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The synthesis and SAR of 2-arylsulfanyl-phenyl piperazinyl acetic acids as glyT-1 inhibitors

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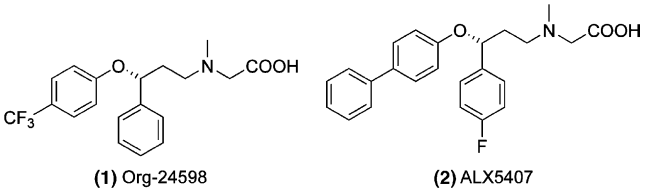
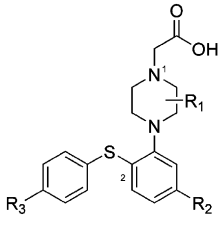
Abstract—Elevation of glycine levels and activation of the NMDA receptor by inhibition of the glycine transporter 1 (GlyT-1) is a potential strategy for the treatment of schizophrenia. A novel series of GlyT-1 inhibitors have been identified containing the 2-arylsulfanyl-phenylpiperazine motif. The most prominent member of this series, (*R*)-4-[5-chloro-2-(4-methoxy-phenylsulfanyl)-phenyl]-2-methyl-piperazin-1-yl-acetic acid (**31**) is a potent glycine transporter-1 inhibitor ($IC_{50} = 150$ nM), which elevated glycine levels in rat ventral hippocampus as measured by microdialysis in vivo at doses of 1.2–4.6 mg/kg s.c.

1. Introduction

Hypofunction of the glutamatergic system has been suggested to be an underlying factor behind schizophrenia.[1] Thus, a potential therapeutic approach is to enhance glutamatergic function by modulating the function of the NMDA receptor.[2] A strategy for achieving this is to increase the amount of the co-agonist glycine in the synapse by inhibiting the reuptake of glycine into glial cells by the glycine transporter 1 (GlyT-1).[3] This transporter has been shown to be co-localised with NMDA receptors.[4] The low potency GlyT-1 inhibitor sarcosine displays clinical benefits as add-on therapy to risperidone.[5] It has been reported in the literature that sarcosine derivatives such as Org-24598[6] (**1**) and ALX5407[7] (**2**) are potent GlyT-1 inhibitors. Structural considerations in finding a replacement for the 3-aryloxy-3-arylpropyl motif in these reference structures lead us to the identification of the (2-arylsulfanyl)-phenyl-1-piperazine moiety. In this letter, we describe novel GlyT-1 inhibitors of the general formula **3**.



(1) Org-24598 **(2)** ALX5407



(3)

2. Chemistry

The solid phase synthesis of Ruhland et al.[8] (method a) was used to synthesise piperazine intermediates (e.g., **9**)

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as illustrated in Scheme 1. 4-[(4-Nitrophenoxy)carbonyl-oxymethyl]phenoxymethyl polystyrene **4** was treated with *trans*-2,5-dimethyl piperazine to afford resin-bound piperazine **5**. Reaction with 1,2-dichlorobenzene cyclopentadienyl iron complex gave resin-bound 2,5-dimethyl piperazinyl 2-chlorobenzene cyclopentadienyl iron complex **6**. Treatment with 4-methoxythiophenol under basic conditions and subsequent decomplexation using phenanthroline and light afforded resin-bound 2-(4-methoxyphenylsulfanyl)phenyl-2,5-dimethylpiperazine (**8**). Cleavage from the resin using trifluoroacetic acid (TFA) yielded 1-[2-(4-methoxy-phenylsulfanyl)-phenyl]-2,5-dimethyl-piperazine (**9**).

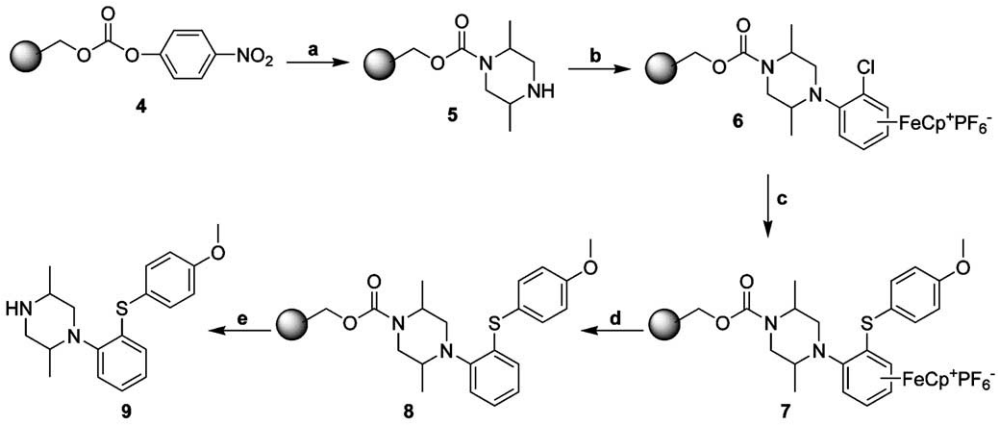
The (2-arylsulfanyl)-phenyl-1-piperazine intermediates were then employed in a second solid phase synthesis as illustrated in Scheme 2 for the preparation of the final product **13**. Chloroacetyl chloride was esterified onto Wang resin **10**, and the solid supported chloroacetate **11** was subsequently reacted with the piperazine **9** under basic conditions. The resulting alkylated product **12** was cleaved from the resin using trifluoroacetic acid to afford the final piperazine acetic acid **13**.

An alternative, solution phase, synthesis (method b) is shown by the synthesis of **18** in Scheme 3. 4-Chloro-2-fluoro-nitrobenzene was reacted with 2-methylpiperazine and then reduced to the aniline **16**. Diazotisation and reaction with 4-methoxythiophenol gave the 1-(2-arylsulfanyl)-phenylpiperazine **17**. Alkylation with ethyl bromoacetate followed by aqueous hydrolysis yielded the piperazine acetic acid **18**.

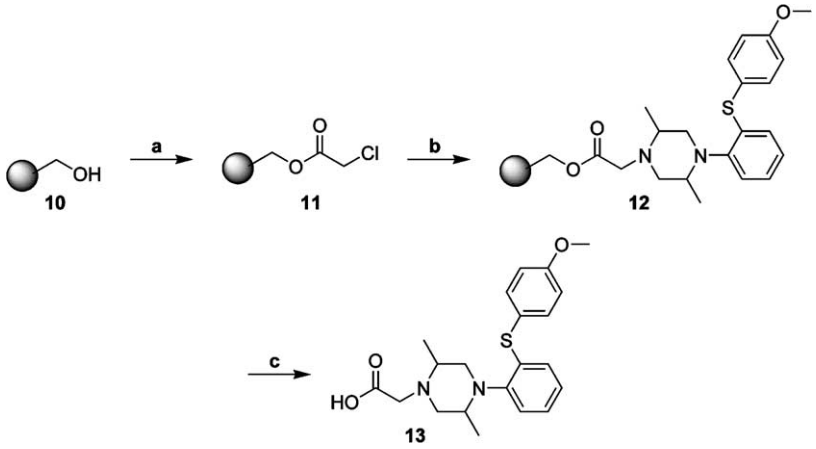
3. Results and discussion

The prepared compounds were tested for inhibition of uptake of ^{3}H glycine into cells transfected with the human GlyT-1_b transporter.[9] The racemic forms of compounds **1** (Org-24461) and ALX5407 (**2**) had IC_{50}'s of 320 and 26 nM, respectively.

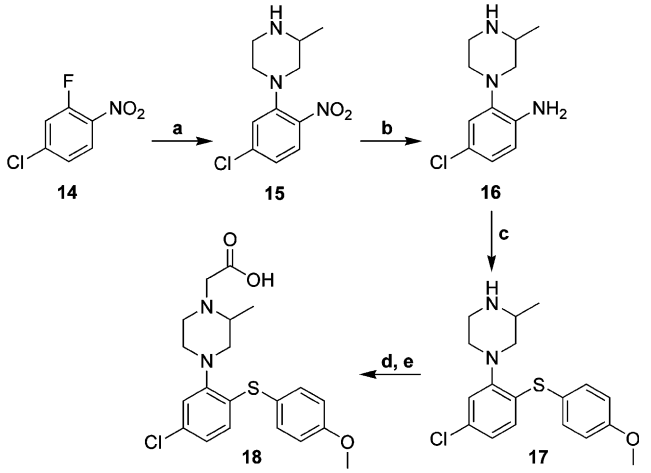
As can be seen in Table 1, introduction of a methyl group in the 2-position of the piperazine ring appears to be important for activity as witnessed by the assay results of compounds **13**, **24** and **26** compared to that of compound **25**. In contrast, introduction of a methyl



Scheme 1. Reagents and conditions: (a) *trans*-2,5-dimethyl-piperazine, *N*-ethyldiisopropylamine, THF, DMF, 70 °C, 16 h; (b) Cp(1,2-dichlorobenzene)Fe, THF–DMF 1:1, 70 °C, 16 h; (c) 4-methoxythiophenol, NaH, THF, 60 °C, 6 h; (d) h*v*, phenanthroline, HCl, CH_3CN, H_2O, 12 h; (e) TFA, DCM, 2.5 h.

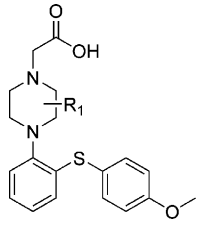


Scheme 2. Reagents and conditions: (a) $ClCH_2COCl$, DIEA, DCM, 16 h; (b) 1-[2-(4-methoxy-phenylsulfanyl)-phenyl]-2,5-dimethyl-piperazine, DIEA, DMF, 70 °C, 16 h; (c) TFA, DCM, 1 h, 14% over two steps.



Scheme 3. Reagents and conditions: (a) 2-methylpiperazine, DIEA, DMF, 80 °C, 16 h, 88%; (b) H_2, Pd/C, MeOH, 3 h, 98%; (c) 1. $NaNO_2$, H_2SO_4, H_2O, 0.5 h; 2. Cu, NaOH, 4-methoxythiophenol, H_2O, 60 °C, 0.5 h, 11%; (d) $BrCH_2CO_2Et$, Et_3N, CH_3CN, 16 h, 48%; (e) NaOH, H_2O, EtOH, 16 h, 100%.

Table 1. Effect of R_1 piperazine ring substitution on inhibition of (^{3}H)-glycine uptake at the GlyT-1_b transporter



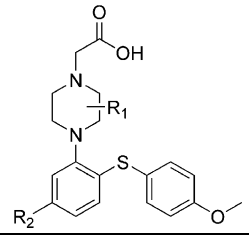
Compound	R_1	GlyT-1_b IC_{50} (nM)
13[a]	*trans*-2,5-Dimethyl	580
19[b]	2*R*,5*S*-Dimethyl	160
20[b]	2*S*,5*R*-Dimethyl	53,000
21[b]	2*S*,5*S*-Dimethyl	18,000
22[b]	2*R*,5*R*-Dimethyl	16,000
23[a]	3-Methyl	>10,000
24[a]	2-Methyl	1700
25[a]	—	>10,000
26[b]	2,2-Dimethyl	1500

[a] Synthesised by method a.
[b] Synthesised by method b.[10]

group at the 3-position (compound **23**) did not lead to activity. However, incorporating a 5-methyl group onto the 2-methylpiperazine unit appears to enhance potency (**13**). The four stereoisomers of the 2,5-dimethyl substitution demonstrate high stereoselectivity for the 2*R*,5*S*-dimethyl analogue **19** with the other stereoisomers showing much weaker potency.

As can be seen in Table 2 where R_1 is 2-methyl, varying R_2 indicates that the presence of the electron withdrawing chlorine (**18**) confers better potency than methyl (**27**) or methoxy (**28**) substitution. The preference for R_2 to be Cl (**30**) compared with methoxy (**29**) is not reflected in the 2,5-dimethyl piperazine analogues where neither compounds (**29**, **30**) are more potent than the

Table 2. Effect of R_2 phenyl ring substitution on inhibition of (^{3}H)-glycine uptake at the GlyT-1_b transporter (compounds were synthesised by method b)

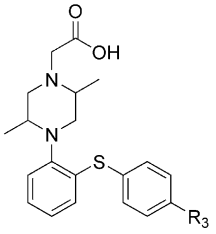


Compound	R_1	R_2	GlyT-1_b IC_{50} (nM)
18	2-Methyl	Cl	290
27	2-Methyl	CH_3	1600
28	2-Methyl	MeO	4600
29	*trans*-2,5-Dimethyl	MeO	>50,000
30	*trans*-2,5-Dimethyl	Cl	1700
31	2*R*-Methyl	Cl	150
32	2*S*-Methyl	Cl	1400

unsubstituted (**13**). Stereochemistry at the 2-methyl position of the piperazine ring is important for affinity with the *R*-isomer **31** displaying an order of magnitude greater potency than the corresponding *S*-isomer **32**. This observation correlates with the preferred stereochemistry of the 2-methyl group in compound **19**.

Variation of R_3 in Table 3 shows that substitution is necessary for submicromolar potency as can be seen in comparing the unsubstituted compound **33** with the methyl-substituted analogue (**34**). This position appears to be tolerant to small neutral groups as can be seen with analogues **35**, **36** and **13**, but intolerant of polar groups as seen with analogues **38** and **39** as well as phenyl substitution (**37**).

Table 3. Effect of R_3 on inhibition of (^{3}H)-glycine uptake at the GlyT-1_b transporter



+/- trans stereochemistry

Compound	R_3	GlyT-1_b IC_{50} (nM)
33[a]	—	2900
34[a]	CH_3	550
35[a]	Cl	360
36[a]	F	1900
37[b]	Ph	>10,000
38[b]	OH	3600
39[b]	$NHCOCH_3$	22,000

[a] Synthesised by method a.
[b] Synthesised by method b.

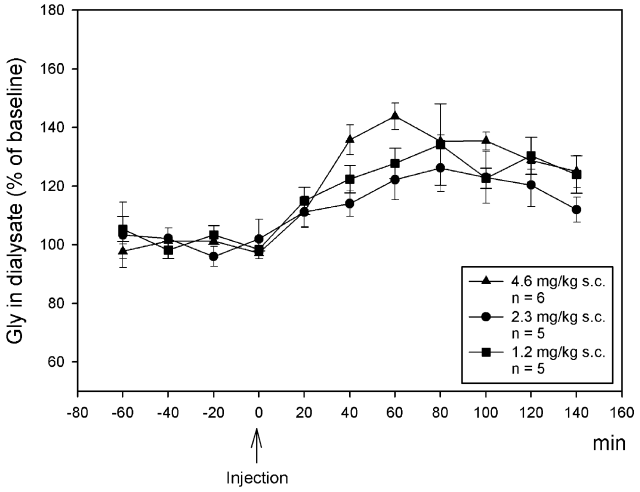


Figure 1. Study of the effects of compound **31** on extracellular glycine levels in the rat ventral hippocampus. Mean basal pre-injection values normalised to 100%; *n*: number of animals.

Compound **31** was profiled further in vitro against the GlyT-2 transporter and a representative set of 75 targets including dopaminergic and serotonergic receptors with no appreciable inhibition at a concentration of 10 μM but compound **31** did show some affinity towards GABA receptors as it increased ^{35}S-TBPS binding to rat brain membranes. From in vitro assessment in Caco-2 cells, penetration through the blood–brain barrier was estimated to be good. The permeability coefficient was 51×10^{-6} cm/s, comparable to clozapine (34×10^{-6} cm/s) and risperidone (31×10^{-6} cm/s) and in the bi-directional Caco-2 set-up the permeability coefficients were equal in both directions, indicating that compound **31** is probably not a substrate for efflux transporters such as *p*-glycoprotein. Assessment of pharmacokinetics in the rat for compound **31** revealed an excellent oral bioavailability of 100% when administered as a solution with a low plasma clearance of 0.4 L/h/kg and a terminal half-life of 4 h.

In order to assess the effect of compound **31** in vivo, a microdialysis study on freely moving rats was undertaken. After subcutaneous administration, a dose-dependent increase in extracellular levels of glycine in the ventral hippocampus was observed, giving an increase to 140% of baseline levels after 60 min with a 4.6 mg/kg dose (Fig. 1).

4. Conclusions

Novel 2-phenylsulfanyl-phenylpiperazinyl acetic acids have been identified as centrally acting GlyT-1 inhibitors with in vitro potencies for **13**, **18** and **31** comparable to Org-24598, **1**. It has been demonstrated that extracellular glycine levels are increased in the ventral hippocampus after subcutaneous administration with compound **31** and thus provides further tool compounds for examining the potential use of GlyT-1 inhibitors as novel antipsychotics. The identification of the novel 2-phenyl-sulfanyl-phenyl piperazinyl motif provides a novel template for further lead optimisation.

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9. Cells transfected with the human GlyT-1_b transporter were seeded in 96-well plates. The cells were pre-washed twice with HBS (10 mM Hepes-Tris (pH 7.4), 2.5 mM KCl, 1 mM $CaCl_2$, 2.5 mM $MgSO_4$, and preincubated with the test compound for 6 min. Afterwards, 10 nM of ^{3}H-glycine was added, and the cells were incubated for 15 min. The cells were washed twice in HBS. Scintillation fluid was added, and the plates were counted on a Trilux (Wallac) scintillation counter.
10. Compounds **19**, **20**, **21** and **22** were prepared by a modification of method b. *N*-Benzyl-2*R*,5*S*- and 2*S*,5*R*-dimethyl piperazine were prepared as described in patent application WO/0071525 and Aicher, T. D. et al. *J. Med. Chem.* **2000**, *43*, 236–249. These intermediates were then employed in the S_NAR reaction with 2-fluoronitrobenzene (method b), and the resulting product was subjected to catalytic hydrogenation at 1.5 bar using Pd/C in methanol for removal of the benzyl group. The 2*R*,5*R*- and 2*S*,5*S*-dimethyl piperazines were prepared by borane mediate reduction of the commercially available diketopiperazines, cyclo-L-ala-L-ala and cyclo-D-ala-D-ala as described by: Jung, M. E.; Rohloff, J. C. *J. Org. Chem.* **1995**, *50*, 4909–4913, followed by monobenzylation and then used as described for the *trans* stereoisomers.