Bioorganic & Medicinal Chemistry Letters 19 (2009) 962–966

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Positive allosteric modulators of the metabotropic glutamate receptor subtype 4 (mGluR4). Part II: Challenges in hit-to-lead

Richard Williams [a,c,†], Colleen M. Niswender [a,c,†], Qingwei Luo [a,c], Uyen Le [b,c], P. Jeffrey Conn [a,c], Craig W. Lindsley [a,b,c,*]

[a] Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[b] Department of Chemistry, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[c] Vanderbilt Program in Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 5 November 2008
Revised 18 November 2008
Accepted 20 November 2008
Available online 25 December 2008

Keywords:
mGluR4
PAM
Positive allosteric modulator
Parkinson's Disease

ABSTRACT

This Letter describes the synthesis and SAR of two mGluR4 positive allosteric modulator leads, **6** and **7**. VU001171 (**6**) represents the most potent (EC_{50} = 650 nM), efficacious (141% Glu Max) and largest fold shift (36-fold) of any mGluR4 PAM reported to date. However, this work highlights the challenges in hit-to-lead for mGluR4 PAMs, with multiple confirmed HTS hits displaying little or no tractable SAR.

The metabotropic glutamate receptors (mGluRs) are members of the GPCR family C, characterized by a large extracellular amino-terminal agonist binding domain. To date, eight mGluRs have been cloned, sequenced and assigned to three groups (Group I: mGluR1 and mGluR5; Group II: mGluR2 and mGluR3; Group III: mGluRs 4, 6, 7, 8) based on their sequence homology, pharmacology and coupling to effector mechanisms.[1] The Group III mGluRs are the least explored and characterized of the mGluRs, but despite this fact, mGluR4 has garnered a great deal of attention as a therapeutic target for multiple indications.[2]

The reason for the slower pace of development within Group III mGluRs concerns the availability of selective ligands.[2,3] Most pharmacological studies employ prototypical Group III agonists such as L-(+)-2-amino-4-phosphonobutryic acid, L-AP4, **1** or functionalized carboxyphenylglycines **2**, which have limited CNS penetration (Fig. 1).[4] A major breakthrough in the field occurred when Maj and co-workers reported on the discovery of *N*-phenyl-7-(hydroxyimino)cyclopropa(*b*)chromen-1*a*-carboxamide, ((−)-PHCCC) **3**, the first mGluR4 positive allosteric modulator (PAM), derived from the mGluR1 negative allosteric modulator (NAM) 7-hydroxyimino-cyclopropan[*b*]chromen-1*a*-carboxylic acid ethyl ester ((−)-CPCCOEt).[5] PHCCC possesses an EC_{50} of 4.1 μM, with a 5.5-fold leftward shift of the glutamate response curve and selectivity versus mGluRs 2, 3, 5, 6, 7, 8.[5,6] However, PHCCC is a partial antagonist (30%) of mGluR1. Despite this, PHCCC has been a very important proof of concept (POC) compound demonstrating a therapeutic role

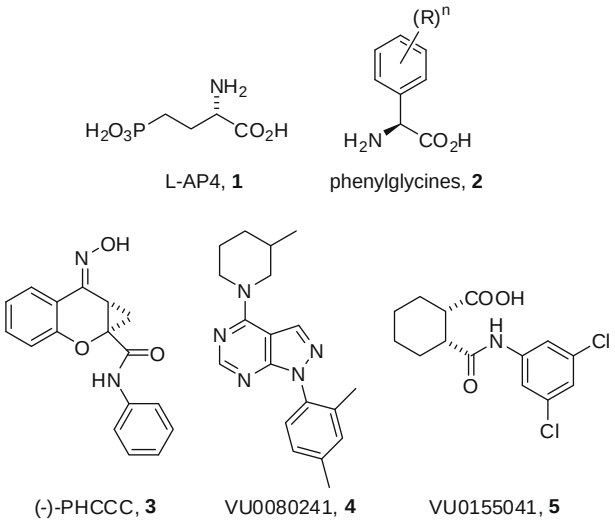


Figure 1. Chemical structures of orthosteric mGluR4 agonists L-AP4 (**1**), functionalized phenylglycines (**2**) and the mGluR4 PAMs (−)-PHCCC (**3**), VU0080241 (**4**) and the mGluR4 ago-potentiator VU0155041 (**5**).

* Corresponding author. Tel.: +1 615 322 8700; fax: +1 615 343 6532.
E-mail address: craig.lindsley@vanderbilt.edu (C.W. Lindsley).
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.104

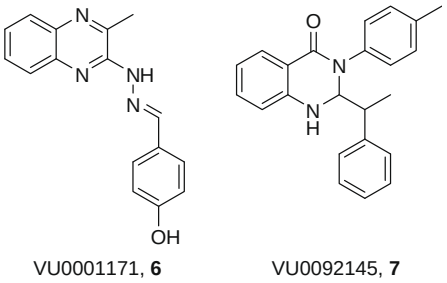
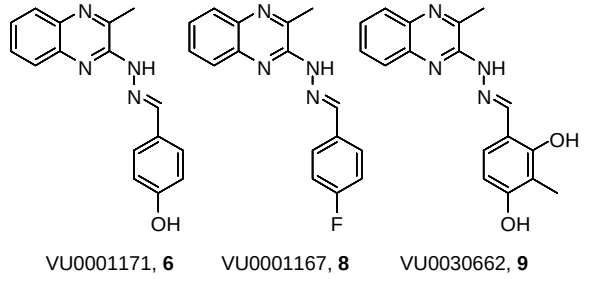


Figure 2. Chemical structures of new mGluR4 PAM series VU0001171 (**6**) and VU0092145 (**7**) identified in HTS.

for selective mGluR4 activation in Parkinson's disease,[6,7] anxiety,[8] depression,[9] neuroprotection[10] and oncology.[11] We recently reported on two new series of mGluR4 PAMs: **4**, with improved fold shift versus **3** (EC_{50} = 5 μM, 11.8-fold shift), and **5**, a more potent (EC_{50} = 750 nM, 6.4-fold shift) mGluR4 PAM than **3**, which also displayed efficacy in preclinical Parkinson's disease models.[12,13] Despite these advances, SAR for **4** was shallow, and, akin to **3** was a full antagonist of mGluR1 (IC_{50} = 2.6 μM).[12] In contrast, **5** was highly selective for mGluR4 versus mGluR1, 2, 5, 7 and 8 as well as a full panel of GPCRs, ion channels and transporters. However, **5** possessed significant allosteric agonist activity, and is therefore more accurately described as an ago-potentiator.[13] In order to evaluate the in vivo efficacy of selective, pure potentiation of mGluR4, versus agonist activation, new small molecule tools are required.

In this Letter, we describe the discovery and structure activity relationship (SAR) of two novel mGluR4 PAMs, VU0001171 (**6**) and VU0092145 (**7**), based on a functionalized benzylidene hydrazinyl-3-methylquinazoline scaffold or a bis-2,3-dihydroquinazolin-4(1*H*)-one core, respectively (Fig. 2). While 'flat' SAR is a staple of allosteric ligands,[14–16] we have never encountered such shallow SAR as in the hit-to-lead campaign from our mGluR4 high throughput screen (HTS). This report will detail the challenges in



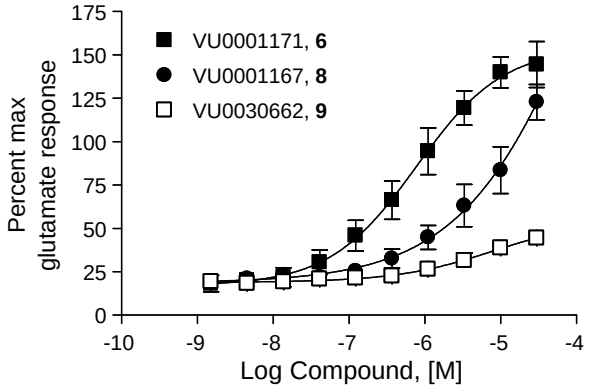


Figure 3. Concentration-dependent potentiation of glutamate in mGluR4/Gqi5 CHO cells by **6**, **8** and **9** identified via high throughput screening campaign (HTS). In the absence of an EC_{20} of glutamate, **6**, **8** and **9** do not elicit receptor activation. Data represent mean ± SEM of four independent experiments performed in triplicate.

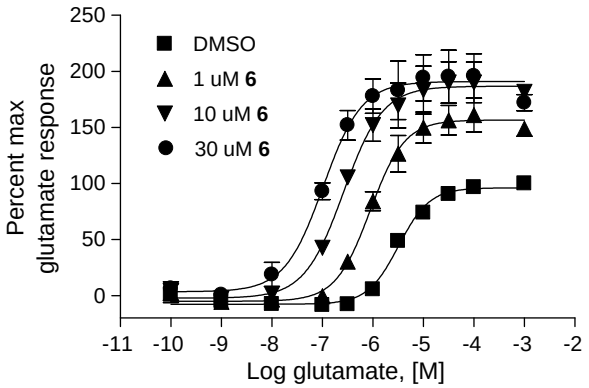


Figure 4. VU0001171 (**6**) shifts the glutamate agonist response curves to the left 36-fold at 30 μM with an elevation in % Glu Max. Data represents the average of at least three independent determinations performed in triplicate.

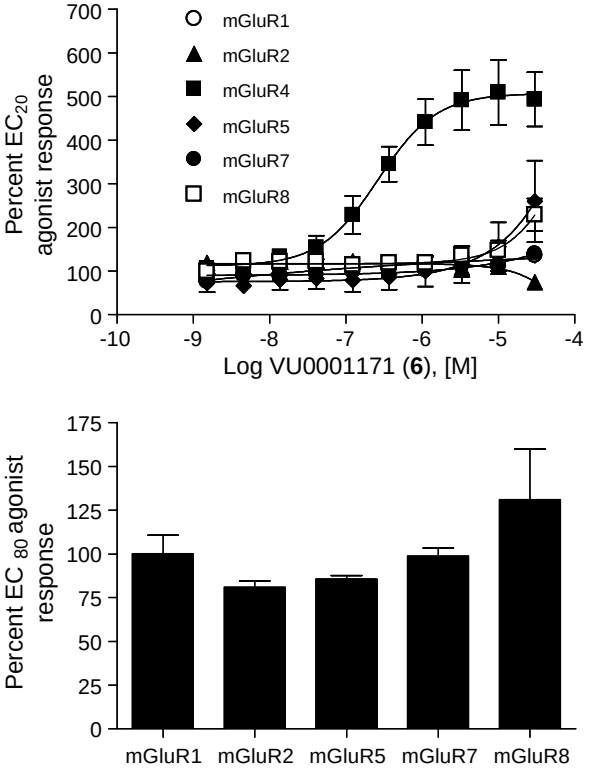
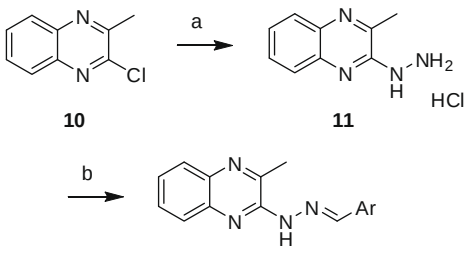


Figure 5. Activation and inhibition responses of VU0001171 (**6**) in both EC_{20} and EC_{80} assays to determine selectivity versus the other mGluRs. VU0001171 was not an antagonist of any mGluR and afforded only moderate activation of mGluR1 and mGluR8 at 30 μM.

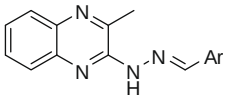
the hit-to-lead process when multiple HTS hits confirm after resynthesis, but virtually any structural modification affords a complete loss of mGluR4 PAM activity.

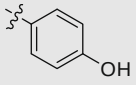
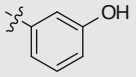
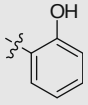
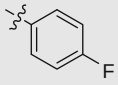
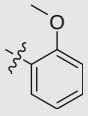
Our mGluR4 PAM HTS identified three 3-methylquinazolines that afforded a concentration-dependent potentiation of an EC_{20} of glutamate in human mGluR4/Gqi5 CHO cells (Fig. 3).[13] When HTS stocks were evaluated with full concentration–response curves, **6**, (*E*)-4-((2-(3-methylquinoxalin-2-yl)hydrazono)methyl) phenol, was a stand-out compound with an EC_{50} for potentiation of 1.7 μM while having no effect on mGluR4 in this assay in the absence of glutamate. While **6** was not a particularly attractive chemical scaffold from a drug discovery perspective, it exhibited a 36-fold shift of the glutamate response at 30 μM, the largest fold shift reported to date for a Class C GPCR (Fig. 4). Additionally, when



Scheme 1. Reagents and conditions: (a) NH_2NH_2, EtOH, MW, 135 °C, 35 min, 95–98%; (b) ArCHO, EtOH, DIPEA, MW, 135 °C, 35 min, 40–86%.

Table 1
Potency values for VU0001171 analogs **12**



Compound	Ar	EC_{50}[a] (μM)	% Glu Max
6, VU0001171		1.7	144
		0.65	141
12a		2.4	136
12b		4.1	57
12c		>5	87
12d		4.4	129
12e		Inactive	—

[a] Potency values represent one experimental performed in triplicate.

screened across the mGluRs, **6** was observed to be highly selective for mGluR4 in both activation (EC_{20} glutamate potentiation) and inhibition (depression of EC_{80}) assays (Fig. 5). This was a marked improvement over both PHCCC (**3**) and our previous reported mGluR4 PAM, **4** which had significant mGluR1 antagonist activity.[5,16] It is possible that **6** binds at a second allosteric site, distinct from the PHCCC (**3** and **4**) allosteric site, which appears to be conserved with mGluR1.

With a *bona fide* novel mGluR4 PAM hit in hand, we employed an iterative analog library synthesis approach to rapidly evaluate SAR for **6**. First, we altered the aromatic group at the hydrazino imine terminus to determine what functional groups would be tolerated. From commercially available **10**, a microwave-assisted S_NAr reaction was performed to displace chlorine with hydrazine to afford **11** (Scheme 1). Imine formation by the addition of an aldehyde to **11** in EtOH followed by microwave irradiation at 135 °C afforded analogs **12a–e** (Table 1).[17]

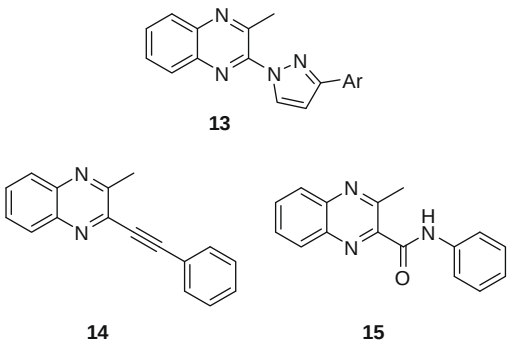


Figure 6. Alternative linkers to replace the hydrazinoimine moiety.

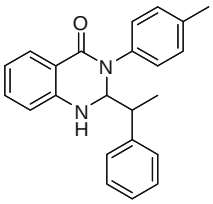
Re-synthesis of the hit compound **6** afforded improved potency with an EC_{50} of 650 nM as compared to the HTS deck sample with an EC_{50} of 1.7 μM. Walking the hydroxyl group around the phenyl ring to the *meta* position (**12a**) gave >3-fold loss in potency, and the *para* analog, **12b**, afforded a >6-fold loss in potency as well as a diminished glutamate response. Of the 24-member library that was synthesized and tested, only the 4-fluorophenyl (**12c**) and phenyl (**12d**) analogs, outside of the phenolic congeners, afforded mGluR4 PAM activity. Overall, a modest SAR was observed with no improvement in potency. Attention turned to replacing the hydrazino imine moiety with a more stable linker. To retain the "diamino" linker region, a series of pyrazoles were synthesized (Fig. 6). The commercially available 3-phenylpyrazoles were deprotonated using NaH in DMF and reacted with **10** to afford analogs **13**; however, these analogs were devoid of mGluR4 PAM activity.

Replacing the hydrazine imine moiety with either an acetylene (**14**) or an amide (**15**) linker was also unsuccessful (Fig. 6). As the labile hydrazino imine linker could not be replaced with a more stable linker, and coupled with the 'flat' SAR, this series was not advanced further. Still, **6** is an important tool compound, devoid of mGluR1 antagonist activity. Moreover, **6** is the most potent mGluR4 PAM reported to date (EC_{50} = 650 nM, 141% Glu Max) and possesses the largest fold shift (36-fold shift of the glutamate CRC) ever reported for a Class C GPCR.

From the HTS screen an attractive hit was identified, **7**, which has some structural features similar to that of PHCCC (Fig. 7). Compound **7** (VU0092145) has a potency value (EC_{50} = 1.8 μM) that is comparable to that of PHCCC (EC_{50} = 4.1 μM), however **7** represents another fundamentally novel mGluR4 chemotype. Unlike **6** with a massive 36-fold shift of the glutamate CRC, **7** possessed only a 2.7-fold shift of the glutamate response curve.

The hit **7** was re-ordered from a commercial vendor and tested side by side with in-house synthesized compound (VU0092145-2) (Fig. 7) employing a novel, microwave-assisted three component coupling reaction cascade (Scheme 2). With the re-synthesis, roughly equivalent potency was observed (EC_{50} = 3.0 μM vs EC_{50} = 1.8 μM); however glutamate response differed (129% Glu Max for commercial vs 80% Glu Max for the re-synthesized material).

The microwave-assisted organic synthesis chemistry was highly amendable to rapid SAR analysis, and two 24-member libraries were synthesized were either the 4-methylaniline or the 2-phenylpropanal were held constant.[18] Of the 48 compounds that were tested only three compounds, re-synthesized **7** and **17a–b**, (6.25%) exhibited any mGluR4 PAM activity (EC_{50}s > 10 μM with increases in % Glu Max), and all with lower potencies than the original hit **7** (Table 2). This was disappointing, but not at all that surprising. There are some structural similarities to that of PHCCC,



VU0092145, **7**

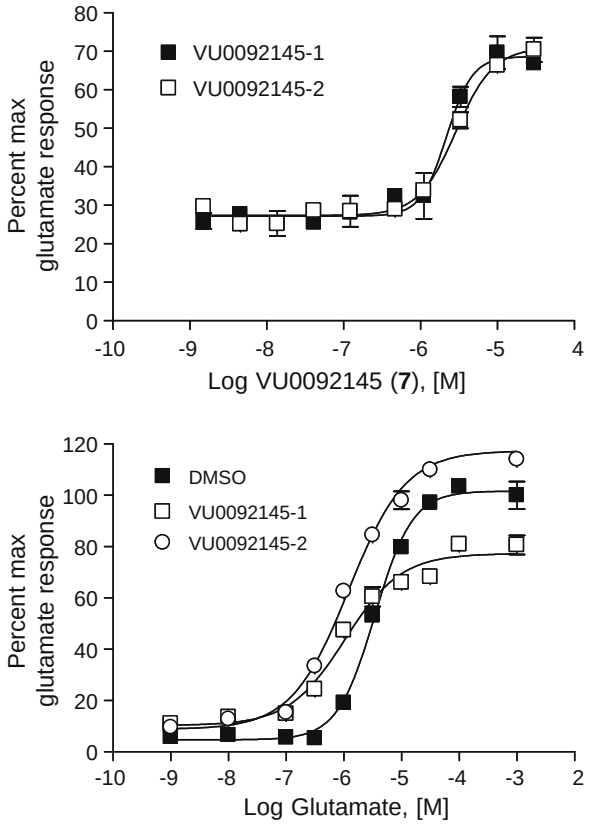
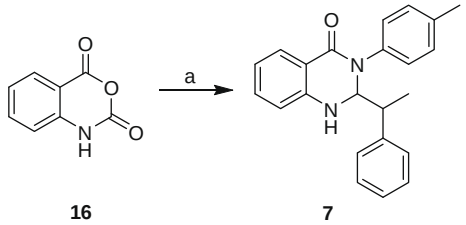


Figure 7. Concentration-dependent potentiation of glutamate in mGluR4/Gqi5 CHO cells by one commercial lot of **7** (VU0092415-1) and a re-synthesized lot (VU0092415-2) along with fold shift of glutamate at 30 μM. Data represent mean ± SEM of three independent experiments performed in triplicate.



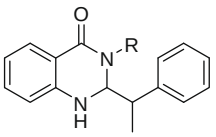
16 **7**

Scheme 2. Reagent and condition: 4-Methylaniline, 2-phenylpropanal, *p*-TSA, H_2O, MW, 150 °C, 35 min, 95–98%.

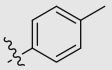
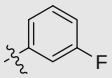
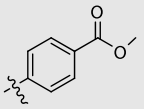
which has also reported to have a very flat SAR. One explanation for the 'flat' SAR is that the allosteric binding sites which both **6** (VU0001171) and **7** (VU0092145) as well as PHCCC occupy are very shallow, similar to the second, non-MPEP, allosteric binding site on mGluR5 that *N*-{4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) occupies.[14,15]

Whilst the SAR of both VU0001171 (**6**) and VU0092145 (**7**) are flat, they represent the third and fourth literature disclosure of positive allosteric modulators of mGluR4. VU0001171 (**6**) represents the most potent (EC_{50} = 650 nM), efficacious (141% of Glu Max) human mGluR4 PAM reported to date. Moreover, VU0001171 (**6**) afforded a significant 36-fold shift of the glutamate response, the largest reported to date, and the compound did not exhibit mGluR1 antagonist activity. Unfortunately, due to the 'flat' SAR and the inability to replace labile, non drug-like moieties, both the VU0001171 (**6**) and VU0092415 (**7**) scaffolds were discontinued. However, both series highlight a major challenge in the development of mGluR4 allosteric ligands—the common theme of HTS hits confirming upon re-synthesis, but displaying little to no SAR (typically less than 7% actives) upon the slightest structural modifications. Clearly, ligands that potentiate mGluR4, and their allosteric binding sites, represent significant challenges in the hit-to-lead stage of drug discovery.

Table 2
Potency values for VU0092145 analogs **17**



17

Compound	R	EC_{50}[a] (μM)	% Glu Max
7, VU0092145		3.0	129
17a		>10	87
17b		>10	69

[a] Represent the average of one experiment performed in triplicate.

Acknowledgments

The authors thank C. David Weaver, Emily L. Days, Tasha Nalywajko, Cheryl A. Austin and Michael Baxter Williams for their critical contributions to the HTS portion of the project as well as the National Institute of Mental Health, the Michael J. Fox Foundation, the Vanderbilt Department of Pharmacology and the Vanderbilt Institute of Chemical Biology for support of this research.

References and notes

1. (a) Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431; (b) Conn, P. J.; Pin, J.-P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
2. (a) Marino, M. J.; Hess, J. F.; Liverton, N. *Curr. Top. Med. Chem.* **2005**, *5*, 885; (b) Marino, M. J.; Conn, P. J. *Curr. Opin. Pharmacol.* **2006**, *8*, 98.
3. (a) Yang, Z.-Q. *Curr. Top. Med. Chem.* **2005**, *5*, 913; (b) Niswender, C. M.; Jones, J. K.; Conn, P. J. *Curr. Top. Med. Chem.* **2005**, *5*, 847.
4. (a) Thomsen, C.; Kristensen, P.; Mulvihill, E.; Haldeman, B.; Suzdak, P. D. *Eur. J. Pharmacol.* **1992**, *227*, 361; (b) Gasparini, F.; Bruno, V.; Battaglia, G.; Lukic, S.; Leonhardt, T.; Inderbitzen, W.; Laurie, D.; Sommer, B.; Varney, M. A.; Hess, S. D.; Johnson, E. C.; Kuhn, R.; Urwyler, S.; Sauer, D.; Poret, C.; Schmutz, M.; Nicoletti, F.; Flor, P. J. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1678.
5. Maj, M.; Bruno, V.; Dragic, Z.; Yamamoto, R.; Battaglia, G.; Inderbitzin, W.; Stoehr, N.; Stein, T.; Gasparini, F.; Vranesic, I.; Kuhn, R.; Nicoletti, F.; Flor, P. J. *Neuropharmacology* **2003**, *45*, 895.
6. Marino, M. J.; Williams, 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. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13668.
7. Battaglia, G.; Busceti, C. L.; Molinaro, G.; Biagioni, F.; Traficante, A.; Nicoletti, F.; Bruno, V. *J. Neurosci.* **2006**, *26*, 7222.
8. Stachowicz, K.; Klak, K.; Klodzinska, A.; Chojnacka-Wojcik, E.; Pilc, A. *Eur. J. Pharmacol.* **2004**, *498*, 153.
9. Klak, K.; Palucha, A.; Branski, P.; Pilc, A. *Amino Acids* **2007**, *32*, 169.

10. Canudas, A. M.; Di Giorgi-Gerevini, V.; Iacovelli, L.; Nano, G.; D'Onofrio, M.; Arcella, A.; Giangaspero, F.; Busceti, C.; Ricci-Vitiani, L.; Battaglia, G.; Nicoletti, F.; Melchiorri, D. *J. Neurosci.* **2004**, *24*, 10343.
11. Iacovelli, L.; Arcella, A.; Battaglia, G.; Pazzaglia, S.; Aronica, E.; Spinsanti, P.; Caruso, A.; De Smaele, E.; Saran, A.; Gulino, A.; D'Onofrio, M.; Giangaspero, F.; Nicoletti, F. *J. Neurosci.* **2006**, *26*, 8388.
12. Niswender, C. M.; LeBois, E. P.; Luo, C.; Kim, K.; Muchalski, H.; Yin, H.; Conn, J. P.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5626.
13. Niswender, C. M.; Johnson, K. A.; Weaver, C. D.; Jones, C. J.; Luo, Q.; Rodriguez, A. L.; Marlo, J.; de Paulis, T.; Thompson, A.; Days, E.; Nalywajko, N. T.; Austin, C.; Williams, M.; Ayala, J. E.; Williams, R.; Lindsley, C. W.; Conn, P. J. *Mol. Pharmacol.* **2008**, *74*, 1345.
14. Zhao, Z.; Wisnoski, D. D.; O'Brien, J. A.; Lemiare, W.; Williams, D. L., Jr.; Jacobson, M. A.; Wittman, M.; Ha, S.; Schaffhauser, H.; Sur, C.; Pettibone, D. J.; Duggan, M. E.; Conn, P. J.; Hartman, G. D.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1386.
15. O'Brien, J. A.; Lemaire, W.; Wittmann, M.; Jacobson, M. A.; Ha, S. N.; Wisnoski, D. D.; Lindsley, C. W.; Schaffhauser, H. J.; Sur, C.; Duggan, M. E.; Pettibone, D. J.; Conn, J.; Williams, D. L. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 568.
16. Lindsley, C. W.; Wisnoski, D. D.; Leister, W. H.; O'Brien, J. A.; Lemiare, W.; Williams, D. L., Jr.; Burno, M.; Sur, C.; Kinney, G. G.; Pettibone, D. J.; Tiller, P. R.; Smith, S.; Duggan, M. E.; Hartman, G. D.; Conn, P. J.; Huff, J. R. *J. Med. Chem.* **2004**, *47*, 5825.
17. *Experimental for the synthesis of* ***12a***: To a solution of 2-chloro-3-methylquinoxaline (100 mg, 0.56 mmol) in EtOH (1 mL) was added hydrazine (18 mg, 0.56 mmol), sealed and heated in microwave at 135 °C for 35 min. The reaction mixture was cooled to room temperature. The solvent was removed under vacuum to afford **11** as a light brown solid (117 mg, 99%); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.45 (br s, 1H), 7.71 (br s, 1H), 7.49 (s, 1H), 7.31 (br s, 1H), 2.43 (s, 3H); LC–MS, single peak, 2.45 min, *m*/*e*, 175.2 (M+1); a solution of **11** (110 mg, 0.52 mmol), DIPEA (67 mg, 0.52 mmol) and 3-hydroxybenzaldehyde (63 mg, 0.52 mmol) in EtOH (1 mL) was irradiated at 135 °C for 35 min in a microwave. The reaction mixture was cooled to room temperature, concentrated under vacuum and purified by mass directed preparative HPLC to afford **12a** as a pale yellow solid; ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.63 (br s, 1H), 8.49 (s, 1H), 7.71 (br s, 2H), 7.50 (br s, 1H), 7.41 (br s, 1H), 7.38–7.32 (m, 1H), 7.30–7.26 (m, 2H), 6.84 (d, J = 13.0 Hz, 1H), 2.55 (s, 3H); LC–MS, 4:1 *E*/*Z*, 2.18 and 2.29 min, *m*/*e*, 279.1; HRMS calc. 279.1246 (M+H), $C_{16}H_{15}N_4O$, found 279.1246, $C_{16}H_{15}N_4O$.
18. Thermal one pot synthesis of bis-2,3-dihydroquinazolin-4(1*H*)-ones; Baghbanzadeh, M.; Salehi, P.; Dabiri, M.; Kozehgary, G. *Synthesis* **2005**, 344.