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The identification of novel orally active mGluR5 antagonist GSK2210875

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ABSTRACT

The identification of novel orally active mGluR5 antagonist GSK2210875 is described.

Antagonism of the type 5 metabotropic glutamate receptor (mGluR5) has therapeutic potential in a wide range of disease states,[1,2] including, for example, pain, anxiety, and drug dependency. Since the discovery of archetypal selective mGluR5 antagonist MPEP, more correctly described as a Negative Allosteric Modulator (NAM), there has been a huge effort industry wide to identify and develop mGluR5 antagonists suitable for clinical use (Fig. 1).[1,2] Much effort has focussed on acetylenic compounds related to MPEP itself, for example, Addex report[3] a positive PoC in migraine and gastroesophogeal reflux disease (GERD) for acetylenic/MPEP analogue compound ADX10059, and also on compounds bearing an acetylene equivalent or isostere, such as compound **1** exemplified by workers at Merck.[4] The previously known anxiolytic Fenobam has been demonstrated to be an mGluR5 antagonist[5] and a number of chemotypes containing its structural features have appeared in the patent literature.[1,2]

Latterly further distinct chemotypes such as the orally active piperidyl amides[6] (**2**) have been reported. However despite the substantial activity in this area the identification of safe tolerated and clinically efficacious mGluR5 antagonists remains an unfulfilled challenge and the search for novel and potential superior chemotypes continues. In this context, herein we report GSK2210875, a novel orally active low molecular weight mGluR5 antagonist.

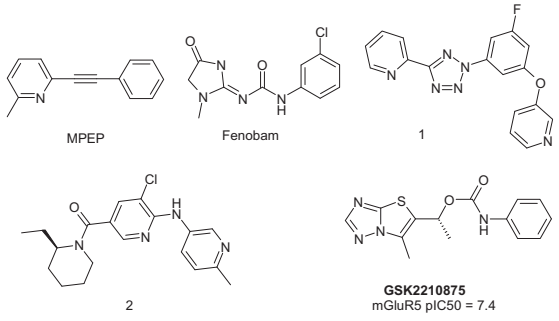

Figure 1. mGluR5 antagonists.

The GSK2210875 chemotype was identified from a High Throughput Screen (HTS) of the GlaxoSmithKline compound collection, and represented an attractive starting point for iteration based on its low molecular weight, relatively high polarity, ligand efficiency[7] (0.42), and selectivity over other mGluRs.

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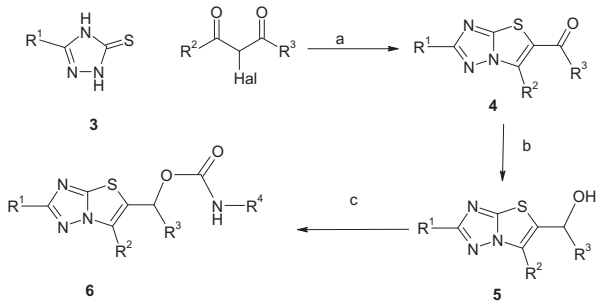
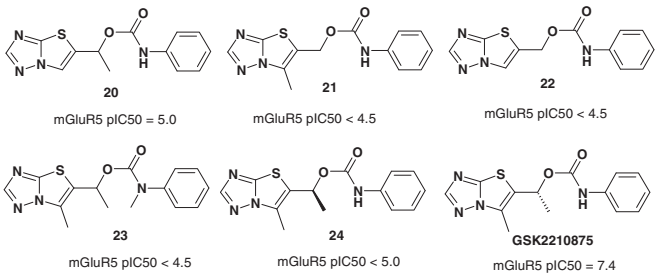


Scheme 1. Reagents and conditions: (a) Ethanol, μWave, 130 °C, 1 h; (b) $NaBH_4$, rt, 1 h; (c) RNCO, μWave, 80 °C, 20 min, or RNH_2, CDI, μWave, 120 °C, 20 min, 20–75%. nb product from (a) typically progressed directly to (b) without work-up or purification steps: Where appropriate enantiomers were separated by chiral HPLC as final compound or alcohol precursor.

Structure–activity relationships[8] (SAR) were established via synthesis of analogues according to Scheme 1 or simple variants thereof. 1,2,4-Triazole-thiones (**3**) were reacted with the appropriate α-halo-diketone (**4**) to afford heteroarylmethylketones (**5**) which were reduced to the corresponding alcohols and capped to afford final compounds (**6**).

It was established that mGluR5 potency (relative to racemic parent compound (**8**)) could be readily modulated by substitution of the triazolo ring (e.g., enhanced potency of thiophene (**16**) and cyclopropane (**15**)), and that whilst potency was not readily enhanced via substitution of the carbamate aryl ring, this region of the molecule was sensitive to structural changes (e.g., lower potency of 4-Cl phenyl analogue (**11**) (see Table 1)

Intriguingly both methyl groups of the chemotype play a key role in interactions with the mGluR5 receptor. Removal of either or both of the methyl groups essentially ablates activity (**20**–**22**), such a profound role for a methyl group suggests more than a simple lipophilic binding interaction with the receptor, but also likely a role in pre-organising the conformation of the ligand. N-Methylation of the carbamate likewise ablates activity (**23**). The single enantiomers of structure **8**, compounds **24** and GSK2210875, were separated by chiral HPLC and activity shown to reside in exclusively GSK2210875 (the R enantiomer as confirmed by VCD).[9]



Modifications to the triazole region of compounds of type **6** afforded modest gains in potency in the case of the corresponding imidazole (**25**), a modest decrease in potency in the case of the corresponding triazole isomer (**26**) and complete ablation of activity in the corresponding oxygen analogue (**27**).

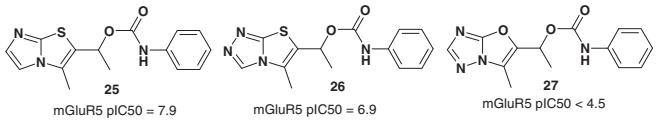
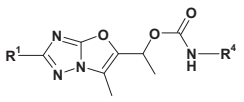


Table 1
Potency of carbamate and triazole variants



	R^1	R^4	mGluR5[8] pIC_{50}
7	H	Cyclohexyl	5.5
8	H	Phenyl	7.2
9	H	2-Cl-Phenyl	6.3
10	H	3-Cl-Phenyl	7.4
11	H	4-Cl-Phenyl	5.6
12	H	3,4-di-Cl Phenyl	5.3
13	H	3-F-Phenyl	7.3
14	Cl	Phenyl	8.0
15	Cyclopropyl	Phenyl	7.9
16	Thiophen-2-yl	Phenyl	8.1
17	4-F-phenyl	Phenyl	8.0
18	Methyl	Phenyl	7.4
19	Trifluoromethyl	Phenyl	7.4

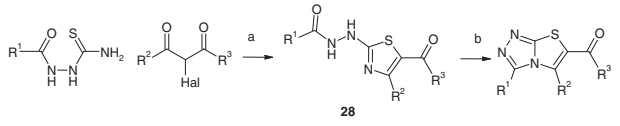
Alternative triazole isomers of type **26** were prepared according to the chemistry of Scheme 2, where the 2-hydrazido-thiazole (**28**) was cyclised with formic acid to form the key bicycle. Reduction of the ketone and capping of the alcohol to afford final compound proceeds as described in Scheme 1. The corresponding oxygen analogue (**27**) was prepared according novel chemistry developed in these laboratories.[10]

The mouse neophobia/marble burying anxiety model[11,12] was chosen as the principal pharmacodynamic (PD) model to establish oral activity. Experiments with MPEP and Fenobam had suggested that a free brain concentration of antagonist corresponding to ca. 10 times its pIC_{50} (i.e., ca. 90% theoretical receptor occupancy) would be required to achieve a significant effect in this model. We thus sought to identify compounds which could afford this profile at doses of <10 mg/kg.

The key interplay between potency and brain free fraction in achieving receptor occupancy has been elegantly demonstrated by Watson et al.[13] in dopamine D2 antagonists. We sought to obtain an optimal balance of these parameters by maximising a hybrid parameter pIC_{50}eff (Eq 1) which corrects the in vitro potency of a compound for the fraction which will be free/unbound in the brain[13] (fub) to afford a more realistic measure of potency in vivo.

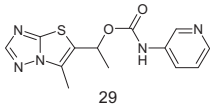
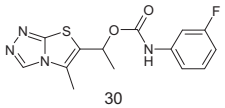
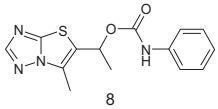
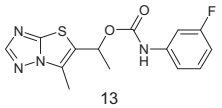
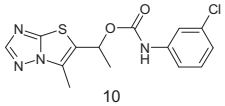
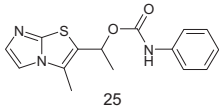
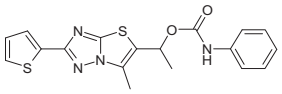
$$pIC_{50}\text{eff} = pIC_{50} + \log_{10}[\text{fub}] \quad (1)$$

The data of Table 2 demonstrate a clear positive correlation between mGluR5 potency and lipophilicity, and a negative correlation between potency and brain free fraction in compounds of type **6** such that the more polar compounds **8** and **30** afford the highest effective potency pIC_{50}eff, that is, the best balance between potency and free fraction. In more potent compounds such as **16** the higher potency does not compensate for the reduction in brain free fraction that the increased lipophilicity causes.



Scheme 2. Reagents and conditions: (a) EtOH, μwave, 130 °C, 1 h; (b) HCO_2H, PPA, 120 °C, 8 h, ~50%.

Table 2
Relationship between potency, brain free fraction (fub) and pIC_{50}eff

Compound (all as racemates)	mGluR5[8] pIC_{50}	log *D* (pH 7.4)	fub[14]	pIC_{50}eff
 29	6.5	1.0	28	5.9
 30	7.2	2.0	6.8	6.0
 8	7.1	2.3	8	6.0
 13	7.3	2.6	1.7	5.5
 10	7.4	2.9	0.9	5.4
 25	7.9	2.4	0.3	5.4
 16	8.1	3.7	<0.1	<5

Based on their effective potency **8** and **30** were progressed for further evaluation as their corresponding single enantiomers GSK2210875 and **31**.

In mouse astrocytes[19] concentration response curves (CRC) of L-quisqualate generated against increasing concentrations of compound **8** were indicative of a non-competitive interaction with the receptor as the dextral displacement of the agonist CRC occurred with a concomitant decrease of the maximal response are best described by an insurmountable profile[16] (Fig. 2). The potency of 7.3 ± 0.2 was determined using the operational model for non-competitive antagonists ($\alpha \ll 1$). Indeed MPEP radioligand[17] binding studies performed on the corresponding demonstrated that compound **8** binds to the classical MPEP allosteric site.[18]

Both GSK2210875 and **31** showed good solubility and selectivity over other metabotropic glutamate receptors (e.g., mGluRs1,2 and 4 in agonist and antagonist mode) and against another 40 targets in the GSK in-house selectivity panel (data not shown). In addition, in anticipation of progression to the neophobia model, both compounds demonstrated essentially indistinguishable potency at mouse[14] and human receptors.

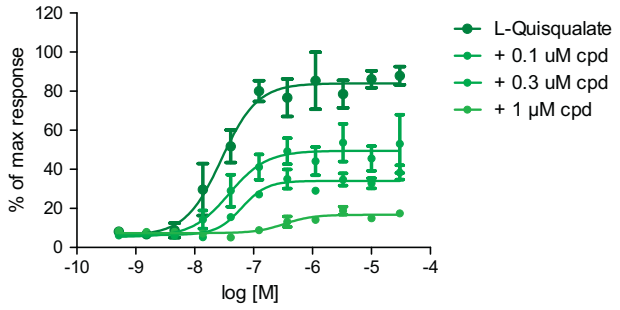
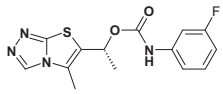
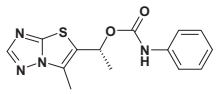


Figure 2. Concentration response curves of orthosteric mGluR5 agonist quisqualic acid raised to increasing concentrations of compound **8**.[15]

Table 3
Profile of **31** and GSK2210875

Compound	Human[8] mGluR5 pIC_{50}	Murine[15] mGluR5 pIC_{50}	mGluR1 pIC_{50}	Solubility (μg/mL)
 31	7.4	7.5	<4.3	31
 GSK2210875	7.4	7.5	4.5	166

Both GSK2210875 and **31** demonstrated excellent oral exposure and bioavailability in the rat (Table 3). Dissappointingly **31** showed poor brain penetration, despite no evidence for PGP efflux hence we hypothesize some other efflux mechanism, however GSK2210875 demonstrated excellent brain exposure. Indeed the values in Tables 3 and 4 would suggest a 1 mg/kg oral dose affords a brain C_{max} of ca. 250 nM and a free brain C_{max} of 30 nM equivalent to the compound's pIC_{50}, and hence that a 5–10 mg dose would afford the 5–10× pIC_{50} or 90% theoretical receptor occupancy appropriate for activity in the neophobia model.

In the mouse neophobia model[11,12] GSK2210875 did indeed demonstrate oral activity (Fig. 3) with a minimum effective dose of 5 mg/kg consistent with that predicted from potency/free fraction/exposure calculations above.

In summary a novel, selective, orally active MPEP competitive mGluR5 negative allosteric modulator GSK2210875 has been identified. Functional studies clearly demonstrated the insurmountable interaction with the native mGluR5 receptor and binding studies with the [^{3}H] MPEP radioligand evidenced the allosteric nature of this interaction. However our initial molecular modeling could

Table 4
In vivo pharmacokinetic data in rat (1.0 mg/kg po 0.5 mg/kg iv)

Compound	Clb (mL/min/Kg)	Oral blood C_{max} (ng/mL)	AUC brain:blood	*F* (%)
GSK2210875	48	63	2.0	28
31	33	210	0.2	100

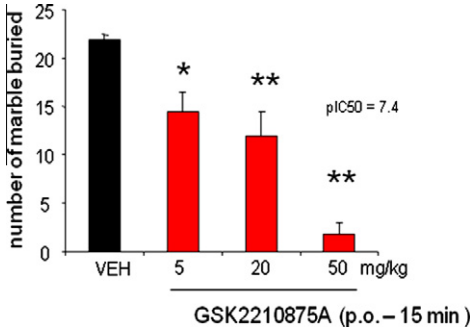


Figure 3. Effect of GSK2210875 in mouse neophobia after oral administration (statistical analysis: ANOVA $F(3,43) = 21.07$, $p < 0.001$; Dunnett's post hoc test, * = $p < 0.05$, ** = $p < 0.001$).

generate no plausible conformations which afforded a convincing overlap with known ligands and we thus postulate GSK2210875 exhibits a novel pharmacophoric interaction with the receptor.

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7. Ligand efficiency = $1.36 pIC_{50}$/#heavy atoms, see: Abad-Zapatero, C.; Metz, J. T. *Drug Discovery Today* **2005**, *10*, 464.
8. mGluR5 pharmacology characterised using an aequorin calcium mobilisation assay on the Lumilux. Assay utilises CHO cells stably expressing apo-aequorin and induced to express the human mGluR5 receptor using doxycycline. Eleven point concentration-response curves were generated in the presence of an EC_{80} of glutamate. pIC_{50} values are average of at least two curves.
9. The absolute configuration of GSK2210875 was determined by ab initio Vibrational Circular Dichroism (VCD). Solution-phase VCD and IR spectra were measured in $CDCl_3$ using a BioTools BOMEM Chiralir™ FT-VCD spectrometer operating at 4 cm^{-1} resolution and 50 scans per minute. Spectral data were acquired in the 2000–950 cm^{-1} region of the mid-infrared spectrum. The absolute configuration was assigned by comparing the experimental VCD spectrum to the VCD spectrum calculated for a full structure model with (*R*)-configuration. The model VCD spectrum was largely coincident with GSK2210875, indicating that this molecule has the same absolute stereochemistry as the model. Based on these data, GSK2210875A was assigned as the (1*R*)-enantiomer. See for example: Stephens, P. J., Devlin, F. J., Pan, J.-J. *Chirality* **2008**, *20*, 643–663.
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12. Fifteen minutes after oral administration of GSK2210875 (5, 20, 50 mg/kg) or vehicle (0.5% HPMC in water, 10 mL/kg), mice were individually placed in separate plastic cages (42.5 × 26.6 × 18.5 cm) containing 5 cm thick sawdust bedding. Twenty-four glass marbles were arranged evenly spaced onto the bedding (three rows of eight 1.5 cm marbles each). Thirty minutes later, mice were removed from the cage and the number of marbles buried (by at least two-thirds) was registered.
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15. Astrocyte cultures were plated for 2 days in 384 well plates (Greiner, black well, clear bottom). For mode of action studies cells were pre-incubated for 30 min with increasing concentrations of antagonist compounds. mGluR5 receptors were then challenged with concentration response curves of L-quisqualate and the intracellular Calcium release monitored in real-time on a FLIPR$^{TETRA®}$ system. For fpK_i determination cells were pre-incubated with antagonist CRCs and then challenged with the EC_{80} of L-quisqualate, determined from the agonist response curve of that day.
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19. CD1 mice were obtained from Charles River, Lecco, Italia. Astrocyte cultures were prepared from mouse pups aged 1–3 days. Mixed primary cultures were established from mouse cortices as previously described by Miller S. et al. (*J. Neurosci.* **1995**, 6103–6109) with the following modifications. Mouse pups were used instead of rat pups. After 5 days in culture cells were detached by trypsinization and re-plated to eliminate neurons from the mixed culture. Cultures were kept at 37 °C in a humidified CO_2 atmosphere until they reached confluence (10–14 d in vitro) prior being used in experiments.