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A novel, non-substrate-based series of glycine type 1 transporter inhibitors derived from high-throughput screening

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Abstract—The synthesis and structure–activity relationships (SAR) of a series of indane and tetralin inhibitors of the type 1 glycine transporter, derived from a high-throughput screening (HTS) hit, are described. Key modifications that reduced the 5HT1B receptor affinity of the HTS hit and the P450 2D6 inhibition of subsequent analogues are delineated. While these modifications led to potent and selective GlyT1 inhibitors, HERG affinity and human microsomal clearance remain an issue for this series of compounds.

A prevailing neurochemical hypothesis of schizophrenia posits a deficit in NMDA receptor-mediated glutamatergic neurotransmission. For example, NMDA antagonists (e.g., PCP or ketamine) can reproduce the positive, negative, and cognitive symptoms of schizophrenia in healthy volunteers and exacerbate these symptoms in patients.[1,2] In addition, NMDA blockade can reproduce the dysregulation of the subcortical dopamine system observed in schizophrenia—hence the clinical activity of D2 antagonists is also consistent with this hypothesis.[3] Therefore, treatments enhancing NMDA function may be of benefit in schizophrenia. Numerous placebo-controlled double-blind trials have reported improvements in negative, cognitive, and positive symptoms following the addition of NMDA/glycine site agonists such as glycine, D-serine or D-cycloserine to ongoing therapy with typical or atypical antipsychotic drugs.[4] Limited preclinical and clinical data are also suggestive of a decrease in EPS liability/tardive dyskinesia with glycine augmentation.[5]

Glycine augmentation elevates NMDA receptor transmission since it is a co-agonist, binding to the NR1 subunit of the receptor.[6] One strategy for augmenting glycine levels is inhibition of the type 1 glycine transporter (GlyT1), which regulates glycine levels in the vicinity of the NMDA receptor.[7,8] GlyT1 is a member of the sodium/chloride-dependent transporter family, which includes transporters for dopamine, noradrenalin, and serotonin.[9] There is a rapidly growing literature describing inhibitors of GlyT1, including substrate-based (sarcosine series) and non-substrate-based compounds.[10] We describe herein a novel, non-substrate-based series of GlyT1 inhibitors useful as reagents for studying the effects of GlyT1 inhibition in vivo.

Our efforts began with a high-throughput screen (HTS) using radiolabeled (R)-(−)-NPTS, a GlyT1 ligand we described previously.[11] The HTS yielded compound **1** (Table 1) as a hit, with a GlyT1 K_i value of 500 nM. Compound **1**, however, showed poor selectivity for GlyT1, with, for example, a 5HT1B K_i value of <1 nM.[12] In addition, **1** shows binding to the HERG channel, as measured by displacement of the HERG ligand dofetilide, inhibition of cytochrome P450 2D6, and rapid degradation in the presence of human microsomes (see Table 1). Our goals for improving compound **1** were to achieve HERG binding in the dofetilide assay of >1000 nM, <10% inhibition of P450 2D6, and >60 min half-life in the presence of human liver microsomes.

The 5HT1B receptor activity was recognized as likely arising from the naphthyl piperazine pharmacophore. Compound **2** replaces the naphthalene with an indane ring, and adds an isopropyl group to the piperazine to afford similar binding potency at GlyT1 (Table 1). GlyT1 potency was improved with cyclopentyl substituents, as in compounds **3** and **4** (Table 1). The hydroxymethyl substituent in **4** was designed to improve

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Table 1. Biological profiling of compounds in the indane and tetralin series

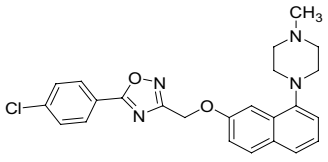
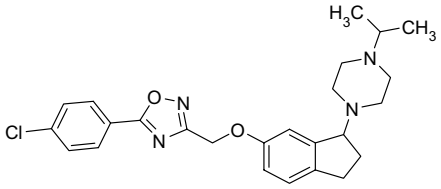
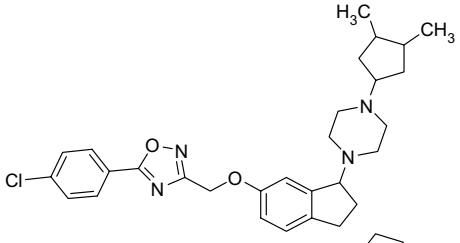
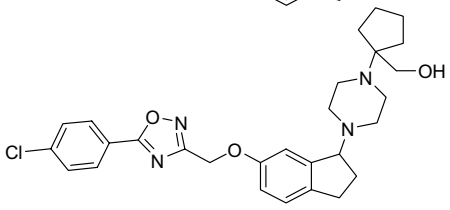
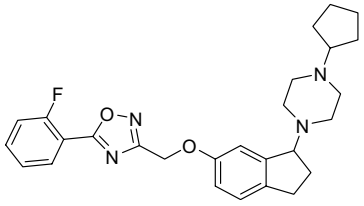
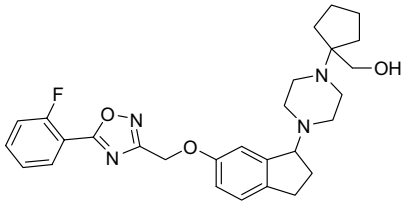
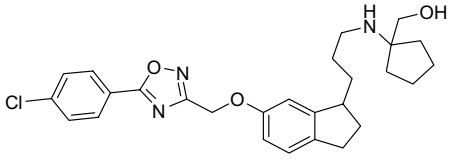
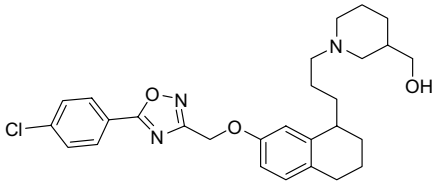
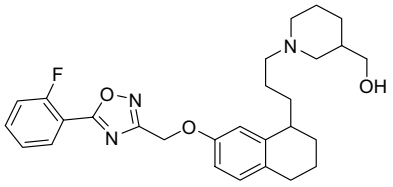
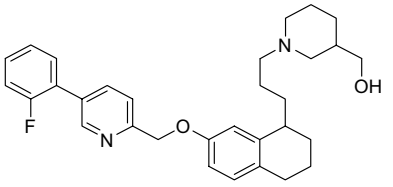
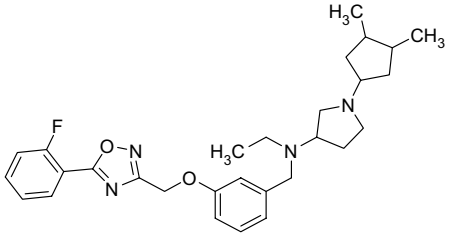
Compound	Structure	GlyT1 K_i (nM)	GlyT2 IC_{50}	5HT1B K_i (nM)	Dofetillide K_i (nM)	CYP2D6 % I at 1.5 μM	hMic $t_{1/2}$ (min)
1		500	ND	<1	1280	75	22
2		394 ± 205	ND	31	98.1	43	13
3		134 ± 103	ND	414	261	29	7
4		26.8 ± 7.05	ND	1490	205	47	9
5		66.8 ± 47.9	531 nM	ND	693	94	12
6		61.4 ± 33.8	31.5 nM	ND	709	85	14
7		108 ± 80	ND	>300	525	70	12
8		15.9 ± 7.21	319 nM	>1000	271	96	22

Table 1 (*continued*)

Compound	Structure	GlyT1 K_i (nM)	GlyT2 IC_{50}	5HT1B K_i (nM)	Dofetillide K_i (nM)	CYP2D6 % I at 1.5 μM	hMic $t_{1/2}$ (min)
9		10.2 ± 2.12	385 nM	ND	1300	43	17
10		11.0 ± 2.46	401 nM	ND	410	30	24
11		85.3 ± 43.1	857 nM	ND	222	84	26

GlyT1, measured as the K_i value ± SEM for displacement of tritiated NPTS[11] from the human GlyT1c transporter expressed in HEK293 cells; GlyT2, measured as the IC_{50} value for blockade of tritiated glycine uptake into HEK293 cells expressing the human GlyT2 transporter; 5HT1B, measured as the K_i value for displacement of tritiated 5-hydroxy-trytamine from cloned human 5HT1B receptors expressed in HEK293 cells; Dofetilide K_i, measured as the K_i for displacement of tritiated Dofetilide from human HERG channel (IKr) expressed in HEK293 cells; Cyp 2D6, measured as the % inhibition of the disappearance of the 2D6 substrate aminoethyl-methoxy-methylcoumarin (AMMC) in the presence of recombinant human cytochrome 2D6 at a concentration of 1.5 μM of the test compound, hMic $t_{1/2}$, human liver microsomal clearance, measured as the half-life in minutes for the disappearance of compound in the presence of human liver microsomes; ND, not determined.

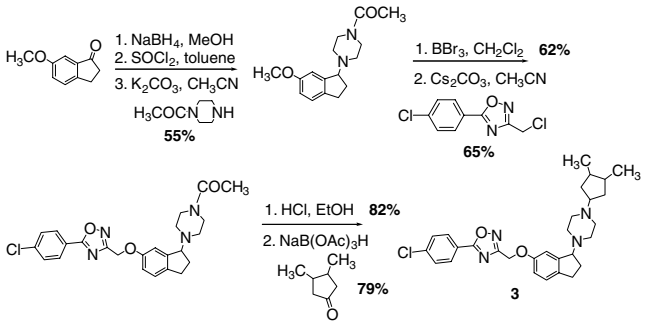
solubility and also reduced 5HT1B binding affinity to above 1 μM. Further SAR studies in this series identified the ortho-fluoro analogues **5** and **6** as potent GlyT1 inhibitors.

The synthesis of these indanyl piperazine compounds was carried out as shown in Scheme 1.

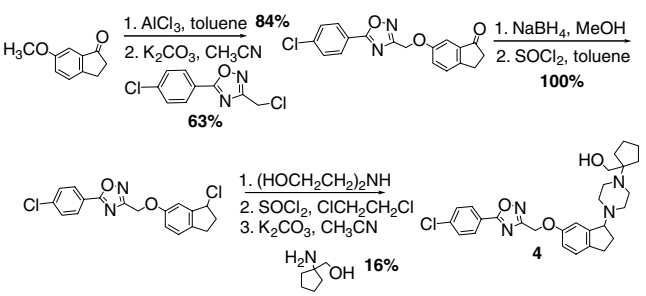
As shown in Scheme 1, the preparation of 1-(4-acetyl-piperazinyl)-5-methoxyindane was carried out in analogy with the known procedure for the corresponding tetralin,[13] followed by removal of the methyl group using BBr_3 and addition of the oxadiazole side chain.[12] This intermediate, which is inactive in the GlyT1 binding assay, was then hydrolyzed to the free piperazine and elaborated by reductive amination to the final product, **3**.

An alternative synthetic route was used for compound **4**, as shown in Scheme 2.

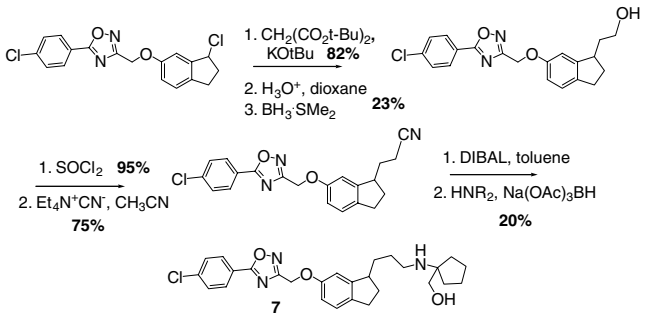
In the alternate synthesis, the oxadiazole side chain is added first, followed by conversion to the indanyl chloride. The addition of the piperazine side chain follows a literature precedent,[14] ending with double alkylation with 1-hydroxymethyl aminocyclopentane.



Scheme 1. Synthetic scheme for indanyl piperazine analogues exemplified by compound **3**.



Scheme 2. Synthetic scheme for indanyl piperazine analogues exemplified by compound **4**.



Scheme 3. Synthetic scheme for indanyl propylamine analogues exemplified by compound **7**.

While the indane ring system resolves the selectivity issue, affording potent GlyT1 binding activity, it does not address either the HERG channel affinity or the clearance in human microsomes. The next structural modification was to replace the piperazine with an acyclic propylamine chain. The synthesis of this series of analogues is shown in Scheme 3.

The key step is the condensation of di-*t*-butyl malonate with the indanyl chloride prepared in Scheme 2. Following hydrolysis and decarboxylation, conversion to the chloride and condensation with cyanide afforded the nitrile. Reduction to the aldehyde and reductive amination completed the synthesis of compound **7**. Compounds **8**–**10** were prepared in a similar manner starting from the corresponding tetralin.

Although this series introduces further structural novelty, it unfortunately does not resolve the HERG and clearance issues. Changing the oxadiazole for a pyridine, in compound **10**, does reduce 2D6 inhibition, but does not reduce HERG binding. A final attempt at structural diversity was the preparation of compound **11**, from 3-hydroxybenzaldehyde by straightforward methods. This approach also did not resolve the HERG or clearance issues.

Many of the compounds depicted herein are stereoisomeric, and all are indicated as mixtures of enantiomers and/or diastereomers. To examine the role of stereochemistry in the SAR of this series, compound **8** was separated by chiral HPLC into its four isomeric components (all data cited are for $N = 1$): isomer **8A**, $\alpha_D = +3.5°$, GlyT1 $IC_{50} = 38$ nM, Dofetilide $K_i = 381$ nM; isomer **8B**, $\alpha_D = +6.5°$, GlyT1 $IC_{50} = 24$ nM, Dofetilide $K_i = 214$ nM, isomer **8C**, $\alpha_D = -6.1°$, GlyT1 $IC_{50} = 31$ nM, Dofetilide $K_i = 219$ nM; isomer **8D**, $\alpha_D = -4.3°$, GlyT1 $IC_{50} = 26$ nM, Dofetilide $K_i = 206$ nM. Thus not only does the GlyT1 affinity differ little between isomers, but also the affinity for dofetilide displacement from the HERG channel is similar, and hence this structural feature did not offer any opportunity to separate GlyT1 affinity from HERG affinity.

Despite the structural diversity of these series, the combination of a basic head group with a long lipophilic tail, required for GlyT1 binding activity, predisposes these compounds to HERG binding and rapid degradation by human microsomes. These structural motifs may be a consideration for chemists when they triage hits from high-throughput screening.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.109.

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