a significantly increased brain to plasma ratio of 2.3, and exhibited a lower plasma Occ_{50} of 0.35 μM in the rat GlyT1 in vivo binding assay as compared to compound **2**. No significant off-target activity was observed for compound **16** in a broad ancillary pharmacology panel screen.

A GlyT1 inhibitor would be expected to lead to an increase in the levels of extracellular glycine in the brain. This has been demonstrated in the literature, and at Merck[1] using proof of concept compounds, by in vivo dialysis through a probe inserted into the rat frontal cortex. Glycine levels were determined up to 4 h postdose with compound **16** at 20 and 3 mg/kg po. Both doses significantly elevated extracellular glycine levels above basal concentrations (mean % peak glycine efflux as a % basal ± SEM; 20 mg/kg = 184.0 ± 17.0%; 3 mg/kg = 151.0 ± 25.0%). The increase in glycine levels at the 3 mg/kg po dose of compound **16** is consistent with that observed for other GlyT1 inhibitors shown to be efficacious in animal models of schizophrenia.[21]

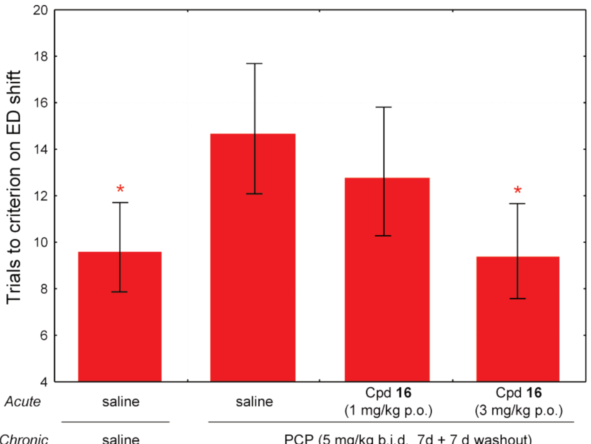

Figure 2. Trials to criterion for ED shift. Asterisk-marked columns denote significance at $p < 0.05$ vs PCP and vehicle for ED shift.

Impairments in executive function have long been considered to be a core feature of schizophrenic illness,[22] with the attentional set shifting aspect of executive function commonly assessed in patients using the Wisconsin Card Sorting Test.[23] The intra/extra dimensional (ED/ID) rodent model of executive function can be used, following impairment in perceptual attentional set shifting by PCP administration, as a model for the set shifting deficits observed in schizophrenic patients.[24] In the rat ED/ID assay,[25] compound **16** dosed at 3 mg/kg po reversed the PCP-induced cognitive deficit in the ED shift (Figure 2). Although all discriminations were performed, for clarity, only the ED data are shown as subchronic dosing of PCP induced a deficit exclusively in this aspect of the assay.

The availability of the GlyT1 PET tracer [^{18}F]MK-6577[26] facilitated the evaluation of the plasma occupancy relationship for compound **16** in higher species. Figure 3 shows the time−activity curves and PET/MRI coregistered images from the PET scans.[27] The differences in tracer uptake under baseline and blockade conditions can be used to determine GlyT1 occupancy at different doses/plasma concentrations. A plasma occupancy curve for **16** was generated giving rise to an estimated plasma Occ_{50} concentration of 120 nM in rhesus monkey, which is in good agreement with the plasma Occ_{50} concentration generated in rat.

In summary, replacement of the piperidine ring of compound **1** generated a series of cyclohexyl sulfone inhibitors of hGlyT1 and removed the in vivo covalent binding observed with the former series. Reoptimization of the sulfone moiety

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DOI: 10.1021/ml1001085 | *ACS Med. Chem. Lett.* **2010,** *1,* 350–354

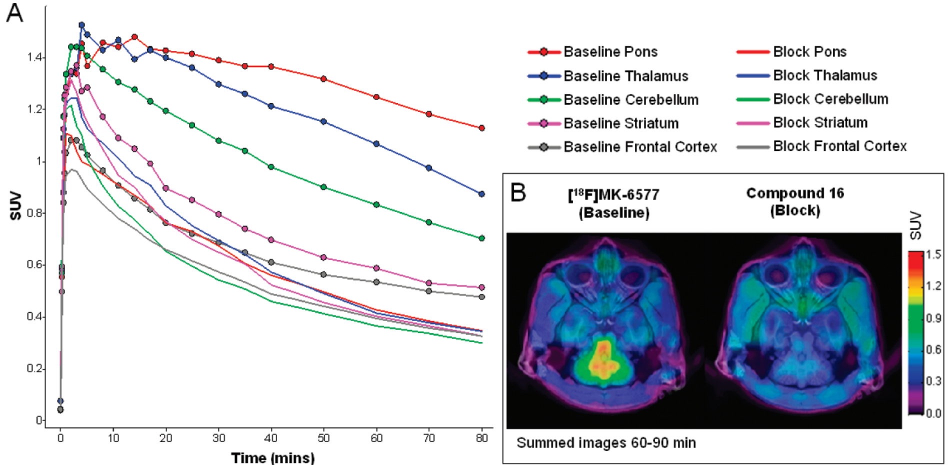

Figure 3. (A) Time−activity curves for baseline ([^{18}F]MK-6577 treated; filled markers) and for blockade ([^{18}F]MK-6577 and cpd **16** treated; no markers) conditions. (B) PET/MRI coregistered images in rhesus monkey brain under baseline (left) and blockade (right) conditions with cpd **16**.

removed the P-gp liability observed for the heterocyclic sulfone derivatives and led to the identification of compound **16**. The clinical evaluation of compound **16** will be published in the near future.

SUPPORTING INFORMATION AVAILABLE Experimental procedures for the preparation of compound **16** including analytical and spectral characterization data (^{1}H and ^{13}C NMR, HR-MS, and HPLC). This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT W.B. thanks Scott Wolkenberg for his help in the preparation of this manuscript.

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$$\mathrm{TAC\ (SUV)} = 1000 \times \mathrm{TAC\ (Bq)} \times \mathrm{weight\ (kg)/injected\ tracer\ dose\ (Bq)}$$

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DOI: 10.1021/ml1001085 | *ACS Med. Chem. Lett.* **2010,** *1,* 350–354