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The synthesis and SAR of 2-arylsulfanylphenyl-1-oxyalkylamino acids as GlyT-1 inhibitors

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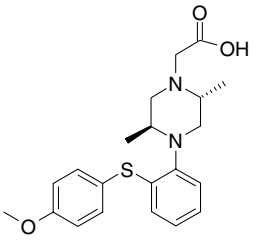
Abstract—Elevation of glycine levels by inhibition of the glycine transporter-1 (GlyT-1) and activation of the NMDA receptor is a potential strategy for the treatment of schizophrenia. A novel series of 2-arylsulfanylphenyl-1-oxyalkyl amino acids have been identified. The most prominent member of this series *S*-1-{2-[3-(3-fluoro-phenylsulfanyl)biphenyl-4-yloxy]ethyl}pyrrolidine-2-carboxylic acid (**38**) is a potent GlyT-1 inhibitor (IC_{50} = 59 nM). In vitro and in vivo assessment of CNS exposure indicates this compound is a likely substrate for active efflux transporters.

Glycine is a major neurotransmitter with both excitatory and inhibitory roles in the central nervous system. Extracellular levels are in part governed by the glycine transporters[1] of which there are two subtypes, the glycine transporter-1 (GlyT-1) and the glycine transporter-2 (GlyT-2). Five GlyT-1 splice variants are known (a,b,c,d,e), whilst two have been identified for GlyT-2 (a,b). Blockade of the GlyT-1 elevates glycine levels and enhances glutamatergic neurotransmission due to the coagonistic action of glycine at the NMDA receptor[2] which is thought to be of benefit in schizophrenia.[3]

Further understanding of the physiological role of the GlyT-1 has come from gene deletion studies.[4] Heterozygote GlyT-1 +/− mice display behaviour characteristic of enhanced NMDA receptor response, whilst homozygote GlyT-1 −/− mice display neonatal lethality apparently due to sustained activation of the inhibitory glycine receptor. There is at present widespread interest in the pharmaceutical industry in understanding how to develop safe and effective therapies based on GlyT-1 blockade.[5,6]

We have previously reported the identification of the biaryl thioether piperazine acetic acids,[7] for example, **1** which were based on the first GlyT-1 sarcosine-based inhibitors reported in the literature.[8,9] In this letter, we wish to report further optimisation of **1** in which we replaced the piperazine linker with an oxyalkylamino chain as shown by the generic structure **2**. The objective of this work was to improve further the potency against GlyT-1 and explore the role of the amino acid fragment in the interaction with GlyT-1.

Three synthetic approaches were taken to prepare the compounds of the general structure **2**. Variations in R^1 and R^2 were achieved in a parallel fashion as shown



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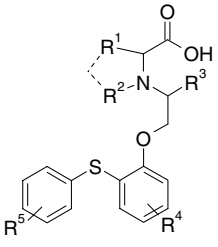

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Keywords: Glycine transporter-1; GlyT-1 inhibitor; Amino acids; Schizophrenia.
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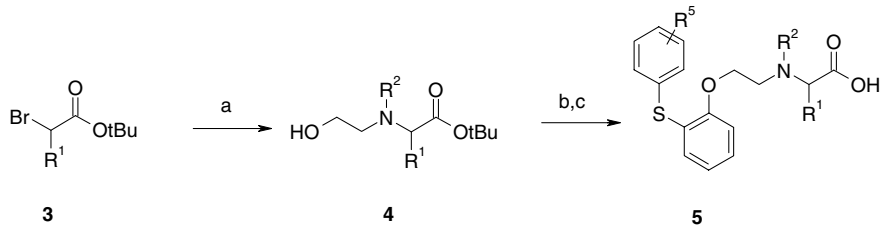
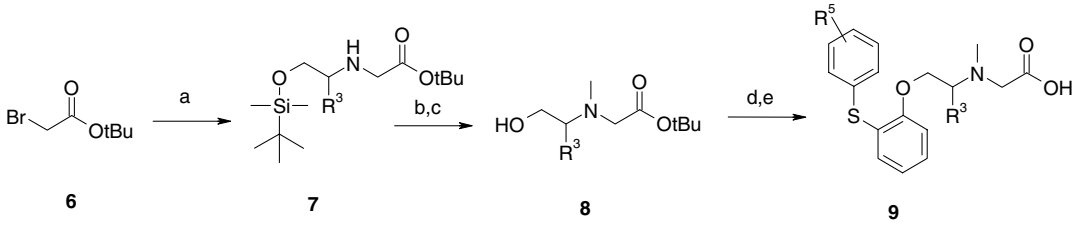
in Scheme 1. *N*-Alkyl amino ethanol was used to alkylate *tert*-butyl bromoacetate derivative **3** under basic conditions and the resulting alcohol **4** was reacted with the appropriately substituted 2-(arylsufanyl)phenol[10] under Mitsunobu conditions. Cleavage of the *tert*-butyl ester gave the final product, **5**. Examination of R^3 was achieved also in a parallel fashion as shown in Scheme 2. Bromo *tert*-butyl acetate **6** was reacted with *tert*-butyldimethyl silyl (TBDMS) protected alcohols and the secondary amine **7** was alkylated with methyl iodide. Removal of the TBDMS group gave the alcohol **8**. Coupling with the appropriately substituted 2-(arylsufanyl)phenol under Mitsunobu conditions, followed by cleavage of the *tert*-butyl ester, gave final product **9**.

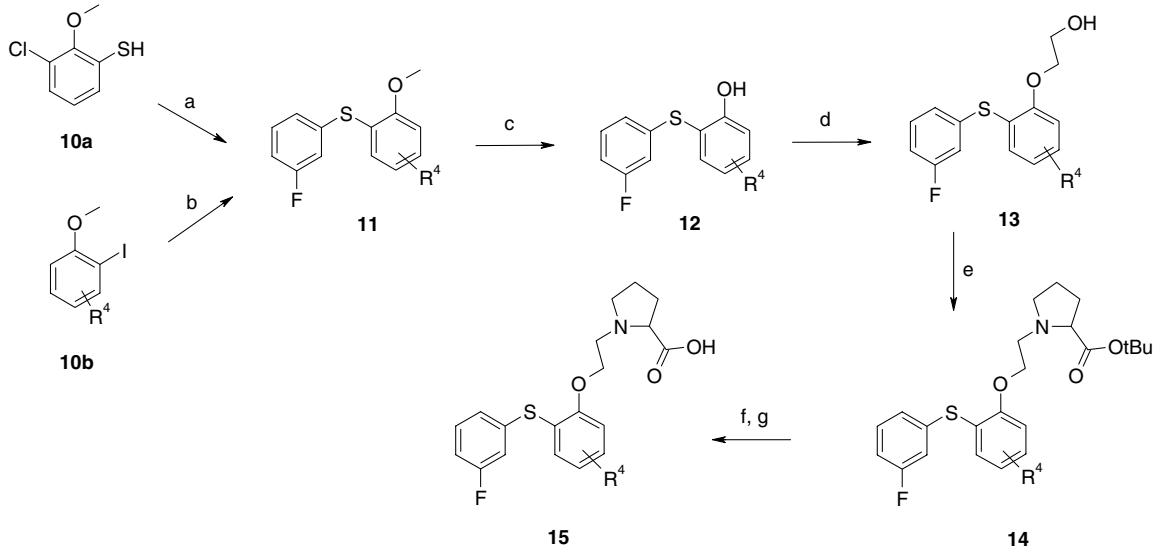
In order to vary R^4 in the *S*-proline series, a synthetic sequence was employed as illustrated in Scheme 3. Coupling of 2-methoxy-3-chlorophenyl thiol **10a** with 3-fluoroiodobenzene gave access to chlorine substitution in the 6 position. Otherwise appropriately substituted halo-2-iodo-anisoles **10b**[11] were coupled with 3-fluorobenzenethiol using palladium catalysis to give the biaryl sulfanyl derivatives **11**. Removal of the methyl group gave the corresponding 2-(3-fluorophenylsulfanyl)-phenol derivatives **12** which were then treated with ethylene carbonate to give the 2-arylsulfanylphenyloxyethyl alcohol **13**. Activation to the triflate and then displacement with D- or L-proline *tert*-butyl ester gave **14**. In the case of R^4 being bromide, Suzuki coupling with aryl boronic



Scheme 1. Reagents and conditions: (a) $HN(R^2)CH_2CH_2OH$, *N*-ethyldiisopropylamine (DIPEA), DMF, 50 °C, 16 h; (b) 2-(R^5-Ph–S)–Ph–OH, DEAD, PS–PPh_3, THF, rt; (c) HCl/AcOH, rt.



Scheme 2. Reagents and conditions: (a) $NH_2CH(R^3)CH_2OTBDMS$, DIPEA, DMF, 50 °C, 16 h; (b) MeI, DIEA, DMF, 50 °C, 16 h; (c) $Et_3N{\cdot}3HF$, AcCN; (d) 2-(R^5-Ph–S)–Ph–OH, DEAD, PS–PPh_3, THF, rt; (e) HCl/AcOH.



Scheme 3. Reagents and conditions: (a) 3-fluoroiodobenzene, Pd_2dba_3, DPEPhos, K^{t}BuO, toluene, 100 °C, 90 min, 55%; (b) 3-fluorobenzenethiol, K^{t}BuO, Pd_2dba_3, toluene; (c) BBr_3, toluene, 0 °C, 16 h; (d) ethylene carbonate, K_2CO_3, DMF; (e) Tf_2O, Et_3N, D- or L-proline-O^{t}Bu; (f) where, R^4 = Br, Ar-$B(OH)_2$, $Pd(PPh_3)_2Cl_2$, K_2CO_3, DME, 85 °C, 2 h; (g) HCl/AcOH, 16 h, rt.

acid and cleavage of the *tert*-butyl ester yielded final product **15**.

The prepared compounds were tested for inhibition of uptake of [^{3}H]glycine into cells transfected with the human GlyT-1_b transporter. The cells were pre-washed twice with HBS (10 mM Hepes–Tris (pH 7.4), 2.5 mM KCl, 1 mM $CaCl_2$ and 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.

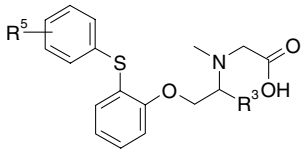
Replacement of the 2*R*,5*S*-dimethyl piperazine moiety in compound **1** with a oxyethylamino linker resulted initially in compounds with micromolar potency as listed in Table 1. A slight advantage was observed for fluorine in the meta position, (**23**) compared to the para position (**18**) with other substitutions weaker in the para position, (**17**, **19** and **21**) or no potency in the case of acetamide (**20**). With R^5 as 3-F no beneficial effects were observed with alkyl substitution in the 2 position of the oxyethyl chain (**24**, **25**, **26** or **27**).

Investigation of the amino acid fragment as listed in Table 2 revealed that the *N*-methyl and *N*-ethyl alanine analogues (**28** and **30**, respectively) showed considerably reduced potency compared to the sarcosine analogue **22**. Intolerance of *N*-ethyl was also seen with the glycine analogue **29**. Surprisingly the ring closed *S*-proline analogue **32** showed significantly improved potency.

Within the *S*-proline series the effect of R^4 substitution in the central benzene ring was examined as listed in Table 3. Introduction of chlorine displayed intolerance for the 6 position (**34**) and the 3 position (**35**) was also disfavoured, whereas both the 4 and 5 positions were tolerated (**36** and **33**, respectively). Further space for substitution was observed with a 4-phenyl group (**38**) significantly improving potency though not with the isosteric 4-(3-thiophene) analogue **39** or in the 5 position (**37**). Substitution on the additional 4-phenyl was also tolerated with 4 and 3-methoxy groups (**40** and **41**), whilst 4-chlorine was less favourable in (**42**).

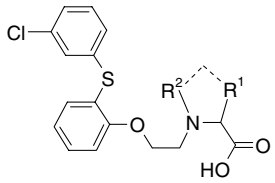
Compound **38** was found to be inactive against the GlyT-2 at a concentration of 10 μM. Key compounds were profiled in vitro and in vivo for their ability to penetrate the CNS as summarised in Table 4. Caco-2 cells, exhibiting tight and confluent monolayers, serve as a model of cell permeability.[12] Thus, transport studies using Caco-2 cells may be applied as an initial indication of blood–brain barrier (BBB) transport characteristics. Compound **1** showed good Caco-2 cell properties with no indication of active efflux and this was corroborated with good CNS penetration in the mouse with a brain/plasma (B/P) ratio[13] of 0.54. Compounds **38** and **40** both displayed high apparent permeability coefficients (P_{app}) in the Caco-2 assay. However, their transport ratios

Table 1. Effect of R^5 and R^3 substitution on inhibition of [^{3}H]glycine uptake at the GlyT-1_b transporter



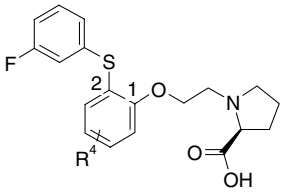
Compound	R^5	R^3	GlyT-1_b IC_{50} (μM)
1			0.16
16	4-Cl	H	7.8
17	4-MeO	H	30
18	4-F	H	15
19	4-Me	H	47
20	4-$NHCOCH_3$	H	>50
21	4-*tert*-Butyl	H	9.2
22	3-Cl	H	3.9
23	3-F	H	5.1
24	3-F	Et	11
25	3-F	Isopropyl	18
26	3-F	*R*-Me	12
27	3-F	*S*-Me	8.9

Table 2. Effect of R^1/R^2 substitution on inhibition of [^{3}H]glycine uptake at the GlyT-1_b transporter



Compound	R^1	R^2	GlyT-1_b IC_{50} (μM)
28	Me	Me	26
29	H	Et	7.3
30	Me	Et	50
31	(*R*)-$CH_2CH_2CH_2$–		22
32	(*S*)-$CH_2CH_2CH_2$–		0.38

Table 3. Effect of R^4 substitution on inhibition of [^{3}H]glycine uptake at the GlyT-1_b transporter



Compound	R^4	GlyT-1_b IC_{50} (μM)
33	5-Cl	0.6
34	6-Cl	19
35	3-Cl	1.2
36	4-Cl	0.4
37	5-Phenyl	0.95
38	4-Phenyl	0.059
39	4-(3-Thiophene)	0.52
40	4-(4-MeO–Ph)	0.16
41	4-(3-MeO–Ph)	0.3
42	4-(4-Cl–Ph)	1.4

Table 4. In vitro and in vivo assessment of CNS penetration for key compounds

Compound	Caco-2 P_{app} ($\times 10^{-6}$ cm/s)	Transport ratio (b-a/a-b)	B/P Mice
1	33.4	0.8	0.54
38	25.1	2.2	0.17
40	26.3	2.9	0.09

(basal–apical(b-a)/apical–basal(a-b)) of >2 in Caco-2 cells pointed towards a possible involvement of active efflux from the brain.

As such, compounds **38** and **40** could thus be substrates for active efflux transporters such as P-glycoprotein. These in vitro data appeared to be predictive of in vivo brain exposure where modest exposure in the CNS was observed. Thus, 70 min following sc administration of 5 mg/kg, **38** and **40** displayed average brain exposures of only 60 and 63 ng/ml, compared to plasma levels of 381 and 723 ng/ml for the two compounds ($n = 2$). This resulted in B/P ratios of 0.17 and 0.09 for **38** and **40**, respectively.

In summary, further optimisation of the 2-phenylsulfanylphenyl piperazine acetic acid series leads to the identification of the *S*-proline derivatives **38** and **40** with improved in vitro potency in blocking the GlyT-1. In vitro and in vivo assessment of these compounds showed that CNS utility of these compounds might be diminished due to active efflux transporter activity relative to **1**.

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11. Preparation of noncommercially available starting materials **10b** was as follows: 5-chloro-2-iodo-anisole/5-bromo-2-iodo-anisole were prepared from the respective anilines using the Sandmeyer reaction employing (1) $NaNO_2/H_2SO_4/H_2O$, 5–10 °C, 30 min; (2) KI, rt, 1 h. 3-Chloro-2-iodo-anisole was prepared by treatment of 3-chloro-anisole with *s*-BuLi (1.3 M in cyclohexane)/THF, −95 °C , 1 h followed by addition of I_2, 16 h, rt. 4-Bromo-2-iodo-anisole was prepared by treatment of 4-bromo-anisole with $PhI(OAc)_2$, I_2, CH_3CN, N_2, 16 h.
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13. Compounds were dosed at 5 mg/kg sc and plasma and brain samples were taken after 70 min. Brains were collected by cervical dislocation, homogenised 1:4 (w/w) with 50% acetonitrile/water solution using an Ultraturrax homogenizer. The supernatant was separated by centrifugation (2000*g*, 10 min). Plasma and extracted brain supernatant were analysed by LC–MS/MS.