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Design and synthesis of substituted *N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)-benzamides as positive allosteric modulators of the metabotropic glutamate receptor subtype 5

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This manuscript is dedicated to Professor James M. Cook on the occasion of his 65th birthday

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ABSTRACT

Based on SAR in the alkyne class of mGlu5 receptor negative allosteric modulators and a set of amide-based positive allosteric modulators, optimized substitution of the aryl 'b' ring was used to create substituted *N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamides. Results from an mGlu5 receptor functional assay, using calcium fluorescence, revealed varying efficacies and potencies that provide evidence that subtle changes in compounds within a close structural class can have marked effects on functional activity including switches in modes of efficacy (i.e., negative to positive allosteric modulation).

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Glutamate (L-Glutamic acid) is the major excitatory neurotransmitter within the mammalian central nervous system and regulates a variety of neuronal activities through ionotropic glutamate receptors and metabotropic glutamate receptors (mGlu receptors).[1] mGlu receptors are G protein-coupled receptors (GPCRs) that have been cloned, sequenced, and classified into Group I (mGlu1 and mGlu5 receptors), Group II (mGlu2 and mGlu3 receptors), or Group III (mGlu4, 6, 7, 8 receptors) based on sequence homology, pharmacology and 2nd messenger coupling.[1] The mGlu5 receptor is primarily located postsynaptically and is coupled with phospholipase C. Activation of mGlu5 receptor stimulates phospholipase C, which results in the hydrolysis of phosphoinositide and increases intracellular calcium concentrations.[1,2] The mGlu5 receptors have been targeted in the development of drugs to treat a variety of neurological and psychiatric illnesses, including anxiety, depression, pain, Parkinson's disease, schizophrenia and Fragile X syndrome.[2–8] Preclinical studies suggest that mGlu5 receptors may also play a role in drug abuse and addiction.[9] A large number of potent noncompetitive antagonists for mGlu5 receptor have been developed based on the structure of the leading compounds 2-methyl-6-(phenylethynyl)pyridine (MPEP, **1**, Fig. 1) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP). However, cross-target activity and in vivo metabolism may limit further development of these alkynes as medications.[10] In our attempts to design nonalkynyl mGlu5 receptor antagonists (negative allosteric modulators), several moderately active diarylamides were discovered (e.g., **2b**, in Fig. 1).[11] Based on the binding and functional data for amide-linked derivatives, we previously considered that the marked differences in functional potency as compared to binding affinities might be due to different conformational states of the mGlu5 receptor.[11a] The discovery of amide-linked positive allosteric modulators of mGlu5 receptor, such as 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide[12] (CDPPB; **3**, Fig. 1), provided further support of this idea. Thus, we hypothesized that the 'b' ring in our amide series primarily influences affinity at the mGlu5 receptor, whereas the 'a' ring determines efficacy that leads to potentiation rather than inhibition of glutamate stimulation.

In this study, we explore (i) the substituent effects at the 6-position of the pyridyl 'a' ring of the 3-CN, 5-F phenyl 'b' ring amide (Scheme 1, Table 1), and (ii) structural modifications of the 'b' ring of CDPPB by incorporating previously described[11b,c] and optimized substitutions of the 'b' ring (Schemes 2 and 3, Table 2). 1-(2-Chlorophenyl)-3-phenyl-1*H*-pyrazol-5-yl was used to replace the 'a' ring of CDPPB since a chloro substitution at

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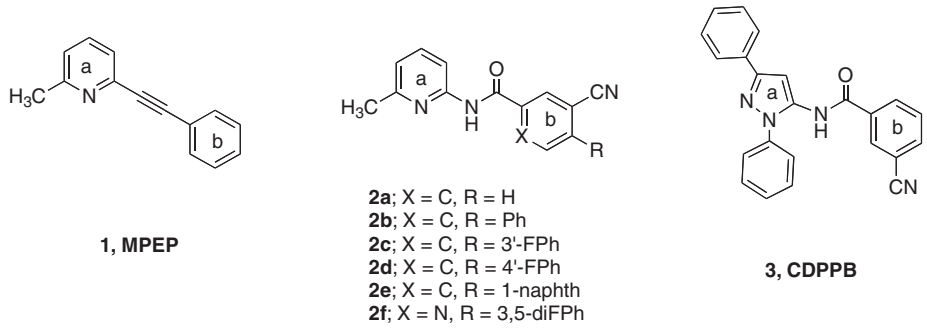
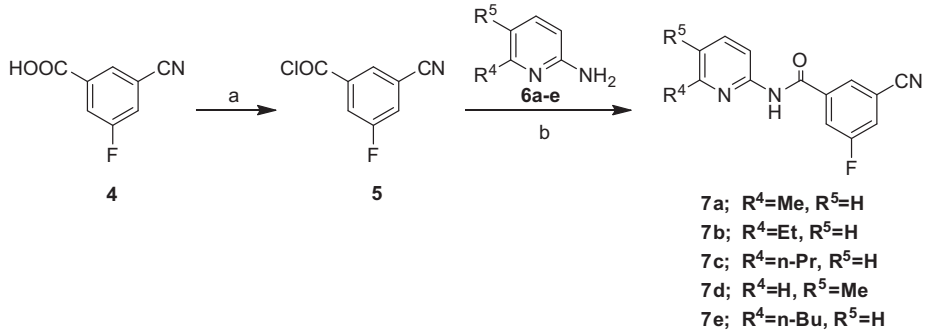
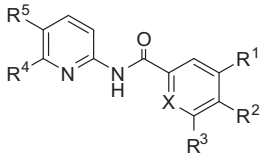


Figure 1. Structures of mGlu5 receptor allosteric modulators.



Scheme 1. Synthesis of (6-substituted pyridine-2-yl)benzamides. Reagents and conditions: (a) $SOCl_2$, reflux, 2 h; (b) 5- or 6-substituted 2-aminopyridines (**6a**–**e**), Et_3N, CH_2Cl_2, rt, 1 h.

Table 1
In vitro data for amide-linked mGlu5 receptor antagonists



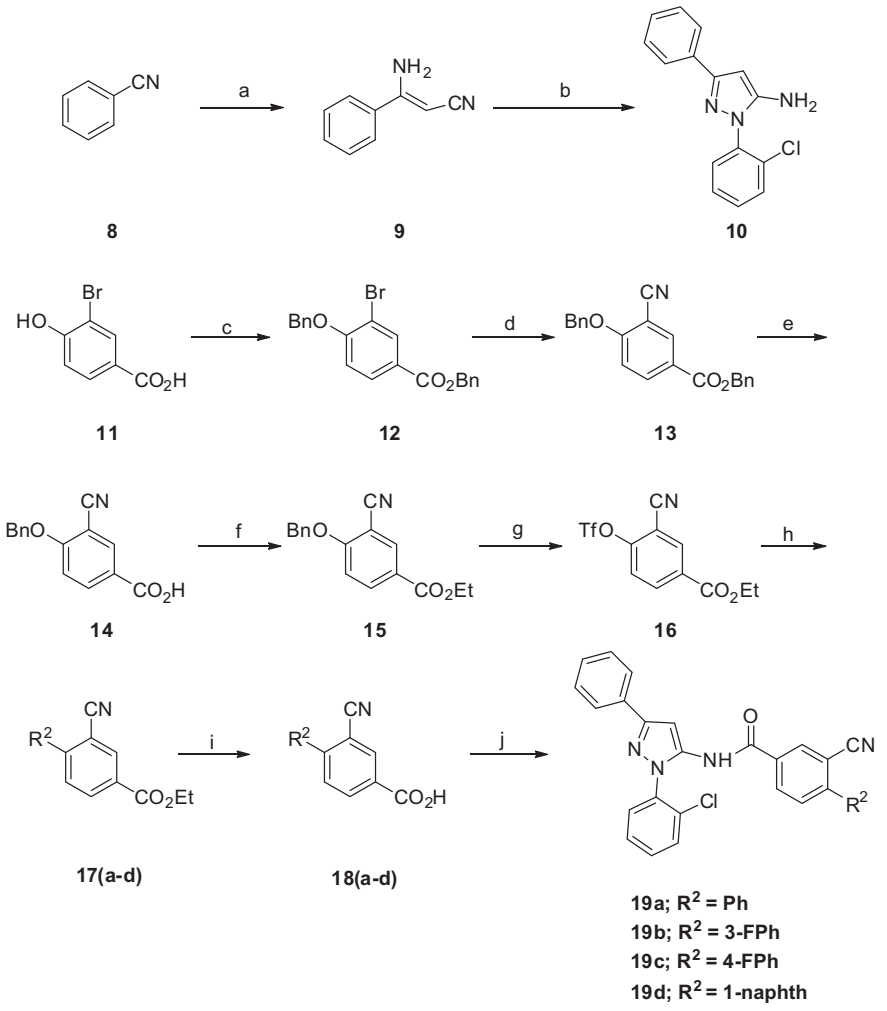
Compd	X	R^1	R^2	R^3	R^4	R^5	*c* Log *P*[f]	mGluR5 binding (K_i, nM)[a]	mGluR5 function IC_{50} (nM) (Ca^{+2} flux)
2a[d,e]	C	CN	H	H	Me	H	2.1	330 ± 20[c,d]	490 ± 94
2b[e]	C	CN	Ph	H	Me	H	4.0	9.8 ± 2.1 [b]	13.7 ± 2.54
2c[e]	C	CN	3′FPh	H	Me	H	4.1	22 ± 5.3 [b]	25.3 ± 1.90
2d[e]	C	CN	4′FPh	H	Me	H	4.1	134 ± 31[c]	4.57 ± 0.38
2e[e]	C	CN	1-Naphth	H	Me	H	5.1	72 ± 12[c]	640 ± 32.2
2f[e]	N	H	3,5-DiFPh	H	Me	H	3.8	43 ± 10	98.1 ± 18.9
7a	C	CN	H	F	Me	H	2.2	65.5 ± 20[c]	21.7 ± 5.41
7b	C	CN	H	F	Et	H	2.8	762 ± 177[c]	169 ± 21.7
7c	C	CN	H	F	*n*-Pr	H	3.3	325 ± 62[c]	165 ± 21.7
7d	C	CN	H	F	H	Me	2.2	2831 ± 567[c]	4170 ± 528
7e	C	CN	H	F	*n*-Bu	H	3.8	1054 ± 262[c]	1550 ± 146
1, MPEP[c]	—	—	—	—	—	—	3.8	13 ± 1	3.54 ± 1.39

[a] Data provided by NIMH-PDSP.
[b] Cloned.[15]
[c] Rat brain (http://pdsp.med.unc.edu).
[d] Compound previously reported.[11a]
[e] Compound and data reported previously.[11b,c]
[f] Determined using Sybyl 7.2.3, Tripos Inc.

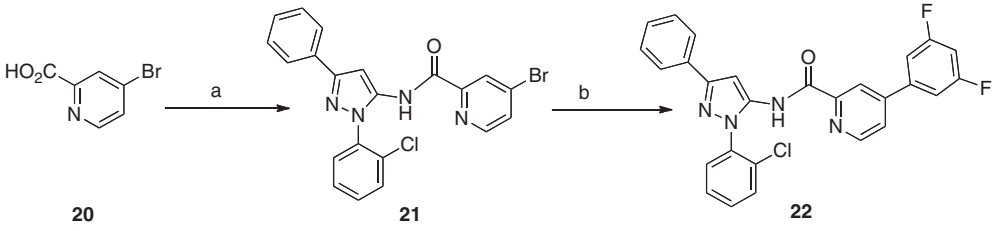
that position was reported to increase mGlu5 receptor binding affinity of CDPPB.[13]

Synthesis of the N-(5- or 6-substituted pyridin-2-yl)-3-cyano-5-fluorobenzamides **7** started from 3-cyano-5-fluorobenzoic acid **4** (Scheme 1). The acid **4** was first converted to the corresponding acid chloride **5** followed by reaction with 2-aminopyridines **6** to give the benzamides **7(a–e)** in good yields.

Synthesis of substituted *N*-[1-(2-chlorophenyl)-3-phenyl-1*H*-pyrazol-5-yl]benzs amide **19** is shown in Scheme 2. The preparation of 1-(2-chlorophenyl)-3-phenyl-5-amino-1*H*-pyrazol **10** was achieved according to a literature procedure.[13] Benzylic protection of both the OH and COOH groups in compound **11** gave intermediate **12**. Cyanation of **12** with $Zn(CN)_2$ in the presence of $Pd(PPh_3)_4$ catalyst gave intermediate **13**. Hydrolysis of **13** under basic conditions resulted in selective deprotection to give the carboxylic acid **14**. The free acid **14** was protected as the ethyl ester **15** via its acid chloride. Pd/C catalyzed hydrogenation successfully deprotected **15,** followed by treatment with trifluoromethanesulfonic



Scheme 2. Synthesis of substituted *N*-(1,3-diphenyl-1*H*-pyrazol-5yl]benzamides. Reagents and conditions: (a) $KOBu^t$, CH_3CN, C_6H_6; (b) *p*-$ClC_6H_4NHNH_2$, HCl; (c) BnBr, K_2CO_3, acetone; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF; (e) NaOH(aq), H_2O/EtOH; (f) (i) $SOCl_2$, DMF(cat.), CH_2Cl_2; (ii) EtOH, Et_3N, CH_2Cl_2; (g) (i) H_2, Pd/C, EtOH; (ii) Tf_2O, pyridine, CH_2Cl_2; (h) $R^2B(OH)_2$, $Pd(PPh_3)_4$, $KF{\cdot}2H_2O$, DME/H_2O; (i) NaOH(aq),H_2O/EtOH; (j) (i) $SOCl_2$, DMF(cat.), CH_2Cl_2; (ii) **10**, Et_3N, CH_2Cl_2.



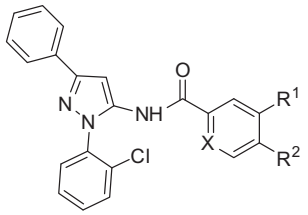
Scheme 3. Synthesis of substituted *N*-(1,3-diphenyl-1*H*-pyrazol-5yl]pyridylamide **22**. Reagents and conditions: (a) (i) $SOCl_2$, DMF(cat.), CH_2Cl_2; (ii) **10**, Et_3N, CH_2Cl_2, 60%; (b) 3,4-difluorophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 80 °C, 78%.

anhydride to give triflate **16,** which was reacted with various arylboronic acids under Suzuki-coupling conditions to give the desired ethyl (3-cyano-4-aryl)benzoates **17**(**a**–**d**). Hydrolysis of the esters **17**(**a**–**d**) under basic conditions gave the free acids **18**(**a**–**d**). Finally, **18**(**a**–**d**) were converted to the amides **19**(**a**–**d**) as shown.

Synthesis of substituted *N*-[1-(2-chlorophenyl)-3-phenyl-1*H*-pyrazol-5yl]pyridyl-amides **22** is depicted in Scheme 3. 4-Bromopicolinic acid **20** was converted to amide **21** by refluxing with $SOCl_2$ in CH_2Cl_2 to form the acid chloride, followed by treatment of the acid chloride with pyrazolamine **10**. Suzuki-coupling of **21** with 3,5-difluorophenyl-boronic acid in the presence of $Pd(PPh_3)_4$ catalyst gave the product **22**.

All the compounds synthesized were assessed in a radioligand displacement binding assay for mGlu5 receptor, using [^{3}H]MPEP as the radioligand in rat brain membranes or HEK293-T cells transfected with cloned rat mGlu5 receptor cDNA. An assay utilizing calcium fluorescence was employed to test functional activity of compounds by measuring receptor-induced intracellular release of calcium utilizing a kinetic imaging plate reader that makes simultaneous measurements of calcium levels in each well of a 384-well plate. Briefly, either vehicle or a test compound was added to cells expressing rat mGluR5 that were loaded with calcium-sensitive fluorescent dye. After a 2.5 min incubation period, an EC_{20} concentration of glutamate was added followed by an EC_{80} concentration added 1 min later. The methods for this triple add protocol, which allows simultaneous testing of antagonists and potentiators, are described in detail.[14] The results of these in vitro tests for the amides are in Tables 1 and 2.

Table 2
In vitro data for amide-linked mGlu5 receptor potentiators



Compd	X	R^1	R^2	*c* Log *P*[b]	mGluR5 binding (K_i, nM)[a]	mGluR5 function EC_{50} (nM) (Ca^{+2} flux)	% Glu max
19a	C	CN	Ph	7.5	37 ± 9	4140 ± 807	61.84 ± 3.48
19b	C	CN	3′FPh	7.7	2060 ± 520	>30,000	
19c	C	CN	4′FPh	7.7	23 ± 5	2430 ± 481	67.98 ± 1.9
19d	C	CN	1-Naphth	8.7	>10,000	2170 ± 1000	50.23 ± 12.62
22	N	H	3,5-DiFPh	7.5	344 ± 63	646 ± 242[c]	67.6 ± 7.28
3, CDPPB[d]				4.8	3670 ± 430	77 ± 15	

[a] Data provided by NIMH-PDSP using rat brain (http://pdsp.med.unc.edu).
[b] Determined using Sybyl 7.2.3, Tripos Inc.
[c] Agonist-potentiator.
[d] Compound and data reported previously.[13]

We previously optimized the substitution of the phenyl 'b' ring of a series of *N*-(6-methylpyridin-2-yl)benzamides and found that a CN at the 3-position and an aromatic substituent at the 4-position resulted in compounds with high binding affinity at the mGlu5 receptor.[11] In addition, the 3-CN, 5-F substitution in the amide 'b' ring demonstrated high affinity at mGlu5 receptor (e.g., **7a**) and was chosen for exploration of other substitutions in the pyridyl 'a' ring. If the substitutions were well-tolerated, the 3-CN, 5-F substituent would also be utilized to synthesize CDPPB analogues, with reduced lipophilicity.

Several amides were synthesized with substitutions larger than methyl (**7b**, **7c**, **7e**) or at another position of the pyridyl ring (e.g., **7d**). mGlu5 receptor binding results showed a 5–43-fold decrease in affinities compared to the parent compound **7a**, and functional test results demonstrated a 8–192-fold decrease. This trend was especially noted with compound **7d**, which has a methyl group at the 5-position of the pyridyl 'a' ring and showed the lowest potencies in both mGlu5 receptor binding affinity and functional assays. The results clearly showed that pyridyl 'a' ring substitutions in the amide series, other than the 6-methyl group, were not well-tolerated at mGlu5 receptor and hence the 3-CN, 5-F substituent was not chosen for the CDPPB series of compounds.

In the CDPPB analogue series, **19a** (R^2 = Ph) and **19c** (R^2 = 4-FPh) showed high binding affinities, while **19d** (R^2 = naphth) did not (K_i >10,000). Moreover, all the CDPPB analogues showed lower mGlu5 receptor binding affinities than their corresponding *N*-(6-methylpyridin-2-yl)benzamides except the 4-fluorophenyl substituted compound **19c**, which showed a 5.8-fold increase in binding affinity. Several *N*-(6-methylpyridin-2-yl)benzamide analogs were potent antagonists in the functional assay. However, all the CDPPB analogs showed lower potency in the functional assay, especially **19b**, which was completely inactive. In addition, their intrinsic activities differed from the benzamide series and included mGluR5 pure potentiators (e.g., **19a, 19c, 19d**) and agonist-potentiator (**22**), which induced a small response when added alone (EC_{50} >10 μM) and potentiated the response to an EC_{20} concentration of glutamate.

In superimposing **2b** and **19a** (Fig. 2), the two molecules fit quite well on the 'b' ring side, and showed similar mGlu5 receptor binding affinities. On the other hand, there were big differences on the 'a' ring side of the two molecules. These differences may contribute to their different intrinsic activities corresponding to our initial hypothesis that the 'b' ring in the amide series primarily influences affinity at mGlu5 receptor, whereas the 'a' ring determines efficacy and may be stabilizing a protein conformation that leads to potentiation rather than inhibition of glutamate stimulation.

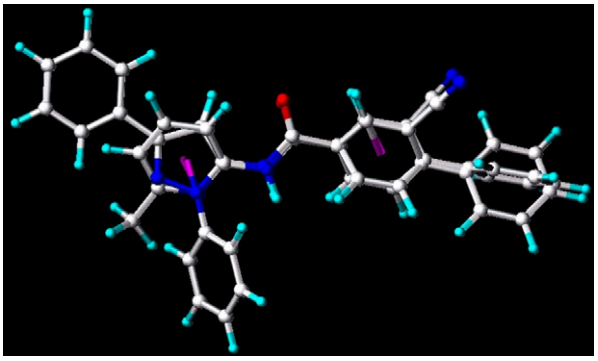


Figure 2. 3D superimposition of **2b** and **19a**.

All the CDPPB analogues, except **19d**, showed relatively high mGlu5 receptor binding affinities, especially **19a** and **19c** that were 99- and 169-fold higher than CDPPB, respectively. However, all the analogues showed much lower potency in the functional assay than CDPPB and their relatively high *c* Log *P* values render these compounds poorly water soluble with predicted poor bioavailability.

This affinity-potency disconnect has been previously observed and may be explained by differing degrees of positive cooperativity between the potentiator and glutamate.[12a] It is also possible these potentiators do not act entirely through the MPEP binding site and instead bind to an overlapping or distinct allosteric binding site (in the case of **19d**) as is the case for mGlu5 receptor potentiator CPPHA.[12b]

In summary, a series of *N*-(6-methylpyridin-2-yl)benzamide analogues (**7a**–**e**) were synthesized to explore substitution on the pyridyl 'a' ring of the *N*-(6-methylpyridin-2-yl)benzamides. In vitro testing showed that a 6-methyl substituent in the pyridyl 'a' ring is optimal. Incorporating the previously discovered optimal Ar-substitution into the phenyl 'b' ring of CDPPB (**19a**–**d, 22**) revealed varying efficacies and potencies in the calcium fluorescence mGlu5 receptor functional assay suggesting a difference in intrinsic activity that may reflect distinct binding modes at the protein, as reported recently for a series of mGlu5 receptor alkynyl analogues.[16]

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.110.

References and notes

1. Conn, P. J.; Pin, J. P. *Ann. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
2. Pin, J.-P.; Acher, F. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 297.
3. Brodkin, J.; Busse, C.; Sukoff, S. J.; Varney, M. A. *Pharmacol. Biochem. Behav.* **2002**, *73*, 359.
4. Cosford, N. D. P.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N. D.; Anderson, J. J.; Bristow, L.; Brodkin, J.; Jiang, X. H.; McDonald, I.; Rao, S.; Washburn, M.; Varney, M. A. *J. Med. Chem.* **2003**, *46*, 204.
5. (a) Walker, K.; Reeve, A.; Bowes, M.; Winter, J.; Wotherspoon, G.; Davis, A.; Schmid, P.; Gasparini, F.; Kuhn, R.; Urban, L. *Neuropharmacology* **2001**, *40*, 10; (b) Walker, K.; Bowes, M.; Panesar, M.; Davis, A.; Gentry, C.; Varney, M. A.; Urban, L.; Kuhn, R. *Neuropharmacology* **2001**, *40*, 1.
6. (a) Marino, M. J.; William, D. L., Jr.; O'Brien, J. A.; Valenti, O.; McDonald, T. P.; Clements, M. K.; Wang, R.; DiLella, A. G.; Hess, J. F.; Kinney, G. G.; Conn, P. J. *PNAS* **2003**, *100*, 13668; (b) Battaglia, G.; Busceti, C. L.; Molinaro, G.; Biagioni, F.; Traficante, A.; Nicoletti, F.; Bruno, V. *J. Neurosci.* **2006**, *26*, 7222.
7. Pietraszek, M.; Nagel, J.; Gravius, A.; Schafer, D.; Danysz, W. *Amino Acids* **2006**, *32*, 173.
8. Dölen, G.; Carpenter, R. L.; Ocain, T. D.; Bear, M. F. *Pharmacol. Ther.* **2010**, 78.
9. Kenny, P. J.; Markou, A. *Trends Pharmacol. Sci.* **2004**, *25*, 265.
10. (a) Pagano, A.; Ruegg, D.; Litschig, S.; Stoehr, N.; Stierlin, C.; Heinrich, M.; Floersheim, P.; Prezeau, L.; Carroll, F.; Pin, J. P.; Cambria, A.; Vranesic, I.; Flor, P. J.; Gasparini, F.; Kuhn, R. *J. Biol. Chem.* **2000**, *275*, 33750; (b) Malherbe, P.; Kratochwil, N.; Zenner, M. T.; Piussi, J.; Diener, C.; Kratzeisen, C.; Fischer, C.; Porter, R. H. *Mol. Pharmacol.* **2003**, *64*, 823.
11. (a) Kulkarni, S. S.; Nightingale, B.; Dersch, C. M.; Rothman, R. B.; Newman, A. H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3371; (b) Kulkarni, S. S.; Newman, A. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2074; (c) Kulkarni, S. S.; Zou, M. F.; Cao, J.; Deschamps, J. R.; Rodriguez, A. L.; Conn, P. J.; Newman, A. H. *J. Med. Chem.* **2009**, *52*, 3563.
12. (a) Chen, Y.; Nong, Y.; Goudet, C.; Hemstapat, K.; de Paulis, T.; Pin, J.-P.; Conn, P. J. *Mol. Pharmcol.* **2007**, *71*, 1389; (b) Chen, Y.; Goudet, C.; Pin, J. P.; Conn, P. J. *Mol. Pharmacol.* **2008**, *73*, 909.
13. De Paulis, T.; Hemstapat, K.; Chen, Y.; Zhang, Y.; Saleh, S.; Alagille, D.; Baldwin, R. M.; Tamagnan, G. D.; Conn, P. J. *J. Med. Chem.* **2006**, *49*, 3332.
14. Rodriguez, A. L.; Grier, M. D.; Jones, C. K.; Herman, E. J.; Kane, A. S.; Smith, R. L.; Williams, R.; Zhou, Y.; Marlo, J. E.; Days, E. L.; Blatt, T. N.; Jadhav, S.; Menon, U. N.; Vinson, P. N.; Rook, J. M.; Stauffer, S. R.; Niswender, C. M.; Lindsley, C. W.; Weaver, C. D.; Conn, P. J. *Mol. Pharmacol.* **2010**, 1105.
15. Iso, Y.; Grajkowska, E.; Wroblewski, J. T.; Davis, J.; Goeders, N. E.; Johnson, K. M.; Sanker, S.; Roth, B. L.; Tueckmantel, W.; Kozikowski, A. P. *J. Med. Chem.* **2006**, *49*, 1080.
16. Sharma, S.; Kedrowski, J.; Rook, J. M.; Smith, R. L.; Jones, C. K.; Rodriguez, A. L.; Conn, P. J.; Lindsley, C. W. *J. Med. Chem.* **2009**, *52*, 4106.