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# Carbamoyloximes as novel non-competitive mGlu5 receptor antagonists

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ABSTRACT

Hit-to-lead optimization of a HTS hit led to new carbamoyloxime derivatives. After identification of an advanced hit (**8d**) the CYP enzyme inhibitory activity of this class of compounds was successfully eliminated. Systematic exploration of different parts of the advanced hit led us to some promising lead compounds with mGluR5 affinities comparable to that of MPEP.



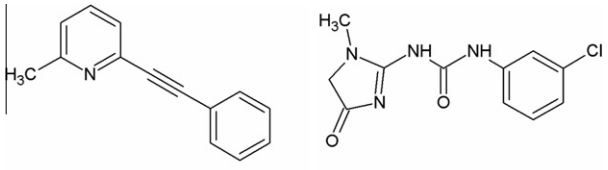
In the early 1990s, a new family of receptors mediating the intracellular metabolic effects of glutamate via coupling to secondary messenger systems was cloned. There are eight metabotropic glutamate (mGlu1–mGlu8) receptor subtypes known to date which have been clustered into three groups (I–III)[1] according to their amino acid sequence, pharmacology, and second-messenger coupling. In contrast to the glutamate-gated ion channels (NMDA, AMPA, and kainate receptors), which are responsible for fast excitatory transmission, mGlu receptors have been shown to play a modulatory role in the glutamatergic synaptic transmission either by modulating the ion channel activity or by influencing neurotransmitter release.[2] Since mGlu receptors are G-protein coupled, they obviously constitute a new attractive group of 'druggable' targets for the treatment of various CNS disorders.[3] The recent discovery of small molecules that selectively bind to group I (mGlu1 and mGlu5) and group II (mGlu2 and mGlu3) receptors[4] has significantly facilitated the understanding of their roles in brain physiology and pathophysiology.

Identification of the first selective, non-competitive mGluR5 antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP, **1**)[5] (Fig. 1) initiated the pharmacological evaluation of this class of compounds. Subsequent behavioral studies revealed that mGlu5 receptor antagonists exhibit profound anxiolytic effects in animal models and are remarkably free of the adverse effects characteristic of either benzodiazepines or iGlu receptor antagonists.[6] These preclinical data added support for the mGlu5 receptor as a potentially important therapeutic target for anxiety.[7]

Before metabotropic glutamate receptor subtypes were cloned, the non-GABAergic agent fenobam (**2**) (Fig. 1) was investigated in a double-blind, placebo-controlled clinical trial in which it showed efficacy and onset of action comparable with that of diazepam.[8] In 2005 Roche reported that fenobam, like MPEP, is a negative allosteric modulator of mGluR5[9] that provided clinical proof of principle for the mGluR5 approach in anxiety.

There is a large unmet medical need for new anti-anxiety agents that relieve symptoms quickly yet lack (benzodiazepine-like) side-effects. Recent findings have suggested an important role for the mGlu5 receptor in anxiolysis. Consequently, several pharmaceutical companies have initiated mGluR5 discovery programmes. The most important chemotypes of negative allosteric modulator of mGluR5 were reviewed recently.[10]

High throughput screening (HTS) of our corporate compound collection resulted in several hit clusters, which were further optimized as reported in our previous communication[11] and several patent applications.[12] This Letter describes the optimization of one structural family, the carbamoyloximes, represented by com-




MPEP, **1**
hmGluR5 $K_i$ = 36 nM

Fenobam, **2**
hmGluR5 $K_i$ = 6.7 nM

**Figure 1.** Proof of concept mGluR5 antagonists.

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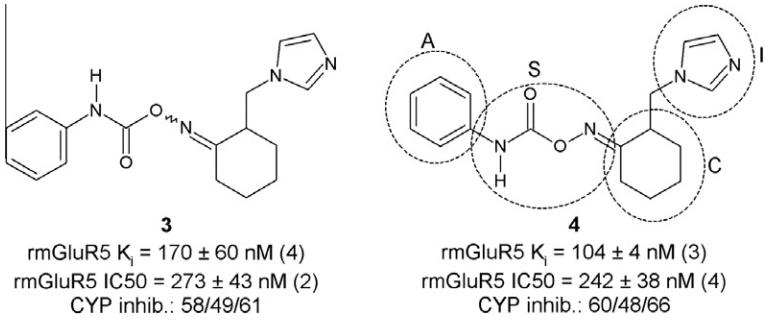
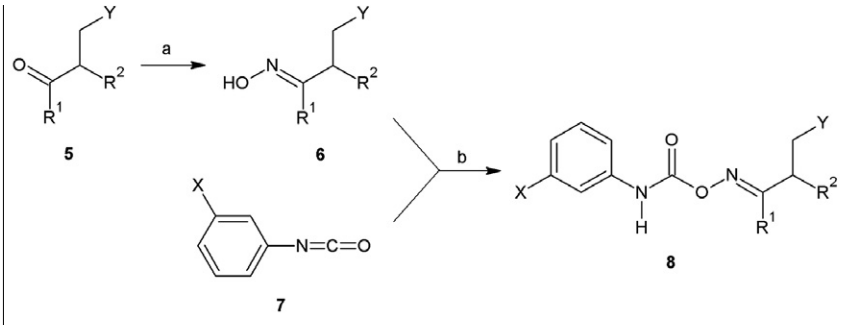



**Figure 2.** The HTS hit and its isomer served as starting point of hit-to-lead optimization. Binding and functional data (value ± SD (N)) and CYP inhibitory activity (inhibition%) were obtained as described in Refs. 13–15.

pound **3** (Fig. 2), which showed moderate affinity to mGluR5.[13] From functional assays these compounds were shown to be non-competitive antagonists.[14] The original HTS hit (**3**) was resynthesized yielding a 4:1 mixture of *E* and *Z* isomers having a $K_i$ value of 156 nM. Isomers were separated and the *E* isomer (**4**) shown to be the more active. The hit-to-lead process was started with compound **4**.

Multiple objectives were set for our hit-to-lead optimization, for this purpose, the hit compound **4** was divided into four regions (Fig. 2). These included identification of the optimal substitution pattern of the aromatic ring (A), optimal replacements for the imidazole ring (I) and the cyclohexane moiety (C), and finding the best spacer unit (S) and the active enantiomer. In order to answer these questions we systematically investigated the respective regions of our hit.

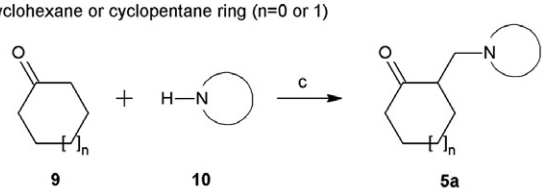
The synthesis of carbamoyloximes **8** was realized by preparing oximes **6** from suitable ketones **5** stirred under mild conditions in aqueous methanol with hydroxylamine (Scheme 1). The *Z* and *E* isomers of oximes **6** were then separated by crystallization or by column chromatography. In the second step, carbamoyloximes (**8**) were prepared from oximes **6** and commercially available appropriate phenylisocyanates **7** in dichloromethane. The final products were purified by crystallization or by column chromatography to >95% purity (HPLC, $^1$H, and $^{13}$C NMR). Depending on the Y substituents, the cyclic ketones **5** were synthesized by different pathways. If Y was a nitrogen heterocycle connected via its nitro-



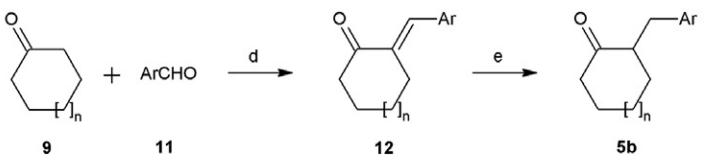

where
Y = aryl, (un)saturated heterocyclyl
$R^1$, $R^2$ = H, alkyl, or with the intermediate two carbon atoms together form a cyclohexane or cyclopentane ring

**Preparation of starting ketones:**

**a.** if Y = (un)saturated heterocyclyl connected via nitrogen; $R^1$ and $R^2$ with the intermediate two carbon atoms together form a cyclohexane or cyclopentane ring (n=0 or 1)




**b.** if Y = aryl or heteroaryl; $R^1$ and $R^2$ with the intermediate two carbon atoms together form a cyclohexane or cyclopentane ring (n=0 or 1)




**Scheme 1.** Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOAc, MeOH, $H_2O$, 45 °C, 2–8 h, then separation of *Z* and *E* isomers, 50–80%; (b) dichloromethane, rt, 6–24 h, 40–70%; (c) $CH_2O$, AcOH, 75 °C, 10 h, 50–70%; (d) NaOH, $H_2O$, rt, 12 h, 50–70%; (e) $H_2$ (1 bar), Pd/C, MeOH, rt, 3 h, 80–90%.

**Table 1**
Early optimization of **4**

| Compd | X | mGluR5 | | CYP inhib.[15] |
|---|---|---|---|---|
| | | $K_i$[13] (nM) | $IC_{50}$[14] (nM) | |
| **8a** | $CF_3$ | 78 ± 26 (2) | 775 ± 175 (3) | 61/57/58 |
| **8b** | CN | 38 ± 10.8 (2) | 561 ± 115 (3) | 50/32/53 |
| **8c** | F | 30.6 ± 4.78 (2) | 92.3 ± 37 (7) | 62/52/69 |
| **8d** | Cl | 10.8 ± 2.28 (2) | 116.5 ± 60 (9) | 61/53/61 |
| (+)-**8d** | Cl | 8.78 (1) | 76 ± 0 (3) | 50/51/45 |
| (−)-**8d** | Cl | >1000 | — | — |

Y = 1-imidazolyl; $R_1$, $R_2$ = cyclohexane, formed with the intermediate two carbon atoms; value ± SD (N).

**Table 3**
Replacement of imidazole to different heteroaryl groups

| Compd | X | Y | rmGluR5 | | CYP inhib.[15] |
|---|---|---|---|---|---|
| | | | $K_i$[13] (nM) | $IC_{50}$[14] (nM) | |
| **8o** | Cl | 2-Furanyl | 438 (1) | 130 ± 26 (9) | 7/0/0 |
| **8p** | Cl | 3-Thiophenyl | 13.8 ± 4.78 (3) | 477 ± 89 (3) | 14/0/0 |
| **8q** | Cl | 2-Thiophenyl | 9.1 ± 1.18 (2) | 109 ± 15 (4) | 0/0/0 |
| **8r** | F | 2-Thiophenyl | 12.3 ± 3.38 (2) | 46 ± 16 (5) | 17/0/1 |
| **8s** | Me | 2-Thiophenyl | 8.3 ± 1.88 (2) | 48 ± 14 (4) | 13/0/1 |
| **8t** | MeO | 2-Thiophenyl | 88 ± 7.8 (2) | 181 ± 43 (4) | 18/0/4 |

$R_1$, $R_2$ = cyclohexane, formed with the intermediate two carbon atoms; value ± SD (N).

gen atom (**5a**), cyclic ketones **9** and N-containing heterocycles **10** were reacted with formaldehyde in acetic acid. If Y was an aryl or heteroaryl group (**5b**), the reaction of cyclic ketones **9** and suitable arylaldehydes **11** was performed with NaOH in water to afford α,β-unsaturated ketones **12**, that were reduced with palladium under hydrogen in methanol. The structures of all intermediates and end products were confirmed by IR, NMR, and MS spectroscopy.[16]

Early optimization of **4** yielded compound **8d** as an advanced hit. In accord with the results of others,[10,17] we found that substituents at position 3 of the phenylcarbamoyl moiety increased the affinity to mGlu5 receptors (Table 1). Compound **8d** showed improved affinity relative to MPEP and was active in vivo as well (Fig. 3).[18] Its enantiomers were separated by preparative HPLC revealing the (+) enantiomer as the eutomer.

Although both the in vitro and in vivo activity of **8d** was promising, its CYP inhibitory activity[15] prompted us to either substitute the imidazole moiety or to replace it by other N-heterocycles.

Unfortunately, most modifications of the imidazole ring resulted in either inactive compounds (**8e–i**) or active compounds with retained CYP inhibitory activity (**8k, 8l** and **8n**) (Table 2). Substitution of the imidazole by pyridine resulted in active compounds (**8k–m**) in which the activity depended on the position of the pyridine nitrogen. The 2-pyridyl analog **8m** was the only compound lacking CYP inhibitory property that showed moderate in vitro activity. Compound **8l** was the most active derivative, but showed moderate CYP inhibition.

Another option was to substitute the imidazole for different heteroaryl groups that—in most cases—eliminated the CYP inhibition yet maintained, or in some cases increased, the affinity to mGluR5 receptors (Table 3). The good activity of furan (**8o**) and the excellent activities of the thiophene (**8p–s**) derivatives were somewhat surprising. These results suggested that the presence of at least one well positioned H-bond acceptor could replace the basic N (H-bond donor when protonated) in active compounds. Elimination of the CYP inhibitory activity was successful in the case of the highly active compounds **8q–s**.

It was interesting that mGluR5 affinity was maintained by replacing the imidazole with (substituted)phenyl groups. In these compounds essentially hydrophobic forces are expected to play a major role in binding. Non-specific hydrophobic contacts resulted in moderately active compounds (**8u**, **8w**). Substituents on the phenyl ring decreased activity (**8v**, **8x**, **8y**) (Table 4).

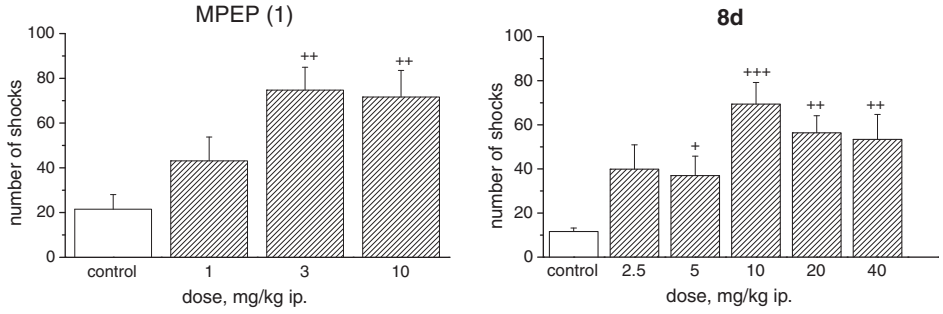



**Figure 3.** Effect of MPEP (**1**) and **8d** in the Vogel punished drinking test in rats. +, ++, and +++: $p$ <0.05, $p$ <0.01, and $p$ <0.001 versus control, respectively.

**Table 2**
Replacement of imidazole to N-heterocycles

| Compd | X | Y | rmGluR5 | | CYP inhib.[15] |
|---|---|---|---|---|---|
| | | | $K_i$[13] (nM) | $IC_{50}$[14] (nM) | |
| **8e** | H | 1-Piperidinyl | Inactive | — | — |
| **8f** | H | 4-Phenyl-piperazin-1-yl | >1000 | — | — |
| **8g** | H | Tetrahydro-isoquinoline-2-yl | >1000 | — | — |
| **8h** | Cl | 4-Morpholinyl | >1000 | — | — |
| **8i** | Cl | 2-Methyl-imidazol-1-yl | >1000 | — | 6/0/44 |
| **8j** | Cl | 4-Methyl-imidazol-1-yl | 43.7 ± 19.5 (4) | 311 ± 56 (4) | 42/0/58 |
| **8k** | Cl | 4-Pyridyl | 117 (1) | — | 56/60/46 |
| **8l** | Cl | 3-Pyridyl | 3.35 ± 0.5 (2) | 15 ± 7 (9) | 22/12/50 |
| **8m** | Cl | 2-Pyridyl | 40.4 ± 16.2 (7) | 65 ± 14 (3) | 0/0/27 |
| **8n** | Cl | 4-Pyrrolyl | 16.1 ± 4.6 (2) | 194 (1) | 44/0/14 |

$R_1$, $R_2$ = cyclohexane, formed with the intermediate two carbon atoms; value ± SD (N).

Next we aimed to investigate the role of the cyclohexane moiety in mGluR5 binding. The analog of **8d** with an unsubstituted cyclohexane ring (**13**) lost activity. Changing the benzylcyclohexane moiety to tricyclic systems (**14–16**) also eliminated mGluR5 affinity. Other modifications of the cyclohexane ring (e.g., tetrahydronaphthyl **17**, cyclopentyl **18**, and propyl **19**, **20**) generally resulted in much weaker compounds (Fig. 4). These observations support our hypothesis that in addition to diastereomeric and enantiomeric constraints this part of the carbamoyloximes is rather sensitive to conformational changes.

In the final part of our hit-to-lead program we modified the carbamoyloxime spacer. Thus, reduction of the oxime to hydroxylamine (**21**) decreased the affinity. Modification of the carbamoyloxime to semicarbazone (**22**) also resulted in an inactive compound (Fig. 5), indicating the carbamoyloxime moiety as a privileged moiety for mGluR5 binding.

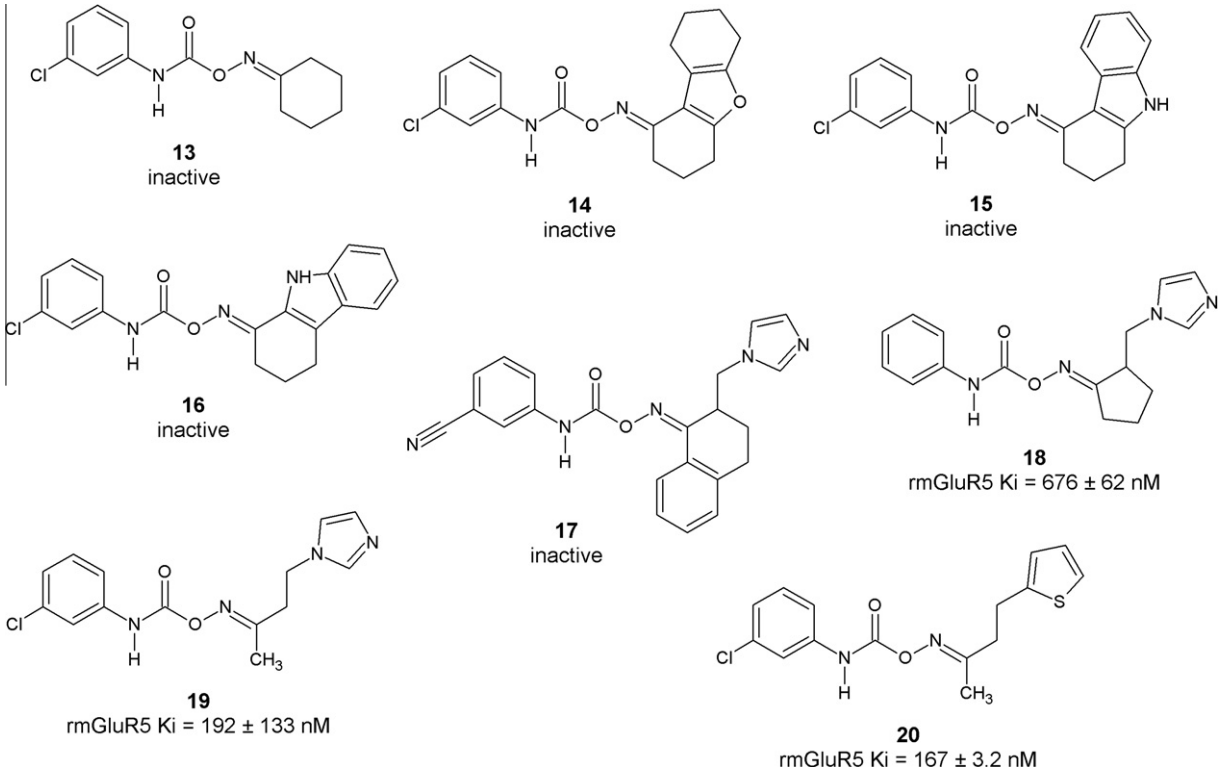



**Figure 4.** Modification of the cyclohexane ring; value ± SD ($n = 2$).

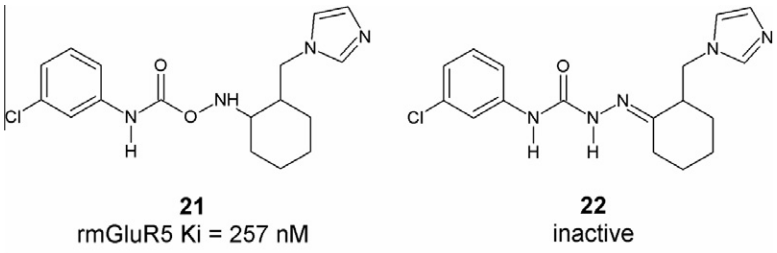



**Figure 5.** Modifications of the spacer ($n = 2$).

**Table 4**
Replacement of imidazole to (substituted) phenyl groups

| Compd | X | Y | rmGluR5 $K_i$ (nM) | | CYP inhib.[15] |
|---|---|---|---|---|---|
| | | | $K_i$[13] (nM) | $IC_{50}$[14] (nM) | |
| **8u** | Me | Phenyl | 29.7 ± 10.8 (3) | 152 ± 39 (4) | 0/0/0 |
| **8v** | Me | 3-F-phenyl | 56.4 ± 4 (2) | 224 ± 67 (15) | 9/0/0 |
| **8w** | Cl | Phenyl | 16 ± 1.4 (2) | 151 ± 37 (3) | 9/0/0 |
| **8x** | Cl | 3-F-phenyl | 39.5 ± 8.8 (2) | 127 ± 27 (5) | 0/0/0 |
| **8y** | Cl | 4-$Me_2N$-phenyl | >1000 | — | — |

$R_1$, $R_2$ = cyclohexane, formed with the intermediate two carbon atoms; value ± SD (N).

**Table 5**
Functional activity at mGluR1 (group I) and mGluR2 (group II)[19]

| Compd | mGluR1 inhib.% (10 μM) | mGluR2 inhib.% (10 μM) |
|---|---|---|
| **MPEP** | 15.7 ± 8.1 (2) | 10.0 ± 3.5 (4) |
| **8d** | 30.6 ± 10.1 (2) | −6.2 ± 3.0 (4) |
| (+)-**8d** | 26.9 ± 14.2 (2) | −6.7 ± 2.9 (4) |
| **8o** | 20.9 ± 11.9 (2) | 33.3 ± 7.6 (4) |
| **8p** | 15.2 ± 4.3 (2) | 22.8 ± 9.3 (4) |
| **8q** | 11.0 ± 4.2 (2) | 32.0 ± 4.9 (4) |

Value ± SD (N).

Functional activity data show high selectivity against other mGluR subtypes both in the same group I and also in group II as exemplified by mGluR1 and mGluR2, respectively (Table 5).

In summary, hit-to-lead optimization of a HTS hit identified carbamoyloxime derivatives as a novel class of non-competitive mGlu5 receptor antagonists. Early optimization of the validated hit (**4**) by identification of the optimal substitution pattern of the aromatic ring, resulted in an advanced hit (**8d**). Further optimization of **8d** successfully eliminated its CYP enzyme inhibitory activity. Modification of the cyclohexane ring, or the spacer diminished the affinity. Replacement of the imidazole ring by aryl or heteroaryl groups yielded active mGluR5 antagonists one of which (**8q**) was identified as a promising lead with affinity, and selectivity comparable to that of MPEP.

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13. The mGluR5 receptor binding was determined according to Gasparini et al. (Gasparini, F.; Andres, H.; Flor, P. J.; Heinrich, M.; Inderbitzin, W.; Lingenhöhl, K.; Müller, H.; Munk, V. C.; Omilusik, K.; Stierlin, C.; Stoehr, N.; Vranesic, I.; Kuhn, R. *Bioorg. Med. Chem. Lett.* **2002**, *2*, 407.) with modifications. Rat cerebrocortical membrane preparation was used to determine the binding characteristics of reference compounds and novel compounds to the rat mGluR5. As radioligand [$^3$H]-M-MPEP (2 nM) was used. The non-specific binding was determined in the presence of 10 μM M-MPEP. The ligand displacement by the compounds was determined in duplicates or triplicates. For $IC_{50}$ ($K_i$) determinations concentration–displacement curves were generated consisting of minimum of six concentrations. $K_i$ values (i.e., inhibition constants) were calculated using the Cheng–Prusoff equation: $K_i = IC_{50}/[1 + (L/K_d)]$, where [L] is the radioligand concentration and $K_d$ the affinity of the labeled ligand for receptor. $K_d$ was determined from the Scatchard plot.
14. Functional activity at mGluR5 was measured in primary rat neuronal cultures of neocortical origin, taking advantage of the high expression level of mGluR5 in this brain area (Romano, C.; Sesma, M. A.; McDonald, C. T.; O'Malley, K.; Van den Pol, A. N.; Olney, J. W. *J. Comp. Neurol.* **1995**, *355*, 455.). The neocortical cell cultures were prepared as described in Nagy et al. (Nagy, J.; Horváth, C.; Farkas, S.; Kolok, S.; Szombathelyi, Z. *Neurochem. Int.* **2004**, *44*, 17.) Functional activity at mGluR5 was measured by $Ca^{2+}$-fluorometry according to Nagy et al. with modifications. Briefly, cells were isolated from E17 rat embryos, seeded in 96-well plates and cultured at least for 5 days before being subjected to $Ca^{2+}$-measurements. For the $Ca^{2+}$-measurements cells were loaded with the $Ca^{2+}$-sensitive dye, fluo-4/AM. Baseline and agonist evoked signals were recorded with a plate reader fluorometer. Agonist was (*S*)-3,5-dihydroxyphenylglycine. For $IC_{50}$ determination sigmoidal (4-parameter) concentration–inhibition curves were fitted to the percent inhibition data derived from at least three independent experiments using GraphPad Prism software.
15. The CYP enzyme inhibition was determined according to Schoene et al. (Schoene, B.; Fleischmann, R. A.; Remmer, H. *Eur. J. Clin. Pharmacol.* **1972**, *4*, 65.) and Chhabra et al. (Chhabra, R. S.; Gram, T. E.; Fouts, J. R. *Toxicol. Appl. Pharmacol.* **1972**, *22*, 50.). CYP (cytochrome P450) enzyme inhibition is calculated as % of inhibition of CYP mediated enzyme reactions such as aminopyrine-N-demethylation/*p*-nitroanisole-O-demethylation/aniline-hydroxylation, respectively, in rat liver microsomes at nominal 10 μM test compound concentration.
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18. The Vogel punished drinking test was according to Vogel et al. (Vogel, J. R.; Beer, B.; Clody, D. E. *Psychopharmacology* **1971**, *100*, 138.) with modifications. Rats are deprived of drinking water for 48 h prior to test. 24 h prior to test they are placed into the test chambers equipped with a metal water spout mounted on the wall of the chamber and a metal grid floor for delivering electric shocks. During a 5-min adaptation period, they have free access to the drinking spout. On the day of the measurement, the animals are treated with the test compounds then placed into the test chambers where they have free access to drinking water for a 30-s unpunished period. After that, electric shocks (1 mA, 1 s) are applied through the drinking spout following every 10 licks during a 5-min punished period. Number of licks and shocks delivered are recorded and stored in a computer. Anxiolytic or anxiogenic activity is reflected by increased or decreased number of accepted shocks, respectively. Test compounds are studied minimally at three dose levels because of the occurrence of bell-shaped dose–response curves.
19. Functional activity at mGluR1 and mGluR2 was measured by $Ca^{2+}$-fluorometry according to Kurkó et al. (Kurkó, D.; Bekes, Z.; Gere, A.; Baki, A.; Boros, A.; Kolok, S.; Bugovics, G.; Nagy, J.; Szombathelyi, Z.; Ignácz-Szendrei, G. *Neurochem. Int.* **2009**, *55*, 467.) with slight modifications. Briefly, cells were seeded in 96-well plates 24 h before being subjected to $Ca^{2+}$-measurements. For the $Ca^{2+}$-measurements cells were loaded with the $Ca^{2+}$-sensitive dyes, fluo-4/AM (mGluR1) or FLIPR Calcium 5 kit (mGluR2). All buffers contained glutamate–pyruvate transaminase (3 μg/ml) and sodium pyruvate (2 mM). Baseline and agonist evoked signals were recorded with a plate reader fluorometer. Agonist was (*S*)-3,5-dihydroxyphenylglycine (mGluR1) or glutamate (mGluR2), administered at their respective $EC_{80}$ concentrations.