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GlyT-1 Inhibitors: From Hits to Clinical Candidates

Roderick A. Porter and Lee A. Dawson

Abstract The treatment of schizophrenia has long been dominated by aminergic receptor antagonist-based therapeutics largely founded on the dopamine hypothesis of schizophrenia. More recently the glutamatergic theory has come to the fore which may potentially address some of the deficiencies of current therapies. While there are many approaches to manipulating the glutamatergic system, the most advanced approach is to increase synaptic concentrations of the NMDA receptor co-agonist glycine via inhibition of the glycine transporter 1 (GlyT-1). Here we will describe the background biological rationale for this approach and review the diverse classes of compounds which have been identified as GlyT-1 inhibitors with particular focus on the identification of those molecules which have entered the clinical stages of development. The role of target kinetics in drug action, a review of the rich vein of PET ligand development and their use in clinical development and the status of clinical-stage compounds will be addressed. Finally there is a discussion of some of the issues that have arisen with the discovery and development of GlyT-1 inhibitors and the prospects for the future of this mechanistic approach.

Keywords Bitopertin, Glutamate, Glycine transporter, GlyT-1, NMDA receptor, Schizophrenia

Contents

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Abbreviations

ADMET	Adsorption, distribution, metabolism, excretion, toxicity
ASST	Attentional set-shifting task in rats
CADSS	Clinician administered dissociative symptoms scale
CAR	Conditioned avoidance response
CBT	Cognitive-behavioural therapy
CSF	Cerebrospinal fluid
DAAO	D-Amino acid oxidase
DAT	Dopamine transporter
EEG	Electroencephalogram
EPSCs	Excitatory postsynaptic currents
ErbB4	Receptor tyrosine-protein kinase erbB-4
Gly	Glycine
GlyB	Strychnine-insensitive glycine-B subunit
GlyR	Glycine receptor
GlyT-1	Glycine transporter-1
GlyT-2	Glycine transporter-2
h	Hour(s)
hERG	Human ether-à-go-go-related gene
HTS	High-throughput screening
i.v.	Intravenous
kg	Kilogram
LeuT	Leucine transporter

LeuTAa	Leucine transporter from *Aquifex aeolicus*
LTP	Long-term potentiation
mPFC	Medial prefrontal cortex
MED	Minimum effective dose
MEST	Maximal electroshock test
NMDA	*N*-Methyl-D-aspartic acid
NR1	NMDA receptor subunit-1
NRG-1	Neuregulin receptor 1
ORD	Object retrieval–detour
p.o.	Per os
PANNS	Positive and negative syndrome scale
PCP	Phencyclidine
PDSS	Panic Disorder Severity Scale
PET	Positron emission tomography
PFC	Prefrontal cortex
P-gp	P-glycoprotein
PK	Pharmacokinetics
PPB	Plasma protein binding
s.c.	Subcutaneous
SAR	Structure activity relationship
SSRI	Selective serotonin reuptake inhibitor
SRR	Serine racemase
TCA	Tricyclic antidepressant
TM	Transmembrane helix
WT	Wild type

1 Introduction

The NMDA receptor (and more specifically a dysfunction of NMDA-mediated neurotransmission) has gained significant interest as the mediator of the dysfunction seen in schizophrenia. This hypothesis has been derived from a number of observations: firstly, both of the NMDA receptor blockers phencyclidine (PCP) and ketamine exacerbate psychotic symptoms in schizophrenic individuals, reinstate schizophrenic-like symptoms in remitting patients [1] and induce schizophrenic-like psychotic states and impairments in cognitive performance in healthy individuals [1–3] and secondly the ever-increasing genetic observations which are continuing to cluster around the NMDA receptor [4–6]. For example, neuregulin-1 (NRG-1)-mediated suppression of NMDA receptor function, possibly via enhanced activation of ErbB4 [7], has been demonstrated in postmortem prefrontal cortex from schizophrenic subjects. Moreover, mice with mutations in NRG-1 transmembrane regions produced selective imbalances in glutamatergic and dopaminergic neurotransmission [8]. G72 and G30 were identified in a segment from chromosome 13q34 that has been shown to be genetically linked to schizophrenia [9]. The G72 gene product was found

to increase the activity of D-amino acid oxidase (DAAO), the enzyme responsible for the breakdown of the NMDA co-agonists D-serine and D-alanine ([9]; for reviews see [10, 11]). Burnet and colleagues reported that DAAO activity and expression were upregulated in tissue from schizophrenic patients [12, 13]. Furthermore, there is evidence that serine racemase (SRR), which is responsible for the catalytic conversion of L-serine to D-serine [14], may also be a susceptibility gene [15, 16] and its expression may also be altered in the disorder [13, 17]. Thus, there is increasing evidence of a variety of potential subtle dysfunctions around the NMDA receptor synapse which may increase susceptibility to progression into schizophrenia and be an underlying substrate for the symptoms of the disease. Targeting the NMDA receptor synapse has, therefore, become an obvious focus for novel therapeutic approaches to treat schizophrenia. Direct activation of the NMDA receptor by an "orthosteric agonist" or elevation of the endogenous agonist, glutamate, can result in overexcitation, potentially leading to seizurogenesis and/or excitotoxicity and as such is not a viable approach. However, the NMDA receptor is unique in that it requires the presence of both an agonist (i.e. glutamate) and an obligatory co-agonist (i.e. glycine (Gly), D-serine and/or D-alanine) which bind at a distinct site, known as the strychnine-insensitive glycine-B (GlyB) site located on the NR1 subunit. It is only in the presence of both agonists that the ligand-gated ion channel opens. Therefore, one such approach for enhancing NMDA receptor function has been to increase occupancy and activity at this co-agonist/regulatory GlyB site.

There are now a number of clinical observations that at least partially provide credence to this approach. Javitt and colleagues have carried out a series of double-blind placebo-controlled studies of high-dose Gly given adjunctively to current antipsychotics [18–21]. The general outcomes were a significant reduction in negative symptoms and improvements in cognition and general psychopathology but with no effect on positive symptom domains. Similarly, a double-blind placebo-controlled trial of D-serine (30 mg/kg/day) in chronic schizophrenia patients who were poorly responsive to neuroleptics (for 6 weeks) produced significant reductions in negative and cognitive symptoms and, in this case, also positive symptoms [22]. Gly and D-serine are not suitable drugs for long-term therapy due to the requirement for very high doses (due to their poor pharmacokinetics (PK) and low brain penetration). In addition, D-serine has shown some evidence of toxicity [23]. However, taken together these studies do substantiate the hypothesis that co-agonism at the GlyB site may modulate NMDA receptor function and thus have therapeutic potential in schizophrenia. Of course the alternative strategy, and perhaps more viable approach, would be to elevate the various endogenous co-agonists within the synapse.

2 Glycine Transporters

Extracellular Gly levels are regulated by two specific sodium-dependent solute carrier family 6 (SLC6) transporters present either in the plasma membrane of the presynaptic nerve terminals or in astrocytes [24]. The two transporters, Gly transporter 1 (GlyT-1 [25]) and Gly transporter 2 (GlyT-2 [26]), share ~50% amino acid

sequence homology but can display quite different pharmacology and function [27]. There are currently five identified variants of GlyT-1 (GlyT-1a, GlyT-1b, GlyT-1c, GlyT-1d and GlyT-1e) and three of GlyT-2 (GlyT-2a, GlyT-2b and GlyT-2c). These originate from differing promoter controls and/or splice variants (for review see [28]). Unfortunately to date the relative CNS distribution of the various variants have not been systematically reported, with the possible exception of GlyT-1e which appears to be largely retinal [29]. Thus, the exact functional consequence of this diversity is not clear; however, functional consequences have been demonstrated, e.g. GlyT-2b does not appear to transport Gly [30].

In situ hybridisation studies have revealed that GlyT-1 expression appears to be localised in astrocytes and widely expressed across the brain, while GlyT-2 is predominantly expressed in glycinergic nerve terminals co-localised with Gly receptors (GlyR) [24]. This differential cellular localisation leads to differing functional roles. Glial GlyT-1 ensures the removal of Gly from the synaptic cleft leading to the termination of Gly-mediated neurotransmission; GlyT-1's presence in glutamatergic synapse and co-localisation with NMDA receptors [24, 31] suggests a critical role in regulating excitatory neurotransmission. GlyT-1 also mediates the clearance of Gly from the synaptic cleft of inhibitory synapses and concurrently participates in the regulation of Gly concentrations at excitatory synapses and thus plays a key role in balancing excitatory vs. inhibitory activity. In contrast, GlyT-2 ensures the refilling of presynaptic vesicles of glycinergic neurons [32, 33]. It is worth noting that data from knockout mouse models have shown that constitutive disruption of GlyT-1 or GlyT-2 is lethal [32, 33], presumably as a result of excessive or deficient glycinergic inhibition, respectively. Heterozygous GlyT-1 knockdowns are, however, viable and show ~50% reductions in tissue uptake of Gly, an increased NMDA receptor function and subsequent behavioural improvements in cognitive and "psychosis" models [34–36]. Furthermore, this delineated localisation and function may not be as clear-cut as originally thought [37, 38], and the dual excitatory vs. inhibitory role of GlyT-1 may lead to unwanted outcomes or limitations in tolerance to pharmacological intervention (see Sect. 8).

3 Molecular Structure of GlyT-1

The Na^+Cl^- coupled solute carrier SLC6 gene family includes monoamine (dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT)) and GABA transporters (GAT1-4) alongside GlyT-1 and GlyT-2. GlyT-1's stoichiometry is bidirectional transport of Gly which involves a two Na^+, one Cl^- symporter dependency. This stoichiometry is however somewhat different for GlyT-2 which is unidirectional in its transport of Gly [28]. An excellent review [39] covers early structural work on SLC6 neurotransmitter transporters; however, until recently there has been only limited crystallographic data for this family class.

This section will briefly review more recent crystallographic data for SLC6 family members and its relevance to GlyT-1, alongside some results of mutagenesis studies.

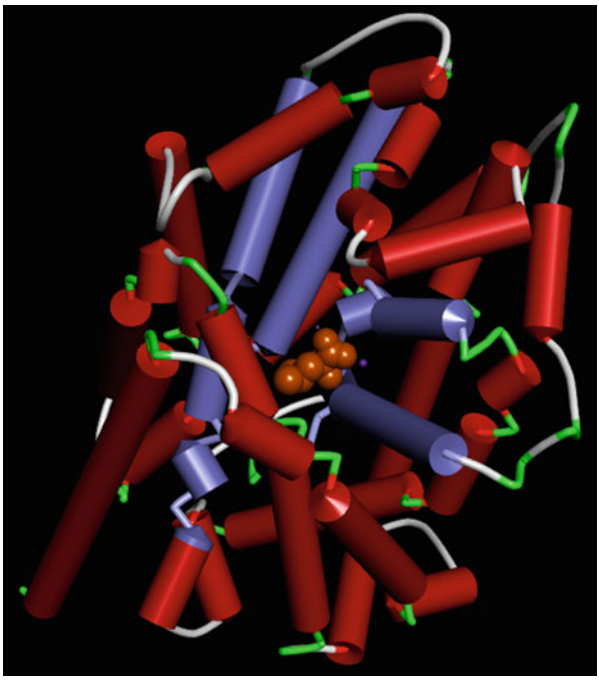


Fig. 1 A side on view of leucine (*orange*) bound in LeuTAa with helices 1, 3, 6 and 8 highlighted in *magenta* from pdb 2A65 [40]

3.1 Crystallography

Several structures of SLC6 family member proteins have now been reported:

- The bacterial Na^+/Cl^--dependent leucine transporter from *Aquifex aeolicus* (LeuTAa; Fig. 1 [40])
- A dopamine transporter from *Drosophila melanogaster* [41] with nortriptyline bound
- Multiple structures of LeuTAa with bound tryptophan (a competitive inhibitor [42]), selective serotonin reuptake inhibitors (SSRIs [43]) and tricyclic antidepressants (TCAs [44, 45])
- Structures of LeuTAa Δ13 mutants in which key residues have been changed to those corresponding to SERT, which recapitulates the pharmacology of SSRIs with several different SSRIs bound (e.g. fluoxetine (**1**) [46])

These crystal structures have confirmed a 12 transmembrane (TM) helical arrangement of this family with corresponding intracellular and extracellular loops and intracellular N- and C-termini (Fig. 1). TM1 and TM6 are partially unwound to give TM1a, TM1b and TM6a, TM6b and, in the LeuTAa, provide the majority of interactions with bound leucine along with TM3 and TM8 residues, including the highly conserved TM3 Tyr108 (=Tyr 128 GlyT-1, Tyr289 GlyT-2a). GlyT-2a Tyr289 has been implicated in substrate binding and ion coupling [47]. Analysis of residues involved in binding leucine to LeuTAa and equivalent

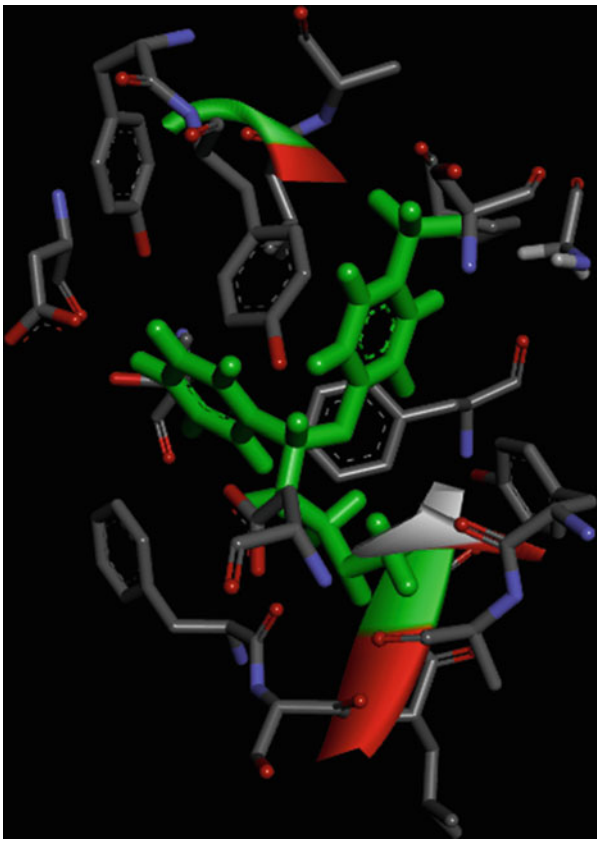


Fig. 2 Fluoxetine (**1**) (*green*) bound to LeuTAa Δ13 showing residues within 4A from pdb 4MM8 [46]

residues in GlyT-1 highlights the more bulky side chains of the latter. It has been postulated that the change from the less sterically demanding Gly305 (hGlyT-1b) to the corresponding bulkier Ser481 (GlyT-2) accounts for the selectivity of sarcosine (**2**) for GlyT-1 vs. GlyT-2 [48]. Furthermore, sarcosine (**2**) can be transported by a Ser481Gly mutant of GlyT-2 [49].

Nortriptyline is bound in an outward-open transporter conformation in the DAT, blocking isomerisation to an inward facing conformation and engaging with residues from TMs 1, 3, 6 and 8 [41]. Also present in this structure is a molecule of cholesterol which has been previously proposed [50] to regulate neurotransmitter transporters and specifically for DAT stabilisation of an outward-open state [51]. Both GlyT-1 [52] and GlyT-2 [53] have also been reported to associate with cholesterol-rich lipid rafts which affects transporter activity.

As fluoxetine **1** (Fig. 3) was used as an initial design motif for early sarcosine (**2**)-based GlyT-1 inhibitors (see Sect. 4.1), the LeuTAa mutant/fluoxetine (**1**) complex is of particular interest (Fig. 2) ([46]; pdb accession code 4MM8). However, the well-documented non-competitive nature of these sarcosine analogues differs from the competitive nature of SSRI binding to SERT, a difference which

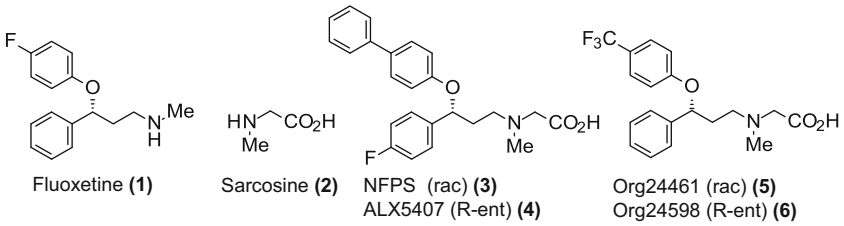


Fig. 3 NFPS/ALX5407, Org24598/Org24461 and their progenitor ligands

remains to be explained. It is noteworthy that in the structures discussed here Na^+ is bound, while NFPS (**3**) (Sect. 4.1) binding has been reported to be Na^+ independent. Transporter inhibitors, being substantially larger than the endogenous amino acid or bioamine cargoes, interact with additional residues, in particular those within TM10.

Consistent with these crystallographic findings, data have been generated demonstrating that TM domains 1 and 3 of GlyT-1 are determinants of NFPS (**3**) (Sect. 4.1) activity. When GlyT-1 TM3 or particularly TM1 were replaced with the equivalent helices from GlyT-2, the activity of NFPS (**3**) was substantially reduced. Conversely, a chimeric GlyT-2 receptor with TM1 and TM3 from GlyT-1 showed sensitivity to NFPS (**3**). While a GlyT-1/TM1/GlyT-2 chimera retained non-competitive kinetics for NFPS (**3**), the GlyT-1/TM3/GlyT-2 chimera had mixed kinetics [54]. TM-1 has also been implicated in the binding of Gly and sarcosine (**2**) [55].

GlyT-1 exists in N-terminal (pre-helix 1) splice variants that are reported to effectively show no variation in ligand pharmacology [48]. This is readily understood as no residue outside the 12TM bundle is invoked in ligand (or ion) binding from the reported structural studies. However, these splice variants may still influence biological function due to differences in cell tracking or protein–protein associations.

3.2 Structure-Based Design

While homology models have been built based on crystallographic data, for use in virtual screening [56], there have been few reports on homology models of GlyT-1 [48]. Furthermore, the detail of conformational changes involved in function of SLC6 class neurotransmitter transporters is not immediately clear from the crystallographic studies reported thus far, with structures tending to only show inhibitors or substrates bound in a common "open-out" conformation. Accelerated molecular dynamics simulations of the LeuT +/− leucine and Na^+ [57] suggest that there are seven unique conformations, only two of which have been seen in crystallography studies. A further challenge is the uncertainty over the binding mode needed to give preferred target kinetics for optimal efficacy and safety (Sects. 5 and 8).

4 Reported Inhibitors: Structural Classes and Pharmacology

Many companies have pursued GlyT-1 inhibitors resulting in a diverse range of structures and mechanisms of action. These have been identified from high-throughput screening (HTS) activities and structural analogy with other SLC transport inhibitors (e.g. SERT and DAT inhibitors) and more recently via pharmacophore-based rational design. This section will discuss the development of the various classes of GlyT-1 inhibitor and their biological profiles, focussing on those classes that have ultimately resulted in molecules which have progressed into the clinic. Broadly the discussion will be categorised based on ionisation state of the molecules, namely, zwitterionic sarcosine-derived inhibitors, basic compounds and non-physiologically ionisable compounds; although the reader will note that significant overlaps between these categories are emerging. For more detail several reviews on GlyT-1 inhibitors have been published [58–68].

4.1 Sarcosine and Sarcosine Analogues

4.1.1 Aryloxyphenylpropyl Substituted Glycines

Sarcosine (**2**) is a very weak competitive substrate/inhibitor of GlyT-1 (IC_{50} 91 μM [69]) but with selectivity over GlyT-2. Despite this, the compound is undergoing clinical evaluation (Table 10). By analogy with fluoxetine (**1**), hydrophobic sarcosine *N*-substituents gave high affinity non-substrate inhibitors (Fig. 3). The earliest examples of this approach are NFPS (racemate) (**3**) or ALX5407 (single enantiomer) (**4**) [70] and Org24461 (racemate) (**5**) and Org24598 (single enantiomer) (**6**) [71] (Fig. 3). ALX5407 (**4**) showed low nM activity ($IC_{50} = 3$ nM) and excellent selectivity over GlyT-2 ($IC_{50} > 75$ μM), other glycine binding proteins and other transporters including SERT.

[^{3}H]NFPS has been shown to have saturable rapid binding to rat forebrain membranes Kd 7.1 nM, Bmax 3.14 pmol/mg protein and a dissociation $t_{1/2}$ of 28 min [72].

Functional studies revealed that NFPS (**3**) was a non-competitive inhibitor of [^{3}H]Gly uptake and did not interact with Na^+ and Cl^- binding sites of GlyT-1 [72] but inhibited uptake of [^{3}H]Gly in hippocampal synaptosomes (IC_{50} of ~20 nM [73]). In vivo NFPS (**3**) increased cerebrospinal fluid (CSF) levels of Gly and elevated, transiently, extracellular levels of Gly in the prefrontal cortex (PFC) of rats at 10 mg/kg p.o. [73]; interestingly a concurrent and much more sustained increase was seen in the cerebellum of rats [74]. Functionally NFPS (**3**) potentiated NMDA receptor-mediated responses and long-term potentiation (LTP, a marker of glutamate-induced synaptic plasticity) both in vitro [75–77] and in vivo [76, 78]. These neurophysiological changes resulted in behavioural reversal of

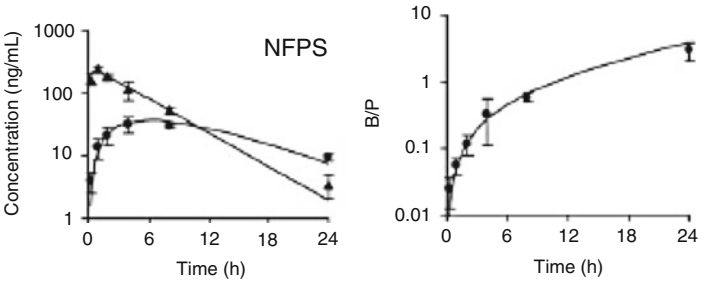


Fig. 4 Brain and plasma concentration-time profiles and brain/plasma (B/P) concentration profile for NFPS (**3**). *Triangle* and *circle symbols* are observed plasma and brain concentrations (mean +/− S.D. $n = 3$–4), respectively. Liu et al. [89] published with permission

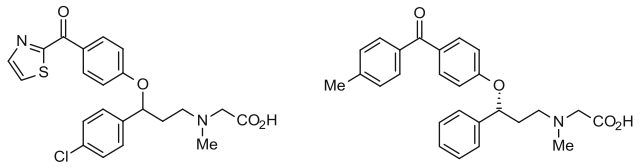


Fig. 5 Pfizer sarcosine analogues

NMDA receptor antagonist-induced impairments in models of cognition (such as novel object recognition [79, 80] and social working memory [81]), significantly reversed PCP-induced alterations in striatal dopamine efflux [82–84] and inhibited PCP and D-amphetamine-induced hypermotility in mice [73]. Taken together these data support the proof of mechanism of NFPS (**3**) (i.e. elevations in synaptic Gly, enhancement of NMDA function) and supports the hypothesis that GlyT-1 inhibition may have therapeutic utility across a number of schizophrenic symptom domains. Unfortunately, NFPS (**3**) was generally poorly tolerated in vivo, potentially due to its irreversible GlyT-1 inhibition [74, 85]. Other sarcosine-based structures such as Org24461 (**5**), CP-802,079 (**7**) (Fig. 5) and LY2365109 (**9**) (Fig. 6) produced largely similar profiles in these types of model [73, 86, 87]. Org24461 (**5**) has also been shown to prevent chronic D2 receptor antagonist-mediated increases in striatal dopamine when co-administered with risperidone, possibly indicating that adjunctive GlyT-1 inhibition may also attenuate classic antipsychotic-induced motor side effects [88].

Structure activity relationship (SAR) studies have been published for Org24598 (**6**) [71]. Key observations from these studies are (1) extending the *N*-methyl substituent (to, e.g. ethyl) results in a tenfold reduction in activity, (2) the corresponding 3-propionic acid was essentially inactive, (3) the phenoxy ether can be replaced with a methylene with only a threefold loss in activity and (4) *ortho*-ring substitution was poorly tolerated with an electron-withdrawing

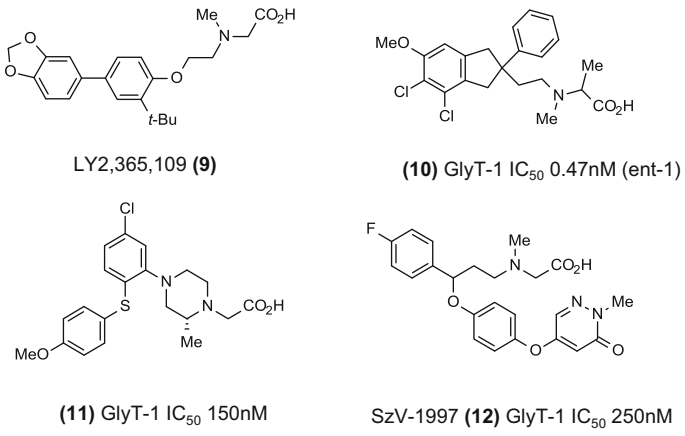


Fig. 6 Miscellaneous sarcosine analogues

para-substituent preferred for target activity in the phenoxy ring. Finally the R(−) enantiomer was most active with a eudismic ratio of approximately tenfold and resulted in Org24598 (**6**) which had a pIC_{50} 6.9 in a Gly uptake assay (GlyT-1 expressing CHO cell line) and excellent selectivity over GlyT-2 ($pIC_{50} < 4.0$).

In a detailed study of "time to reach brain equilibrium" in the rat [89], it was shown that NFPS (**3**) took ~6 h to reach brain C_{max}, while the brain plasma ratio was still rising at 24 h post dose (Fig. 4). Noteworthy is that brain levels at 24 h are comparable to brain concentrations at around the 1 h time-point.

In a binding assay, with cloned human GlyT-1c expressed in HEK293 cells, [^{3}H]-(*R*)-NPTS (**8**) (Fig. 5) had a Kd 1 nM [90] making it a useful tool to be used to support HTS studies. A close analogue of (*R*)-NPTS (**8**), CP-802,079 (**7**) ($IC_{50} = 16$ nM in a rat synaptosomal uptake assay [86]), gave a concentration-dependent increase in extracellular Gly levels when administered to rats via reverse microdialysis (0.1 or 1 μM). At the concentration estimated as IC_{50} (based on an assumed 10–20% efficiency at crossing the microdialysis membrane), a 59% increase in Gly was observed. Effects on LTP in the CA1 region of rat hippocampal slices were determined using 25 nM CP-802,079 (**7**) and 750 μM sarcosine (**2**). Increases in amplitude of response, following induction of LTP, were 195 and 213% above baseline, respectively.

4.1.2 Other Research Stage Sarcosine Analogues

More structurally diverse sarcosine-based GlyT-1 inhibitors have also been described (Fig. 6). Examples include the work from Lilly reporting 1,2,4-trisubstituted aromatics leading to LY2365109 (**9**) [74, 91, 92]. NFPS (**3**) (0.1–10 mg/kg p.o.) and LY2365109 (0.3–30 mg/kg p.o.) gave comparable increases in CSF Gly.

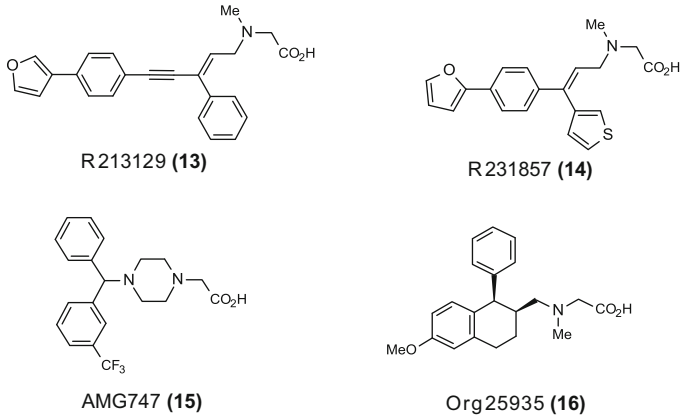


Fig. 7 Clinical-stage sarcosine analogues

Indandiones, e.g. (**10**) reported by Merck [93], give excellent target activity. This template evolved from in-house screening and derivatisation of published inhibitors, although no specifics were disclosed – unusually it was noted that an α-substituent was well tolerated.

The Lundbeck team have reported arylthio-substituted analogues such as (**11**) (Fig. 6 [94]). Compounds showed good permeability, although in some cases P-glycoprotein (P-gp) substrate activity was evident [95]. Pharmacokinetics of (**11**) in the rat were also encouraging with 100% oral bioavailability (dosed as a solution) and low clearance (0.4 L/h/kg) and a terminal $t_{½}$ of 4 h. In freely moving mice a dose-dependent increase in Gly was seen with an increase to 140% of baseline levels 60 min post 4.6 mg/kg s.c.

While few polar-substituted sarcosine analogues have been reported, one exception was the arylpyridazinone SzV-1997 (**12**) [48]. SzV-1997 (**12**) showed modest potency in a rat cortical synaptosomal [^{3}H]Gly uptake assay (IC_{50} 250 nM cf. NFPS (**3**) IC_{50} 12 nM), but despite this a 10 mg/kg i.p. dose produced a robust increase in striatal Gly at 2 h post dose, while an equivalent dose of NFPS (**3**) took 4 h to achieve a significant increase. In contrast, in a mouse hyperlocomotion model, NFPS (**3**) gave profound locomotor and behavioural changes at 10 mg/kg i.p. [74], while the same dose of SzV-1997 (**12**) was without effect. The authors suggested that the substantially increased polarity of SzV-1997 (**12**) (cLog*P* 2.55 unionised species, cLog$D_{7.4}$ − 0.18), relative to NFPS (**3**) (cLog*P* 4.95 unionised species, cLog$D_{7.4}$ 2.21), may be contributing to a change in target kinetics and a consequent change in side-effect profile (see Sect. 5); however, neither exposure nor kinetic data were presented.

4.1.3 Sarcosine Analogues and the Clinic

Several sarcosine-derived compounds have progressed to the clinic with little preclinical data published (Fig. 7). Early clinical data has been reported for the

Allelix/Johnson and Johnson compound R213129 (JNJ-17305600) (**13**) [96, 97] and R231857 (**14**) [98].

Benzhydryl piperazines from Amgen (e.g. AMG747 (**15**)) have shown excellent target activity [99] and this compound has been reported to be in PhII clinical studies. AMG747 (**15**) has approximate IC_{50}s of 75, 79 and 205 nM against human, rat and dog GlyT-1 with >100-fold selectivity over GlyT-2 and reportedly good PK. Rat CSF Gly is elevated with an MED of 0.3 mg/kg and AMG747 (**15**) is active in a model of object recognition in naïve rats (MED 0.3 mg/kg p.o.) and in a subchronic PCP-induced model of cognitive impairment also in rats (MED 0.1 mg/kg, p.o.) [204].

Org25935 (**16**) (renamed as SCH900435 a conformationally constrained analogue of Org24598 (**6**)) is thought to be the sarcosine analogue that has been advanced furthest into the clinic (see Sect. 7). Org25935 (**16**) is known to be a selective inhibitor of GlyT-1 vs. GlyT-2 and monoamine transporters. Scientists at Organon Laboratories Ltd. have demonstrated high radioligand binding of Org25935 (**16**) in several brain regions [101]. Functionally Org25935 (**16**) has been demonstrated to specifically increase Gly levels in rat frontal cortex (for up to 4 h [101]) and in the nucleus accumbens and striatum [102] using in vivo microdialysis in the rat. No concurrent change in extracellular levels of taurine or β-alanine (both GlyR agonists) was observed [100], suggestive of at least some selective action on the Gly uptake mechanism. Furthermore, an increase in Gly can also be demonstrated in the CSF of both rats and primates [101]. Org25935 (**16**) has also been shown electrophysiologically to enhance NMDA-induced currents in pyramidal cells of the rat mPFC neurons [103]. Further neurochemical evaluation demonstrated that, like previous sarcosine-like molecules, Org25935 (**16**) produced increases in nucleus accumbens dopamine levels while attenuating PCP- [104] and ethanol-induced increases in subcortical dopamine [100, 105]. In a conditioned avoidance response (CAR) paradigm in rats, Org25935 (**16**) (1, 3 and 6 mg/kg s.c.) produced a very small but statistically significant suppression of CAR at 6 mg/kg. Org25935 (**16**) (6 mg/kg) adjunctively produced a small augmentation of haloperidol but not olanzapine- or risperidone-induced suppression of CAR. Finally, Org25935 (**16**) (0.1, 0.3 and 1 mg/kg, p.o.) produced a significant attenuation of the scopolamine-induced impairment in a primate object retrieval task, but a higher dose (3 mg/kg) was ineffectual [106]. The predicted GlyT-1 occupancies of Org25935 (**16**) at effective doses ranged from 16 to 80% [106]. These data suggest that GlyT-1 inhibitors have the potential to improve performance in PFC-dependent tests, but that efficacy is lost when higher occupancies are achieved. This "bell-shaped" dose phenomenon was also seen with bitopertin (**58**) (Sect. 4.3.3), the Roche clinical GlyT-1 molecule, in this task and seems to have been borne out by the clinical findings with this molecule (Sect. 7).

4.2 Basic GlyT-1 Inhibitors

Several series of basic GlyT-1 inhibitors have been reported but here particular focus will be on work resulting in clinical candidates.

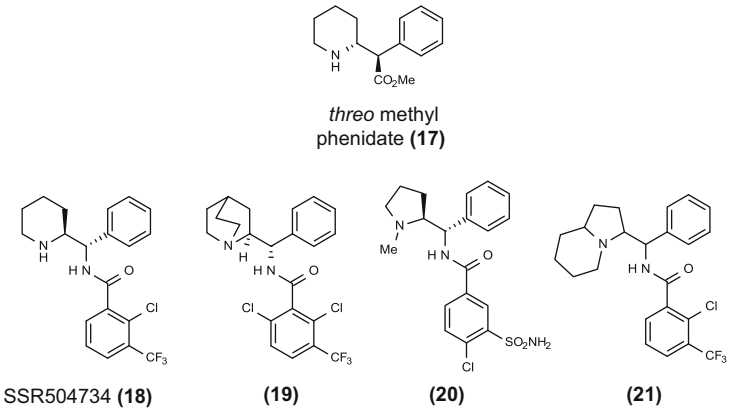


Fig. 8 Examples of Sanofi aminophenethylbenzamides

4.2.1 Aminophenethylbenzamides SSR504734, SSR103800 and GSK1018921

This class of compound has generated intense interest from several companies and resulted in at least three molecules being progressed to clinical evaluation. The initial template can be seen to emerge from the DAT inhibitor methylphenidate (**17**) from which Sanofi researchers identified SSR504734 (**18**) [107] (Fig. 8) and subsequently SSR103800. While the structure of SSR103800 has not been disclosed, a recent patent from Sanofi ([108] also see [109]) discloses novel polymorphic forms of (*S*,*S*)azabicyclooctane (**19**) (Fig. 8).

SSR504734 (**18**) is a selective (vs. 120 different receptors, ion channels, enzymes or transporters including GlyT-2) and reversible inhibitor at human, rat and mouse GlyT-1 (IC_{50}'s of 18, 15 and 38 nM, respectively). SSR504734 (**18**) blocked Gly uptake ex vivo and produced an increase in extracellular Gly and dopamine levels in the rat PFC (MED 3 mg/kg i.p.). SSR504734 (**18**) potentiated NMDA-mediated excitatory postsynaptic currents (EPSCs) in rat hippocampal slices and increase electroencephalography (EEG) spectral power in mice and rats. SSR504734 (**18**) prevented ketamine-induced metabolic activation in mice and reversed MK-801-induced hyperactivity in rats and mice. Additionally, it normalised the endogenous deficit in pre-pulse inhibition seen in DBA/2 mice (MED, 15 mg/kg i.p.) and reversed *d*-amphetamine-induced hyperlocomotion [110]. This compound also showed additional activity in chronic mild stress in mice and rat pup maternal separation models of anxiety [110]. In the attentional set-shifting task in rats (ASST), a model of cognitive flexibility/executive function, SSR-504734 (**18**)-treated animals required significantly less trials to criteria during the extra-dimensional shift (EDs) phase of the ASST, an effect completely prevented by the Gly/NMDAR site antagonist, L-687,414 [111], demonstrating that this molecule can enhance executive function performance via activation of

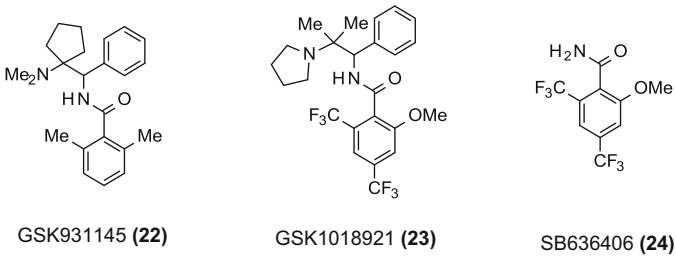


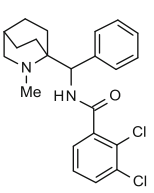
Fig. 9 Examples of GlaxoSmithKline *gem*-dialkylaminophenethylbenzamides

the Gly site of the NMDA receptor. Similar enhancements of activity have been seen in other models of cognitive performance [112–114].

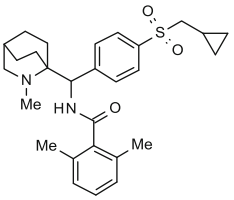
SSR103800 behaved in a similar manner to previous Sanofi molecules with human, rat and mouse IC_{50} values of 1.9, 5.3 and 6.8 nM, respectively and showing reversible inhibition of Gly uptake in mouse cortical homogenates. Using in vivo microdialysis SSR130800 increased extracellular levels of Gly in the rat PFC and potentiated NMDA-mediated EPSCs in rat hippocampal slices. SSR103800 (30 mg/kg, p.o.) attenuated MK-801- and PCP-induced hyperlocomotor behaviour and neonatal PCP-induced deficits in social recognition in rats. SSR103800 (30 mg/kg, i.p.) also normalised the pre-pulse inhibition of the startle reflex seen in DBA/1 J mice. In addition, in putative models of anxiety SSR103800 decreased defensive- and despair-related behaviours' in the gerbil tonic immobility test (10 and 30 mg/kg, p.o.) and in the rat forced-swimming test (1 and 3 mg/kg, p.o.) suggestive of anxiolytic behaviour [115, 116]. Both SSR504734 (**18**) and SSR103800 have been reported to have progressed to the clinic for schizophrenia, although interestingly not anxiety.

Sanofi has disclosed other aminophenethylbenzamide templates (Fig. 8) such as pyrrolidines, e.g. (**20**) [117], and pyrrolizine; indolizine, e.g. (**21**); and quinolizines [118] with low nM activity. *Gem*-dialkylaminophenethylbenzamides have been disclosed by GlaxoSmithKline (GSK; Fig. 9), including GSK931145 (**22**) (see Sect. 6.1 [119]) and GSK1018921 (**23**) (see Sect. 7 [120]). Preclinical data on these compounds are sparse; however, detailed metabolism of GSK1018921 (**23**) in both human and preclinical species has been disclosed. Notable was the extensive metabolism in humans relative to preclinical species and in particular the high level of the primary amide metabolite SB636406 (**24**). Other metabolites generated included *N*,*N*-debutylated and *O*-demethylated analogues and various permutations of oxidation and glucuronidation [121].

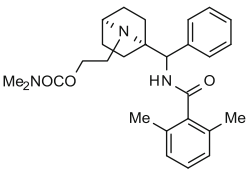
Numerous patent disclosures from other companies have emerged exploring the aminophenethylbenzamide motif. AstraZeneca (Fig. 10) has investigated further basic bicyclic motifs such as (**25**) [122] and notably successful approaches to introduce polarity either via an arylsulphone, e.g. (**26**) [123], or via an *N*-alkyl substitution such as urethane (**27**) [124]. Nonbasic cyclohexylsulphones will be discussed in Sect. 4.3.1.



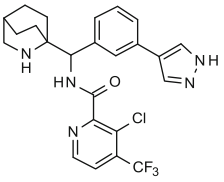
(25) AstraZeneca
GlyT-1 IC_{50} 1nM



(26) AstraZeneca
GlyT-1 IC_{50} 9nM

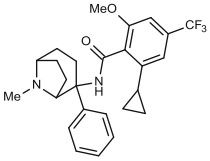


(27) AstraZeneca
GlyT-1 IC_{50} 1nM

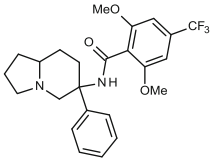


(28) Taisho
GlyT-1 IC_{50} 0.2nM

Fig. 10 Examples of AstraZeneca and Taisho aminophenethylbenzamides



(29) GlyT-1 IC_{50} 4nM



(30) GlyT-1 IC_{50} 13nM

Fig. 11 Examples of templates from Roche with a quaternary carbon adjacent to the benzamide

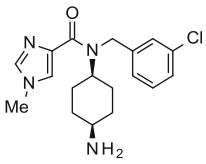
In a further approach to increase polarity, Taisho [125] have identified heterobiaryls (**28**) with excellent target activity (Fig. 10) by retaining the core 3-azabicyclo[2.2.2]octan-4-yl of (**25**) but now introducing a polar pyrazole heterocycle and nicotinamide.

While all these templates retained a secondary carbon adjacent to the benzamide, Roche have reported templates with a corresponding quaternary carbon centre (Fig. 11). Particularly potent were 4-substituted 8-azabicyclo[3.2.1]octanes such as (**29**) [126]. Structures based on a 6-substituted 2,3,5,7,8,8a-hexahydro-1H-indolizine core (**30**) also retained good target activity [127].

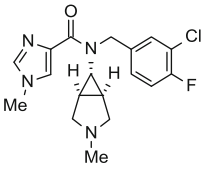
Little literature data has appeared describing the SAR of aminophenethylbenzamides; however, some general trends can be discerned from analysis of the data and claims disclosed in patents in this field, i.e.:

- A two-carbon amide/amine spacer is maintained in all the examples described.
- Preferred benzamide substitution, at least for target activity, appears to be 2,3-, 2,6- or 2,4,6- and is generally hydrophobic. Exceptions to this include the

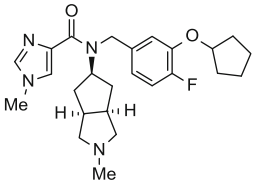
Table 1 Pfizer amines



(31)



PF3463275 **(32)**



(33)

Compound	GlyT-1 Ki (nM)	GlyT-2 Ki (nM)	HLM % $t_{1/2}$ (min)	MDCK AB-C[a]	CSF Gly ED_{200}[b]
(**31**)	2	1,150	>120	0.5	3.9
PF3463275 (**32**)	12	>10,000	80	13.2	3.5
(**33**)	4	>10,000	78	10.4	181% at 1 mg/kg

Data from [128, 129]

[a]MDCK AB-C apparent permeability through MDCK cell membrane units 10^{-6} cm/s

[b]Dose of drug (in mg/kg) that doubles the endogenous CSF Gly concentration (90 min post dose)

2-nicotinamides such as (**28**) (Fig. 10) and the unusual 3-sulphonamide of the 3,4-disubstituted benzamide (**20**) (Fig. 8). However, exemplification of this pattern is sparse, suggesting it is generally disfavoured.

- A secondary benzamide is present in almost all molecules disclosed suggesting a key role for this motif.
- Despite SSR504734 (**18**) being a secondary amine, the majority of compounds disclosed are tertiary amines and considerable effort has gone into identifying a range of tertiary amine templates.
- The phenyl ring is not obligate (see Sect. 4.2.3 for an example of an ethylenediamine benzamide) but is present in the great majority of published patents and is generally unsubstituted. However, examples of polar substituents have been reported, e.g. compounds (**26**) and (**28**) (Fig. 10).
- Substitution of the basic nitrogen has generally not been widely explored, with only one report identified (**27**) (Fig. 10) in which polar substitution was reported to give robust target activity.

4.2.2 4-Aminocyclohexanes/Piperidines and Isosteres, PF-03463275

An HTS at Pfizer identified the 4-substituted aminocyclohexane (**31**) (Table 1 [128]). Compound (**31**) had a very encouraging overall profile with significant in vivo activity, as determined by increases in CSF Gly following oral dosing, and no hERG (patch clamp) or cytochrome P4502D6 or 3A4 liabilities. However, permeability was poor; thus, effort was focussed on preparing metabolically stable tertiary amines and modulating aryl ring substitutions. This resulted in the tetrahydrocyclopropyl[c]pyrrole, PF-03463275 (**32**), which has progressed to the clinic, and the octahydro-cyclopenta[c]pyrrole (**33**) [129]. PF-03463275 showed

good target affinity and selectivity and good in vivo activity coupled with improved permeability. A kilogramme scale synthesis of PF-03463275 (**32**) has been reported [130].

PF-03463275 (**32**) has been shown [131], in the non-human primate, to alleviate the deficit in spatial working memory induced by ketamine at all doses tested (0.01–0.17 mg/kg s.c.). However, ketamine-induced hallucinatory effects were not reversed. PF-03463275 (**32**) is primarily metabolised by cytochrome P450 3A4 and 2D6 and may be effluxed by P-gp ([132]).

4.2.3 Other Basic GlyT-1 Inhibitors

In this section a miscellaneous selection of basic GlyT-1 inhibitors will be briefly surveyed demonstrating the diversity of structures with activity at the target.

Ethylenediamines

Roche have reported a range of cyclo(hetero)alkylethylenediamine benzamides such as (**34**) [133]. Noteworthy again is the *ortho*-substituted benzamide.

In an alternative approach to hit identification, a virtual screening approach was adopted by a group from AstraZeneca [134]. Compounds with pharmacophoric similarities to literature GlyT-1 inhibitors were identified using 2D topological fingerprints to encode pharmacophoric features. Compounds that met fingerprint criteria were clustered and sample members from each cluster filtered by CNS drug-like properties (e.g. molecular weight and lipophilicity). After this process 15,000 compounds were assayed leading, after optimisation, to (**35**) that demonstrated modest CNS exposure (brain 61 ng/g 1 h post 0.99 mg/kg s.c.) but a reasonable brain/plasma ratio of 2.1 (Fig. 12).

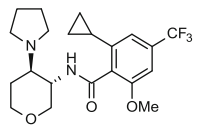
Ethylenediamine Piperazinesulphonamides

In a design strategy invoking a tethered benzamide and propyl sulphonamide held in appropriate proximity to reflect pharmacophoric similarities to other GlyT-1 inhibitors, a team at AMRI [135] identified metabolically labile spirosubstituted ethylenediamines such as (**36**) (Table 2). As previously observed *ortho*-substitution of the benzamide was found to be essential for good target activity. Introduction of a difluorocyclohexane and heterocyclic sulphonamides (see Sect. 4.3.1) leads to compounds such as (**37**) with excellent target activity and selectivity; (**37**) gave prolonged in vivo activity increasing CSF Gly levels. An example from this series (structure unknown) has been reported [136] to bind competitively with Gly to GlyT-1.

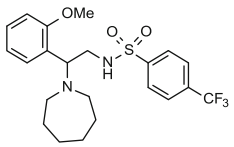
Spiropiperidines

Optimising a spiropiperidine HTS hit [137] that had multiple developability issues led to compounds such as RO4543338 (**38**) (Fig. 13). Issues that were addressed, with some success, during the optimisation included increasing target activity,

Fig. 12 Roche and AZ ethylenediamines

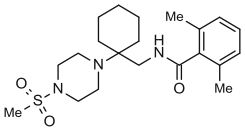


(34) GlyT-1 IC_{50} = 8nM

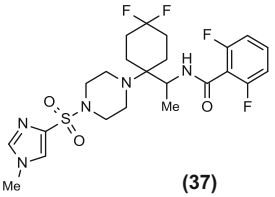


(35) GlyT-1 IC_{50} = 37nM

Table 2 AMRI ethylenediamine piperazinesulphonamides



(36)



(37)

	GlyT-1 Ki (nM)	GlyT-2 Ki (nM)	HLM % remaining 60 min	PTT (min)	Dose (mg/kg)	% increase CSF glycine
(**36**)	15	>75,000	29			
(**37**)	1	>75,000	–	360	3	227
					10	339

Data from [135]

selectivity over the μ-opioid receptor, metabolic stability and reducing hERG channel interaction [138–141]. While substantial progress was made in addressing multiple issues, it remained challenging to retain good target activity while maintaining acceptable adsorption, distribution, metabolism, excretion, toxicity (ADMET) and PK properties. Despite this RO4543338 (**38**) was shown to have activity in models of cocaine-seeking behaviours, albeit at relatively high doses (i.e. 30 or 45 mg/kg i.v. [142]).

Phenethylamines

Abbott/Abbvie has reported potent phenethylamines (Fig. 13) such as compounds (**39**) [143] and (**40**) [144]. The nonbasic carboxamide (**41**) was only marginally active indicating the key importance of the basic centre. Heteroarylsulphonamides are particularly preferred for target activity, e.g. *N*-methylimidazoles and *N*-methylpyrazoles, while scope for substituting the pendant phenyl ring appeared limited. Some compounds, e.g. (**40**), showed evidence of being effluxed from the brain.

Arylsulphonamides

Amongst the earliest non-sarcosine-based GlyT-1 inhibitors were 1,4-disubstituted piperidine arylsulphonamides reported by NPS/Allelix, e.g. (**42**) (Fig. 14 [145]) and can be seen as an early template of series of more recent interest.

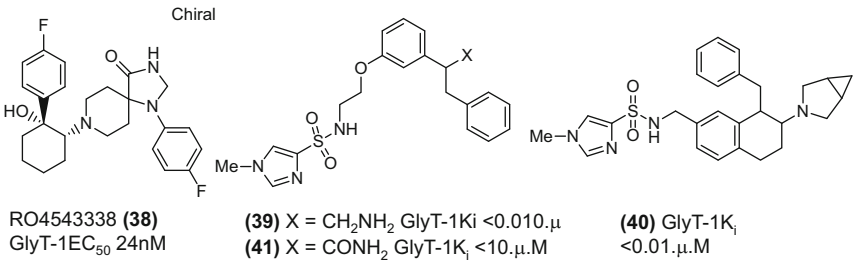


Fig. 13 Roche spiropiperidines and Abbvie phenethylamines

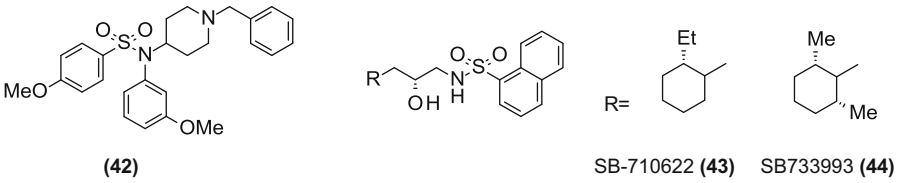


Fig. 14 Basic arylsulphonamides – NFPS/Allelix and GSK

Diaminopropan-2-ol sulphonamides were identified from an HTS campaign of which SB710622 (**43**) (GlyT-1 Ki 100 nM) was an optimised example [146] albeit still retaining multiple ADMET issues. Despite modest exposure (brain 564 ng/g 1 h post 5 mg/kg s.c. with a brain/plasma ratio of 0.4) SB710622 (**43**) demonstrated in vivo efficacy following s.c. dosing in the maximal electroshock threshold test (MEST [147]). An analogue, SB733993 (**44**), was reported to have a Kd of 2 nM in HEK293 membranes expressing human GlyT-1 [148].

4.3 Nonbasic GlyT-1 Inhibitors

A diverse range of nonbasic GlyT-1 inhibitors have been disclosed and have led to several clinical candidates, including the most advanced inhibitor bitopertin (**58**) which is currently in phase III (January 2014).

4.3.1 1,4-Disubstituted Piperidines/Cyclohexanes: DCCCyB (48)

In an extensive optimisation campaign by Merck, the HTS hit (**45**) led initially to the more potent competitive [149] inhibitor ACPPB (46) (Fig. 15). ACPPB (46) (1, 3 and 10 mg/kg i.v.) selectively increased Gly levels in rat PFC. ACPPB (46) (3–100 mg/kg s.c.) also significantly increased prepulse inhibition in DBA/2 mice without affecting basal startle amplitude [66, 150]. However, ACPPB

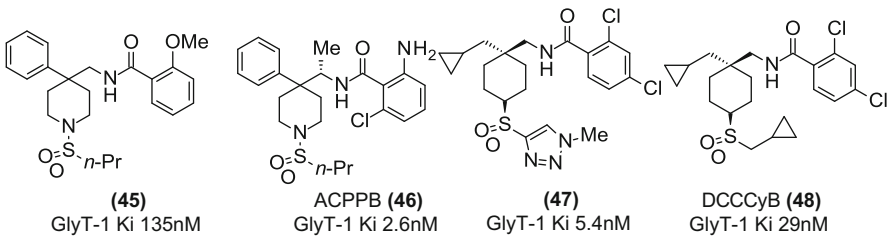


Fig. 15 GlyT-1 inhibitory data for selected (Merck) 1,4-disubstituted piperidines/cyclohexanes

Table 3 Pharmacokinetic data for (Merck) 1,4-disubstituted piperidines/cyclohexanes

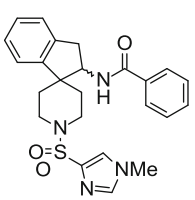
Compound	Species	CL[a]	Vdss (L/kg)	$T_{1/2}$ (h)	%Fpo	C_{max} (μM)
(**47**)	Rat	34	2.6	1.1		
DCCCyB (**48**)	Rat	36	4.2	2.4	65	0.14
	Rhesus	24	2.3	1.5	2	0.04
	Dog	4.9	3.1	10	48	1.39

Data from [153, 154]
[a]Clearance in mL/min/kg

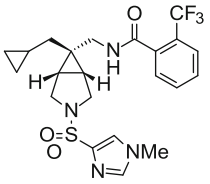
(46) suffered from time-dependent cytochrome P450 inhibitory activity and poor water solubility, limiting further progression. In a series of papers, the Merck team outlined issues addressed, and new ones identified, as they progressed the structural class to a clinical candidate. Issues solved included time-dependent inhibition of cytochrome P450s [151], P-gp substrate and subsequent poor CNS exposure [152] and poor oral bioavailability [153–155]. *N*-Dealkylation of *N*-alkylheterocycle sulphonamides resulted in potent P450 inhibitors and piperidine ring oxidation [154]. This work led to the identification of (**47**).

Exemplar (**47**) had a good rat PK profile (Table 3) resulting in good receptor occupancy (Occ_{50} of 3.4 mg/kg p.o. [154]). However, (**47**) was a P-gp substrate, giving a low brain/plasma ratio (0.16), and had potential to form *N*-demethylated NH triazole metabolites which were shown to be potent cytochrome P4502C9 inhibitors (IC_{50} 10 nM [153]). Returning to an alkylsulphonamide gave the clinical candidate DCCCyB (**48**). This ligand represented the best compromise of target activity and ADMET properties with good rat and dog PK. Removal of the P-gp liability resulted in a rat blood/plasma ratio of ~2.3. In vivo 3 mg/kg p.o. increased Gly levels in rat frontal cortex. In the rhesus monkey, using the PET ligand [^{18}F]MK-6577 (**76**) (Sect. 6.2), DCCCyB (**48**) gave an estimated plasma EC_{50} of 120 nM which was comparable to that seen in the rat (plasma EC_{50} of 350 nM at 3 mg/kg p.o.).

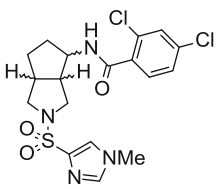
Further scaffold hopping led to novel nonbasic piperidine bioisosteres sulphonamides (Fig. 16), e.g. (**49**) [149], (**50**) [156] and (**51**) [157]. Examples showed excellent selectivity, significant free fraction, CNS exposure and activity in pre-pulse inhibition in mice. It is however unclear if examples of these *N*-methylheterocycle sulphonamides suffer from *N*-dealkylation to potent P450 inhibitors identified by the Merck team vide supra.



(49) GlyT-1 IC_{50} 15nM

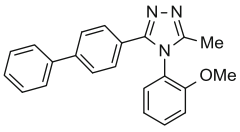


(50) GlyT-1 IC_{50} 5nM

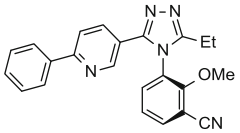


(51) GlyT-1 IC_{50} 25nM

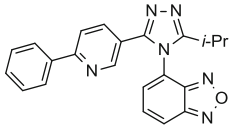
Fig. 16 Vanderbilt University GlyT-1 inhibitors



(52) GlyT-1 IC_{50} 1.8uM
sel. GlyT-2 0.9 fold



(53) GlyT-1 IC_{50} 0.064uM
sel. GlyT-2 >450 fold



ASP2535 **(54)** GlyT-1 IC_{50} 0.09uM
sel. GlyT-2 50 fold

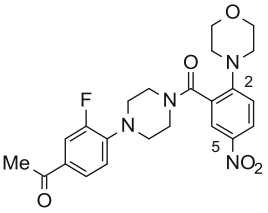
Fig. 17 Astellas triazoles

4.3.2 Biaryl Triazoles ASP2535 (54)

A series of biaryl triazoles (Fig. 17) have been identified by the Astellas group, optimised from their HTS hit (**52**) [158, 159]. (**52**) lacked selectivity over GlyT-2, but optimisation resulted in (**53**) identified as the more active (*R*)-atropisomer (the (*S*)-atropisomer was ~100-fold less active [160]). Target activity was much improved as was the selectivity over GlyT-2. Despite moderate oral bioavailability (32% in mouse and 23% in monkey) the molecule showed activity in vivo. The synthesis of the atropisomers has also been reported [161]. From the same series of compounds, ASP2535 (**54**) [162] progressed into phase 1.

ASP2535 (**54**) showed minimal affinity for 54 receptors, 7 ion channels and 4 transporters with the closest cross-reactivity being to the μ-opioid receptor (IC_{50} 1.83 μM). It inhibited ex vivo [^{3}H]-Gly uptake in mouse cortical homogenates with an MED of 10 mg/kg p.o. and attenuated an MK-801-induced working memory deficits in mice and visual learning deficits in a mouse neonatal PCP model (0.3–3 mg/kg, p.o. and 0.3–1 mg/kg, p.o., respectively). ASP2535 (**54**) also improved the PCP-induced deficit in pre-pulse inhibition in rats (1–3 mg/kg, p.o.) and scopolamine-induced deficits in working memory in mice and spatial learning deficits in aged rats (0.1–3 mg/kg, p.o. and 0.1 mg/kg, p.o. respectively [162]). Interestingly in all of these models, there was evidence of the bell-shaped dose response that appears to be common across GlyT-1 inhibitor compounds.

Table 4 Roche benzoylpiperazines and the invention of RG1678 (bitopertin) (**58**)



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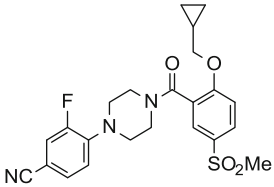

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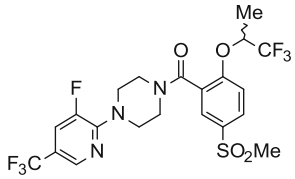


(57) = (R)
RG1678 = (S) **(58)**

Compound	EC_{50} GlyT-1 (μM)	hERG (μM)	ID_{50} (mg/kg)[a]	PK[b]	Cl[c]	Fpo (%)	$T_{1/2}$ (h)	B/P
(**55**)	0.015							
(**56**)	0.016	0.6	3.0	m	9.5	100	5.8	0.1
(**57**)	0.057	28	3.0					
RG1678 (**58**)	0.03	17	0.5	m				0.5
				r	4.3	78	5.8	0.7
				mk	3.6	56	6.4	–

Data from [164]
[a]Reversal of L-687,414-induced hyperlocomotion in mouse (p.o.)
[b]PK species
[c]Clearance in mL/min/kg
m mouse, *r* rat, *mk* monkey

4.3.3 Benzoylpiperazines: Bitopertin (RO4917838/RG1678) (58)

Benzoylpiperazines initially identified through HTS (Table 4) have received intense scrutiny with the most clinically advanced compound, bitopertin (**58**) (aka RO4917838 or RG1678), deriving from this work. The primary hit (**55**) was potent, selective (vs. GlyT-2 > 320-fold) but metabolically labile [163]; the 5-nitro group was seen as a potential liability and important to remove. Preliminary SAR studies showed that the 5-nitro substituent could be effectively replaced by primary or *N*-methylsubstituted sulphonamides and methylsulphones, which gave the best balance of target activity vs. polarity and metabolic stability. The 2-substituent morpholine could be replaced by a range of *N*-, *S*-, *O*- or *C*-linked substituents, but at least 4 heavy atoms were required to maintain target activity. Electron-donating arylpiperazine aromatic ring substituents were generally not tolerated, while electron-withdrawing 4-substituents proved to be beneficial for target activity. Exploration of this SAR resulted in the lead compound (**56**) which showed a good balance of target activity, excellent selectivity (over GlyT-2; $IC_{50} > 30$ μM) and physicochemical properties/solubility with negligible cytochrome P450 activity ($IC_{50} > 29$ μM), modest (88%) plasma protein binding (PPB) and an encouraging PK profile (Table 4). This profile gave in vivo proof of mechanism with increased striatal Gly levels following a 10 mg/kg p.o. dose. Unfortunately (**56**) had unacceptable hERG activity, attributed to direct interaction of the polar cyano substituent with the hERG channel, and only modest brain exposure.

An optimisation campaign to address these issues led to the identification of the clinical candidate RG1678, redesignated bitopertin (**58**) and its less active enantiomer (**57**) [164]. Bitopertin had no interactions with cytochrome P450s ($IC_{50} > 24$ μM) and was inactive in Ames and micronucleus genotoxicity assays.

Bitopertin (**58**) non-competitively inhibited Gly uptake at human recombinant GlyT-1 with an IC_{50} of 25 nM while having no affinity for GlyT-2 (up to 30 μM). In hippocampal CA1 pyramidal neurons, bitopertin (**58**) enhanced NMDA-dependent LTP at 100 nM but interestingly not at 300 nM. A dose-dependent increase in both CSF and striatal extracellular levels of Gly were observed in vivo with a plateau effect reached at 10 mg/kg p.o. (as measured using microdialysis in rats). Bitopertin (**58**) attenuated D-amphetamine or L-687,414-induced hyperlocomotion in both rats and mice [165]. Bitopertin (**58**) was also characterised in the object retrieval–detour (ORD) task in scopolamine-impaired rhesus monkeys and concurrently assessed using PET [106]. Low doses of bitopertin (**58**) (0.3 and 1.0 mg/kg, p.o.) significantly attenuated the scopolamine-induced impairments, while the highest dose tested (1.8 mg/kg) was ineffectual. The predicted GlyT-1 occupancies at these effective doses were ~10 and 30%, respectively. Interestingly these are the approximately equivalent to those occupancies [166] which resulted in clinical efficacy in the proof of concept study (see Sect. 7 [167]).

4.3.4 Isoindolines

Further exploration of benzoylpiperazines particularly focussed on the structural diversity tolerated by the left-hand side arylpiperazine. This led to the identification of a series of potent isoindolines (Table 5). The initial chloroisoindoline lead (**59**) [168] had good target activity and over 200-fold selectivity over GlyT-2, had moderate to low in vitro clearance in mouse and human liver microsomes (23 and 2 μL/min/mg, respectively) and, despite poor solubility (<1 μg/mL), was active in vivo. Introduction of a pyran, alongside manipulation of the 2-substituent, gave RO5013853 (**60**) (solubility 76 μg/mL) and RO5013852 (**61**) (solubility 191 μg/mL). Both RO5013853 (**60**) and RO5013852 (**61**) had good rat PK profiles and elevated extracellular Gly levels in rat striatum at 10 mg/kg p.o. Both RO5013853 (**60**) and RO5013852 (**61**) have been investigated as PET ligands (Sect. 6.3).

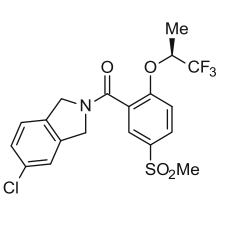
4.3.5 Miscellaneous Nonbasic GlyT-1 Inhibitors

Further diverse structures have been reported as GlyT-1 inhibitors, although none of the following classes have apparently progressed beyond research leads and tools.

Heterocycles

Several heterocyclic structures (Fig. 18) have been described. These include benzodiazepine-2-ones, e.g. (**62**) [169], and aminoimidazolinones, e.g. (**63**) [170], both described by Roche. Imidazolinones, e.g. (**64**) (GSK [171]), have also been

Table 5 Roche benzoylisoindolines



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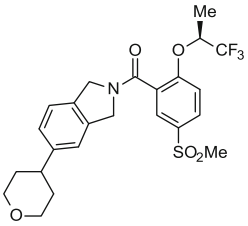
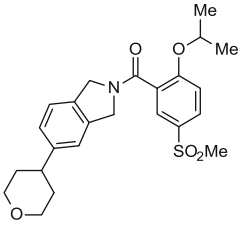


RO5013853 **(60)**



RO5013852 **(61)**

Compound	EC_{50} GlyT-1 (μM)	ID_{50} (mg/kg)[a]	Cl[b]	Vss (L/kg)	Fpo (%)	$T_{1/2}$ (h)	B/P
(**59**)	0.135	20					
RO5013853(**60**)	0.014	0.5	21	3.7	65	3.2	0.6
RO5013852(**61**)	0.028	0.5	22	2.0	40	1.6	0.6

Data from [168]
[a]Reversal of L-687,414-induced hyperlocomotion in mouse (p.o.)
[b]Clearance in mL/min/kg

extensively exemplified in the patent literature along with unusual difluorinated benzoxazinones disclosed by GSK, e.g. (**65**) [172].

Linear Amides

Linear amides (Fig. 19) have been generated from these heterocyclic systems by Roche, e.g. (**66**) from (**62**) [169], while GSK have described linear tertiary amides such as (**67**) from (**64**) [173].

From the limited data disclosed, particularly for structures such as (**66**) and (**67**), it is evident that there are a number of developability issues including solubility and high metabolic clearance. Following the amide theme cyclic tetrapeptides showing nM inhibitory activity and 100-fold selectivity over GlyT-2 (Fig. 19) e.g. (**68**) have been isolated from *Nonomuraea sp.* TA-0426 [174].

4.4 Marketed Drugs as GlyT-1 Inhibitors

Some marketed drugs have been reported to show marginal GlyT-1 inhibitory activity and debatably may achieve sufficient total brain concentrations to be pharmacologically relevant.

The "typical" antipsychotics chlorpromazine (**69**) and haloperidol (**70**) (Fig. 20) are reported to have weak GlyT-1 inhibitory activity with IC50s between 9 and 21 μM but were nonselective with respect to GlyT-2 [175]. Clozapine (**71**) an "atypical" antipsychotic (IC_{50} 100 μM [83]) has been suggested to be able to achieve up to a modest but arguably clinically relevant [166] 30% GlyT-1 occupancy (see Sect. 7) at clinical exposures.

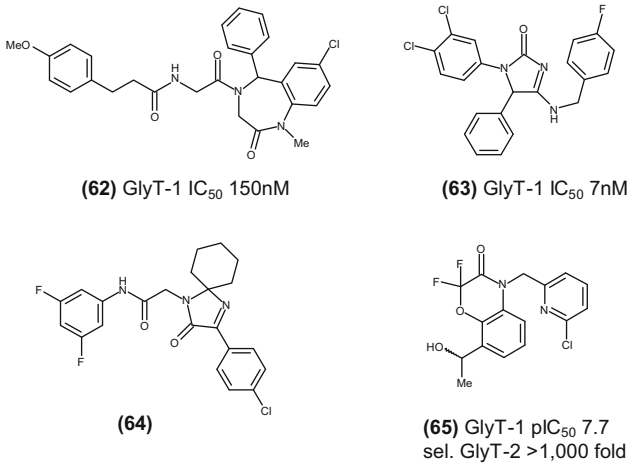


Fig. 18 Miscellaneous heterocycles

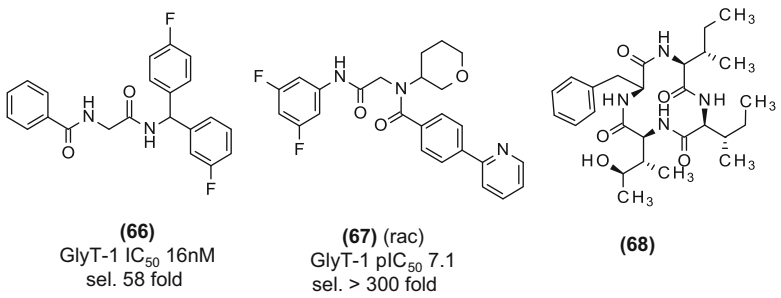


Fig. 19 Linear amides – Roche and GSK and cyclic tetrapeptides

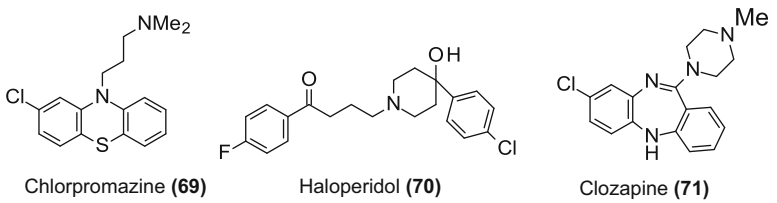
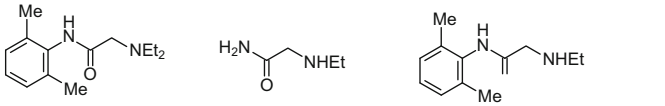


Fig. 20 Marketed antipsychotics as GlyT-1 inhibitors

From the anaesthetic lidocaine (**72**) (Fig. 21), the metabolite *N*-ethylglycine (**73**) was found to be a GlyT-1 substrate, while monoethylglycinexylidide (**74**), also a lidocaine metabolite, was shown to inhibit GlyT-1 activity both in primary astrocytes and in GlyT-1-expressing *Xenopus laevis* oocytes [176]. While these effects appear modest (i.e. μM), a cocktail of lidocaine (**72**) and its metabolites at clinically relevant concentrations gave comparable inhibition of Gly uptake in vitro to that achieved by NFPS (**3**) at 200 nM.



Lidocaine **(72)** N-Ethylglycine **(73)** Monoethylglycinexylidide **(74)**

Fig. 21 Anaesthetic metabolites as GlyT-1 inhibitors

Table 6 Calculated physicochemical properties of clinical candidates

Clinical compounds	Mol (Wt)	pIC_{50}	cLog*P*	cLog$D_{7.4}$	LLE[a]	PSA A^2
Sarcosine analogues						
Alx5407 (**4**)	393	8.5	4.95[b]	2.21	3.55	54
R231857 (**14**)	353		3.92[b]	1.20		58
R213129 (**13**)	371		4.70[b]	1.96		58
Org25935 (**16**)	339		3.80[b]	1.07		54
AMG747 (**15**)	378	7.2	3.90[b]	1.15	3.3	48
Basic inhibitors						
GSK1018921 (**23**)	488		5.55	4.26		43
SSR504734 (**18**)	397	7.8	4.86	2.97	2.94	46
PF3463275 (**32**)	363	7.9	1.87	0.76	6.03	43
Nonbasic inhibitors						
Bitopertin (**58**)	543	7.5	3.46	3.46	4.04	80
DCCCyB (**48**)	494	7.5	4.53	4.53	2.97	63
ASP2535 (**54**)	382	7.1	4.01	4.01	3.09	83

[a]*LLE* lipophilic ligand efficiency pIC_{50}−cLog*D*, all calculations using the ChemAxon calculator plug-in
[b]cLog*P* nonionic species

4.5 Physicochemical Properties of Clinical GlyT-1 Inhibitors

Despite wide structural diversity and substantial lead optimisation, clinical-stage GlyT-1 inhibitors are often showing somewhat unfavourable physicochemical properties (Table 6). This is perhaps reflected in the multiple developability challenges that have had to be overcome to identify these clinical-stage compounds. Reducing lipophilicity has often been hampered by the presence of lipophilic-substituted benzamides important for target affinity and to achieve effective CNS exposures/avoiding efflux transporter activity. Also it has been suggested that higher lipophilicity may be influencing kinetics at the target and in particular target off-rates (see Sect. 5); however, data is too limited to draw definitive conclusions at this stage.

Table 7 Compilation of structures with their target kinetics

Compound	Clinical analogue	Gly Comp.[a]	Na^+ dependent	Reference
Sarcosine (**2**)	Sarcosine (**2**)	C	Yes	Herdon et al. [148]
NFPS (**3**)	Org25935 (**16**)	NC	No	Herdon et al. [148]
Org25935 (**16**)	Alx5407 (**4**) R231857 (**14**) R213129 (**13**) AMG747 (**15**)			Mallorga et al. [72]
GSK931145 (**22**)	GSK1018921 (**23**)	C	No	Herdon et al. [148]
SSR504734 (**18**)	SSR504734 (**18**) SSR103800	C		Deportère et al. [110]
SB733993 (**44**)	–	C	Yes	Herdon et al. [148]
ACPPB (**46**)	DCCCyB (**48**)	C	Yes	Lindsley et al. [149]
(**55**)	Bitopertin (**58**)	NC		Alberati et al. [165]
RO5013853 (**60**)				
(**49**)	–	C		Lindsley et al. [149]
(**37**)	–	C		Mhyre et al. [136]
Clozapine (**71**)	Clozapine (**71**)	NC	No	Williams et al. [175]

[a]Gly Comp., competitive with glycine (C) or non-competitive (NC)

5 Target Kinetics

Kinetics of inhibition of GlyT-1 are complex with possible differences in competitiveness with endogenous ligand and dependencies on Na^+ and Cl^- concentrations coupled with differences in k_{off}. These differences for selected inhibitors are summarised with the relevant tool compound and the structurally closest clinical compound (Table 7). Less clear are the pharmacological consequences of these variations. The clinically most advanced GlyT-1 inhibitor bitopertin (**58**) is non-competitive with respect to Gly and competitive with respect to the sarcosine analogue [^{3}H]ORG24598 (**6**), However, other non-competitive and competitive compounds alike have all been terminated at the early clinical stages of development. Although reasons for the various terminations are largely unknown, it would appear that simple competitive vs. non-competitive pharmacology is not a differentiator with respect to clinical progression at least.

While the competitive vs. non-competitive nature of drug target interaction has not been directly implicated in issues with compounds, it has been suggested [177] that GlyT-1 inhibitors with a short residence time at the target are less likely to induce the unwanted hyperlocomotion effects which have been observed in preclinical species (Table 8). Interestingly from the limited available data, reducing lipophilicity tends to give faster K_{off}-rates which may be something which can be optimised in future pharmacophore-based virtual screening campaigns.

Table 8 Summary of effects of target kinetics on hyperlocomotion and pre-pulse inhibition

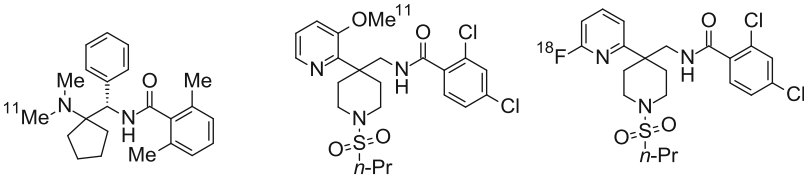
Compound	K_i nM	Functional $k_{off\ min}$	Comp/non-comp	OP[a]	cLog*P* (cLog*D*)[b]
Sarcosine (**2**)	39,000	<10	C	N	−0.8 (−2.8)
ALX-5407(**4**)	0.9	294	NC	Y	4.95 (2.2)
ACPPB (**46**)	3.8	103	C	Y	4.2
(**55**)	25	<10	NC	N	2.5 (2.5)
SB733933 (**44**)	2	5[c]	C	–	3.0 (2.9)
GSK931145 (**22**)	2	40[c]	C	–	5.3 (3.6)

Data from [148, 177]

[a]OP – hyperlocomotion – obstinate progression observed at doses efficacious in increasing prepulse inhibition

[b]cLog*P*/Log*D* calculated using ChemAxon calculator plug-in

[c]K_{off}



[11C]GSK931145 **(22)** [11C]CMPyPB **(75)** [18F]MK-6577 **(76)**

Fig. 22 Early GlyT-1 PET ligands – GSK and Merck

6 Positron Emission Tomography (PET) Ligands

One feature of the development of clinical GlyT-1 inhibitors has been the parallel development of several PET ligands. This has been of particular utility as, with all targets, there is the importance of demonstrating target engagement vs. exposure relationships, but of particular concern for GlyT-1 is the need to more fully understand the occupancy required for efficacy vs. potential adverse effects/tolerability.

Of particular advantage for GlyT-1 is the high Bmax in the cortex, with one estimate of 3,000 fmol/mg protein in rat cortical membranes [148] which reduces the necessity for very high affinity ligands.

Synthesis of the sarcosine-based [11C]NFPS (**3**) has been reported [178], but characterisation data is sparse, probably reflecting slow brain uptake with this class of inhibitor. However, several non-sarcosine-derived ligands have been reported.

6.1 [11C]GSK931145

[11C]GSK931145 (**22**) (Fig. 22) was selected based on brain penetration, heterogeneous distribution consistent with known GlyT-1 distribution and the level of specific binding as determined in the pig [179, 180]. Biomathematical modelling also identified [11C]GSK931145 (**22**) as a preferred ligand from a set of four

Table 9 Comparison of PET ligand tracer characteristics

Compound	hIC_{50} (nM)	Species[a]	K_1[b]	V_T thal	V_T pons	V_T cereb	BP thal	BP pons	BP cereb
GSK931145 (**22**)	3	p	0.07	4.8		3.46	1.71		0.97
		bab	0.13	4.55		4.34	2.83		2.53
		h	0.03	0.75		0.7	1.74		1.55
MK-6577 (**76**)	2	rh		4.9	6.79	6.53			
		h		5		6			
RO5013853 (**60**)[c]	1.7 (K_d)	bab		1.66	1.80	1.36	1.48	1.78	1.01
		h	0.04	~2.1	2.59	1.86	1.43	1.93	1.26

V_T observed distribution vol mL cm^{-3} BPND V_T/fp distribution normalised to free tracer concentration

[a]species: *p* pig, *bab* baboon, *h* human, *rh* rhesus

[b]K_1 tracer delivery mL cm^{-3} min^{-1}

[c]Data from two-tissue 5-parameter model

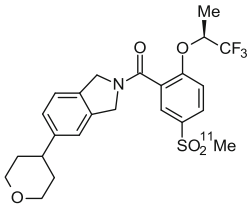
analogues [181]. [^{11}C]GSK931145 (**22**) has been dosed to baboons and to humans; the limiting organ with highest radiation absorbed dose was the liver in both species [182, 183].

In a study of 13 healthy volunteers, radioactivity was taken up rapidly into the cerebellum, brainstem and thalamus which was similar to that seen previously in the baboon [184]. Plasma EC_{50} for target occupancy by GSK1018921 (**23**) in the baboon and human were 22.5 and 45.7 ng/mL, respectively. One caveat to the use of [^{11}C]GSK931145 (**22**) is that it shows unusually large differences in plasma protein binding across species [184] with 8% fraction unbound in primate but only 0.8% fraction unbound in human which translates to a reduced tracer delivery K_1 and reduced V_T of the ligand in humans (Table 9). Furthermore, test/retest reproducibility was poor with [^{11}C]GSK931145 (**22**).

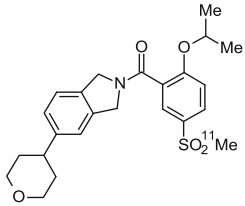
6.2 *Disubstituted Piperidine Sulphonamides [^{11}C]CMPyPB and [^{18}F]MK-6577*

Both [^{11}C]CMPyPB (**75**) and [^{18}F]MK-6577 ([^{18}F]CFpyPB) (**76**) [185] (Fig. 22) showed good uptake in rhesus monkey brain with, as expected, highest uptake in the pons, thalamus and cerebellum and lowest uptake in the striatum and grey matter of the cerebral cortex. However, [^{18}F]MK-6577 (**76**) showed higher brain uptake than [^{11}C]CMPyPB (**75**) (1.5 vs. 1.0 SUV in the pons) and a higher ratio of tracer uptake in the pons relative to the striatum (2.2 vs. 1.6). In both rhesus monkey and human brain slices, [^{18}F]MK-6577 (**76**) showed high binding to cerebellum, thalamus and white matter of the frontal cortex with low binding in the caudate and grey matter of the frontal cortex. Two more polar metabolites of [^{18}F]MK-6577 (**76**) were observed in the rhesus monkey with 68% of radioactivity due to parent 90 min post dose.

Fig. 23 Roche PET ligands



[^{11}C]RO5013853 **(60)**



[^{11}C]RO5013852 **(61)**

Detailed in vivo kinetics have been established [186] in the rhesus monkey for [^{18}F]MK-6577 (**76**). Human studies have been undertaken with [^{18}F]MK-6577 (**76**) ([^{18}F]CFpyPB [187]) revealing highest exposures in the lower intestine, ovaries and liver. Rapid brain penetration was achieved with the highest uptake in brainstem ($V_T = 7$) with cerebellum, thalamus and midbrain also showing good exposure. As expected low binding was seen in cortical grey matter ($V_T = 2$)

In a head-to-head comparison of [^{11}C]GSK931145 (**22**) and [^{18}F]MK-6577 (**76**) in anaesthetised baboons [188], [^{18}F]MK-6577 (**76**) gave more reliable measurement of binding parameters. It showed higher baboon plasma free fraction 12.8 vs. 5.6% for [^{11}C]GSK931145 (**22**), higher brain uptake SUV ~4 vs. ~2 and faster kinetics. Mean distribution volumes were at least twofold higher in relevant brain regions for [^{18}F]MK-6577 (**76**) vs. [^{11}C]GSK931145 (**22**).

6.3 Benzoylindolines [^{11}C]RO5013853 and [^{11}C]RO5013852

Roche have disclosed two [^{11}C] PET ligands, [^{11}C]RO5013853 (**60**) and [^{11}C] RO5013852 (**61**) (Fig. 23), which have been evaluated in baboons [189] and the latter in humans [190]. In the baboon, tracer uptake of both compounds was rapid and high in the thalamus, cerebellum and pons with the lowest level seen in the occipital cortex which was used as the reference brain region; kinetics appeared to be reversible. Pretreatment with unlabelled RO5013853 (**60**) or bitopertin (**58**) almost completely blocked binding of both ligands. In the baboon [^{11}C] RO5013853 (**60**) did show slightly higher retention and slower clearance and demonstrated higher contrast between regions with high and low levels of GlyT-1 than those seen with [^{11}C]RO5013852. [^{11}C]RO5013853 (**60**) was 30–35% metabolised in the baboon over the course of a 90 min experiment. [^{11}C] RO5013853 (**60**) was safe and well tolerated in humans [190, 191]. Using a two-tissue 5-parameter model, as expected, volumes of distribution were higher in the cerebellum, pons and thalamus (1.99–2.59 mL/mL) and lower in putamen, caudate and cortical areas (0.86–1.13 mL/mL) with test/retest showing good reproducibility with $< 10\%$ difference between scans. As in the baboon bitopertin (**58**) effectively blocked tracer binding.

Comparing [^{11}C]RO5013853 (**60**) with [^{11}C]GSK931145 (**22**) and [^{18}F]MK-6577 (**76**), the authors conclude that while brain uptake of [^{11}C]RO5013853 (**60**)

may be lower than the other ligands, this was compensated for by several other factors. These are as follows: (1) a lower dose of radiation was required for [^{11}C]RO5013853 (**60**) (0.013 rem/mCi) compared with (0.015 rem/mCi) and (0.095 rem/mCi) for [^{11}C]GSK931145 (**22**) and [^{18}F]MK-6577 (**76**), respectively; (2) V_T test/retest reliability was substantially greater for [^{11}C]RO5013853 (**60**) relative to [^{11}C]GSK931145 (**22**); and (3) synthesis of [^{11}C]RO5013853 (**60**) via methylation of the sulphinate was efficient [192] and high specific activity could be achieved (> 49GBq/umol) along with high chemical purity (100%).

Cortical GlyT-1 occupancy studies in the baboon, using [^{11}C]RO5013853 (**60**) [189] and bitopertin (**58**), gave 50% occupancy at plasma concentrations of 150–300 ng/mL. This level of occupancy is considered to be an efficacious level in a range of preclinical models [165, 193, 194] and subsequently in man [166].

6.4 PET Ligand Comparison

Some of the comparative characteristics of the various PET ligands have been summarised in Table 9.

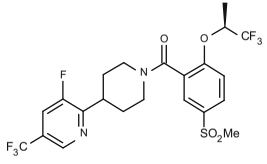
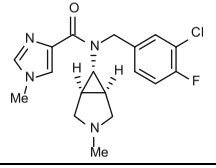
7 Clinical Evaluation of GlyT-1 Modulators

A number of synthetic GlyT-1 inhibitors have been assessed in schizophrenic patients (Table 10), but Roche's bitopertin (**58**) (also known as RG1678 or RO4917838) is the most advanced in development.

7.1 Bitopertin

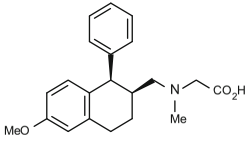
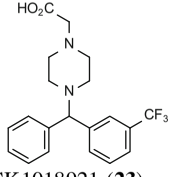
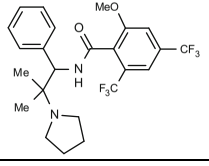
In a proof-of-mechanism study in healthy volunteers, once-daily oral doses of 3–60 mg bitopertin (**58**) for 10 days resulted in a dose-dependent increase in CSF Gly levels [195]. Steady state (10–12 days dosing) transporter occupancy was also evaluated using PET and [^{11}C]RO5013853 (**60**) in healthy male volunteers [166]. At steady state, occupancy amounted to 30, 40, 60, 74 and 85% after 5, 15, 30, 60 and 175 mg doses, respectively; Emax was 92%, with an ED50 of 15 mg which equated to a plasma EC50 of 190 ng/mL. Subsequently, a 320 patient phase II proof-of-concept study (NCT0616798) evaluated the efficacy of bitopertin (**58**) (10, 30 or 60 mg daily) in the treatment of negative symptoms in patients with schizophrenia whose symptoms were stabilised by an antipsychotic medication. The molecule was well tolerated at all doses. Improvements were seen in negative symptoms and patients' personal and social performance, reaching statistical significance on primary and secondary end points at the 10 mg dose group; interestingly, in comparison efficacy was reduced at the 30 mg dose and absent in the group receiving 60 mg [167]. It would therefore appear that the mechanism seems to have therapeutically relevant efficacy at transporter occupancy levels as low as 30–40%, with the caveat that this is extrapolated from the earlier study. As mentioned

Table 10 Compounds with reported clinical trial data

Compound	Study	Dosing regimen	Measures and outcome	Reference/sponsor
Bitopertin (**58**) (aka RO4917838 or RG1678) 	**Phase III** (ongoing) Evaluating the efficacy and safety of adjunctive bitopertin (58)	5, 10 or 20 mg daily, 52 weeks followed by an optional 3 year	Efficacy and safety of bitopertin (58) in antipsychotic – stabilised patients with persistent, predominant negative symptoms of schizophrenia (ongoing)	NCT01192867, NCT01192906, NCT01192880, NCT01235520, NCT01235559 Hoffmann-La Roche
	Evaluated the efficacy of adjunctive bitopertin (58)	10, 30 or 60 mg daily; 3 months	Efficacy and safety of bitopertin (58) in antipsychotic – stabilised patients with persistent, predominant negative symptoms of schizophrenia Efficacy consistently improved at 10 mg, to a lesser extent in the 30 mg and no efficacy in the 60 mg dose group	NCT00616798 Hoffmann-La Roche
	Phase II (ongoing) Effect of adjunctive bitopertin (58)	10 mg once daily; 6 weeks	Changes in biomarkers of cognitive dysfunction in schizophrenia patients stabilised on antipsychotic medication (study ongoing)	NCT01116830 Hoffmann-La Roche
Sarcosine (**2**) 	**Phase II** (ongoing) Evaluating the efficacy of adjunctive sarcosine (2)	2 g once daily; 6 months	Positive and negative symptom, quality of life and cognitive and sexual function measures in patients with schizophrenia stabilised on antipsychotic medication (ongoing)	NCT01503359 Medical University of Lodz
	Phase II (ongoing) Evaluating the efficacy and safety of adjunctive sarcosine (2)	2 g once daily; 12 weeks	Positive, negative and cognitive symptom measures in schizophrenia patients stabilised on antipsychotic medication (ongoing)	NCT01047592 Chang-Hua Hospital
PF03463275 (**32**) 	**Phase II** Effects of adjunctive PF03463275	30 mg twice daily; 12 weeks	Add-on therapy in outpatients with persistent negative symptoms of schizophrenia (study terminated September 2010; reasons unknown)	NCT00977522 Pfizer

(continued)

Table 10 (continued)

Compound	Study	Dosing regimen	Measures and outcome	Reference/sponsor
Org25935 (**16**) (aka SCH 900435) 	Phase II	4–16 mg twice daily; 12 weeks	Effects on negative symptoms in schizophrenia patients stabilised on second generation antipsychotics (completed 2008; no data reported)	NCT00725075
	Effects of adjunctive Org25935 (16)		Study was withdrawn prior to recruitment (reasons unknown)	NCT00988728 Merck Sharp & Dohme
AMG747 (**15**) 	Phase II Effects of adjunctive AMG747	3 doses (not specified) once daily; 12 weeks	Effects on negative symptoms in schizophrenia patients stabilised on antipsychotics (study terminated June 2013)	NCT01568229 NCT01568216 Amgen
GSK1018921 (**23**) 	Phase I	Not specified	Assess safety, tolerability, pharmacokinetics, pharmacodynamics in healthy volunteers and patients with schizophrenia and to evaluate its effect on PK of midazolam (study terminated June 2009)	NCT00929370 GlaxoSmithKline

previously these occupancies appear to be equivalent to those which have been shown to be efficacious in preclinical models.

A subsequent study has been carried out in patients experiencing acute exacerbations of schizophrenic symptoms (NCT01234779). Here 10 and 30 mg doses were dosed for 4 weeks (followed by a 4-week follow-up) and compared to the positive comparator, olanzapine (15 mg). Positive and negative PANNS scores were used as the primary end point. This study has completed but no data have been released to date (as of December 2013). However, several phase III studies have commenced: NCT01192867, NCT01192906 and NCT01192880 are phase III, 24-week, double-blind placebo-controlled studies to evaluate efficacy and safety of RO4917838 in stable patients with persistent, predominant negative symptoms of schizophrenia treated with antipsychotics, followed by a 28-week, double-blind treatment period; NCT01235520 and NCT01235559 are phase III, 12-week placebo-controlled studies to evaluate the efficacy and safety of bitopertin (**58**) in patients with suboptimally controlled (with antipsychotics) symptoms of schizophrenia followed by a 40-week double-blind, placebo-controlled treatment period. No dose information is currently available and none of these studies appear to have a positive comparator component. Early phase III studies are reporting out as this document is completing; unfortunately initial results have not proved encouraging [196]. All studies will complete towards the end of 2014/early 2015 with full data expected in 2015.

7.2 Sarcosine

Interestingly, based on its likely very poor pharmacokinetics/CNS penetration and the subsequent need for very high doses, the only other molecule currently undergoing clinical evaluation is sarcosine (**2**). Two separate studies are evaluating 2 g doses for 12 weeks (NCT01503359) and 6 months (NCT01047592) in antipsychotic-stabilised schizophrenic patients. Positive and negative symptom, quality of life and cognitive and sexual function measures are all being used as assessment end points.

7.3 Other GlyT-1 Inhibitors

A number of other small molecule GlyT-1 inhibitors have been assessed in the clinic for schizophrenia (Table 10) but have all generally failed to progress beyond phase I. On the whole the reasons for this have not been released, but one may speculate that this is due to adverse events/tolerability of the compounds.

7.3.1 Org25935 (SCH900435)

Early stage clinical evaluation of Org25935 (**16**) (0.5–30 mg) was examined for its observable central nervous system effects and pharmacokinetic profile in healthy

male volunteers [197]. Pharmacokinetics were dose-linear over the dose range studied and peak concentrations of Org25935 (**16**) (1,170 ng/mL at 30 mg) were reached between 30 and 50 min after dosing. EEG measures demonstrated small statistically significant reductions in alpha2 power spectra (10.5–12.5 Hz) suggestive of central penetration and pharmacodynamic activity. The compound was generally well tolerated, although some increases in dizziness were reported and again dose-related visual disturbances were clearly evident.

Org25935 (**16**) has been assessed for its effects on ketamine-induced schizophrenia-like psychotic symptoms and perceptual alterations in 12 healthy male subjects [198]. Ketamine produced behavioural, subjective and cognitive effects consistent with its NMDA receptor antagonist activity. Org 25935 (**16**) reduced the ketamine-induced increases in psychosis (PANSS positive and negative) and perceptual alterations (Clinician Administered Dissociative Symptoms Scale (CADSS)). None of the behavioural effects of ketamine were increased/augmented by Org25935 (**16**). These data fundamentally support the mechanism of action of GlyT-1 and demonstrate the interaction at the level of the NMDA receptor in human subjects.

In addition to its assessment in schizophrenia, Org25935 (**16**) was also assessed for its efficacy in panic disorder as an augmentation strategy to cognitive-behavioural therapy (CBT; NCT00725725 [199]). Patients ($n = 40$) diagnosed (DSM-IV) with panic disorder receive either a dose of Org 25935 (4 or 12 mg) or placebo 2 h prior to an assessment. The primary end point was symptomatic change as measured by the Panic Disorder Severity Scale (PDSS) vs. CBT alone. Although mean PDSS total scores did decrease significantly from baseline, no statistically significant benefit vs. placebo was observed for either dose of Org 25935 (**16**). Org 25935 (**16**) showed no major safety issues at either dose but was much better tolerated at the 4 mg cf. 12 mg.

7.3.2 R213129 and R231857

R213129 (**13**) and R231857 (**14**) have been assessed for their effects on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. Despite scopolamine producing the predicted and reproducible anticholinergic CNS impairments, R231857 lacked any consistent dose-related effects, probably due to a low CNS penetration and exposure at the doses tested [97, 98]. Both compounds are believed to have been discontinued [67].

7.3.3 GSK1018921

A single oral dose PhI study with GSK1018921 (**23**) (0.5–280 mg) showed a dose proportional pharmacokinetic profile and a dose-dependent increases in reported frequency of dizziness format doses from 70 to 280 mg. Despite this the authors predicted that receptor occupancy up to 80% would be well tolerated [200].

8 The Safety vs. Efficacy Dilemma

GlyT-1 knockout animals provided some of the early indications that this target may have some safety concerns. Knockout animals showed deficits in locomotor behaviour and respiratory function prior to postnatal death [201]. Heterozygote animals are however viable and show the characteristic profile [201] seen with most of the small molecule inhibitors describe here. Although the reported adverse events with higher doses of GlyT-1 inhibitors vary across research groups and compounds, they generally agree with the findings in the knockout animals, i.e. behavioural disturbances, locomotor deficits and ultimately respiratory issues [61, 74, 177].

Recent preclinical and clinical assessments of the efficacy vs. occupancy relationship has revealed that low levels of transporter occupancy (10–40%) are actually required to gain efficacy both in preclinical models ([106] and in the Roche clinical studies [166, 167]. Furthermore, high occupancy levels (>50%) appear to be detrimental to efficacy both clinically [166, 167] and across a wide range of preclinical models of function and behaviour [106, 162, 165].

Based on the NMDA receptor hypofunction hypothesis of schizophrenia, it is presumed that blockade of GlyT-1 transport in the forebrain, and perhaps more specifically prefrontal regions, results in enhanced NMDA function and hence efficacy. However GlyT-1 is expressed across the brain and particularly in the hindbrain regions, such as the brainstem and cerebellum. Here NMDA receptor function is more restricted and the glycinergic system prevails; thus, concurrent blockade of GlyT-1 here will increase glycinergic overflow onto inhibitory strychnine-sensitive GlyRs. This hypothesis is partially substantiated by the observations that the respiratory depression seen in the knockout animals could be attenuated by strychnine. Perry et al. [74] demonstrated concurrent elevations in Gly in the cerebellum of animals treated with NFPS (**3**) (Fig. 3) and with LY2365109 (**9**) (Fig. 6). These increases appeared to be of a greater magnitude than those seen in the PFC and correlated with the onset of the locomotor and respiratory side effects. Again locomotor effects of LY2365109 (**9**) were attenuated by strychnine [74]. Behavioural effects were observed including hunched back posture and a tendency to walk on their toes, such that animals were unable to perform on the rotarod and showed laboured, shallow breathing, lateral recumbency, tremors, chromodacryorrhoea, hypothermia and tachycardia [74]. Interestingly, Kalinichev et al. [147] reported anticonvulsant activity of a range of GlyT-1 inhibitors in MEST. It is somewhat counterintuitive that this effect is mediated by forebrain NMDA function and doses which produced robust efficacy were on the high side. Thus, these anticonvulsant effects may also be mediated via the glycinergic receptor system.

A further potential issue is the now multiple reports of visual disturbances with different GlyT-1 inhibitors in clinical trials [197, 200]. Similar effects have also been observed preclinically using PF-03463275 (1, 3 or 10 mg/kg s.c) which produced significant alterations in the electroretinogram of albino rats [202]. Gly is certainly one of the essential neurotransmitters which appear to modulate visual

signals in the retina [203]. It is not clear if GlyT-1 per se is the modulator of retinal Gly levels, but the dose ranges and time course of effects in the two clinical studies are definitely commensurate with an acute pharmacological effect [197, 200]. Thus, this may be another safety concern which needs consideration for this target, although to date no visual disturbances have been reported with bitopertin (**58**). Liem-Moolenaar et al. [197] did suggest early evidence of tolerance to these visual disturbances, so perhaps this may be the reason.

9 Issues and Challenges in Inventing Clinical GlyT-1 Inhibitors

From the lack of successful clinical outcomes with GlyT-1 inhibitors, it is clear that potential mechanistic liabilities are complicating the development of molecules targeting this approach. However, clearly the phase II clinical observations with the Roche compound have suggested that a therapeutic window is achievable and therapeutic benefit can be delivered, although within a relatively narrow dose range. Why this molecule has been able to achieve this when so many of the others have failed is not clear, perhaps the differences in chemotype have resulted in subtle differences in binding kinetics vs. other molecules? The community obviously hopes for positive phase III data with bitopertin (**58**) and a novel therapeutic for schizophrenic suffers; however, we should be cognisant that efficacy and long-term tolerability have not yet been proven. Early indications released during the writing of this chapter are unfortunately not encouraging.

The complexity of target kinetics has until recently hampered the successful use of rational or pharmacophore-based design strategies, although there are some examples (Sect. 4). The increasing availability of structural information for SLC6 transporters may suggest that alternative routes to defining new chemotypes are available, but caveats still remain with respect to the interpretation of these (Sect. 3.2).

The relatively high lipophilicity of many clinical agents and frequent reports of efflux liabilities reflects the difficulty in achieving a balance between effective CNS exposure and manageable physicochemical properties. It has been noted [177] that functional K_{off} of some of these inhibitors may be able to limit at least the motor function deficits seen with GlyT-1 inhibitors and as such may be a strategy to enhance the therapeutic window, a hypothesis yet to be proven. If it is confirmed that target off-rates are an important indicator of clinical efficacy and safety, then there will be a need to rationally design compounds with more rapid K_{off}-rates. Reducing lipophilicity may be a useful guide (Sect. 5), but again this awaits confirmation.

Finally, both preclinical and now clinical data are suggesting that the level of transporter occupancy required to achieve efficacy is relatively low. However, the apparent bell-shaped dose relationships in efficacy and potential adverse events suggest that the optimal pharmacokinetic/occupancy level is within a quite narrow

range. This puts further constraints on defining an acceptable pharmacokinetic profile with a predictable low peak-trough inhibitor ratio or that appropriate controlled release formulations may be required.

There is now an extensive amount of information available to help the medicinal chemist to design new GlyT-1 inhibitors. If close attention is paid to physicochemical properties, target kinetics and pharmacokinetic profile, there is still scope to potentially differentiate the next generation of GlyT-1 inhibitors from existing compounds. However, the concern must now be that with the extensive effort already dedicated to this target and the paucity of clinical success to date that, despite the major unmet medical need in schizophrenia, it may be difficult for the community to justify significant new research investment in this target.

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