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Molecular pharmacology of homologues of ibotenic acid at cloned metabotropic glutamic acid receptors

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Abstract

We have studied the effects of the enantiomers of 2-amino-3-(3-hydroxyisoxazol-5-yl)propionic acid (homoibotenic acid, HIBO) and analogues substituted with a methyl, bromo or butyl group in the four position of the ring at cloned metabotropic glutamate (mGlu) receptors expressed in Chinese hamster ovary (CHO) cells. In contrast to the parent compound ibotenic acid, which is a potent group I and II agonist, the (*S*)-forms of homoibotenic acid and its analogues are selective and potent group I antagonists whereas the (*R*)-forms are inactive both as agonists and antagonists at group I, II, and III mGlu receptors. Interestingly, (*S*)-homoibotenic acid and the analogues display equal potency at both $mGlu_{1\alpha}$ and $mGlu_{5a}$ with K_i values in the range of 97 to 490 μM, (*S*)-homoibotenic acid and (*S*)-2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid [(*S*)-4-butylhomoibotenic acid] displaying the lowest and highest potency, respectively. The homoibotenic acid analogues thereby differ from mGlu receptor antagonists derived from phenylglycine such as (*S*)-4-carboxyphenylglycine which only antagonizes $mGlu_{1\alpha}$ ($K_i = 18$ μM) showing no effect at $mGlu_{5a}$ ($K_i > 300$ μM).

Keywords: Metabotropic glutamate receptor; $mGlu_{1\alpha}$ receptor antagonist; $mGlu_{5a}$ receptor antagonist; Ibotenic acid; Homoibotenic acid; Homoibotenic acid analogue

1. Introduction

(*S*)-Glutamic acid [(*S*)-Glu] is the major excitatory amino acid in the central nervous system and is involved in many important neural processes such as learning, memory and plasticity (Krogsgaard-Larsen et al., 1996; Knöpfel et al., 1995). (*S*)-Glu mediates these effects through ionotropic glutamate (iGlu) receptors, which belong to the family of ligand-gated ion channels, and metabotropic glutamate (mGlu) receptors, which belong to the family of G-protein coupled receptors. Based on pharmacological and cloning studies the, so far identified, eight mGlu receptors have been sub-divided into three groups (Knöpfel et al., 1995; Nakanishi and Masu, 1994). The $mGlu_1$ and $mGlu_5$ receptors, which form group I, are positively coupled to hydrolysis of phosphatidylinositol 4,5-biphosphate and are selectively activated by (*S*)-quisqualic acid and (*RS*)-3,5-dihydroxyphenylglycine (Knöpfel et al., 1995; Brabet et al., 1995). $mGlu_2$ and $mGlu_3$, which belong to group II, are negatively coupled to adenyl cyclase and are selectively activated by (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) and LY354740 (Hayashi et al., 1993; Schoepp et al., 1997). Finally, group III comprises the $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors which are also negatively coupled to adenyl cyclase and are selectively activated by (*S*)-2-amino-4-phosphonobutyric acid (L-AP4) (Knöpfel et al., 1995).

In our search for new selective mGlu receptor ligands we have previously reported the pharmacological consequences of elongating the backbone chain-length of (*S*)-Glu and some heterocyclic analogues by one carbon. Thus, (*S*)-2-aminoadipic acid, the homologue of (*S*)-Glu, selectively activates $mGlu_2$ and $mGlu_6$ but lacks activity at $mGlu_{1\alpha}$ and $mGlu_{4a}$ (Bräuner-Osborne et al., 1996). (*S*)-2-Amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid [(*S*)-Homo-AMPA], the 3-hydroxyisoxazole bioisostere of (*S*)-2-aminoadipic acid and homologue of (*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)-propionic acid [(*S*)-AMPA], is a specific $mGlu_6$ receptor agonist and is devoid

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of any iGlu receptor activity (Bräuner-Osborne et al., 1996; Ahmadian et al., 1997). Furthermore, (*S*)-homoquisqualic acid, the homologue of the potent agonist at $mGlu_{1\alpha}$, $mGlu_{5a}$, and iGlu receptors, (*S*)-quisqualic acid, is an agonist at $mGlu_{5a}$ and $mGlu_2$ and an antagonist at $mGlu_{1\alpha}$ (Bräuner-Osborne and Krogsgaard-Larsen, 1998). Finally, (1*S*,3*R*)-homo-ACPD is a selective $mGlu_2$ receptor partial agonist, whereas the parent compound, (1*S*,3*R*)-ACPD, displays full agonism at $mGlu_{1\alpha}$ and $mGlu_2$ (Bräuner-Osborne et al., 1997). In order to further investigate the pharmacological effects of elongating the backbone chain-length, we here report the pharmacological profile of the (*S*)- and (*R*)-forms of 2-amino-3-(3-hydroxyisoxazol-5-yl)propionic acid (homoibotenic acid, HIBO) and a series of homoibotenic acid analogues. These compounds were shown to display pharmacological profiles distinctly different from that of the parent compound, ibotenic acid.

2. Materials and methods

2.1. Cell culture

The Chinese hamster ovary (CHO) cell lines stably expressing $mGlu_{1\alpha}$, $mGlu_2$, $mGlu_{4a}$ and $mGlu_{5a}$ receptors have previously been described (Aramori and Nakanishi, 1992; Tanabe et al., 1992, 1993; Abe et al., 1992). Cell cultures were maintained at 37°C in a humidified 5% CO_2 incubator in Dulbecco's Modified Eagle Medium (DMEM) containing a reduced concentration of (*S*)-glutamine (2 Mm) and supplemented with 1% proline, penicillin (100 U/ml), streptomycin (100 mg/ml), and 10% dialyzed fetal calf serum (all GIBCO, Paisley, Scotland). For phosphatidylinositol 4,5-biphosphate hydrolysis assays, 1.8×10^6 cells were divided into the wells of 48 well plates 2 days before assay. For cyclic AMP assays, 1×10^6 cells were divided into the wells of 96 well plates 2 days before assay.

2.2. Second messenger assays

Phosphatidylinositol 4,5-biphosphate hydrolysis was measured as described previously (Hayashi et al., 1992, 1994). Briefly, the cells were labeled with [^{3}H]inositol (2 μCi/ml) 24 h prior to the assay. For agonist assays, the cells were incubated with ligand dissolved in phosphate buffered saline (PBS)–LiCl for 20 min, and agonist activity was determined by measurement of the level of [^{3}H]-labeled mono-, bis- and tris-inositol phosphates by ion-exchange chromatography. For antagonist assays, the cells were preincubated with the ligand dissolved in PBS–LiCl for 20 min prior to incubation with ligand and 20 μM ($mGlu_{1\alpha}$) or 10 μM ($mGlu_{5a}$) (*S*)-Glu for 20 min. The antagonist activity was then determined as the inhibitory effect of the (*S*)-Glu-mediated response. The assay of cyclic AMP formation was performed as described previously (Hayashi et al., 1992, 1994). Briefly, the cells were incubated for 10 min in PBS containing the ligand and 10 μM forskolin and 1 Mm 3-isobutyl-1-methylxanthine (IBMX) (both Sigma, St. Louis, MO). The agonist activity was then determined as the inhibitory effect of the forskolin-induced cyclic AMP formation. For antagonist assay, the cells were preincubated with ligand dissolved in PBS containing 1 Mm IBMX for 20 min prior to a 10 min incubation in PBS containing the ligand, 30 μM (*S*)-Glu, 10 μM forskolin, and 1 Mm IBMX.

2.3. Data analysis

All experiments were performed in triplicate and the results are given as mean ± S.E.M. of at least two independent experiments. Antagonist potencies were calculated from inhibition curves using the 'functional equivalent' of the Cheng–Prusoff equation; $K_b = IC_{50}/(1 + ([A]/EC_{50}))$ (Craig, 1993), where IC_{50} is the concentration of antagonist required to reduce the response to 50% of the maximal response, EC_{50} is the concentration of agonist which elicits 50% of the maximal response, and [A] is the fixed concentration of the agonist.

2.4. Ligands

All ligands were either purchased from Tocris Cookson (Bristol, UK) or synthesized in our own laboratory according to previously published methods (Bischoff et al., 1995; Hansen et al., 1989, 1992; Johansen et al., 1998).

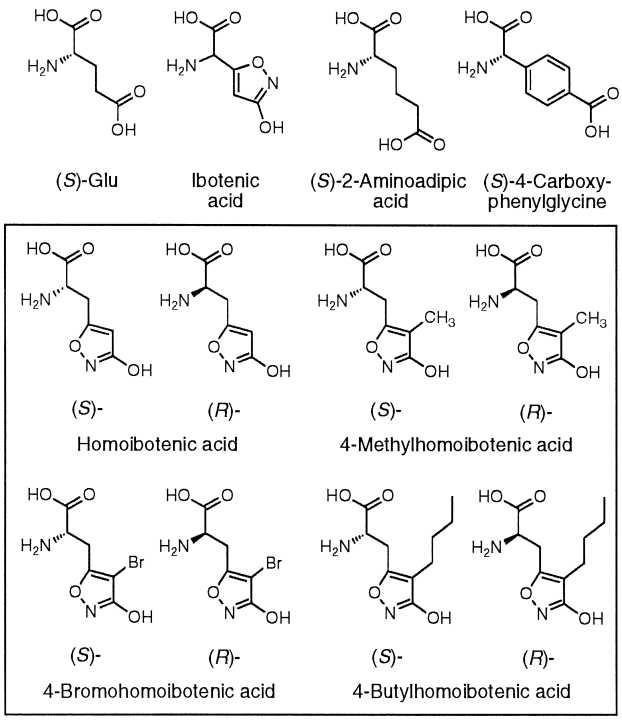

Fig. 1. Structure of the compounds tested in this study.

3. Results

The structures of the compounds tested in this study are shown in Fig. 1. On the basis of the previously published potencies of ibotenic acid and (*S*)-2-amino-3-(4-bromo-3-hydroxyisoxazol-5-yl)propionic acid [(*S*)-4-bromohomoibotenic acid] at mGlu receptors (Abe et al., 1992; Aramori and Nakanishi, 1992; Tanabe et al., 1992, 1993; Thomsen et al., 1994) all compounds were initially tested at a relatively high concentration (1 mM) on CHO cells expressing $mGlu_{1\alpha}$, $mGlu_{5a}$ (group I), $mGlu_2$ (group II) or $mGlu_{4a}$ (group III) receptor subtypes. Both $mGlu_{1\alpha}$ and $mGlu_{5a}$ were selected as representatives of group I due to the previously published antagonistic activity of (*S*)-4-bromohomoibotenic acid at $mGlu_{1\alpha}$ (Thomsen et al., 1994).

In agreement with previous studies (Aramori and Nakanishi, 1992; Tanabe et al., 1992, 1993; Abe et al., 1992) and as shown in Fig. 2 and Table 1, ibotenic acid displayed agonistic effects at group I ($mGlu_{1\alpha}$ and $mGlu_{5a}$) and group II ($mGlu_2$) with lower potency than (*S*)-Glu, whereas it was inactive at group III ($mGlu_{4a}$). As seen in Fig. 2, none of the tested enantiomeric homologues of ibotenic acid displayed any agonistic effect at the four mGlu receptor subtypes at 1 mM concentration.

The ibotenic acid homologue, homoibotenic acid, and the homoibotenic acid analogues were also tested at 1 mM

Table 1
Pharmacological activities at cloned metabotropic glutamic acid receptors expressed in CHO cells

	$mGlu_{1\alpha}$	$mGlu_2$	$mGlu_{4a}$	$mGlu_{5a}$
EC_{50} (μM)				
(*S*)-Glu	13±1	4.4±0.8	13±1	7.4±1.5
(*S*)-2-Aminoadipic acid	>1000[a]	35±1[a]	>3000[a]	>1000
Ibotenic acid	43±1	110±11	>1000	17±7
K_b (μM)				
(*S*)-Homoibotenic acid	250±27	>1000	>1000	490±8
(*R*)-Homoibotenic acid	>1000	>1000	>1000	>1000
(*S*)-4-Methylhomoibotenic acid	190±18	>1000	>1000	180±37
(*R*)-4-Methylhomoibotenic acid	>1000	>1000	>1000	>1000
(*S*)-4-Bromohomoibotenic acid	160±3	>1000	>1000	230±28
(*R*)-4-Bromohomoibotenic acid	>1000	>1000	>1000	>1000
(*S*)-4-Butylhomoibotenic acid[b]	110±3	>1000	>1000	97±9
(*R*)-4-Butylhomoibotenic acid[b]	>1000	>1000	>1000	>1000
(*S*)-4-Carboxyphenylglycine	18±1	nt	nt	>300

[a] From the work of Bräuner-Osborne et al., 1996.
[b] From the work of Johansen et al., 1998.
nt: Not tested.
Data represent the mean±S.E.M. of at least two independent results.

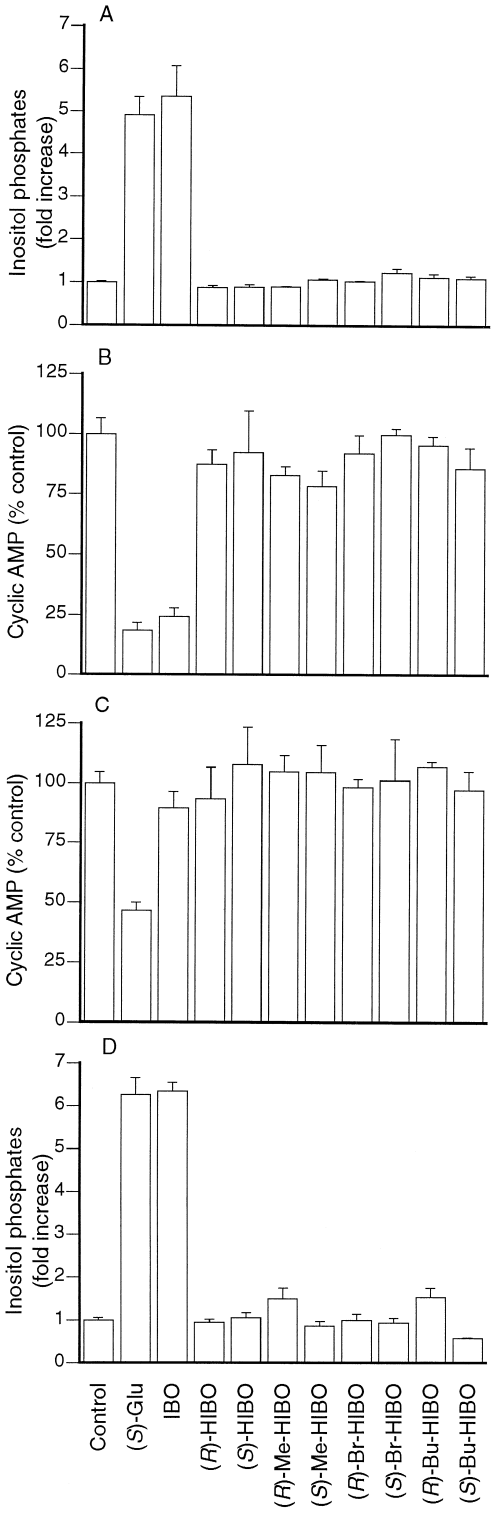

Fig. 2. Agonist activities of (*S*)-Glu, ibotenic acid (IBO), homoibotenic acid (HIBO), 4-methylhomoibotenic acid (Me-HIBO), 4-bromohomoibotenic acid (Br-HIBO) and 4-butylhomoibotenic acid (Bu-HIBO) analogues at CHO cells expressing (A) $mGlu_{1\alpha}$, (B) $mGlu_2$, (C) $mGlu_{4a}$ and (D) $mGlu_{5a}$. $mGlu_{1\alpha}$- and $mGlu_{5a}$-expressing cells were incubated with ligands at a concentration of 1 mM for 20 min. Total IP formation was determined by an ion-exchange chromatography assay and the fold increase in IP level calculated compared to control cells (incubated in buffer only). $mGlu_2$- and $mGlu_{4a}$-expressing cells were incubated with ligands (1 mM) for 10 min in the presence of 10 μM forskolin and 1 mM IBMX. Cyclic AMP levels were measured by a scintillation proximity assay and expressed as percent of cyclic AMP level in control cells (incubated in buffer only). Data are the mean (±S.D.) of representative experiments performed in triplicate.

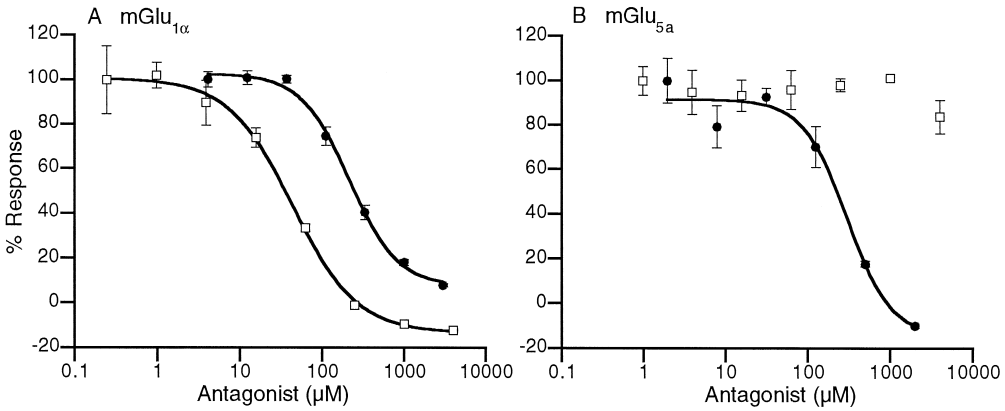

Fig. 3. Antagonistic activities of (*S*)-4-butylhomoibotenic acid (●) and (*S*)-4-carboxyphenylglycine (□) at CHO cells expressing (A) $mGlu_{1\alpha}$ or (B) $mGlu_{5a}$. Cells were preincubated with antagonist alone for 20 min and then co-incubated with 20 μM ($mGlu_{1\alpha}$) or 10 μM ($mGlu_{5a}$) (*S*)-Glu for 20 min. For further details see Fig. 2.

concentration for antagonistic effect against (*S*)-Glu (data not shown). As shown in Table 1, none of the (*R*)-enantiomers of these compounds displayed any antagonistic effects at the four mGlu receptor subtypes. Furthermore, the corresponding (*S*)-enantiomers were inactive as antagonists at $mGlu_2$ and $mGlu_{4a}$ (Table 1). As exemplified by the most potent compound (*S*)-2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid [(*S*)-4-butylhomoibotenic acid] in Fig. 3, all of the (*S*)-enantiomers of the homoibotenic acid analogues were able to antagonize a (*S*)-Glu-mediated response at $mGlu_{1\alpha}$ and $mGlu_{5a}$ with similar potencies. Interestingly, as seen in Table 1, the potency of the analogues at $mGlu_{1\alpha}$ and $mGlu_{5a}$ increased with increasing bulk and lipophilicity of the ring substituent of (*S*)-homoibotenic acid.

It has previously been shown that phenylglycine-derived antagonists, such as (*S*)-4-carboxyphenylglycine and 1-aminoindan-1,5-dicarboxylic acid (AIDA), selectively antagonize $mGlu_{1\alpha}$ whereas they are weak or inactive at $mGlu_{5a}$ (Kingston et al., 1995; Brabet et al., 1995; Moroni et al., 1997). In order to compare these results at the cell lines used in this study, the antagonistic effect of (*S*)-4-carboxyphenylglycine was determined. As seen in Fig. 3 and Table 1, results similar to the previous studies were obtained, confirming the $mGlu_{1\alpha}$ receptor subtype selectivity of phenylglycine derivatives.

4. Discussion

In this study we have shown that the (*S*)-forms of homoibotenic acid and analogues of homoibotenic acid with ring substituents of different sizes are antagonists at $mGlu_{1\alpha}$ and $mGlu_{5a}$, each compound being approximately equipotent at these two receptors. In a previous study of $mGlu_{1\alpha}$, $mGlu_2$ and $mGlu_{4a}$ receptors expressed in baby hamster kidney (BHK) cells, (*S*)-4-bromohomoibotenic acid was found to antagonize $mGlu_{1\alpha}$ with similar potency as reported in this study whereas (*R*)-4-bromohomoibotenic acid was found to be inactive both as an agonist and an antagonist (Thomsen et al., 1994). However, in another study also performed using mGlu receptors expressed in BHK cells, (*RS*)-4-bromohomoibotenic acid has been reported to be a low-efficacious partial agonist at group I and III and (*RS*)-2-amino-3-(4-methyl-3-hydroxyisoxazol-5-yl)propionic acid [(*RS*)-4-methylhomoibotenic acid] to be a low-efficacious partial agonist at group III (Chung et al., 1997). Unfortunately, the antagonistic behavior of (*RS*)-4-bromohomoibotenic acid and (*RS*)-4-methylhomoibotenic acid was not investigated in the latter study (Chung et al., 1997) and the authors had no explanation for the apparent disagreement with the previously reported lack of agonistic activity of (*S*)- and (*R*)-4-bromohomoibotenic acid at BHK cells expressing $mGlu_{1\alpha}$, $mGlu_2$, or $mGlu_{4a}$ (Thomsen et al., 1994).

It is interesting to note the dramatic effect of elongating the backbone chain-length of the group I and II agonist ibotenic acid by one carbon unit to give homoibotenic acid, which shows selective group I antagonism. These results add to a growing list of homologous compounds, such as (*S*)-2-aminoadipic acid (Bräuner-Osborne et al., 1996), (*S*)-homo-AMPA (Bräuner-Osborne et al., 1996; Ahmadian et al., 1997), (*S*)-homoquisqualic acid (Bräuner-Osborne and Krogsgaard-Larsen, 1998) and (1*S*,3*R*)-homo-ACPD (Bräuner-Osborne et al., 1997), which display pharmacological profiles distinctly different from those of their parent compounds. It is also interesting to note the approximately equal potency of (*S*)-homoibotenic acid and each of the analogues at $mGlu_{1\alpha}$ and $mGlu_{5a}$ (Table 1). This is in contrast to the antagonists derived from phenylglycine, which have been shown to selectively antagonize $mGlu_{1\alpha}$ with weak or no effect at $mGlu_{5a}$ (Kingston et al., 1995; Brabet et al., 1995; Moroni et al., 1997). These results for (*S*)-4-carboxyphenylglycine were confirmed in this study, which showed more than 10-fold selectivity of (*S*)-4-carboxyphenylglycine for

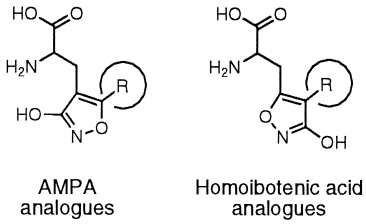

Fig. 4. A comparison of generalized structures of AMPA analogues, which interact with AMPA receptors but not with mGlu receptors, and of homoibotenic acid analogues which show effects at AMPA receptors as well as group I mGlu receptors.

$mGlu_{1\alpha}$ compared to $mGlu_{5a}$. To our knowledge, (*S*)-homoibotenic acid and the analogues are thus the first compounds showing potent antagonism at $mGlu_{5a}$.

Unfortunately, the use of the (*S*)-forms of homoibotenic acid and its analogues as pharmacological tools at the group I receptor subtypes is impaired by their activity at AMPA receptors. We have previously shown that (*S*)-homoibotenic acid, (*S*)-4-methylhomoibotenic acid, (*S*)-4-bromohomoibotenic acid and (*S*)-4-butylhomoibotenic acid are AMPA agonists with IC_{50}-values of 0.80 μM, 0.32 μM, 0.34 μM and 0.48 μM, respectively (Bischoff et al., 1995; Hansen et al., 1989, 1992; Johansen et al., 1998). However, the agonist potency of AMPA analogues with substituents in the 5-position, has previously been shown to decrease at the AMPA receptor, when the size and lipophilicity of the ring substituent (Fig. 4) has been increased (Krogsgaard-Larsen et al., 1996; Sløk et al., 1997). As shown in this study, the opposite is observed for the (*S*)-forms of homoibotenic acid and homoibotenic acid analogues at the group I mGlu receptors, and we are currently in the process of designing new ring substituted homoibotenic acid analogues with increased substituent bulk and lipophilicity (Fig. 4) with the aim of obtaining compounds showing selectivity and increased potency for the group I mGlu receptors.

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References

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N., Nakanishi, S., 1992. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction. J. Biol. Chem. 267, 13361–13368.

Ahmadian, H., Nielsen, B., Bräuner-Osborne, H., Johansen, T.N., Stensbøl, T.B., Sløk, F.A., Sekiyama, N., Nakanishi, S., Krogsgaard-Larsen, P., Madsen, U., 1997. (*S*)-Homo-AMPA, a specific agonist at the $mGlu_6$ subtype of metabotropic glutamic acid receptors. J. Med. Chem. 40, 3700–3705.

Aramori, I., Nakanishi, S., 1992. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. Neuron 8, 757–765.

Bischoff, F., Johansen, T.N., Ebert, B., Krogsgaard-Larsen, P., Madsen, U., 1995. Excitatory amino acid receptor ligands: Asymmetric synthesis, absolute stereochemistry and pharmacology of (*R*)- and (*S*)-homoibotenic acid. Bioorg. Med. Chem. 3, 553–558.

Brabet, I., Mary, S., Bockaert, J., Pin, J.-P., 1995. Phenylglycine derivatives discriminate between mGlu1- and mGlu5-mediated responses. Neuropharmacology 34, 895–903.

Bräuner-Osborne, H., Krogsgaard-Larsen, P., 1998. Pharmacology of (*S*)-homoquisqualic acid and (*S*)-2-amino-5-phosphonopentanoic acid [(*S*)-AP5] at cloned metabotropic glutamate receptors. Br. J. Pharmacol. 123, 269–274.

Bräuner-Osborne, H., Sløk, F.A., Skjærbæk, N., Ebert, B., Sekiyama, N., Nakanishi, S., Krogsgaard-Larsen, P., 1996. A new highly selective metabotropic excitatory amino acid agonist: 2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid. J. Med. Chem. 39, 3188–3194.

Bräuner-Osborne, H., Madsen, U., Mikiciuk-Olasik, E., Curry, K., 1997. New analogues of ACPD with selective activity for group II metabotropic glutamate receptors. Eur. J. Pharmacol. 332, 327–331.

Chung, D.S., Traynelis, S.F., Murphy, T.J., Conn, P.J., 1997. 4-Methylhomoibotenic acid activates a novel metabotropic glutamate receptor coupled to phosphoinositide hydrolysis. J. Pharmacol. Exp. Ther. 283, 742–749.

Craig, D.A., 1993. The Cheng–Prusoff relationship: something lost in the translation. Trends Pharmacol. Sci. 14, 89–91.

Hansen, J.J., Nielsen, B., Krogsgaard-Larsen, P., Brehm, L., Nielsen, E.Ø., Curtis, D.R., 1989. Excitatory amino acid agonists. Enzymatic resolution, X-ray structure, and enantioselective activities of (*R*)- and (*S*)-bromohomoibotenic acid. J. Med. Chem. 32, 2254–2260.

Hansen, J.J., Jørgensen, F.S., Lund, T.M., Nielsen, B., Reinhardt, A., Breum, I., Brehm, L., Krogsgaard-Larsen, P., 1992. AMPA receptor agonists: structural, conformational and stereochemical aspects. In: Krogsgaard-Larsen, P., Hansen, J.J. (Eds.), Excitatory Amino Acid Receptors, Ellis Horwood, London, pp. 216–247.

Hayashi, Y., Tanabe, Y., Aramori, I., Masu, M., Shimamoto, K., Ohfune, Y., Nakanishi, S., 1992. Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 107, 539–543.

Hayashi, Y., Momiyama, A., Takahashi, T., Ohishi, H., Ogawa-Meguro, R., Shigemoto, R., Mizuno, N., Nakanishi, S., 1993. Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. Nature 366, 687–690.

Hayashi, Y., Sekiyama, N., Nakanishi, S., Jane, D.E., Sunter, D.C., Birse, E.F., Udvarhelyi, P.M., Watkins, J.C., 1994. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. J. Neurosci. 14, 3370–3377.

Johansen, T.N., Ebert, B., Bräuner-Osborne, H., Christensen, I.T., Johansen, J.S., Søby, K., Krogsgaard-Larsen, P., Brehm, L., 1998. Resolution, absolute stereochemistry and pharmacology of the (*R*)-(−)- and (*S*)-(+)-isomers of the AMPA receptor agonist (*RS*)-2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid [(*RS*)-Bu-HIBO]. J. Med. Chem. 41, 930–939.

Kingston, A.E., Burnett, J.P., Mayne, N.G., Lodge, D., 1995. Pharmacological analysis of 4-carboxyphenylglycine derivatives: Comparison of effects on $mGlu1\alpha$ and mGluR5a subtypes. Neuropharmacology 34, 887–894.

Knöpfel, T., Kuhn, R., Allgeier, H., 1995. Metabotropic glutamate recep-

tors: Novel targets for drug development. J. Med. Chem. 38, 1417–1426.

Krogsgaard-Larsen, P., Ebert, B., Lund, T.M., Bräuner-Osborne, H., Sløk, F.A., Johansen, T.N., Brehm, L., Madsen, U., 1996. Design of excitatory amino acid receptor agonists, partial agonists and antagonists: Ibotenic acid as a key lead structure. Eur. J. Med. Chem. 31, 515–537.

Moroni, F., Lombardi, G., Thomsen, C., Leonardi, P., Attucci, S., Peruginelli, F., Torregrossa, S.A., Pellegrini-Giampietro, D.E., Luneia, R., Pellicciari, R., 1997. Pharmacological characterization of 1-aminoindan-1,5-dicarboxylic acid, a potent mGluR1 antagonist. J. Pharmacol. Exp. Ther. 281, 721–729.

Nakanishi, S., Masu, M., 1994. Molecular diversity and functions of glutamate receptors. Annu. Rev. Biophys. Biomol. Struct. 23, 319–348.

Schoepp, D.D., Johnson, B.G., Wright, R.A., Salhoff, C.R., Mayne, N.G., Wu, S., Cockerham, S.L., Burnett, J.P., Belegaje, R., Bleakman, D., Monn, J.A., 1997. LY354740 is a potent and highly selective group II metabotropic glutamate receptor agonist in cells expressing human glutamate receptors. Neuropharmacology 36, 1–11.

Sløk, F.A., Ebert, B., Lang, Y., Krogsgaard-Larsen, P., Lenz, S.M., Madsen, U., 1997. Excitatory amino-acid receptor agonists. Synthesis and pharmacology of analogues of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propionic acid. Eur. J. Med. Chem. 32, 329–338.

Tanabe, Y., Masu, M., Ishii, T., Shigemoto, R., Nakanishi, S., 1992. A family of metabotropic glutamate receptors. Neuron 8, 169–179.

Tanabe, Y., Nomura, A., Masu, M., Shigemoto, R., Mizuno, N., Nakanishi, S., 1993. Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. J. Neurosci. 13, 1372–1378.

Thomsen, C., Bau, A., Faarup, P., Foged, C., Kanstrup, A., Suzdak, P.D., 1994. Effects of bromohomoibotenate on metabotropic glutamate receptors. Neuroreport 5, 2417–2420.