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The identification of structurally novel, selective, orally bioavailable positive modulators of mGluR2

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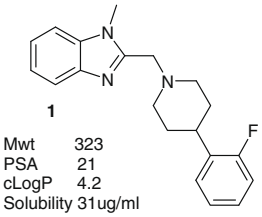
ABSTRACT

The optimisation of an HTS hit series (**1**) leading to the identification of structurally novel, selective, orally bioavailable mGluR2 positive modulators GSK1331258 and GSK1331268 is described. Structure–activity relationships, attenuation of dopaminergic activity, and potentiation of mGluR2 responses in rat hippocampal MPP-DG synapses are also reported.

There is a strong body of evidence that modulation of metabotropic glutamate receptors could afford novel approaches for the treatment of neurodegenerative and neuropsychiatric diseases.[1–4] The type 2 metabatropic glutamate receptor is of particular interest, indeed, a prodrug of the orthosteric mGluR2/3 agonist LY404039 (Lilly) exhibited statistically significant improvements in both positive and negative symptoms of schizophrenia in phase II trials.[5] Additionally a study using receptor deficient mice showed that antipsychotic effects of the mGluR2/3 receptor agonist LY404039 are dependent on mGluR2 and not mGluR3 receptors.[6]

Positive allosteric modulation could offer a superior mechanism of potentiating mGluR2 function than classical orthosteric agonism. The advantages positive modulation may afford, in terms of enhanced selectivity and spatial and temporal control, have been described extensively elsewhere[7,8] suffice it to say that this mechanism has received a great deal of attention in recent patent and scientific literature. Notable recent reports of mGluR2 positive modulators include the pioneering work of Lilly, exemplified by pyridylsulfonamides LY487379[10,9–13] and, a promising report[14] of compounds such as **2** from Pfizer (Fig. 2), which prompted us to report the work from our laboratories.

Herein we describe a novel series of 2-piperidine/piperazine-methylene-benzimidazole mGluR2 positive modulators derived from a high throughput screening (HTS) hit series exemplified by compound **1** (Fig. 1). The synthesis, structure–activity relationships (SAR) investigation, and the control of dopaminergic off-target activities of this series is described: culminating in the identification of the selective, orally bioavailable mGluR2 positive modulators GSK1331258 and GSK1331268.



mGluR2 Pos Mod	pEC50	6.9
mGluR2 Ag	pEC50	5.4
mGluR1 Ag	pEC50	< 4.8
mGluR1 Ant	pEC50	< 4.8
mGluR5 Ag	pEC50	< 4.8
mGluR5 Ant	pEC50	< 4.8
mGluR5 Pos Mod	pEC50	< 4.8
Dopamine D2	pIC50	7.4

Figure 1. Structure and profile of HTS hit compound **1**.

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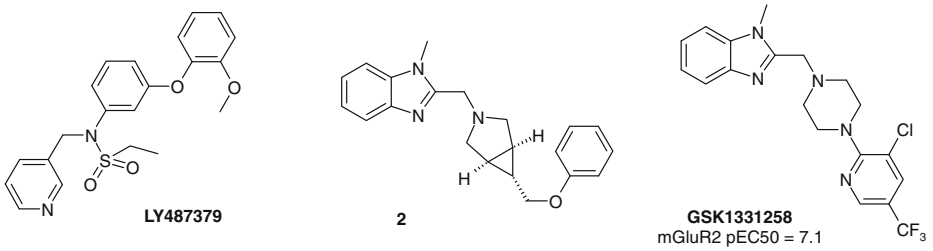
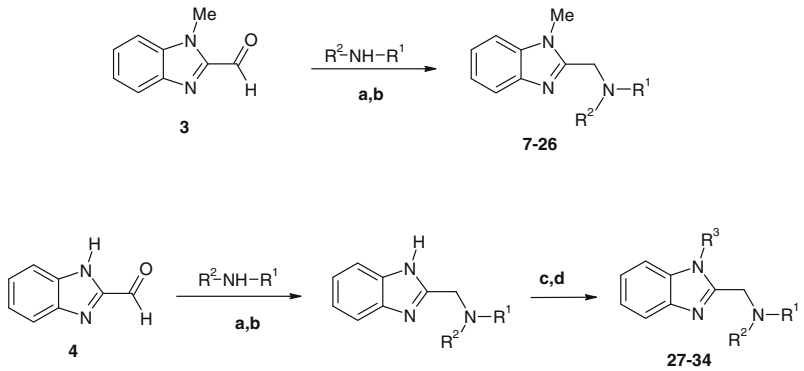


Figure 2. Exemplar mGluR2 positive modulators and GSK1331258.



Scheme 1. Reagents and conditions: (a) DCM/AcOH = 98:2 (V/V), rt, 15 min; (b) MP–$BH(OAc)_3$ (5.0 equiv), microwave irradiation, 110 °C, 10 min; 50–90%; (c) 60% NaH in mineral oil, DMF/THF = 30:70 (V/V), 10 min at rt; (d) R^3X, microwave irradiation, 100 °C, 10 min (X = Cl, Br), 40–75%.

From a HTS in a chimeric CHO cell line using a FLIPR readout[15] several promising mGluR2 modulator chemotypes were identified. Compound **1** was selected for further progression based on its displaying the best balance of pharmacology, selectivity, developability and properties suitable to afford good CNS penetration (Mol. wt <400, *c* Log *P* <4.5 and PSA <80). It was anticipated that dopaminergic activity associated with this template could be readily attenuated.

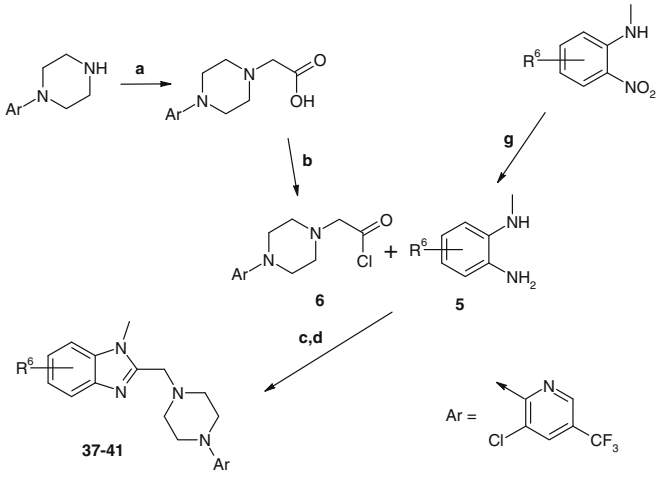
The desired mechanism of action of this compound/chemotype was confirmed by blockade of the positive modulation by the orthosteric mGluR2 antagonist[16] LY341495. A medicinal chemistry programme was initiated with the aim of identifying selective, orally bioavailable and brain penetrant mGluR2 positive modulators suitable for progression to studies in in vivo models.

Compounds were typically prepared according to the chemistry shown in Schemes 1 and 2.[17]

Simple reductive amination of 1-methyl-3-formyl benzimidazole (**3**) with appropriate amines allowed investigation of the aryl-piperazine/piperidine region of the template (Table 1). Variation of the benzimidazole N-substituent was achieved via a final step alkylation of intermediates generated by reductive amination of 2-formylbenzimidazole (**4**) (Table 2). Variation of the benzimidazole benzosubstituents (Table 2) was effected via reaction of *N*-methyl bis-anilines (**5**) and the corresponding piperazine/piperidine derived glycine (**6**).

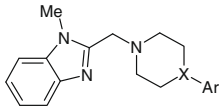
Table 1 illustrates the profiles of a representative set of analogues of lead compound **1**, in which the piperazine, and corresponding piperidine, aryl ring is modified. There are apparent differences in SAR between the piperazines and piperidines. Piperidines are typically more potent mGluR2 positive modulators than the corresponding piperazines (e.g., contrast the potency of the two simple phenyl analogues **7** and **17**) but in some instances potencies are comparable (e.g., 4-trifluoromethyl analogues **11** and **22**). Most compounds also demonstrated weak agonism of the receptor, this could be due to low level allosteric agonism (but effects due to contaminating traces of glutamate in the assay preparation cannot be discounted). Compounds typically potentiated an EC_{20} of glutamate to 70–100% of the glutamate EC_{100} response, but there is no apparent correlation between this efficacy of potentiation and pEC_{50}.

Close analogues of the chemotype exemplified by compound **1** are known to display activities at dopaminergic receptors,[18–20] hence it was important to investigate and attenuate any dopaminergic activity[21] such that dopaminergic pharmacology could not confound readout in any downstream in vivo models. Data in Table 1 illustrates that piperidine based analogues of compound **1** indeed typically demonstrated significant D_2 and D_3 antagonism. However this activity could be successfully attenuated in the



Scheme 2. Reagents and conditions: (a) $BrCH_2COOH$, MeCN, rt, overnight, 62%; (b) 1-chloro-*N*,*N*,2-trimethyl-1-propen-1-amine MeCN, rt, 40 min; (c) MeCN, 20 min at rt; (d) MeCN, microwave irradiation, 140 °C, 20 min; (b–d in one pot-11–25% overall); (g) Fe, NH_4Cl, 50% MeOH/H_2O (V/V), 2 h at reflux, 40–60%.

Table 1
Positive modulation of EC_{20} glutamate[15] at mGluR2 and dopaminergic antagonism[20] by piperazine and piperidine analogues

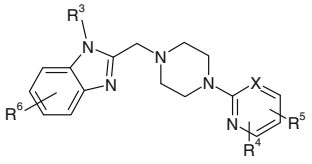


Compound	Ar	X	mGluR2 pos. mod. pEC_{50}	mGluR2 pos. mod. max.	mGluR2 agonist. pEC_{50}	D2 pK_i	D3 pK_i	D4 pK_i
7	Phenyl	CH	6.7	89	5.2	7.6	6.9	7.0
1	2-F-Phenyl	CH	6.9	91	5.4	7.4	7.0	
8	4-Chloro-phenyl	CH	7.1	91	5.3	7.8	6.5	7.2
9	2-Trifluoromethyl-phenyl	CH	6.5	94	5.2	<5.5	6.3	
10	3-Trifluoromethyl-phenyl	CH	7	94	5.6	6.9	<5	6.6
11	4-Trifluoromethyl-phenyl	CH	7	110	5.7	6.6	<5	6.9
12	3-Chloro-phenyl	CH	7	92	5.4	7.4	5.8	6.6
13	1,3-Benzothiazole-2-yl	CH	6.8	76	5.5	6.4	<5	8.1
14	1,2-Benzisothiazole-3-yl	CH	6.7	84	5.6	7.1	5.4	6.7
15	3-Chloro-5-(trifluoromethyl)-2-pyridinyl	CH	7.4	70	6.2	6.1	5.8	5.6
16	2-Pyridyl	CH	<5.5	n/a	<4.8			
17	Phenyl	N	5.3	100	<4.8			
18	2-F-Phenyl	N	6.1	64	5.2			
19	4-Chloro-phenyl	N	6.1		<4.8	<5	<5	6.9
20	2-Trifluoromethyl-phenyl	N	6.5	84	<4.8			6.2
GSK1331258	3-Chloro-5-(trifluoromethyl)-2-pyridinyl	N	7.1	71	5.7	5.3	5.3	6.2
GSK1331268	5-Chloro-2-pyrimidinyl	N	6.9	69	5.9	<5	<5	5.6
21	4-Chloro-2-fluoro-phenyl	N	6.9	69	5.6			6.6
22	4-Trifluoromethyl-phenyl	N	6.7	84	5.2	<5	<5	6.4
23	1,3-Benzothiazole-2-yl	N	6.5	88	5.7	<5	<5	6.1
24	5-(Trifluoromethyl)-2-pyridinyl	N	6.3	81	5.2	<5	<5	6.0
25	2-Pyridyl	N	4.8	100				
26	4-Cyanophenyl	N	<4.9	n/a				

majority of the piperazines. D_4 antagonism was more persistent within the analogues, but was reduced to a very low level in compounds such as GSK1331268.

Based on the data in Table 1, the 5-chloro-2-pyrimidinyl and 3-chloro-5-(trifluoromethyl)-2-pyridinyl substituted piperazine fragments of compounds GSK1331258 and GSK1331268 were selected for use in further studies (see Table 2), as they represented the best balance of high mGluR2 activity and low dopaminergic activity.

Table 2
FLIPR pEC_{50} positive modulator activity as potentiation of EC_{20} glutamate[15]



	X	R^3	R^4	R^5	R^6	mGluR2 pos. mod. pEC_{50}
27	C	–Et	2-Cl	5-CF_3	H	7.2
28	C	–CH_2Ph	2-Cl	5-CF_3	H	6.9
29	N	–CH_2Ph	5-Cl	H	H	6.9
30	C	–$(CH_2)_2OCH_3$	2-Cl	5-CF_3	H	6.8
31	N	–Et	5-Cl	H	H	6.8
32	N	–$(CH_2)_2OCH_3$	5-Cl	H	H	6.3
33	N	–nProp	5-Cl	H	H	6.1
34	C	–nProp	2-Cl	5-CF_3	H	5.6
35	C	–H	2-Cl	5-CF_3	H	4.9
36	N	–H	5-Cl	H	H	4.9
37	C	Me	2-Cl	5-CF_3	5-OMe	6.6
38	C	Me	2-Cl	5-CF_3	5-CN	7.3
39	C	Me	2-Cl	5-CF_3	5-CF_3	5.7
40	C	Me	2-Cl	5-CF_3	6-F	6.9
41	C	Me	2-Cl	5-CF_3	5-F	6.7

Compounds **27**–**34** (Table 2) illustrate that increasing the size of the benzimidazole N-substituent affords little enhancement of mGluR2 pharmacology. Interestingly the two N-unsubstituted analogues **35** and **36** have effectively no mGluR2 activity.

Table 2 also reports data for modifications to benzimidazole ring substitution of 3-chloro-5-(trifluoromethyl)-2-pyridinyl piperazines (**37**–**41**). Similarly to further Benzimidazole N-substitution, these ring substitutions conferred no significant improvement on mGluR2 pharmacology.

In contrast to the majority of analogues GSK1331258 and GSK1331268 demonstrated good in vitro metabolic stability (data not shown) and were selected for progression for in vivo pharmacokinetic investigation. In rat, in a 1 mg/kg oral cassette study both compounds showed a promising profile, viz. GSK1331258 exhibited low clearance (CLb 18.0 ml/min/kg), a half life of 4.1 h, a brain:blood ratio of 3.2 with brain C_{max} of 147 ng/g, and bioavailability of 58%; GSK1331268 exhibited moderate clearance (CLb 32.0 ml/min/kg), a half life of 1.0 h, a brain–blood ratio of 1.3 with brain C_{max} of 32 ng/g and bioavailability of 22%.

Furthermore GSK1331258 and GSK1331268 preserved the mGluR selectivity observed in initial hit compound **1**, with neither compound displaying significant activity at mGluRs1, 4 or 5 in agonist, antagonist or positive modulator modes (data not shown).

The pharmacology of GSK1331268 was characterised further on Medial Perforant-Path Dentate Gyrus (MPP-DG) Synapses in rat hippocampal slices,[22] in which synaptic transmission is inhibited by activation of group II mGluR receptors.[23]

Orthosteric agonist LY354740[24,25] inhibits MPP–DG fEPSP with an EC_{50} of 134 nM. Co-application of LY354740 with GSK1331268 (10 μM) shifts the concentration–response curve for LY354740 to the left by threefold, to 43 nM (see Fig. 3).

In summary a successful SAR investigation of a HTS hit series (**1**) was conducted. Understanding of factors controlling mGluR2 activity and dopaminergic activity allowed the identification of structurally novel, selective,[26] and orally bioavailable mGluR2 positive

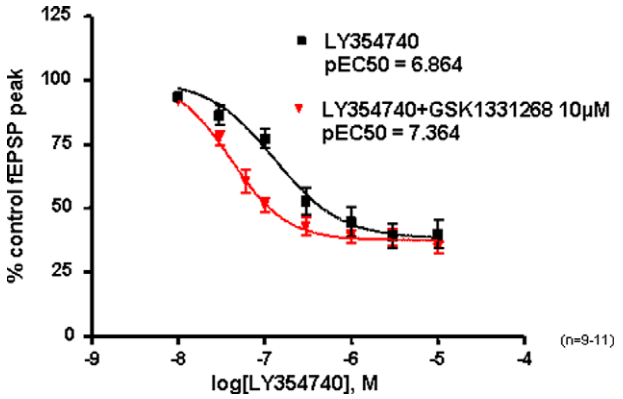


Figure 3. The effect of GSK1331268 on excitatory post-synaptic potentials (fEPSP) at MPP–DG synapses in rat hippocampal slices (data points indicate the mean ± -SEM of fEPSP peak amplitude expressed as a percentage of predrug control baseline; n = 9–11 slices per data point).

modulators GSK1331258 and GSK1331268. Indeed these compounds have suitable profiles for investigation of the effects mGluR2 positive modulation in in vivo disease models.

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