Bioorganic & Medicinal Chemistry Letters 20 (2010) 3737–3741

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Hit-to-lead optimization of disubstituted oxadiazoles and tetrazoles as mGluR5 NAMs

Gábor Wágner *, Csaba Wéber, Olga Nyéki, Katalin Nógrádi, Attila Bielik, László Molnár, Amrita Bobok, Attila Horváth, Béla Kiss, Sándor Kolok, József Nagy, Dalma Kurkó, Krisztina Gál, István Greiner, Zsolt Szombathelyi, György M. Keserű, György Domány

Gedeon Richter Plc, Budapest 10, PO Box 27, H-1475, Hungary

ARTICLE INFO

Article history:
Received 10 March 2010
Revised 16 April 2010
Accepted 16 April 2010
Available online 22 April 2010

Keywords:
mGluR5
Negative allosteric modulator
Parallel synthesis

ABSTRACT

Here we report the discovery and early SAR of a series of mGluR5 negative allosteric modulators (NAMs). Starting from a moderately active HTS hit we synthesized 3,5-disubstituted-oxadiazoles and tetrazoles as mGluR5 NAMs. Based on the analysis of ligand efficiency and lipophilic efficiency metrics we identified a promising lead candidate as a starting point for further optimization.

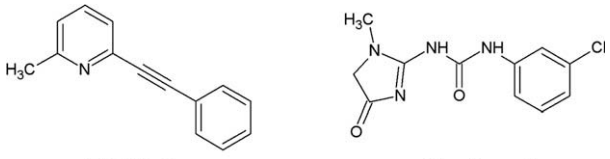
Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system (CNS) and binds to neurons in the CNS, thereby activating cell surface receptors. These receptors can be divided into two major classes, ionotropic and metabotropic glutamate receptors, based on the structural features of the receptor proteins.

Metabotropic glutamate receptors are a family of eight G-protein coupled receptors which are classified into three groups according to their sequence homology, effector coupling and pharmacology. Group I mGlu receptors (mGluR1 and mGluR5) are positively coupled to phospholipase C; group II mGlu receptors (mGluR2 and mGluR3) and group III mGlu receptors (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to adenylate cyclase.[1]

Activation of group I mGlu receptors leads to a transient increase in intracellular calcium via the production of inositol-trisphosphate. Generally, it has been shown that activation of group I receptors enhances or facilitates the excitatory effects of glutamate by modulation of ion channel activity.[2] Although group I mGlu receptors are related phylogenetically, both mGlu1 and mGlu5 receptors have a distinct expression pattern in the brain, which clearly suggests their different roles in nervous system function. mGlu5 receptors are found most abundantly throughout the cerebral cortex, hippocampus, caudate-putamen, nucleus accumbens, and lamina I–III of the spinal cord.[3]

A large body of preclinical data were reported with mGluR5-antagonists and mGluR5 knock-out mice emphasising the mGlu5 receptor as a potentially important therapeutic target for several CNS disorders including anxiety,[4] pain,[5] depression,[6] epilepsy,[7] neurodegeneration,[8] Parkinson's disease[9] and cocaine-dependence.[10] There is a large unmet medical need for new anti-anxiety agents that relieve symptoms quickly and have no (benzodiazepine-like) side effects. Recent findings have suggested an important role for the mGlu5 receptor in anxiolysis.

According to the literature, the first selective non-competitive mGluR5 antagonist compound, 2-methyl-6-(phenylethynyl)pyridine (MPEP, **1**)[11] has a very broad and potent anxiolytic-like activity in rodent models of anxiety. It has a short onset of action and lacks the potential to induce sedation or psychotomimetic effects, in contrast to the benzodiazepines.[12] (Fig. 1) Before cloning of metabotropic glutamate receptor subtypes, the non-GABAergic



MPEP, **1**
hmGluR5 $pK_i = 7.44$

Fenobam, **2**
hmGluR5 $pK_i = 8.17$

Figure 1. Proof of concept mGluR5 antagonists.

* Corresponding author.
E-mail address: g.wagner@richter.hu (G. Wágner).

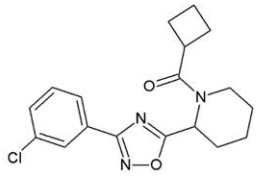
0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.075

agent fenobam (**2**) was investigated in a double-blind placebo-controlled clinical trial and showed efficacy and onset of action comparable with diazepam.[13] In 2005 Roche reported that similar to MPEP, fenobam is a negative allosteric modulator of mGluR5[14] that provided clinical proof of principle for the mGluR5 approach in anxiety. Consequently, several pharmaceutical companies have initiated mGluR5 discovery programmes.[15]

The high throughput screening (HTS) of our corporate compound library resulted in several hits. This Letter describes the hit-to-lead optimization process of 3,5-disubstituted-oxadiazoles represented by compound **3**. (Fig. 2) Optimization of other clusters were reported in several patent applications.[16]

Multiple objectives were set for our hit-to-lead optimization, including the identification of the optimal central heterocycle, the cyclic secondary amide, the substitution pattern of the aromatic ring etc. In order to achieve these goals we utilized a parallel synthesis strategy, synthesizing both oxadiazoles and tetrazoles as racemates. The hit-to-lead process was monitored calculating size independent ligand efficiency (SILE)[19] and a lipophilic ligand efficiency metrics (LELP)[20] recently introduced by us. LELP was defined as the logP/LE ratio indicating the price of ligand efficiency paid in logP. Consequently, the higher the absolute value of LELP the less drug-like the lead compound.

Scheme 1 demonstrates the synthetic pathways leading to the oxadiazole series.[21] The synthesis of amines **8** was realized by preparing amidoximes **6** from suitable nitriles **5** refluxed in methanol with hydroxylamine, followed by acylation with Boc-protected pipecolic acid, nipecotic acid, proline or thioproline under mild conditions.[22] Cyclocondensation of *O*-acylamidoximes using tetrabutylammonium fluoride then provided the oxadiazoles ring[23] (**7**).



3

rmGluR5 pK_i = 6.69 [17]

rmGluR5 pIC50 = 5.93 [18]

SILE = 3.91 [19]

LELP = 3.80 [20]

Figure 2. The HTS hit served as starting point of hit-to-lead optimization.

Finally, amines **8** were obtained by deprotection of **7**. In the last step we used parallel synthesis to prepare final products (**9**). Acylation of the secondary amines **8** can be accomplished with carboxylic acids (RCOOH) activated by EDC in the presence of a base (e.g. TEA). Various commercially available substituted or unsubstituted alkyl-, cycloalkyl-, aryl-, heteroaryl-carboxylic acids were used.

Final products (**9**) can be obtained in appropriate purity therefore after concentration of the solution. Biological experiments were carried out without further purification. All compounds were characterized by LC–MS.

Yields and purity data of the parallel synthetic step are summarized in Table 1. The purity of most oxadiazoles was greater than 95%, and yields were also sufficient.

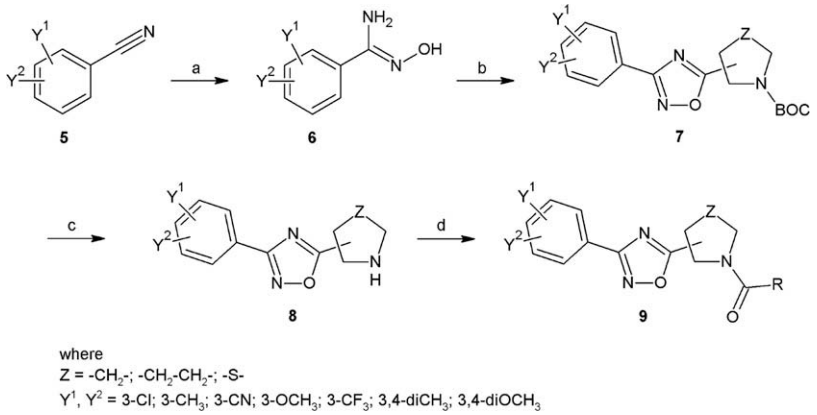
For the final acylation step, 15 amines and 61 acids were selected, 915 reactions were performed and 701 endproducts were isolated, 656 compounds fulfilled the purity criterion (>85%, LC–MS) and were tested. The K_i values of the compounds,[22] with only a couple of exceptions, were above 200 nM, Affinities and functional activities of the most active compounds are presented in Table 2.

The 2-piperidinyl, 2-pyrrolidinyl, and 4-thiazolidinyl derivatives were found to be the most active compounds, while 3-piperidinyl derivatives were generally inferior. The optimal acyl groups depended on the nature of the saturated heterocycles. The 2-piperidines with cycloalkylcarbonyl groups, (like cyclobutylcarbonyl (**9c**, **9e**) and cyclopentylcarbonyl) gave active compounds while 2-pyrrolidines acylated with 2-furoyl and 2-thiophenecarbonyl (**9b**, **9d**) groups had higher affinity towards the target. The

Table 1
Purity and yield data of for the final products (**9**) obtained by parallel synthesis

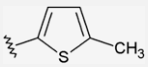
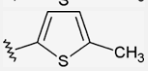
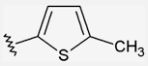
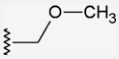
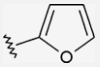
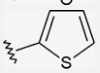
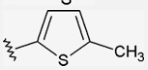
Core	Cyclic sec. Amine (num. of subtypes)	Num. of compound	Purity (%)			Yield (%)		
			>95	85–95	<85	>60	30–60	<30
Oxa	3-Pip (8)	274	71.5	22.3	6.2	57.3	34.7	8
Oxa	2-Pip (4)	244	75	19.7	5.3	43.4	42.2	14.4
Oxa	2-Pyr (3)	183	69.4	22.4	8.2	32.2	48.1	19.7
Oxa	Sum	701	72.1	21.4	6.4	45.9	40.8	13.3
Tet	3-Pip (2)	57	89.5	10.5	0	15.8	63.2	21
Tet	2-Pip (10)	344	78.2	20	1.7	13.4	57.3	29.4
Tet	2-Pyr (9)	228	53.1	45.2	1.7	31.1	53.5	15.4
Tet	Sum	629	70.1	28.3	1.6	20	56.4	23.5
	Total	1330	71.2	24.7	4.1	33.7	48.2	18.1

Oxa, oxadiazoles; Tet, tetrazoles; pip, piperidines; pyr, pyrrolidines; num, number; comp, compounds.



Scheme 1. Reagents and conditions: (a) Hydroxylamine, K_2CO_3, MeOH, rt, 3 h, 90%; (b) Boc-pipecolic acid or Boc-nipecotic acid or Boc-proline or Boc-thioproline, *i*-butylchloroformate, N-Me-morpholine, DMF, 0 °C, 30 min, then tetrabutylammonium fluoride, 0 °C, 3 h, 60%; (c) HCl, EtOAc, rt, 3 h, 50–70%; (d) RCOOH, EDC, TEA, CH_2Cl_2, rt, overnight, parallel synthesis.

Table 2
Structures, activities and ligand efficiencies of the most potent oxadiazoles

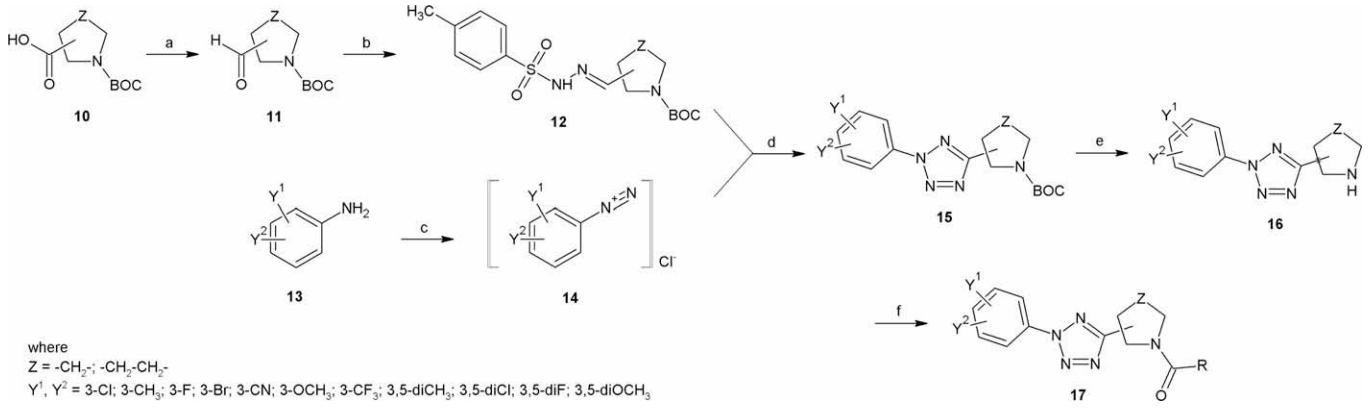
Compound	Y		R	rmGluR5		SILE[19]	LELP[20]
				pK_i[17]	pIC50[18]		
9a	3-Cl	4-Thiazolidinyl		7.08	6.12	3.79	5.70
9b	3-Cl	2-Pyrrolidinyl		6.91	5.18	3.86	5.05
9c	3-Me	2-Piperidinyl		6.90	6.31	4.26	3.02
9d	3-Me	2-Pyrrolidinyl		6.84	6.00	4.00	4.32
9e	3-MeO	2-Piperidinyl		6.74	6.30	3.93	3.09
9f	3-Me	2-Pyrrolidinyl		6.73	4.52	4.15	1.77
9g	3-Cl	4-Thiazolidinyl		6.71	5.98	3.60	4.19
9h	3-Cl	2-Pyrrolidinyl		6.71	4.52	3.74	4.50
9i	3-CN	2-Piperidinyl		6.70	5.76	3.59	5.61

most active compound of this series was, however, a thiazolidine derivative **9a** containing a thiophene-2-carbonyl group.

This compound showed almost a 0.5 pK_i unit improvement in binding affinity while its ligand efficiency and lipophilic efficiency were weaker than that of the HTS hit. Analysing SILE and LELP values we concluded that despite of their somewhat reduced affinity **9c** and **9f** could be considered as more promising leads. Higher SILE and lower LELP of these compounds suggest those being better starting points for further optimizations.

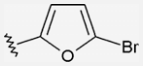
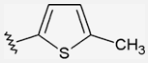
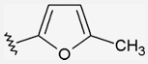
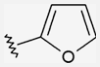
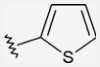
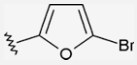
The modification of the central heterocycle of the original HTS hit (**3**) to tetrazole, a known oxadiazole bioisostere, resulted in a second starting point for lead discovery **17c**. Since all the compounds synthesized in the oxadiazole library were racemates, the first question was whether enantiomers have different biological activities. Since the synthetic protocol to **17c** (Scheme 2)[24] was not affected by stereochemical inversion we were able to prepare its pure enantiomers with known absolute configuration. Testing the enantiomers of **17c** revealed that the *R* enantiomer is the active isomer (*R*-**17c**; optical purity: >90%, rmGluR5 pK_i: 7.24; *S*-**17c**; optical purity: >90%, rmGluR5 pK_i: 6.13). After having prepared several other pairs, we concluded that this observation could be extended to the whole chemical series and therefore mostly racemic compounds were prepared during this stage of hit-to-lead optimization.

After having established the SAR of the oxadiazole library, we focused on the synthesis of the more promising tetrazole library (Scheme 2). Aldehydes of formula **11** were prepared by the reduction of Weinreb amides and were not isolated. Weinreb amides were prepared by reacting Boc-protected amino acids (**10**) with *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of DIPEA, DCC and DMAP[25] and were subsequently reduced in anhydrous THF with LAH. Hydrazones **12** were prepared by reacting aldehydes **11** with *p*-toluenesulfonhydrazide in ethanol.[26] Diazonium salts **14** were obtained from suitable substituted anilines **13**, and converted to tetrazoles **15** by reaction with hydrazones **12** in aqueous ethanol with sodium hydroxide.[27] Amines **16** were



Scheme 2. Reagents and conditions: (a) i—*N*,*O*-dimethylhydroxylamine hydrochloride, DIPEA, DCC, DMAP, 0 °C, 30 min, then rt, 3 h, 85%; ii—LAH, THF, 0 °C, 10 min, 90%; (b) *p*-toluenesulfonhydrazide, ethanol, 25 °C, 30 min, 90%; (c) $NaNO_2$, hydrochloric acid, water, 0 °C; (d) NaOH, aqueous ethanol, 0 °C, 30 min, then 25 °C, 1 h, 60%, two steps (c and d); (e) HCl, EtOAc, rt, 3 h, 80–90%; (f) RCOOH, EDC, TEA, CH_2Cl_2, rt, overnight, parallel synthesis.

Table 3
Structures, activities and ligand efficiencies of the most potent tetrazoles.

Compound	Y		R	rmGluR5		SILE[19]	LELP[20]
				pK_i[17]	pIC50[18]		
17a	3-Cl	2-Piperidinyl		7.31	6.67	3.78	4.31
17b	3-Me	2-Piperidinyl		7.14	6.65	4.17	2.87
17c	3-Cl	2-Piperidinyl		7.11	6.47	3.96	3.42
17d	3-Cl	2-Piperidinyl		7.06	6.53	3.94	3.84
17e	3-Cl	2-Pyrrolidinyl		7.05	6.60	3.78	4.72
17f	3-Me	2-Piperidinyl		6.99		4.08	3.28
17g	3-Cl	2-Pyrrolidinyl		6.98		3.74	3.07
17h	3-Cl	2-piperidinyl		6.90		3.70	3.55
17i	3-Cl	2-Piperidinyl		6.86	6.42	3.83	3.65
17j	3-CN	2-Piperidinyl		6.64		3.70	2.92
17k	3-Cl	3-Piperidinyl		6.50		3.48	4.53
17l	3-F	2-Piperidinyl		6.34		3.28	4.43

obtained by deprotecting the basic nitrogen of **15**. Acylation of the secondary amines **16** was accomplished with acids (RCOOH) activated by EDC in the presence of a base (e.g. TEA) in a parallel synthesis setup. The final tetrazole products **17** were characterized by LC–MS (Table 1). Purities of the tetrazoles were similar to those of the oxadiazoles, but yields were somewhat lower.

Before the preparation of the tetrazole library orthogonal combinations of 21 amines and 85 acids were synthesized and tested. Binding affinity data were used selecting the best 24 acids used for the acylation of the amines during library preparation.

Altogether 629 tetrazoles were obtained; those 619 that fulfilled the purity criterion were tested. Binding data of the most active subset are shown in the Table 3. Similarly to the oxadiazole series 2-piperidyl derivatives were more active than the 3-piperidyl analogues. With suitable acyl parts, the 2-pyrrolidinyl derivatives could also have acceptable affinity.

In the piperidinyl series the cyclobutylcarbonyl **17b**, **17c** and the cyclopentylcarbonyl **17d** groups were the best ones, but in one case 5-bromofurane-2-carbonyl substitution (**17a**) resulted in a compound with remarkable affinity. In the pyrrolidinyl series the most active compound was obtained by the acylation with 5-methylthiophene-2-carboxylic acid (**17e**). Substitution at position 3 of the benzene ring was generally tolerated; the best compounds contained Cl or Me at this position.

Despite of its improved binding affinity ligand efficiency and lipophilic efficiency of the most active compound (**17a**) were inferior to that of the HTS hit. Comparative analysis of these metrics revealed that **17b** would be a more promising lead having improved binding affinity and efficiency profile.

In summary, HTS of our corporate compound library identified a 3,5-disubstituted-oxadiazole (compound **3**) as moderately active non-competitive mGluR5 antagonist. Structural modification of the hit led to new 1,3 disubstituted heterocycles with reasonable mGluR5 affinities. Two central heterocycles were investigated in detail. During the hit-to-lead process we improved the binding affinity of the original HTS hit by about 0.4 and 0.6 pK_i unit (2.5-fold and 5-fold) in both the oxadiazole (compound **9a**) and tetrazole (compound **17a**) series, respectively. Our results confirmed that tetrazoles are generally more active than their oxadiazole analogues.[28] Analysing SILE and LELP data we identified the tetrazole **17b** as having improved affinity, ligand efficiency and lipophilic efficiency relative to the original HTS hit. This lead was considered as a promising starting point for further optimization.

References and notes

1. (a) Schoepp, D. D.; Conn, P. J. *Trends Pharmacol. Sci.* **1993**, *14*, 13; (b) Schoepp, D. D. *Neurochem. Int.* **1994**, *24*, 439; (c) Pin, J. P.; Duvoisin, R. *Neuropharmacology* **1995**, *34*, 1; (d) Bordi, F.; Ugolini, A. *Prog. Neurobiol.* **1999**, *59*, 55.
2. Alagarsamy, S.; Sorensen, S. D.; Conn, P. J. *Curr. Opin. Neurobiol.* **2001**, *11*, 357.
3. Spooren, W. P.; Gasparini, F.; Salt, T. E.; Kuhn, R. *Trends Pharmacol. Sci.* **2001**, *22*, 331.
4. Spooren, W. P.; Vassout, A.; Neijt, H. C.; Kuhn, R.; Gasparini, F.; Roux, S.; Porsolt, R. D.; Gentsch, C. J. *Pharmacol. Exp. Ther.* **2000**, *295*, 1267.
5. Walker, K.; Reeve, A.; Bowes, M.; Winter, J.; Wotherspoon, G.; Davis, A.; Schmid, P.; Gasparini, F.; Kuhn, R.; Urban, L. *Neuropharmacology* **2001**, *40*, 10.
6. Tatarczyńska, E.; Klodzińska, A.; Chojnacka-Wójcik, E.; Palucha, A.; Gasparini, F.; Kuhn, R.; Pilc, A. *Br. J. Pharmacol.* **2001**, *132*, 1423.
7. Chapman, A. G.; Nanan, K.; Williams, M.; Meldrum, B. S. *Neuropharmacology* **2000**, *39*, 1567.
8. (a) Bruno, V.; Ksiazek, I.; Battaglia, G.; Lukic, S.; Leonhardt, T.; Sauer, D.; Gasparini, F.; Kuhn, R.; Nicoletti, F.; Flor, P. J. *Neuropharmacology* **2000**, *39*, 2223; (b) Movsesyan, V. A.; O'Leary, D. M.; Fan, L.; Bao, W.; Mullins, P. G.; Knoblach, S. M.; Faden, A. I. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 41.
9. Ossowska, K.; Konieczny, J.; Wolfarth, S.; Wierońska, J.; Pilc, A. *Neuropharmacology* **2001**, *41*, 413.

10. Chiamulera, C.; Epping-Jordan, M. P.; Zocchi, A.; Marcon, C.; Cottiny, C.; Tacconi, S.; Corsi, M.; Orzi, F.; Conquet, F. *Nat. Neurosci.* **2001**, *4*, 873.
11. Allgeier, H.; Auberson, Y.; Biollaz, M.; Cosford, N. D.; Gasparini, F.; Heckendorn, R.; Johnson, E. C.; Kuhn, R.; Varney, M. A.; Velicelebi, G. WO 9902497, **2007**.
12. O'Brien, J. A.; Lemaire, W.; Wittmann, M.; Jacobson, M. A.; Ha, S. N.; Wisnoski, D. D.; Lindsley, C. W.; Schaffhauser, H. J.; Rowe, B.; Sur, C.; Duggan, M. E.; Pettibone, D. J.; Conn, P. J.; Williams, D. L., Jr. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 568.
13. Pecknold, J. C.; McClure, D. J.; Appeltauer, L.; Wrzesinski, L.; Allan, T. J. *Clin. Psychopharmacol.* **1982**, *2*, 129.
14. Porter, R. H.; Jaeschke, G.; Spooren, W.; Ballard, T. M.; Büttelmann, B.; Kolczewski, S.; Peters, J. U.; Prinssen, E.; Wichmann, J.; Vieira, E.; Mühlemann, A.; Gatti, S.; Mutel, V.; Malherbe, P. J. *Pharmacol. Exp. Ther.* **2005**, *315*, 711.
15. Jaeschke, G.; Wettstein, J. G.; Nordquist, R. E.; Spooren, W. *Expert Opin. Ther. Patents* **2008**, *18*, 123.
16. (a) Galambos, J.; Vastag, M.; Bobok, A. Á.; Keserű, G. M.; Gál, K.; Benkő, B.; Rill, A.; Demeter, Á. WO 2007072089, **2007**.; (b) Nógrádi, K.; Wágner, G. A.; Keserű, G. M.; Bielik, A.; Gáti, T.; Háda, V.; Kóti, J.; Gál, K.; Vastag, M.; Bobok, A. Á. WO 2007072090, **2007**.; (c) Nógrádi, K.; Keserű, G. M.; Bielik, A.; Gáti, T.; Gál, K.; Vastag, M.; Bobok, A. Á. WO 2007072091, **2007**.; (d) Keserű, G. M.; Wéber, C.; Bielik, A.; Bobok, A. Á.; Gál, K.; Meszlényiné S. M.; Molnár, L.; Vastag, M. WO 2007072093, **2007**.; (e) Nógrádi, K.; Wágner, G. A.; Keserű, G. M.; Bielik, A.; Gáti, T.; Háda, V.; Kóti, J.; Gál, K.; Vastag, M.; Bobok, A. Á. WO 2007072094, **2007**.; (f) Nógrádi, K.; Wágner, G. A.; Keserű, G. M.; Bielik, A.; Gáti, T.; Háda, V.; Kóti, J.; Gál, K.; Vastag, M.; Bobok, A. Á. WO 2007072095, **2007**.
17. 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 nonspecific binding was determined in the presence of 10 μM M-MPEP. The ligand displacement by the compounds was determined in duplicates or triplicates. For IC50 (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 labelled ligand for receptor. K_d was determined from the Scatchard plot.
18. 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.
19. Nissink, J. W. M. J. *Chem. Inf. Model.* **2009**, *49*, 1617.
20. Keserű, G. M.; Makara, G. M. *Nat. Rev. Drug Disc.* **2009**, *8*, 203.
21. Gál, K.; Wéber, C.; Wágner, G. A.; Horváth, A.; Nyéki, G.; Vastag, M.; Keserű, G. M. WO 2007039781, **2007**.
22. Poulain, R. F.; Tartar, A. L.; Déprez, D. P. *Tetrahedron Lett.* **2001**, *42*, 1495.
23. Gangloff, A. R.; Litvak, J.; Shelton, E. J.; Sperandio, D.; Wang, V. R.; Rice, K. D. *Tetrahedron Lett.* **2001**, *42*, 1441.
24. Gál, K.; Wéber, C.; Wágner, G. A.; Bobok, A. Á.; Nyéki, G.; Vastag, M.; Keserű, G. M.; Háda, V.; Kóti, J. WO 2007039782, **2007**.
25. Balboni, G.; Marastoni, M.; Merighi, M.; Borea, P. A.; Tomatis, R. *Eur. J. Med. Chem.* **2000**, *35*, 979.
26. Chandrasekhar, S.; Reddy, M. V.; Rajaiahet, G. *Tetrahedron Lett.* **2000**, *41*, 10131.
27. (a) Ito, S.; Tanaka, Y.; Kakehi, A.; Kondo, K. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1920; (b) Shawali, A. S.; Fahmi, A. A.; Eweiss, N. F. J. *Heterocyclic Chem.* **1979**, *16*, 123.
28. (a) Merck&Co. Inc.: Cosford, N. D.; Roppe, J.; Chen, C.; Smith, N.; Reger, T. WO 2003029210, **2003**.; (b) Smith, N. D.; Cosford, N. D. P.; Reger, T. R.; Roppe, J. R.; Poon, S. F.; Huang, D.; Chen, C.; Eastman, B. W. WO 2003077918; AstraZeneca AB; (c) Wensbo, D.; Xin, T.; Stefanac, T.; Arora, J.; Edwards, L.; Isaac, M.; Slassi, A.; Stefanac, T. WO 2004014902, **2004**.; (d) Wensbo, D.; Edwards, L.; Isaac, M.; McLeod, D. A.; Slassi, A.; Xin, T.; Stormann, T. M. WO 2005066155, **2005**.; (e) Johansson, M.; Minidis, A.; Staaf, K.; Wensbo, D.; McLeod, D. A.; Edwards, L.; Isaac, M.; O'Brien, A.; Slassi, A.; Xin, T. WO 2005080356, **2005**.; (f) Arora, J.; Edwards, L.; Isaac, M.; Kers, A.; Staaf, K.; Slassi, A.; Stefanac, T.; Wensbo, D.; Xin, T.; Holm, B. WO 2005080386. **2005**.; (g) Isaac, M.; Slassi, A.; Edwards, L.; Xin, T.; Stefanac, T. WO 2007130821, **2007**.; (h) Isaac, M.; Slassi, A.; Edwards, L.; Xin, T.; Stefanac, T. WO 2007130822, **2007**; Addex Pharm. SA: (i) Bessis, A.; Bonnet, B.; Le Poul, E.; Rocher, J.; Epping-Jordan, M. WO 2005044797, **2007**.