Glycine Transporter Inhibitors as Therapeutic Agents for Schizophrenia

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Abstract: Multiple lines of evidence suggest that a dysfunction in the glutamatergic neurotransmission via the *N*-methyl-D-aspartate (NMDA) receptors contributes to the pathophysiology of psychiatric diseases including schizophrenia. The potentiation of NMDA receptor function may be a useful approach for the treatment of diseases associated with NMDA receptor hypofunction. One possible strategy is to increase synaptic levels of glycine by blocking the glycine transporter-1 (GlyT-1) in glia cells, since glycine acts as a co-agonist site on the NMDA receptor. In this article, the author reviews the recent important patents on GlyT-1 inhibitors for treatment of schizophrenia and other psychiatric diseases associated with the NMDA receptor hypofunction.

Keywords: NMDA receptor, glycine, glycine transporter, glia, cognition, schizophrenia.

INTRODUCTION

Glycine (Fig. **1**) was first proposed to act a neurotransmitter in the mammalian spinal cord in 1965 [1]. At present, glycine is a well-characterized amino acid neurotransmitter in the mammalian central nervous system (CNS) where it acts as an inhibitory transmitter *via* its interaction with strychnine-sensitive glycine receptors [2-7]. It also plays an important role in the excitatory neurotransmission *via* strychnine-insensitive glycine sites located on the N-methyl-D-aspartate (NMDA) receptors [4-7]. In the CNS, synaptic levels of glycine are regulated by specific sodium/chloride-dependent transporters. The actions of glycine are terminated by reuptake *via* two high-affinity glycine transporters referred to as GlyT-1 and GlyT-2. GlyT-1 and GlyT-2 possess 12 putative transmembrane spanning domains, and share approximately 50% amino acid sequence identity [4-8]. GlyT-1 is widely expressed in the CNS, where it is present predominantly on glial cells. It is likely that GlyT-1 is responsible for glycine reuptake in forebrain areas, and in some regions it may be co-localized with strychnine-insensitive glycine sites on the NMDA receptors [8-14]. A recent study demonstrated that glycine transport might keep local synaptic glycine levels very low, suggesting that GlyT-1 could play a role in regulating glutamatergic neurotransmission *via* the NMDA receptors [15]. In contrast to GlyT-1, GlyT-2 has a predominantly neuronal distribution and more limited distribution, being mainly restricted to the spinal cord, brainstem and cerebellum [13]. Indeed, GlyT-2 is co-localized with strychnine-sensitive glycine receptors, suggesting that GlyT-2 may be a reliable marker for glycinergic neurons [4-7].

In an attempt to clarify the *in vivo* functional roles of glycine transporters in the CNS, knockout mice deficient in the GlyT-1 gene have been generated [16,17]. Newborn mice deficient in the GlyT-1 gene are anatomically normal but show severe motor and respiratory deficits and die during the first postnatal day [16]. Both glycine and GlyT-1 inhibitor sarcosine (*N*-methyl glycine: Fig. **1**) suppress respiratory activity in slices from wild-type mice. During early postnatal life, GlyT-1 is essential for regulating glycine levels at inhibitory glycine receptors, and GlyT-1 deletion generates symptoms found in human glycine encephalopathy [16]. Furthermore, it has been demonstrated that heterozygous knock-out mice with reduced expression of GlyT-1 have enhanced hippocampal NMDA receptor function and memory retention, and are protected against a disruption of sensory gating by amphetamine, suggesting that GlyT-1 inhibitors might have both cognitive enhancing and antipsychotic effects [17]. Mice heterozygous for the GlyT-1 gene show faster decay kinetics, reduced ifenprodil sensitivity and increased zinc-induced antagonism in NMDA receptor currents [18]. Moreover, the ratio of AMPA receptors to NMDA receptors was decreased in mutant compared to wild-type mice, suggesting that this change was associated with a reduction in the number of AMPA receptors expressed at the CA1 synapses in the mutant mice [18]. These findings highlight the importance of GlyT-1 in regulating glutamatergic neurotransmission. Thus, it is possible that elevation of synaptic glycine levels, through blockade of the GlyT-1, potentiates NMDA receptor function *in vivo*, and that GlyT-1 inhibitors might have both cognitive enhancing and antipsychotic effects.

GLUTAMATE HYPOTHESIS OF SCHIZOPHRENIA

Multiple lines of evidence suggest that a dysfunction in glutamatergic neurotransmission might be involved in the pathophysiology of schizophrenia [19-30]. This hypothesis has evolved from clinical findings that phencyclidine (PCP) and its congener ketamine, which blocks the NMDA receptor ion channel, induce a schizophrenia-like psychosis that includes negative and positive symptoms as well as cognitive dysfunction in normal humans, and that PCP exacerbates symptoms in patients with chronic schizophrenia [31-33]. Furthermore, it is known that the competitive NMDA receptor antagonists are also psychotomimetic, and that other non-competitive NMDA receptor antagonists can induce a spectrum of schizophrenia-like symptoms in proportion to their potency as regards binding to NMDA receptors [19].

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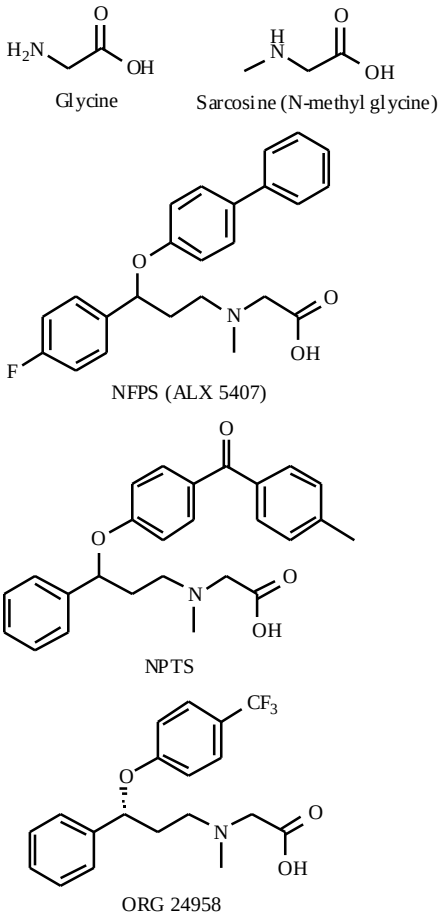


Fig. (1). Chemical structures of glycine, sarcosine, NFPS, NPTS, and ORG24958.

Taken together, it seems that endogenous dysfunction of NMDA receptor-mediated neurotransmission might contribute to the pathophysiology of schizophrenia [29].

It has previously been reported that the plasma levels of total serine and glycine in patients with schizophrenia are higher than those of controls [34], and that the levels of serine and glycine in the brains of schizophrenic patients are higher than those of controls [34,35], suggesting a possible abnormality in serine hydroxymethyltransferase, by which glycine is converted to L-serine. Glycine has been reported to ameliorate persistent negative symptoms of schizophrenia [36-38]. Recent retrospective analysis has suggested that NMDA receptor agonists are effective in treatment of persistent negative symptoms of schizophrenia, and that the full agonist glycine may be more effective than the partial agonist D-cycloserine [39].

Thus, it is likely that the NMDA receptor glycine-modulatory site is a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia [40]. Both glycine and D-serine are endogenous compounds that are actively metabolized and sequestered in the brain. In addition, glycine is extensively metabolized in the liver and crosses the blood-brain barrier poorly. Therefore, the ability of glycine levels to modulate NMDA receptor-mediated neurotransmission suggest that pharmacological manipulation of synaptic glycine might be effective in the treatment of conditions involving a hypofunction of the NMDA receptors [40-45]. In fact, treatment with sarcosine can benefit schizophrenic patients also being treated with antipsychotics including risperidone [46], suggesting that GlyT-1 inhibitors are a novel pharmacotherapeutic target for enhancing NMDA receptor function.

In this article, the author reviews the recent important patents regarding GlyT-1 inhibitors for therapeutic drugs. More than thirty patent applications presenting GlyT-1 inhibitors have been published from 2003 through 2005. Compounds provided in the applications can be divided into two types: substituted glycine derivatives and non-amino-acid derivatives. The latter type has increased more than the former type recently.

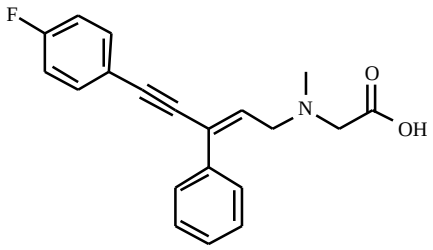
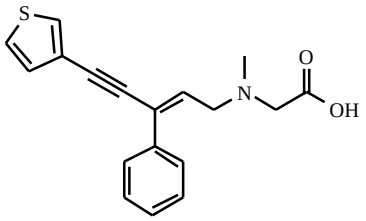
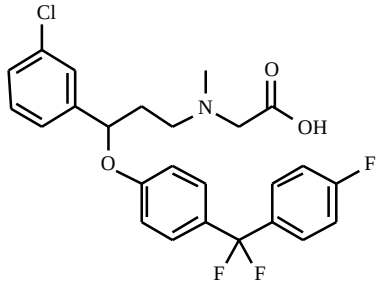
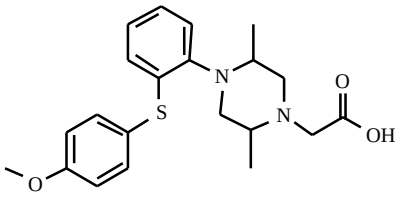
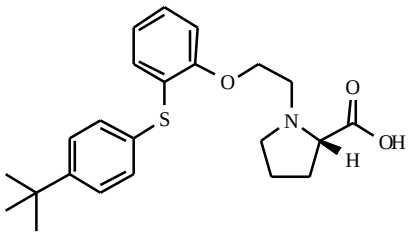
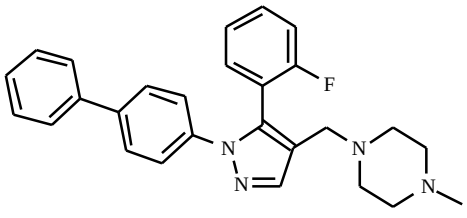
SUBSTITUTED GLYCINE DERIVATIVES AS GLYT-1 INHIBITORS

Researchers at Allelix Neuroscience, Inc. developed a sarcosine derivative, (*R*)-(*N*-[3-(4´-fluorophenyl)-3-(4´-phenylphenoxy)propyl]sarcosine (ALX 5407; NFPS; Fig. **1**) [47-49]. NFPS completely inhibited glycine transport in the GlyT-1 cells with an IC_{50} value of 3 nM, but had little or no activity at the human GlyT-2, at other binding sites for glycine, or at other neurotransmitter transporters [47-49]. NFPS inhibited PCP-induced hyperactivity in mice, and NFPS reversed PCP-induced changes in electroencephalogram (EEG) power spectra in conscious rats [50]. Furthermore, it has been reported that NFPS induced a pattern of c-Fos immunoreactivity comparable with the atypical antipsychotic clozapine, and that NFPS enhanced prepulse inhibition of the acoustic startle response in DBA/2J mice, a strain with low basal levels of prepulse inhibition [51]. Moreover, to an equal extent as clozapine and D-serine, NFPS was able to reverse persistent latent inhibition induced by dizocilpine ((+)-MK-801)[52]. These findings suggest that GlyT-1 inhibitors may have potential importance in treatment of negative symptoms of schizophrenia.

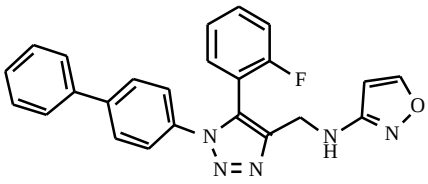
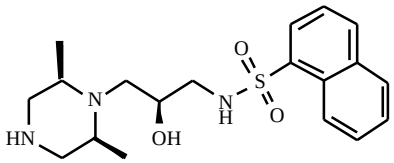
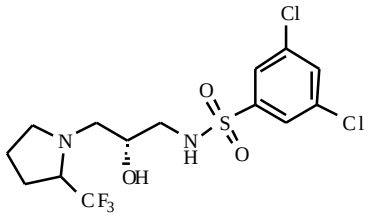
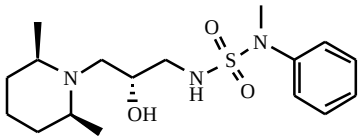
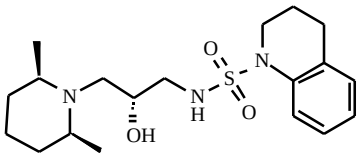
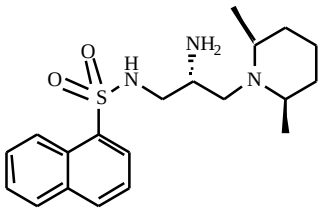
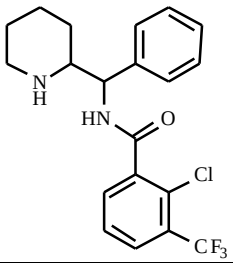
Researchers at Organon reported the selective GlyT-1 inhibitor, ORG 24598 (Fig. **1**), *R*-(-)-*N*-methyl-*N*-[3-[4-fluoromethyl]phenoxy]-3-phenyl-propylglycine [53]. Reverse dialysis of ORG 24598 (0.1 - 10 μM) in the rat dorsal spinal cord induced a concentration-related increase in extracellular levels of glycine accompanied by a progressive increase in citrulline [54]. It has been reported that ORG 24598 inhibited PCP-induced hyperactivity in mice, and that ORG 24598 reversed PCP-induced changes in EEG power spectra in conscious rats [50]. Furthermore, ORG 24598 (10 mg/kg, i.p.) fully and partially reversed neonatal ventral hippocampal lesion-induced prepulse inhibition (PPI) deficits, suggesting that GlyT-1 inhibitors could present promising targets for the development of novel therapies for schizophrenia [55].

Researchers at Pfizer Inc. developed an analogue of NFPS, (*R*)-*N*-[3-phenyl-3-(4´-(4-toluoyl)phenoxy)-propyl] sarcosine ((*R*)-NPTS) (Fig. **1**) [56]. They reported [^{3}H]-(*R*)-NPTS, a radioligand for the GlyT-1. In studies of binding to the cloned human GlyT-1c subtype of the GlyT-1 expressed

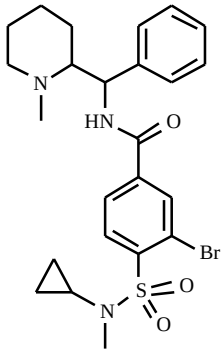
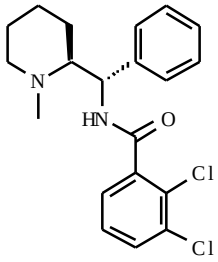
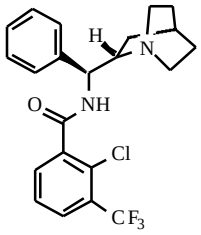
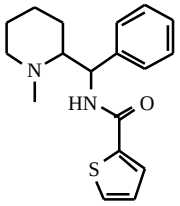
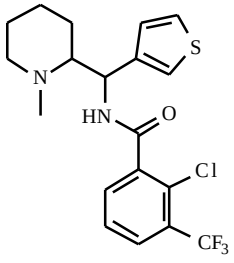
Table 1. Profiles of GlyT-1 Inhibitors

Number of compound	Chemical structure	Patent number	Name of company	Affinity for GlyT-1	Comments	Reference number
1		US2002426364	NPS Allelix Corp.		No biological data is described.	[57]
2		US2002169197 (2002)	NPS Allelix Corp.		No biological data is described.	[58]
3		EP1284257 (2003)	Pfizer Products Inc.		No biological data is described.	[59]
4		WO2003053942	H. Lundbeck		No biological data is described.	[60]
5		WO2004096761	H. Lundbeck		No biological data is described.	[61]
6		WO2003031435	MERCK PATENT GMBH Yamanouchi Pharmaceutical Co. Ltd.		No biological data is described.	[62]

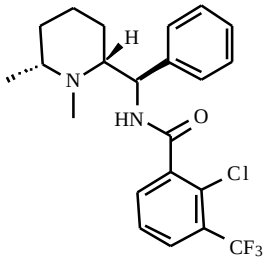
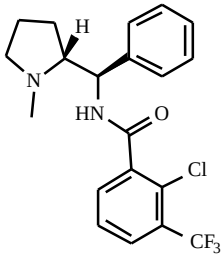
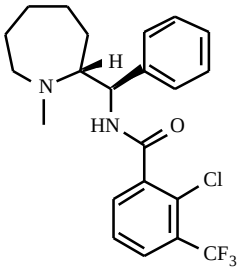
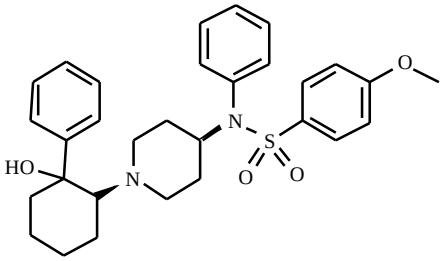
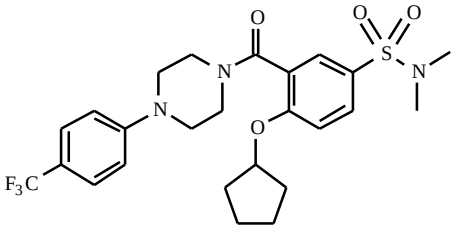
(Table 1) Contd….

Number of compound	Chemical structure	Patent number	Name of company	Affinity for GlyT-1	Comments	Reference number
7		DE10315570 (2004)	MERCK PATENT GMBH Yamanouchi Pharmaceutical Co Ltd		The compounds are stated to act as inhibitors of Glycine transport, particularly for the 1a-c subtypes, and have a low affinity for the 2 subtype. No biological data is described.	[63]
8		WO2003055478	SmithKline Beecham PLC		No biological data is described.	[64]
9		WO2004113280	Glaxo Group Limited		No biological data is described.	[65]
10		WO2004112787	Glaxo Group Limited		No biological data is described	[66]
11		WO2004113301	Glaxo Group Limited		No biological data is described.	[67]
12		WO2005049023	Glaxo Group Limited		No biological data is described.	[68]
13: (*S*,*S*) SSR504734		WO2003089411	Sanofi-Synthelabo	IC_{50} = 26 nM	Effective in animal models of schizophrenia, anxiety and depression	[69]

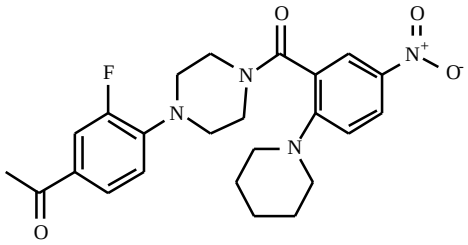
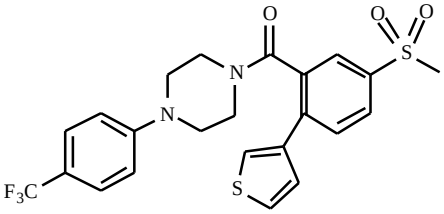
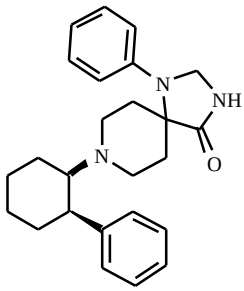
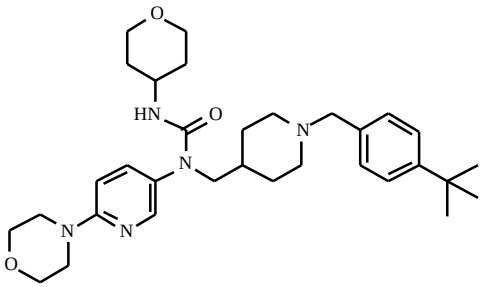
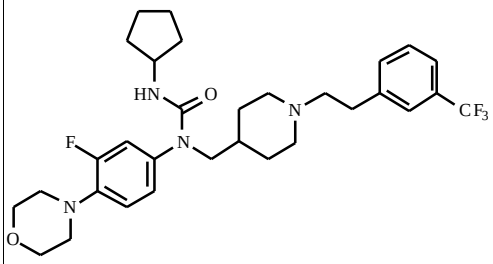
(Table 1) Contd….

Number of compound	Chemical structure	Patent number	Name of company	Affinity for GlyT-1	Comments	Reference number
14		WO2004013100	Sanofi-Synthelabo		No biological data is described.	[70]
15		WO2004013101	Sanofi-Synthelabo		No biological data is described.	[71]
16		WO2005037783	Sanofi-Aventis		No biological data is described.	[72]
17		WO2005037792	Sanofi-Aventis		No biological data is described.	[73]
18		WO2005037781	Sanofi-Aventis		No biological data is described.	[74]

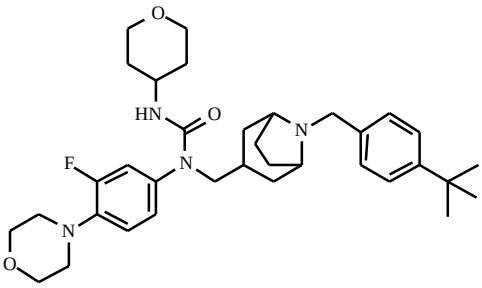
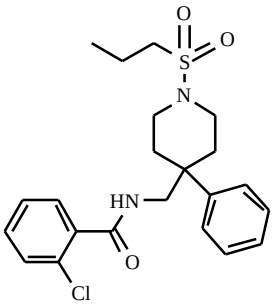
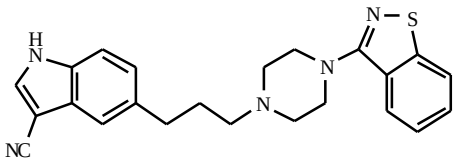
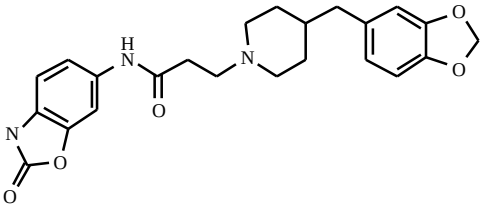
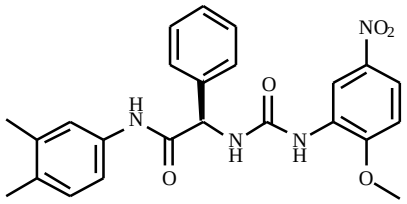
(Table 1) Contd….

Number of compound	Chemical structure	Patent number	Name of company	Affinity for GlyT-1	Comments	Reference number
19		WO2005037782	Sanofi-Aventis		No biological data is described.	[75]
20a		WO2005037785	Sanofi-Aventis		No biological data is described.	[76]
20b		WO2005037785	Sanofi-Aventis		No biological data is described.	[77]
21		WO2004072034	F.Hoffmann-La Roche AG	15 nM (*in vitro*, p 18).		[78]
22		WO2005014563	F.Hoffmann-La Roche AG	IC_{50} = 6nM		[79]

(Table 1) Contd….

Number of compound	Chemical structure	Patent number	Name of company	Affinity for GlyT-1	Comments	Reference number
23		WO2005023260	F.Hoffmann-La Roche AG	IC_{50} = 1.2nM		[80]
24		WO2005023261	F.Hoffmann-La Roche AG	IC_{50} = 6nM		[81]
25		WO2005040166	F.Hoffmann-La Roche AG	IC_{50} = 4nM (mGlyT1b)		[82]
26		WO2005058882	Glaxo Group Limited		No biological data is described.	[83]
27		WO2005058885	Glaxo Group Limited		No biological data is described.	[84]

(Table 1) Contd….

Number of compound	Chemical structure	Patent number	Name of company	Affinity for GlyT-1	Comments	Reference number
28		WO2005058317	Glaxo Group Limited		No biological data is described.	[85]
29		WO2005046601	Merck & Co.Inc.		no compound-specific data is described.	[86]
30		WO2003087086	MERCK PATENT GMBH		No biological data is described.	[87]
31		WO2003076420	MERCK PATENT GMBH		No biological data is described.	[88]
32		WO2004022528	NPS Allelix Corp.	IC50=26nM		[89]

in HEK 293 cells, [^{3}H]-(*R*)-NPTS showed a Kd value of 1 nM with saturable binding by Scatchard analysis. Therefore, [^{3}H]-(*R*)-NPTS is a useful radioligand for labeling GlyT-1 in the brain.

NPS Allelix published GlyT-1 inhibitor-related applications as another series of substituted glycine derivatives in 2002 and 2003. The compounds described in the applications have a diaryleyne system in those structures (e.g. compounds (**1**) and (**2**)) [57,58]. Profiles of these compounds are in (Table **1**). Compounds in patent applications appearing from Pfizer in 2003 contain difluoromethylene aromatic ether derivatives (e.g. compound (**3**)) [59]. Two patent applications published by Lundbeck in 2003 and 2004 describe aryloxyphenyl and arylthiophenyl

derivatives either with or without a heterocyclic linker (e.g. compounds (**4**) and (**5**)) [60,61].

NON-AMINO ACID DERIVATIVES AS GLYT-1 INHIBITORS

Patent applications published by Yamanouchi Pharmaceuticals and Merck patent GMBH in 2003 and 2004, respectively, exemplify compounds possessing heteroaromatic rings: namely, 1-aryl-1H-pyrazole derivatives and 1-aryl-1, 2, 3-triazole derivatives (e.g. compounds (**6**) and (**7**)) [62,63].

Compounds in patent applications published by the Glaxo group (including the Smith Kline Beecham group) from 2003 through 2005 contain hydroxy sulfonamide derivatives (e.g. compounds (**8**) and (**9**)) [64,65], hydroxy sulfamide derivatives (e.g. compounds (**10**) and (**11**)) [66,67], and an amino sulfonamide derivatives (e.g. compound (**12**)) [68].

Sanofi-Aventis (former Sanofi-Synthelabo) published eight patent applications from 2003 through 2005. The first three patent applications appeared in 2003 mad 2004 describe *N*-[Phenyl(piperidin-2-yl)methyl]benzamide derivatives (e.g. (*S, S*)-SSR504734 (**13**), and compounds (**14**) and (**15**)) [69-71], and the other five applications published in 2005 expand the scope of claims of the first three applications, which exemplify *N*-(heterocyclylmethyl) bezamide derivatives (e.g. compound (**16**)) [72], heteroaryl and naphthalene carboxylic acid [phenyl(piperidin-2-yl)methyl] amide derivatives (e.g. compound (**17**)) [73], *N*-[heteroaryl (piperidin-2-yl)methyl]benzamide derivatives (e.g. compound (**18**)) [74], *N*-[phenyl(alkylpiperidin-2-yl)me-thyl] benzamide derivatives (e.g. compound (**19**)) [75], *N*-[phenyl(pyrrolidine-2-yl)methyl]benzamide derivatives (e.g. compound (**20a**)) [76], and *N*-[(azepan-2-yl)phenylmethyl] benzamide derivatives (e.g. compound (**20b**))[76].

Researchers at Sabofi-Santhelabo Rechereche reported a detailed neuropharmacological profile of SSR 504734 (**13**) as part of a chemical effort aimed at developing a selective and reversible GlyT-1 inhibitor [77]. SSR 504734, a selective and reversible inhibitor of GlyT-1, blocked the *ex vivo* uptake of glycine rapidly, reversibly, and for a long duration. In the animal models of schizophrenia, this compound normalized spontaneous PPI deficits in DBA/2 mice, and reversed hyperactivity to locomotor effects of d-amphetamine and selective attention deficits in adult rats treated neonatally with PCP. Furthermore, this compound showed additional activity in depression/anxiety models, such as chronic mild stress in mice, ultrasonic distress calls in rat pups separated from their mother, and the increased latency of paradoxical sleep in rats. These findings suggest that SSR 504734 is a potent and selective GlyT-1 inhibitor, exhibiting activity in animal models of schizophrenia, anxiety and depression, and that this compound could be efficacious not only against positive but also negative symptoms, cognitive deficits, and comorbid depression/anxiety states [77].

Applications published by Hoffmann-La Roche in 2004 and 2005 exemplify compounds possessing various heterocyclyl moieties functioning as linkers between two aromatic rings, including 4-aminopiperidine derivatives (e.g. compound (**21**))[78], piprazine derivatives (e.g. compounds (**22**), (**23**), and (**24**)) [79-81], and triaza-spiropiperidine derivatives (e.g. compound (**25**)) [82].

Compounds (**26**) - (**28**) described in patent applications by the Glaxo group in 2005 have the same structural features: non-substituted or substituted 4-piperidinyl-methylamine moieties, and urea or urethane bonding [83-85]. Compound (**29**) from Merck also has substituted 4-piperidinylmethylamine moieties [86]. Merck patent GMBH published patent applications in 2003; these contain substituted indole derivatives (e.g. compound (**30**)) [87] and heterobicyclyl amide derivatives (e.g. compound (**31**)) [88]. Applications from NPS Allelix include substituted glycine peptide-like derivatives (e.g. compound (**32**)) [89].

As described above, compounds (**8**)-(**12**) are sulfonamide derivatives and sulfamide derivatives. Compounds (**13**)-(**15**) are 2-substituted piperidine derivatives, and compounds (**21**)-(**25**) are 4- substituted piperidine derivatives and piperazine derivatives. Among these compounds, (S, S)-SSR504734 (**13**) is the most promising drug as described above [77]. Therefore, the author noted that the three patents [64, 69, 78] are the most important in these patents.

CURRENT AND FUTURE DEVELOPMENTS

As described above, GlyT-1 is essential for regulating glycine levels at synapses. Therefore, pharmacological modulation of glycine-mediated neurotransmission by GlyT-1 inhibitors might be beneficial in the treatment of cognitive deficits as well as psychosis in several psychiatric diseases including schizophrenia. In a recent 6-week double-blind, placebo-controlled trial of sarcosine (2 g/day), schizophrenic patients who received sarcosine treatment revealed significant improvements in their positive, negative, cognitive, and general psychiatric symptoms [46]. Sarcosine was also well-tolerated, and no significant side effects were noted [46]. Several side effects of GlyT-1 inhibitors have been theoretically suggested although no notable side effects have been reported. These side effects include an increase in pain sensation, a decrease in seizure threshold, and neurotoxicity threshold since inhibition of GlyT-1 may potentiate NMDA receptor function [44]. Given the role of the NMRA receptor in the function of the vertebrate retina [90-93], it is theoretically possible that a blockade of GlyT-1 may lead to unwanted side effects in the retina, where GlyT-1 is highly expressed in amacrine cells [12]. Therefore, further studies on side effects by chronic administration of GlyT-1 inhibitors should be undertaken.

At present, SSR 504734 (compound (**13**)), a potent, selective, and orally active GlyT-1 inhibitor, might be the most promising of the drugs mentioned above, as this compound exhibits activity in animal models of schizophrenia and anxiety/depression [69,77]. Furthermore, it has been suggested that glycine sites on the NMDA receptor play a role in the cognitive function of neurodegenerative disorders including Alzheimer's disease [94-96]. Clinically, administration of D-cycloserine (partial agonist at glycine sites on the NMDA receptor) improved memory deficits in patients with Alzheimer's disease [95,96]. Therefore, it is possible that GlyT-1 inhibitors may

be a useful therapeutic drug for neurodegenerative diseases such as Alzheimer's disease.

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