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3-Phenyl-5-isothiazole carboxamides with potent mGluR1 antagonist activity

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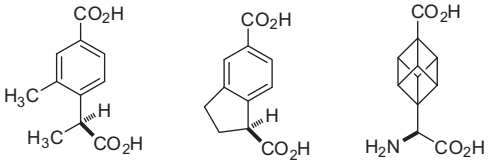
ABSTRACT

The disclosed 3-phenyl-5-isothiazole carboxamides are potent allosteric antagonists of mGluR1 with generally good selectivity relative to the related group 1 receptor mGluR5. Pharmacokinetic properties of a member of this series (1*R*,2*R*)-*N*-(3-(4-methoxyphenyl)-4-methylisothiazol-5-yl)-2-methylcyclopropanecarboxamide (**14**) are good, showing acceptable plasma and brain exposure after oral dosing. Oral administration of isothiazole **14** gave robust activity in the formalin model of persistent pain which correlated with CNS receptor occupancy.

Glutamate is a nonessential amino acid that functions as an excitatory neurotransmitter in the central nervous system (CNS). Biological signaling via glutamate is accomplished by activation of either ionotrophic GRIN (NMDA), GRIA (AMPA), GRIK (kainate) or metabotropic (mGlu) receptors.[1,2] The metabotropic glutamate receptors comprise of a family of 8 Class-C G-protein coupled receptors (GPCR) which are divided into three subfamilies based on differences in molecular structure, pharmacology, and intracellular signaling pathways. Group 1 mGlu receptors consist of mGluR1 and mGluR5, which are predominantly expressed postsynapticaly and signal through Gαq G-proteins. Group 2 (mGluR2 and mGluR3) and Group 3 (mGluR4, 6, 7, 8) are expressed either pre and/or post synaptically and inhibit adenylate cyclase through coupling with Gαi.[3,4] Since the identification and characterization of the mGlu receptors, significant effort has been expended to understand the pharmacology and medicinal utility afforded by this subclass of glutamate receptors.

Of the Group 1 mGlu receptors, considerable interest has focused on mGluR1 receptors because they are expressed in ascending nociceptive pathways, including peripheral nerves and sensory regions of the spinal cord and brain. In addition, activation of mGluR1 receptors has been shown to be critical for the induction and maintenance of long-term increases in glutamatergic signaling in nociceptive pathways which is postulated to contribute to the reduction in pain threshold (i.e., allodynia), amplification of pain responses (i.e., hyperalgesia) and a spread of pain sensitivity to non-injured areas in clinical persistent pain states.[5–7] Support for this hypothesis comes from early preclinical studies demonstrating that intrathecal injection of antisense mGlu1 oligonucleotides or neutralizing antibodies can attenuate nociceptive responding in multiple animal models of pain.[8–11] While these initial data demonstrated that blockade of mGluR1 signaling may have utility for the treatment of chronic pain conditions, practical exploration and subsequent exploitation of this finding required the identification and refinement of bioavailable small molecule antagonists.

Structurally, type 3 GPCR's have a large extracellular domain containing an orthosteric binding site and a familiar 7-transmembrane domain found across GPCR's. Initial strategies for the generation of competitive antagonists targeted the extracellular binding site and consequently yielded compounds structurally similar to glutamate (see compounds **1**–**3**).[12] These analogs provided a means for in vitro studies but lacked the properties required for predictable CNS penetration. They typically possessed low micromolar potency and sub-optimal subtype selectivity for meaningful in vivo work.



1 (LY367385) **2** (AIDA) **3** (s-ACUDA)

In an effort to identify compounds structurally distinct from amino acids, screening strategies employing functional readouts were developed which afforded the opportunity to discover

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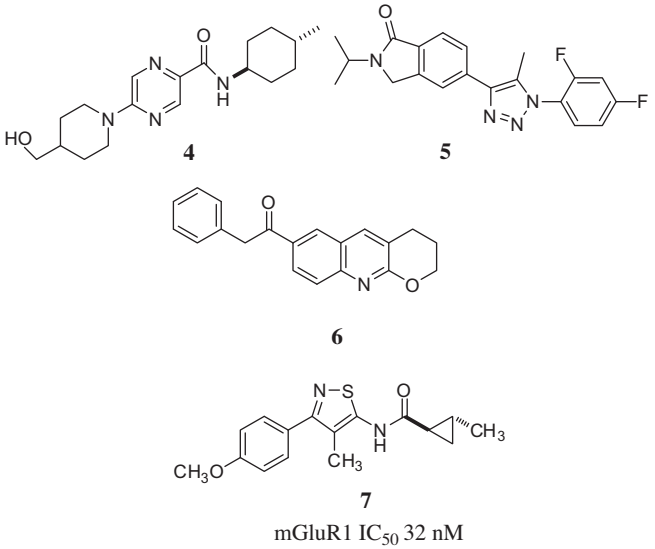
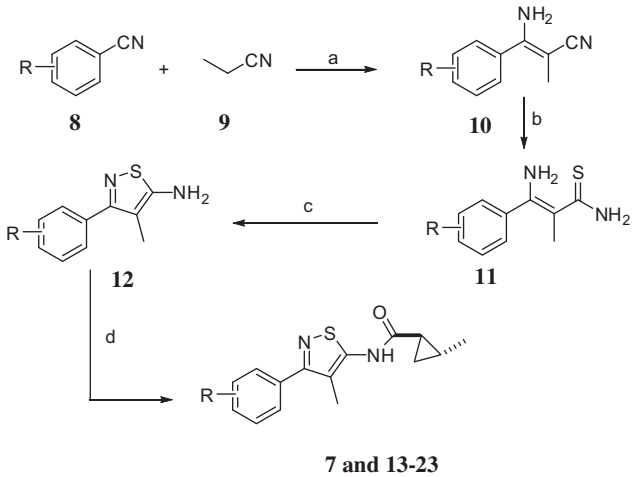
antagonists which engage sites other than the glutamate binding domain.[13–16] Over the years, this approach has yielded a number of potent structurally distinct allosteric mGluR1 antagonists with drug-like properties (see compounds **4**–**6**).[17–21] These tool compounds have helped to solidify the role of mGluR1 in pain as well as other interesting pharmacologies.[22]

During the course of our investigations into bioactive amides of 3-phenylaminoisothiazoles, we found that compound **7** (a mixture of *trans*-enantiomers) had an IC_{50} of 32 nM in an in vitro functional assay designed to detect allosteric antagonists of human mGluR1.[23] Herein we would like to report our structure activity relationship (SAR) findings regarding the aryl portion of this molecule and disclose both the pharmacokinetic (PK) and in vivo properties of one of our most potent molecules.



Construction of the desired molecules begins as shown in Scheme 1.[24] Condensation of a variety of substituted phenyl nitriles **8** with propionitrile **9** afforded the β-amino acrylonitriles **10** in good yield. Reaction of nitriles **10** with thioacetamide yielded the desired isothiazole precursors **11**. Cyclization of these intermediates was accomplished by oxidation with hydrogen peroxide which gave rise to aminoisothiazoles **12**.[25] These materials were converted into the desired product amides **7** and **13–23** by either allowing the free amine to react with the acid chloride of 2-methyl cypropanecarboxylate or by condensation of the isothiazole with and an ester of 2-methylcyclopropane carboxylate in the presence of trimethyl aluminum.

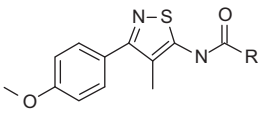


Scheme 1. Reagents and conditions: (a) 1 N NaoBu^t in THF, rt, 4–18 h, 50–85%; (b) thioacetamide, HCl dioxane, rt, 2–4 h; (c) MeOH, 30% H_2O_2, rt, 2–8 h (steps b and c ~30–60%); (d) Me_3Al, toluene, RO_2Et, 50 °C, 18 h,60–80% or pyridine, ROCl, DCM, 3 h, 60–92%.

From an activity perspective, our first concern was to understand if compound **7** displayed an enantiopreference with respect to mGluR1 antagonistic activity. Towards this end, we prepared both antipodes starting from the enantiopure cyclopropyl carboxylates and the data for each is collected in Table 1.[26–28] Compound **13**, with the *S,S* configuration displayed an IC_{50} of 110 nM while the *R,R* enantiomer **14** was significantly more potent with an IC_{50} of 9 nM. This finding allowed us to focus on the *R,R* configuration for subsequent analogs.

Additional SAR reflecting modifications of the phenyl group are captured in Table 2. Using compound **14** as a reference, removal of the ring substituent afforded compound **15** which gave roughly a 7-fold loss in activity. Extension of the methoxy group by one atom provides the ethoxy analog **16**, which was roughly 10-fold less potent than **3**. Addition of fluorine to the ring of compound **14** gives rise to **17** which afforded similar mGluR1 activity. Exchange of the methoxy with fluoro yielded compound **18** which displayed a 10-fold erosion of activity relative to **14** while the chloro analog **19** and bromo analog **20** were essentially equipotent with regard to mGluR1 potency. The one example of double substitution by halogen caused a slight reduction in potency as exemplified by **21**. The

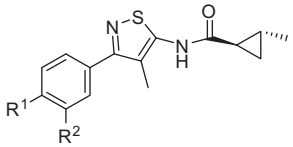
Table 1
mGluR1 antagonist activity relative to cyclopropyl configuration



Compound	R	mGlu1[a] (nM)
7		32 ± 12
13		110 ± 99
14		9 ± 2

[a] Represents the average (±SEM) of at least three separate determinations.

Table 2
SAR findings for phenyl ring substitution



Compound	R^1	R^2	hmGlu1 (nM)[a]	hmGlu5 (nM)[a]
14	OCH_3	H	9 ± 2	158 ± 65
15	H	H	65 ± 32	>12500
16	OCH_2CH_3	H	92 ± 9	19 ± 5
17	OCH_3	F	7 ± 2	159 ± 43
18	F	H	100 ± 69	>12500
19	Cl	H	9 ± 2	1220 ± 182
20	Br	H	7 ± 2	730 ± 157
21	Cl	Cl	39 ± 15	6650 ± 1614
22	CF_2CH_3	H	67 ± 21	>12500
23	OH	H	138 ± 36	>12500

[a] Represents the average (±SEM) of at least three separate determinations.

difluoroethyl analog **22** was similar in potency to the ethoxy analog **16** and the hydroxyl analog **23** was substantially less active than its methoxy congener **14**. Compounds generally showed moderate to good selectivity for mGluR1 with the exception of compound **16**, which was slightly more potent as an mGluR5 antagonist.

In an effort to establish the utility of this series for understanding in vivo mGluR1 pharmacology, we chose to evaluate the species receptor selectivity and in vivo exposure characteristics of compound **14**. Results from an in vitro rat mGluR1 receptor assay, analogous to the one described for determining human mGluR1 receptor activity,[23] demonstrated compound **14** was a comparably potent antagonist of the rat mGluR1 receptor having an IC_{50} of 0.9 nM. Compound **14** was also checked across a panel of mGlu receptors (mGluR 3, 4, 7, and 8) as well as broadly through a panel of 38 receptors and ion channels at CEREP™ and was found to have no additional cross reactivities. The plasma pharmacokinetic profiles of **3** in Sprague Dawley rats was next determined by measuring plasma levels resulting from both intravenous (IV) and oral administration of 3 and 10 mg/kg, respectively. The oral doses were delivered as a suspension consisting of methylcellulose A400 1% plus antifoam 0.1% and the IV doses were delivered as a solution in phosphate buffer 25 mmol/L (pH 3) plus 5% solutol HS15 and 5% pharmasolve. The data indicate that compound **14** was rapidly absorbed, with maximum plasma concentrations (t_{max}) attained at 1.0 h after oral dosing. The C_{max} and $AUC_{0-24\ h}$ after oral dosing were 1050 ng/mL and 3240 ng h/mL, respectively. The IV and oral elimination half-life was 0.41 and 1.24 h, respectively. The observed clearance was 35.9 mL/min/kg and the volume of distribution was 1.27 L/kg. The absolute oral bioavailability was 55.5%. In similar studies, we determined that brain concentration profiles corresponded to those found for plasma, with a brain to plasma ratio of 0.2.

Having demonstrated acceptable peripheral and brain exposure, we next sought to determine if compound **14** would afford measurable CNS receptor occupancy (RO) following oral administration.[29,30] Accordingly, a cohort of fasted Sprague Dawley rats were dosed orally with **14** and a similar cohort dosed with vehicle (1% methyl cellulose). After one hour, both groups received a 30 μg/kg intravenous bolus of a competing mGluR1 antagonist, compound **6**, which was used as a tracer.[31,32] Twenty minutes following administration of the tracer, animals were sacrificed and the cerebellum (mGlu1 receptor-rich brain region) and frontal cortex (little to no mGlu1 receptor) were dissected and weighed. Each tissue sample was then evaluated for concentrations of compound **6** and **14** by mass spectrometry and the concentrations from each group compared. The determined RO was calculated using the well-established ratio method which employs a region of high receptor density, representative of total binding (in this case the cerebellum), normalized by an area without or with very low levels of receptor (in this case the frontal cortex).[33] A dose-occupancy experiment with **14**, with doses of 0.3, 1, 3, 10, 30, and 60 mg/kg, demonstrated that compound **14** afforded good occupancy (ED_{50} value of 3.7 mg/kg) at the doses examined (see Fig. 1).

We next examined the activity of compound **14** in the formalin model of persistent pain.[34] In this assay, oral administration of **14**, 1 h prior to treatment of male Harlan Sprague Dawley rats with an intraplantar injection of formalin, caused dose dependent attenuation of late phase formalin-induced paw-licking behavior (see Fig. 2). Statistically significant effects were observed between the dose range of 3–60 mg/kg po and the absolute ED_{50} for attenuation of late phase formalin-induced paw-licking behavior was determined to be 7 mg/kg. Interestingly, the absolute ED_{50} for efficacy in the formalin model was comparable to the ED_{50} afforded by the RO assay suggesting positive correlation between central occupancy of mGluR1 and efficacy in this model.[35]

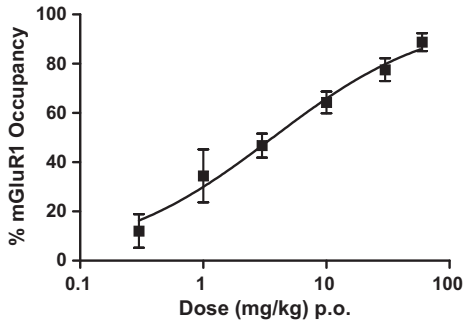


Figure 1. Oral dose–response curve for **14** mGluR1 RO. For each group $n = 4$ except for the 10 mg/kg dose, where $n = 3$. Error bars represent ±SEM.

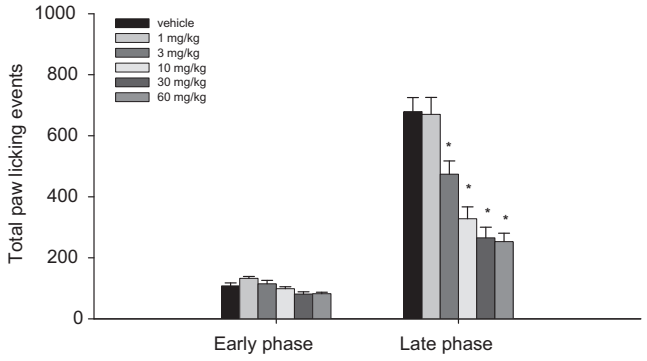


Figure 2. Effects of **14** on formalin-induced paw-licking behavior in fasted male Sprague Dawley rats 1 h after oral administration of drug (1, 3, 10, 30, 60 mg/kg, $N = 8$ per group). Early phase behavior is measured from 0–5 min and late phase behavior is measured from 11–40 min after intraplantar injection of 50 μl of 5% formalin into the right hindpaw. Data are expressed as paw-licking events (mean ± SEM). $^{*}p$ <0.05 significantly different from vehicle group.

Finally, as efficacy in the formalin model can be confounded by impairment, we subjected compound **14** to a standard evaluation in the rotorod test for motor function.[36] At an oral dose of 60 mg/kg, a dose that is 8.5× the formalin absolute ED_{50}, we found that compound **14** did not induce any deficits in rotorod

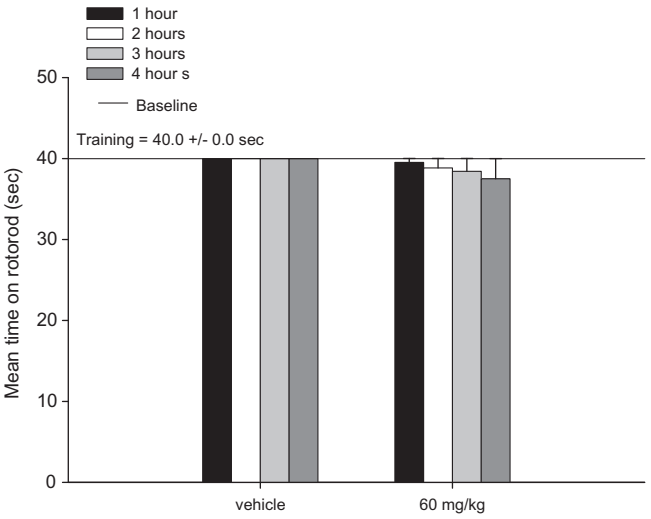


Figure 3. Effects of **14** on rotorod performance at 60 mg/kg in fasted male Sprague Dawley rats at 1, 2, 3 and 4 h after oral administration of drug ($N = 8$ per group). Baseline performance was 40 s. Data are expressed as time on rotorod in seconds (mean ± SEM).

performance for up to 4 h (see Fig. 3). This data supports the notion that the efficacy observed in the formalin model was due to the specific compound related blockade of mGluR1 and not the result of motor impairment.

In summary, we have disclosed a novel series of isothiazole amides with potent mGluR1 antagonist activity. One representative compound (**14**) displayed good peripheral and central PK properties. This compound showed dose responsive CNS receptor occupancy that correlated with activity in the formalin model of persistent pain. These data are consistent with other reports of antagonists of mGluR1 demonstrating efficacy in various pain states.[37–40] Additional data regarding both preclinical and clinical characterization of molecules within this series will be reported in due course.

References and notes

1. Foster, A. C.; Kemp, J. A. *Curr. Opin. Pharmacol.* **2006**, *6*, 7.
2. Meldrum, B. S. *J. Nutr.* **2000**, *130*, 1007S.
3. Schoepp, D. D.; Bockaert, J.; Sladeczek, F. *Trends Pharm. Sci.* **1990**, *11*, 508.
4. Schoepp, D. D.; Conn, P. J. *Trends Pharm. Sci.* **1993**, *14*, 13.
5. Lesage, A. S. *J. Curr. Neuropharmacol.* **2004**, *2*, 363.
6. Bleakman, D.; Alt, A.; Nisenbaum, E. S. *Semin. Cell Devel. Boil.* **2006**, *17*, 592.
7. Schkeryantz, J. M.; Kingston, A. E.; Johnson, M. P. *J. Med. Chem.* **2007**, *50*, 2563.
8. Young, M. R.; Blackburn-Munro, G.; Dickinson, T.; Johnson, M. J.; Anderson, H.; Nakalembe, I.; Fleetwood-Walker, S. M. *J. Neurosci.* **1998**, *18*, 10180.
9. Fundytus, M. E.; Henry, J. L.; Dray, A.; Coderre, T. *J. Prog. Pain Res. Man.* **2000**, *16* (Proceedings of the 9th World Congress on, Pain, 1999), 343.
10. Fundytus, M. E.; Yashpal, K.; Chabot, J.-G.; Osborne, M. G.; Lefebvre, C. D.; Dray, A.; Henry, J. L.; Coderre, T. J. *Br. J. Pharmacol.* **2001**, *132*, 354.
11. Noda, K.; Anzai, T.; Ogata, M.; Akita, H.; Ogura, T.; Saji, M. *Brain Res.* **2003**, *987*, 194.
12. Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431.
13. Litschig, S.; Gasparini, F.; Rueegg, D.; Stoehr, N.; Flor, P. J.; Vranesic, I.; Prezeau, L.; Pin, J.-P.; Thomsen, C.; Kuhn, R. *Mol. Pharmacol.* **1999**, *55*, 453.
14. Lavreysen, H.; Janssen, C.; Bischoff, F.; Langlois, X.; Leysen, J. E.; Lesage, A. S. J. *Mol. Pharmacol.* **2003**, *63*, 1082.
15. Gasparini, F.; Kuhn, R.; Pin, J.-P. *Curr. Opin. Pharmacol.* **2002**, *2*, 43.
16. Hemstapat, K.; de Paulis, T.; Chen, Y.; Brady, A. E.; Grover, V. K.; Alagille, D.; Tamagnan, G. D.; Conn, P. *J. Mol. Pharmacol.* **2006**, *70*, 616.
17. Owen, D. R. *ACS Chem. Neurosci.* **2011**, *2*, 349.
18. Layton, M. E. *Curr. Top. Med. Chem.* **2005**, *5*, 859.
19. Sasikumar, T. K.; Li, Q.; Burnett, D. A.; Greenlee, W. J.; Li, C.; Heimark, L.; Pramanik, B.; Grilli, M.; Bertorelli, R.; Lozza, G.; Reggiani, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3199.
20. Owen, D. R.; Dodd, P. G.; Gayton, S.; Greener, B. S.; Harbottle, G. W.; Mantell, S. J.; Maw, G. N.; Osborne, S. A.; Rees, H.; Ringer, T. J.; Rodriguez-Lens, M.; Smith, G. F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 486.
21. Wu, W.-L.; Burnett, D. A.; Domalski, M.; Greenlee, W. J.; Li, C.; Bertorelli, R.; Fredduzzi, S.; Lozza, G.; Veltri, A.; Reggiani, A. *J. Med. Chem.* **2007**, *50*, 5550.
22. Ferraguti, F.; Crepaldi, L.; Nicoletti, F. *Pharmacol. Rev.* **2008**, *60*, 536.
23. The compounds of this Letter were evaluated in stable AV12 clonal cell lines expressing recombinant human mGlu1 receptors. Responses mediated by the mGlu receptor were determined by changes in intracellular calcium concentrations measured by a fluorescent calcium sensitive dye Fluo-3 using a 96 channel fluorometric imaging plate reader. The antagonist effects of compounds were quantified by comparing the peak fluorescence response to glutamate in the presence and absence of compound. The assay window was defined as the maximal response obtained by glutamate at its predetermined EC90% concentration minus the response obtained by buffer alone. Antagonist effects were calculated as a percent of the assay window. IC_{50} values were calculated using a 4 parameter logistic curve fitting program.
24. Backer, R. T.; Fisher, M. J.; Kuklish, S. L.; Hollinshead, S. P.; Smith, E. C. R.; Takeuchi, K. WO2008/103185A2.
25. Kuklish, S. L.; Fisher, M. J.; Kempema, A. M.; Mauldin, S. C.; Merschaert, A.; Backer, R. T. Abstracts of Papers, 233rd ACS National Meeting, Chicago, IL, United States, March 25–29, 2007.
26. Delhaye, L.; Stevens, C.; Merschaert, A.; Delbeke, P.; Brione, W.; Tilstam, U.; Borghese, A.; Geldhof, G.; Diker, K.; Dubois, A. *Org. Process Res. Devel.* **2007**, *11*, 1104.
27. Delhaye, L.; Merschaert, A.; Delbeke, P.; Brione, W. *Org. Process Res. Devel.* **2007**, *11*, 689.
28. Gajewski, J. J.; Squicciarini, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 6717.
29. Chernet, E.; Martin, L. J.; Li, D.; Need, A. B.; Barth, V. N.; Rash, K. S.; Phebus, L. A. *Life Sci.* **2005**, *78*, 340.
30. Barth, V. N.; Chernet, E.; Martin, L. J.; Need, A. B.; Rash, K. S.; Morin, M.; Phebus, L. A. *Life Sci.* **2006**, *78*, 3007.
31. Lavreysen, H.; Pereira, S. N.; Leysen, J. E.; Langlois, X.; Lesage, A. S. *J. Neuropharmacol.* **2004**, *46*, 609.
32. Mabire, D.; Coupa, S.; Adelinet, C.; Poncelet, A.; Simonnet, Y.; Venet, M.; Wouters, R.; Lesage, A. S. J.; Van Beijsterveldt, L.; Bischoff, F. *J. Med. Chem.* **2005**, *48*, 2134.
33. Wadenberg, M.-L. G.; Kapur, S.; Soliman, A.; Jones, C.; Vaccarino, F. *Psychopharmacology* **2000**, *150*, 422.
34. Moore, S. A.; Nomikos, G. G.; Dickason-Chesterfield, A. K.; Schober, D. A.; Schaus, J. M.; Ying, B.-P.; Xu, Y.-C.; Phebus, L.; Simmons, R. M. A.; Li, D.; Iyengar, S.; Felder, C. C. *Proc. Nat. Acad. Sci.* **2005**, *102*, 17852.
35. Suzuki, G.; Kawagoe-Takaki, H.; Inoue, T.; Kimura, T.; Hikichi, H.; Murai, T.; Satow, A.; Hata, M.; Maehara, S.; Ito, S.; Kawamoto, H.; Ozaki, S.; Ohta, H. *J. Pharmacol. Sci.* **2009**, *110*, 315.
36. Iyengar, S.; Webster, A. A.; Hemrick-Luecke, S. K.; Xu, J.; Simmons, R. M. A. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 576.
37. More, L.; Gravius, A.; Pietraszek, M.; Belozertseva, I.; Malyshkin, A.; Shekunova, E.; Barberi, C.; Schaefer, D.; Schmidt, W. J.; Danysz, W. *Behav. Pharmacol.* **2007**, *18*, 273.
38. Zhu, C. Z.; Baker, S.; El-Kouhen, O.; Lehto, S. G.; Hollingsworth, P. R.; Gauvin, D. M.; Hernandez, G.; Zheng, G. Z.; Chang, R.; Moreland, R. B.; Stewart, A. O.; Brioni, J. D.; Honore, P. *Eur. J. Pharmacol.* **2008**, *580*, 314.
39. El-Kouhen, O.; Lehto, S. G.; Pan, J. B.; Chang, R.; Baker, S. J.; Zhong, C.; Hollingsworth, P. R.; Mikusa, J. P.; Cronin, E. A.; Chu, K. L.; McGaraughty, S. P.; Uchic, M. E.; Miller, L. N.; Rodell, N. M.; Patel, M.; Bhatia, P.; Mezler, M.; Kolasa, T.; Zheng, G. Z.; Fox, G. B.; Stewart, A. O.; Decker, M. W.; Moreland, R. B.; Brioni, J. D.; Honore, P. *Br. J. Pharmaol.* **2006**, *149*, 761.
40. Kohara, A.; Nagakura, Y.; Kiso, T.; Toya, T.; Watabiki, T.; Tamura, S.; Shitaka, Y.; Itahana, H.; Okada, M. *Eur. J. Pharmacol.* **2007**, *571*, 8.