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4-Alkylated homoibotenic acid (HIBO) analogues: Versatile pharmacological agents with diverse selectivity profiles towards metabotropic and ionotropic glutamate receptor subtypes

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Abstract

4-Alkylated analogues of homoibotenic acid (HIBO) have previously shown high potency and selectivity at ionotropic and metabotropic glutamic acid receptor (iGluR and mGluR) subtypes. Compounds with different selectivity profiles are valuable pharmacological tools for neuropharmacological studies, and the series of 4-alkyl-HIBO analogues have been extended in this paper in the search for versatile agents. Pharmacological characterization of five new analogues, branched and unbranched 4-alkyl-HIBO analogues, have been carried out. The present compounds are all weak antagonists at Group I mGluRs (mGluR1 and 5) presenting only small differences in potencies (K_i values ranging from 89 to 670 μM). Affinities were studied at native and cloned iGluRs, and the compounds described show preference for the AMPA receptor subtypes GluR1 and 2 over GluR3 and 4. However, compared to previous 4-alkyl-HIBO analogues, these compounds show a remarkably high affinity for the Kain preferring subtype GluR5. The observed GluR5 affinities were either similar or higher compared to their GluR1 and 2 affinity. Isopropyl-HIBO showed the highest affinity for GluR5 ($K_i = 0.16$ μM), and represents a unique compound with high affinity towards the three subtypes GluR1, 2 and 5. In general, these compounds represent new selectivity profiles compared to previously reported Glu receptor analogues.

Keywords: iGluRs; mGluRs; AMPA receptors; GluR5; Receptor selectivity

1. Introduction

Glutamic acid (Glu) is the major excitatory neurotransmitter in the central nervous system and both ionotropic and metabotropic Glu receptors (iGluRs and mGluRs) are important for the functions of the brain and are implicated in mechanisms leading to neurological disorders (Bräuner-Osborne et al., 2000; Parsons et al., 1998). Compounds with selective activity at the three iGluR families, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), kainic acid (Kain) and *N*-methyl-D-aspartic acid (NMDA) receptors, have been available for many years, whereas compounds with selectivities towards the different subtypes are still in demand. Agonists as well as antagonists selectively interacting with subtypes of GluRs are indispensable tools for characterization of GluRs with respect to physiological functions and as potential therapeutic agents for neurological disorders. The need for good pharmacological agents is applicable for all subtypes; AMPA receptor subtypes GluR1-4, Kain receptor subtypes GluR5-7 and KA1-2 as well as for NMDA receptor subtypes NR1, NR2A–D and NR3A–B. Similarly, the research devoted to the development of

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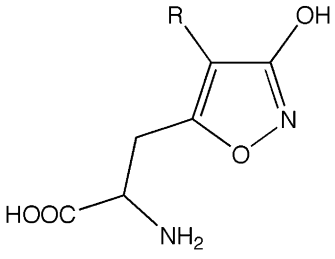
mGluR ligands has not yet filled the need for subtype selective Group I (mGluR1 and 5), Group II (mGluR2 and 3) and Group III (mGluR4 and 6–8) ligands.

4-Substituted homoibotenic acid (HIBO) analogues have previously shown various selectivity profiles at GluRs. Notably Hexyl-HIBO (Fig. 1) have been shown to be a selective antagonist at Group I mGluRs (mGluR1 and mGluR5) (Madsen et al., 2001), and Chloro-HIBO have shown high selectivity and potent agonist activity at the two AMPA receptor subtypes GluR1 and 2 (Bjerrum et al., 2003). Unsubstituted HIBO shows low selectivity between mGluRs and iGluRs, and in contrast to other 4-alkylated analogues HIBO also interacts with Glu uptake mechanisms, leading to low functional activity (Bischoff et al., 1995). In the series of 4-alkylated HIBO analogues the length of the 4-alkyl group has been shown to be of major importance for the activity at AMPA receptors. Methyl- and Butyl-HIBO are potent AMPA receptor agonists (Christensen et al., 1992; Johansen et al., 1998), whereas Pentyl-HIBO is quite weak and all AMPA receptor agonist activity is lost for Hexyl-HIBO (Madsen et al., 2001). Further extension of the side chain leads to Heptyl-HIBO, which in contrast to Hexyl-HIBO shows some preference for mGluR1 over mGluR5. Finally, Octyl-HIBO is virtually inactive, which, at least partially, may be explained by very poor solubility in aqueous as well as lipophilic media (Madsen et al., 2001).



	R:
HIBO	-H
Methyl-HIBO	-CH_3
Ethyl-HIBO	-CH_2-CH_3
Propyl-HIBO	-CH_2-CH_2-CH_3
Isopropyl-HIBO	-CH-$(CH_3)_2$
Isobutyl-HIBO	-CH_2-CH-$(CH_3)_2$
Isopentyl-HIBO	-CH_2-CH_2-CH-$(CH_3)_2$
Butyl-HIBO	-CH_2-CH_2-CH_2-CH_3
Pentyl-HIBO	-CH_2-CH_2-CH_2-CH_2-CH_3
Hexyl-HIBO	-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3
Heptyl-HIBO	-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3
Octyl-HIBO	-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3

Fig. 1. Structure of homoibotenic acid (HIBO) analogues. New compounds in bold.

The pharmacology profiles of 4-substituted HIBO analogues have shown remarkable shifts, based on small structural changes. In this paper a new series of 4-alkyl-HIBO analogues has been tested in order to search for new selectivity profiles. Ethyl- and Propyl-HIBO completes the series of unbranched 4-alkyl-HIBO analogues and the three-branched analogues, Isopropyl-, Isobutyl- and Isopentyl-HIBO have been included to observe the change in molecular pharmacology of these compounds.

These new analogues may lead to new compounds with selectivity profiles different from previous analogues, and thus expand the array of pharmacological tools offering various selectivity profiles for neuropharmacological studies.

2. Methods

2.1. Materials

Ethyl-, Propyl-, Isopropyl-, Isobutyl- and Isopentyl-HIBO were all synthesized in analogy to previously synthesized 4-alkyl-HIBO analogues (Madsen et al., 2001). Briefly, methyl acetoacetate was alkylated with the appropriate alkylbromide in the presence of sodium methoxide as a base. Cyclization of the obtained α-alkylated β-keto esters were performed with hydroxylamine and afforded 4-alkyl-3-hydroxyisoxazoles, which were *O*-ethylated with ethyl bromide. Bromination with neat bromine afforded 5-bromomethyl-isoxazole analogues, which were substituted with diethyl acetamidomalonate. The fully protected forms [ethyl 2-acetamido-2-ethoxycarbonyl-3-(4-alkyl-3-ethoxy-5-isoxazolyl)propionates] were finally deprotected by reflux in concentrated hydrobromic acid. The obtained 4-alkyl-HIBOs were isolated as zwitterions and characterized by ^{1}H NMR and elemental analyses.

2.2. Native receptor binding assays

Affinities for the native AMPA, Kain and NMDA receptors were determined using 5 nM [^{3}H]AMPA (Honoré and Nielsen, 1985), 5 nM [^{3}H]Kain (Braitman and Coyle, 1987) and 2 nM [^{3}H]CGP39653 (Sills et al., 1991), respectively, with modifications previously described (Hermit et al., 2004). Rat brain membrane preparations used in the receptor binding experiments were prepared according to the method described by Ransom and Stec (1988).