Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 57–60
Bioorganic & Medicinal Chemistry Letters

2,3-Ethylene- and 2,3-trimethylene-bridged analogues of the group III metabotropic glutamate receptor ligand 2-amino-4-phosphonobutanoic acid

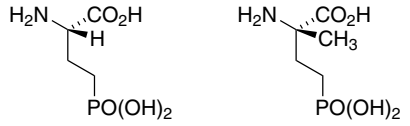
Rodney L. Johnson* and Kolluri S. S. P. Rao

Department of Medicinal Chemistry, University of Minnesota, 308 Harvard Street, SE, Minneapolis, MN 55455, USA

Received 27 August 2004; revised 12 October 2004; accepted 12 October 2004
Available online 2 November 2004

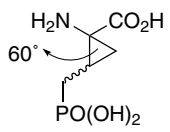
Abstract—The racemic *trans*- and *cis*-isomers of 1-amino-2-phosphonomethyl-cyclobutanecarboxylic acid (**5** and **6**) and 1-amino-2-phosphonomethyl-cyclopentanecarboxylic acid (**7** and **8**) were synthesized as extensions of the mGluR4 agonists *trans*- and *cis*-1-amino-2-phosphonomethyl-cyclopropanecarboxylic acid (**3** and **4**). Although the methylene bridge in **3** and **4** allows for retention of affinity toward the mGluR4 receptor, increasing the bridging unit to the ethylene group as in **5** and **6** or to the trimethylene group as in **7** and **8** introduces sufficient steric hindrance to eliminate affinity for the mGluR4 receptor.

Glutamic acid is the principal excitatory neurotransmitter in the central nervous system. It exerts its effects through both ligand-gated ion channels and G-protein coupled receptors. The latter are referred to as metabotropic glutamate receptors (mGluR) of which eight subtypes have been identified based upon their signal transduction mechanisms and upon their reaction toward different glutamic acid analogues.[1] Group I is made up of mGluR1 and mGluR5, while mGluR2 and mGluR3 make up Group II. Group III consists of mGluR4, mGluR6, mGluR7, and mGluR8. A distinctive characteristic of Group III mGluRs is their activation by the glutamic acid analogue L-2-amino-4-phosphonobutanoic acid (**1**, L-AP4).[2–6] The α-methyl derivative of **1**, α-methyl-2-amino-4-phosphonobutanoic acid (**2**), has been shown to be an antagonist at some Group III mGluRs, in particular mGluR4a.[7]

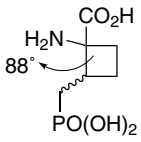


1

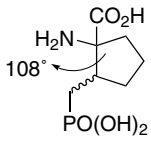
2



3 (*trans*-isomer)
4 (*cis*-isomer)



5 (*trans*-isomer)
6 (*cis*-isomer)



7 (*trans*-isomer)
8 (*cis*-isomer)

Several analogues of **1** have been synthesized to investigate the structure–activity relationships of this molecule.[8–12] Two of the most potent analogues of **1** are the cyclopropyl derivatives **3** and **4**.[10] Both cyclopropyl analogues show potent activity at the mGluR4 receptor with the *cis*-isomer (**4**) being the most active.[13,14] Since **2**–**4** all can be viewed as α-substituted analogues of **1**, the question that arises is why **3** and **4** exhibit agonist activity, while **2** is an antagonist at mGluR4 receptors. Clearly, there are electronic differences between the 2,3-methano group of **3** and **4** and the α-methyl group of **2**. Also, the CH_2–C^{α}–C^{β} bond angle of ~60° seen in **3** and **4** versus the CH_3–C^{α}–C^{β} bond angle of ~109° found in **2** results in the methano group of **3** and **4** being projected into a different area of space than the α-methyl group of **2**. To test the possible effect that such a difference might have on the different activity seen for **2**–**4**, the synthesis of analogues of **1** possessing either an ethylene or trimethylene bridge between the α- and β-carbon atoms was undertaken. This gave rise to the cyclobutyl-AP4 (**5** and **6**) and cyclopentyl-AP4 (**7** and **8**)

Keywords: AP4; Conformational analogues; mGluR4.
* Corresponding author. Tel.: +1 612 624 7997; fax: +1 612 624 0139; e-mail: johns022@umn.edu

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.040

analogues with expected CH_2–C^{α}–C^{β} bond angles of ~88° and ~108°, respectively.

Synthesis of the racemic *trans*- and *cis*-isomers of 1-amino-2-phosphonomethyl-cyclobutanecarboxylic acid (**5** and **6**) and 1-amino-2-phosphonomethyl-cyclopentanecarboxylic acid (**7** and **8**) was carried out as shown in Scheme 1. A Mannich reaction on either cyclobutanone (**9**) or cyclopentanone (**10**) with dimethylamine hydrochloride and aqueous formaldehyde gave the 2-dimethylaminomethyl-cycloalkanones **11** and **12**, respectively.[15] Each of these two compounds was converted quantitatively to the corresponding methiodide salts **13** and **14** by treatment with methyl iodide. Reaction of **13** and **14** with triethylphosphite in benzene under refluxing conditions yielded the ketophosphonates **15** and **16**, respectively, in good yield. A Strecker reaction on each ketophosphonate gave the corresponding amino nitriles **17** and **18**, each as a mixture of diastereoisomers.[9] The amino nitriles were not purified, but immediately benzoylated with benzoyl chloride in pyridine at 0 °C. The *trans*- and *cis*-isomers of the cyclobutyl *N*-benzoylamino nitriles, **19** and **20**,[16] were separated from one another by preparative TLC using EtOAc as the eluting solvent. In the case of the cyclopentyl *N*-benzoylamino nitriles **21** and **22**,[17] separation was accomplished by silica gel column chromatography eluting with hexanes/EtOAC (1:1) initially, followed by EtOAc. Conversion of **19**–**22** to **5**–**8**, respectively, was accomplished through hydrolysis with 6 N HCl. Purification of **5**–**8** was carried out on an AG WX8 cation exchange resin (50–100 mesh).[18]

The relative stereochemistry of the cyclopentyl analogues was determined at the *N*-benzoylamino nitrile stage. Compound **21** was successfully crystallized from hexane/CH_2Cl_2 (1:1) to give crystals from which a crystal structure was obtained (Fig. 1) that showed it was the *trans*-isomer.[19] Crystals suitable for X-ray analysis, however, could not be obtained for either of the two isomers in the cyclobutane series.

The assignment of the stereochemistry for **19** and **20** was made by comparing the relative NMR chemical shifts of selected atoms in these cyclobutyl derivatives with those seen for the same atoms in the corresponding cyclopentyl derivatives. Analysis of the ^1H NMR spectrum of **19** and **20** showed that the chemical shift of

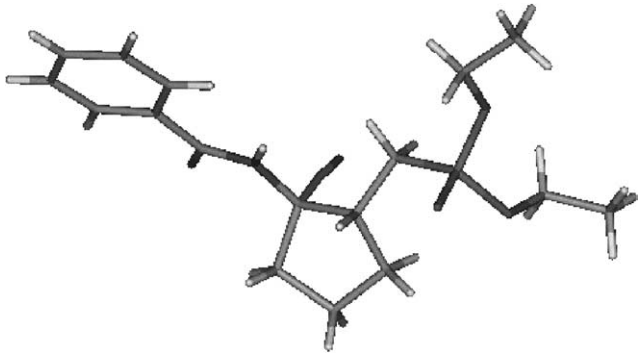
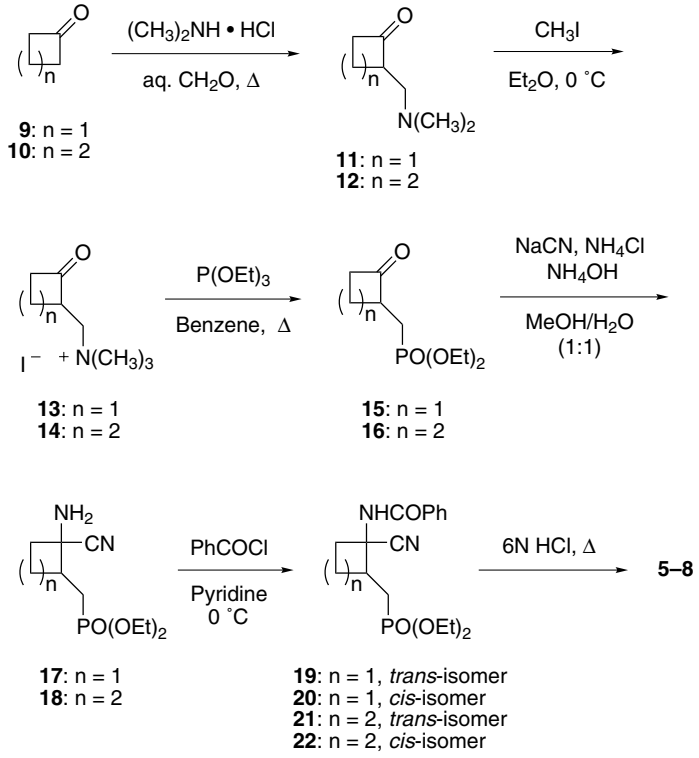


Figure 1. X-ray crystal structure of **21**.



Scheme 1.

the NH peak in **20** (9.04 ppm) was downfield of that in **19** (8.7 ppm), while the phosphorous resonance in **20** (29.2 ppm) was upfield of that in **19** (30.4 ppm). These chemical shift differences were analogous to those seen between the *cis*-cyclopentyl derivative **22** (δNH = 9.8 ppm, δP = 31.6 ppm) and the *trans*-cyclopentyl derivative **21** (δNH = 9.2 ppm, δP = 32.3 ppm). In addition, the chemical shift of the methylene hydrogens next to the phosphorous atom in **5** and **6** showed a distinct pattern that was analogous to that seen for the corresponding methylene hydrogens in **7** and **8**. In the case of **6**, these methylene protons appear as two sets of multiplets at 2.30–2.56 and 2.82–2.93 ppm, whereas in the case of **5**, the methylene protons appear as a single multiplet at 2.28–2.38 ppm. This pattern is analogous to that seen in the cyclopentyl *cis*-isomer **8** (2.20–2.25 and 2.25–2.50 ppm) and the cyclopentyl *trans*-isomer **7** (2.15–2.25 ppm). Molecular modeling (Insight, version 2.0) suggested a possible explanation for the different chemical shift patterns seen between the *trans*- and *cis*-isomers in the cyclobutyl and cyclopentyl series. The energy-minimized structures of both sets of isomers reveal that the CH_2P hydrogens are diastereotopic with respect to the protonated amino group. The distance between the nitrogen atom and the two methylene hydrogens was 4.23 and 4.57 Å in *trans*-isomer **5**, whereas the distance between the nitrogen atom and methylene hydrogens was 2.92 and 4.15 Å in *cis*-isomer **6**. An analogous pattern was observed in cyclopentane *trans*-isomer **7** (4.16 and 4.42 Å) and *cis*-isomer **8** (2.89 and 4.11 Å).

Racemic compounds **5**–**8** were evaluated in a fluorometric imaging plate reader (FLIPR) assay utilizing mGluR1–5, 8 that were stably expressed in AV12 cells co-expressing the rat glutamate transporter.[20] Cells expressing mGluR2–4, 8 also expressed the promiscuous G-protein $G_{\alpha 15}$. The four AP4 analogues, **5**–**8**, did not show significant activity as agonists at the mGluR4 receptor when tested up to a concentration of 200 μM. Neither did they show significant antagonist activity at mGluR4 receptors when tested up to a concentration of 100 μM. Similar results also were seen at another Group III receptor, mGluR8. Not surprisingly, **5**–**8** did not show significant affinity toward either the Group I metabotropic receptors mGluR1 and 5 or the Group II metabotropic receptors mGluR2 and 3.

In contrast to the inactivity of **5**–**8**, the racemic cyclopropyl AP4 analogues **3** and **4** were found to have EC_{50} values of 7.9 and 0.58 μM, respectively, at the mGluR4 receptor.[14] The possible juxtaposition in space of the amino, carboxyl, and phosphonic acid moieties is similar for the corresponding isomers in the cyclopropyl, cyclobutyl, and cyclopentyl AP4 analogues. The difference in these analogues lies in the nature of the bridging unit between the α- and β-carbon atoms of the AP4 backbone. Clearly, these results show that a methylene bridge allows for retention of affinity toward the mGluR4 receptor. Increasing the bridging unit to the ethylene group as in the cyclobutyl isomers or to the trimethylene group as in the cyclopentyl isomers introduces sufficient steric hindrance to eliminate affinity for the mGluR4 receptor. This implies that the area on the mGluR4 receptor that interacts with the α- and β-carbon atoms of AP4 is quite restricted as anything larger than a methylene bridge is not allowed. Also, the fact that the cyclopropyl AP4 analogues with the methylene bridge retain agonist activity, while breaking the methylene bridge to yield the α-methyl analogue of AP4 results in an antagonist further indicates that this region of the receptor is very sensitive to where the steric bulk is projected. The results obtained in this study should prove useful in the design of future ligands for the mGluR4 receptor and they should be of value in the refinement of the molecular models of the mGluR4 receptor that have been developed.[21,22]

Acknowledgements

This work was supported in part from a grant from NIH (NS35608) and a Development Grant in Drug Design from the Department of Medicinal Chemistry, University of Minnesota. The crystallographic analysis carried out by Neil R Brooks and Victor G. Young, Jr. of the University of Minnesota X-ray Crystallographic Laboratory is gratefully acknowledged. The authors thank Dr. James Monn and Renee Emkey of Eli Lilly and Company for arranging and conducting, respectively, the biological testing.

References and notes

1. Conn, P. J.; Pin, J.-P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
2. Tanabe, Y.; Nomura, A.; Masu, M.; Shigemoto, R.; Mizuno, M.; Nakanishi, S. *J. Neurosci.* **1993**, *13*, 1372.
3. Nakajima, Y.; Iwakabe, H.; Akazawa, C.; Nawa, H.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. *J. Biol. Chem.* **1993**, *266*, 11,868.
4. Okamoto, N.; Hori, S.; Akazawa, C.; Hayashi, Y.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. *J. Biol. Chem.* **1994**, *269*, 1231.
5. Duvoisin, R. M.; Zhang, C.; Rammonell, K. A. *J. Neurosci.* **1995**, *15*, 3075.
6. Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431.
7. Johansen, P. A.; Robinson, M. B. *Eur. J. Pharmacol.* **1995**, *290*, R1.
8. Crooks, S. L.; Freund, R. K.; Halsrud, D. A.; Koerner, J. F.; Johnson, R. L. *Brain Res.* **1985**, *329*, 346.
9. Crooks, S. L.; Robinson, M. B.; Koerner, J. F.; Johnson, R. L. *J. Med. Chem.* **1986**, *29*, 1988.
10. Kroona, H. B.; Peterson, N. L.; Koerner, J. F.; Johnson, R. L. *J. Med. Chem.* **1991**, *34*, 1692.
11. Peterson, N. L.; Kroona, H. B.; Johnson, R. L.; Koerner, J. F. *Brain Res.* **1992**, *571*, 162.
12. Gasparini, F.; Inderbitzin, W.; Francotte, E.; Lecis, G.; Richert, P.; Dragic, Z.; Kuhn, R.; Flor, P. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1241.
13. Peterson, N. L.; Thoreson, W. B.; Johnson, R. L.; Koerner, J. F.; Miller, R. F. *Brain Res.* **1991**, *568*, 15.
14. Johansen, P. A.; Chase, L. A.; Sinor, A. D.; Koerner, J. F.; Johnson, R. L.; Robinson, M. B. *Mol. Pharmacol.* **1995**, *48*, 140.
15. Frank, J.; Pierle, M. *J. Am. Chem. Soc.* **1951**, *73*, 724.

16. Spectral properties of (±)-**19** and (±)-**20**. (±)-**19**: ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H, NH), 7.85–7.88 (d, 2H, Ph), 7.40–7.50 (m, 3H, Ph), 4.10–4.18 (m, 4H, $POCH_2$), 2.88–2.95 (m, 2H, PCH_2CH), 2.33–2.45 (m, 2H), 1.98–2.23 (m, 3H), 1.31–1.38 (apparent t, 6H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, DEPT assignment) δ 16.4 (d, $^{3}J_{C-P}$ = 6.3 Hz, $POCH_2CH_3$), 16.5 (d, $^{3}J_{C-P}$ = 5.6 Hz, $POCH_2CH_3$), 24.1 (d, $^{3}J_{C-P}$ = 16.4 Hz, PCH_2CHCH_2), 28.2 (d, $^{1}J_{C-P}$ = 135.8 Hz, PCH_2), 32.9 ($CH_2C(CN)$), 39.6 (d, $^{2}J_{C-P}$ = 4.7 Hz, PCH_2CH), 54.9 (d, $^{3}J_{C-P}$ = 6.8 Hz, $NC(CN)$), 62.1 (d, $^{2}J_{C-P}$ = 6.8 Hz, $POCH_2CH_3$), 62.7 (d, $^{3}J_{C-P}$ = 6.3 Hz, $POCH_2CH_3$), 119.3 (CN), 127.4, 128.7, 132.1, 132.9 (Ph), 167.6 (C=O); ^{31}P NMR ($CDCl_3$) δ 30.4; CIMS *m/z* 351.2 $(M+H)^+$. (±)-**20**: ^{1}H NMR ($CDCl_3$) δ 9.04 (s, 1H, NH), 7.96–8.15 (m, 2H, Ph), 7.45–7.58 (m, 3H, Ph), 4.05–4.18 (m, 4H, $POCH_2CH_3$), 3.05–3.18 (m, 1H, PCH_2CH), 2.81–2.95 (m, 1H, PCH_2CH), 2.05–2.67 (m, 4H), 1.85–2.00 (m, 1H), 1.20–1.35 (m, 6H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 16.8 (d, $^{3}J_{C-P}$ = 5.6 Hz, $POCH_2CH_3$), 21.6 (PCH_2CHCH_2), 29.8 (PCH_2), 32.8 ($CH_2C(CN)$), 41.06 (d, $^{2}J_{P-C}$ = 4.6 Hz, PCH_2CH), 56.6 ($NC(CN)$), 62.2 (d, $^{2}J_{P-C}$ = 6.6 Hz, $POCH_2CH_3$), 117.0 (CN), 128.4, 128.7, 128.8, 130.1, 134.2 (Ph), 164.6 (C=O); ^{31}P NMR ($CDCl_3$) δ 29.2; CIMS *m/z* 351.2 $(M+H)^+$.
17. Spectral properties of (±)-**21** and (±)-**22**. (±)-**21**: ^{1}H NMR ($CDCl_3$) δ 9.20 (s, 1H, NH), 7.95–7.99 (dd, 2H, Ph), 7.35–7.46 (m, 3H, Ph), 3.95–4.15 (m, 4H, $POCH_2CH_3$), 3.05–3.20 (m, 1H, PCH_2CH), 2.43–2.60 (m, 1H), 1.60–2.40 (m, 6H) 1.21 (t, J = 7.5 Hz, 3H, $POCH_2CH_3$), 1.30 (t, J = 7.5 Hz, 3H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$, DEPT assignment) δ 16.5 (d, $^{3}J_{P-C}$ = 6.3 Hz, $POCH_2CH_3$), 20.5 (CH_2), 26.8 (d, $^{1}J_{P-C}$ = 138 Hz, PCH_2), 31.1 (d, $^{3}J_{P-C}$ = 14.3 Hz, PCH_2CHCH_2), 39.0 ($CH_2C(CN)$), 43.2 (d, $^{2}J_{P-C}$ = 3.4 Hz, PCH_2CH), 61.2 (d, $^{3}J_{P-C}$ = 4.6 Hz, $NC(CN)$), 62.7 (d, $^{2}J_{P-C}$ = 6.3 Hz, $POCH_2CH_3$), 119.2 (CN), 127.8, 128.6, 132.0, 133.2 (Ph), 167.6 (C=O); ^{31}P NMR ($CDCl_3$) δ 32.3; CIMS *m/z* 365.2 $(M+H)^+$. (±)-**22**: ^{1}H NMR ($CDCl_3$) δ 9.80 (s, 1H, NH), 7.95–7.99 (dd, 2H, Ph), 7.35–7.46 (m, 3H, Ph), 3.95–4.15 (m, 4H, $POCH_2CH_3$), 2.75–2.85 (m, 2H), 2.50–2.60 (m, 2H), 2.30–2.40 (m, 2H), 2.00–2.20 (m, 1H), 1.9–2.00 (m, 2H), 1.20–1.35 (m, 6H); ^{13}C NMR ($CDCl_3$, DEPT assignment) δ 16.6 (d, $^{3}J_{P-C}$ = 6.3 Hz, $POCH_2CH_3$), 21.0 (CH_2), 24.3 (d, $^{1}J_{P-C}$ = 139.7 Hz, PCH_2), 29.8 (d, $^{3}J_{P-C}$ = 6.9 Hz, PCH_2CHCH_2), 36.9 ($CH_2C(CN)$), 43.7 (d, $^{2}J_{P-C}$ = 3.4 Hz, PCH_2CH), 59.1 (d, $^{3}J_{P-C}$ = 4.6 Hz, $NC(CN)$), 62.7 (d, $^{2}J_{P-C}$ = 6.9 Hz, $POCH_2CH_3$), 121.2 (CN), 127.9, 128.7, 132.2, 133.8 (Ph), 167.8 (C=O); ^{31}P NMR ($CDCl_3$) δ 31.6; CIMS *m/z* 365.2 $(M+H)^+$.
18. Spectral properties for **5**–**8**. (±)-**5**: ^{1}H NMR (D_2O) δ 1.58–1.74 (m, 2H), 1.78–1.92 (m, 2H), 2.00–2.10 (m, 1H, PCH_2CH), 2.28–2.38 (m, 2H, PCH_2); ^{31}P NMR (D_2O) δ 26.4; FABMS *m/z* 210.0 $(M+H)^+$. (±)-**6**: ^{1}H NMR (D_2O) δ 1.68–1.95 (m, 3H), 2.00–2.25 (m, 2H), 2.30–2.56 (m, 1H, PCH_2), 2.82–2.93 (m, 1H, PCH_2); ^{31}P NMR (D_2O) δ 27.0; FABMS *m/z* 210.0 $(M+H)^+$. (±)-**7**: ^{1}H NMR (D_2O) δ 1.40–1.80 (m, 6H), 1.80–2.00 (m, 1H, PCH_2CH), 2.15–2.25 (m, 2H, PCH_2); ^{31}P NMR (D_2O) δ 24.4; FABMS *m/z* 224.0 $(M+H)^+$. (±)-**8**: ^{1}H NMR (D_2O) δ 1.40–1.80 (m, 6H), 2.00–2.10 (m, 1H, PCH_2CH), 2.20–2.25 (m, 1H, PCH_2), 2.25–2.50 (m, 1H, PCH_2); ^{31}P NMR (D_2O) δ 27.0; FABMS *m/z* 224.0 $(M+H)^+$.
19. Crystallographic data (excluding structure factors) for the structure in this paper has been deposited with the Cambridge Crystallographic Data Centre as the supplementary publication number CCDC 248532. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
20. Cells were seeded at 50,000 per well in poly D-lysine coated 96-well black walled clear bottom plates one day prior to the assay. The day of the FLIPR assay cells were dye loaded with 8 μM Fluo-3AM (Molecular Probes, F-1241) in FLIPR Buffer (HBSS Biowhittaker, 10-527F) supplemented with 20 mM HEPES (Biowhittaker, 17-737E) at 25 °C for 1.5 h (mGluR1, 2, 5) or 2 h (mGluR3, 4, 8). The dye was removed from the plate and replaced with 50 μL per well of FLIPR Buffer, which was pre-warmed to 37 °C. The stage of the FLIPR was set to 34.5 °C and the compounds and glutamate (Sigma, G-1626) used in the assay was also pre-warmed to 37 °C. A two addition FLIPR assay was performed by addition of compound (50 μL of 50 μM compound in 2.5% DMSO for a final concentration of 25 μM in 1.25% DMSO) in the first addition. The second addition was either an EC_{10} concentration of glutamate (for potentiator assays) or an EC_{90} concentration of glutamate (for antagonist assays). In both cases the volume of the second addition was 100 μL resulting in a compound concentration of 12.5 μM (0.625% DMSO) in the second read. Data was collected every sec for the first 30 s and then every 3 s until the second addition was made at 2 min. Following the second addition data was collected every second for 30 s and then every 3 s until the end of the run at total time of 3.25 min.
21. Bertrand, H.-O.; Bessis, A. S.; Pin, J.-P.; Acher, F. C. *J. Med. Chem.* **2002**, *45*, 3171.
22. Rosemond, E.; Peltekova, V.; Naples, M.; Thogersen, H.; Hampson, D. R. *J. Biol. Chem.* **2002**, *277*, 7333.