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SYNTHESIS AND PHARMACOLOGY OF *N*-ALKYLATED DERIVATIVES OF THE EXCITOTOXIN IBOTENIC ACID

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Abstract

Three amino-alkylated derivatives of the naturally occurring excitatory amino acid (EAA) receptor agonist ibotenic acid (Ibo) have been synthesized and tested pharmacologically. *N*-Methyl-Ibo (**1a**) and *N*-ethyl-Ibo (**1b**) were shown to be agonists at NMDA receptors (EC_{50} =140 and 320 μM, respectively), though with activities considerably lower than Ibo (EC_{50} = 9.6 μM). *N*-Benzyl-Ibo (**1c**) was inactive at ionotropic EAA receptors and all three compounds were, in contrast to Ibo, inactive at metabotropic EAA receptors. Molecular mechanics calculations have been performed on Ibo, **1a-c** and the potent NMDA agonist 2-amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA) in order to elucidate the observed structure-activity data.

Introduction

Glutamic acid (Glu) is the major excitatory amino acid (EAA) transmitter in the mammalian central nervous system.[1,2] The EAA receptors are potential targets for therapeutic intervention in a number of neurodegenerative disorders[3,4] and EAA receptors play a key role in e.g. learning and memory processes.[5] EAA receptors are divided into two main families, ionotropic and metabotropic receptors, each comprising a number of subtypes.[6,7] The ionotropic EAA receptor family consists of three types named by selective agonists: *N*-methyl-*D*-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainic acid receptors. The metabotropic receptors, consist of eight different subtypes subdivided into three groups (group I, II and III) based on signal transduction mechanisms, pharmacology and sequence homology.[8]

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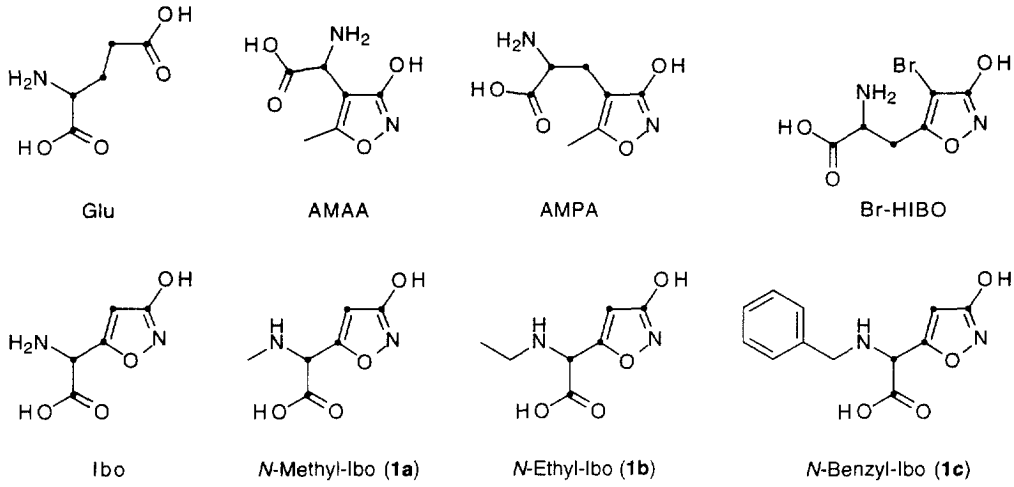
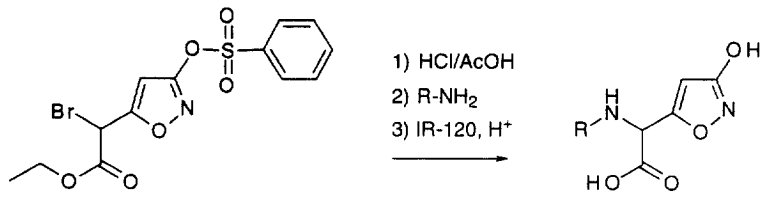


Figure 1

Ibotenic acid (Ibo) is a constituent of the fly agaric mushroom, *Amanita muscaria*, and may be regarded as a conformationally restricted analogue of Glu, in which the 3-hydroxy-isoxazole moiety functions as a bioisostere to the distal carboxylic acid of Glu (Fig. 1). Ibo shows potent neurotoxic properties and interacts potently with NMDA and metabotropic receptors, and to a lesser extent with other EAA receptors.[9] Ibo has been used in multiple studies as a lead compound for the development of selective ligands for EAA receptors, e.g. 2-amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA), AMPA and 2-amino-3-(4-bromo-3-hydroxy-5-isoxazolyl)propionic acid (bromo-homoibotenic acid; Br-HIBO), the former showing potent and selective agonist activity at NMDA receptors and the other two interact potently with AMPA receptors.[10-12] These analogues all contain a primary amino group. However *N*-alkylation may be favorable in order to obtain NMDA receptor selective compounds, as is observed for NMDA itself. In this study we have synthesized three derivatives of Ibo, namely *N*-methyl- (**1a**), *N*-ethyl- (**1b**) and *N*-benzyl-Ibo (**1c**). The pharmacology of these have been tested by electrophysiological experiments and at cloned metabotropic receptors. Molecular mechanics calculations have been performed on Ibo, **1a-c** and AMAA in order to elucidate the structure-activity results obtained.

Chemistry

The starting material for synthesis of the *N*-alkylated Ibo derivatives was ethyl 2-bromo-2-(3-benzenesulfonyloxy-5-isoxazolyl)acetate (**2**), which previously has been used for synthesis of Ibo.[13] The free carboxylic acid was generated by treatment of (**2**) with conc. HCl in glacial acetic acid, and subsequent treatment with methylamine, ethylamine or benzylamine gave the ammonium salts of the three respective *N*-alkylated Ibo derivatives (Scheme 1). The final compounds (**1a-c**) were obtained as zwitterions by ion-exchange chromatography (Amberlite 120, H+-form) and the overall yields ranged from 30-50 %.[14]



2 **1a-c**

R: **a** = CH_3, **b**= CH_2-CH_3, **c** = CH_2-C_6H_5

Scheme 1

In vitro Pharmacology

The activity at ionotropic EAA receptors was studied in an *in vitro* electrophysiological model, the rat cortical slice model.[15] *N*-Methyl-Ibo (**1a**) and *N*-ethyl-Ibo (**1b**) showed agonist activity with EC_{50} = 140 and 320 μM, respectively. The EC_{50} for Ibo was determined to be 9.6 μM (Table 1). The depolarizing activity of all three agonists could be fully antagonized by the competitive NMDA receptor antagonist 3-(2-carboxy-4-piperazinyl)-propyl-1-phosphonic acid (CPP) (10 μM), whereas no antagonist effect was observed with the non-NMDA antagonist 6-nitro-7-sulfamoylbenzo(*f*)quinoxaline-2,3-dione (NBQX) (5 μM). In contrast to this, no significant activity was observed for *N*-benzyl-Ibo (**1c**), neither when given alone (1 mM), nor as an antagonist when co-applied (1 mM) with either NMDA (10 μM), AMPA (5 μM) or kainic acid (10 μM).

Table 1

Agonist activities at metabotropic receptors and in the rat cortical slice model

	EC_{50} (μM)			
	mGluR1α	mGluR2	mGluR4a	Electrophys.
Ibo	43 ± 1[a]	110 ± 11[a]	> 1000[a]	9.6 ± 1.2
N-methyl-Ibo (**1a**)	> 1000	> 1000	> 1000	140 ± 10
N-ethyl-Ibo (**1b**)	> 1000	> 1000	> 1000	320 ± 35
N-benzyl-Ibo (**1c**)	> 1000	> 1000	> 1000	> 1000
AMAA	> 1000	> 1000	> 1000	12[b]

Values ± SEM, n = 3-4. [a]Ref. 17 and [b]ref. 10

The compounds **1a-c** were also tested for activity at metabotropic receptors. Three metabotropic subtypes mGluR1α, mGluR2 or mGluR4a were expressed in Chinese hamster ovary cell lines and used as representatives for group I, II and III metabotropic receptors.[16] The compounds tested showed no activity when added alone or

in combination with a submaximal concentration of Glu (30 μM). Thus, in contrast to Ibo, which is a fairly potent agonist at mGluR1 and mGluR2 receptors, neither of the three derivatives show agonist or antagonist activity at metabotropic receptors (Table 1).

Molecular Mechanics Calculations

A conformational study of Ibo, **1a-c** and AMAA has been performed using Allinger's MM2 force field program.[18] All calculations were done for *R*-enantiomers of the molecules in the uncharged form. For the construction of the isoxazole ring was used the following atomic parameters from the MM2 field: O1 - Osingle, N2 - Nimine, C3, C4 and C5 - sp2 carbon. The geometries of the isoxazole moieties for the calculated low energy conformations of Ibo, **1a-c** and AMAA were compared to the geometry of a similar isoxazole nucleus determined by X-ray crystallographic analysis of the structurally related molecule Br-HIBO.[12] Very small differences were observed in this comparison of bond lengths, valency angles and torsion angles (data not shown), confirming the applicability of the chosen geometry parameters.

Ibo, **1a-c** and AMAA are fairly rigid molecules with conformations primarily determined by rotation around the single bond connecting the α-amino acid moiety and the isoxazole nucleus. A comparison of the distances between the essential functional groups of Ibo, **1a-c** and AMAA in their low energy conformations has been performed. The central atoms of the three pharmacophore groups, C in the carboxylic acid, N in the amino group and C3 in the 3-isoxazolol nucleus, were used for the comparison (Fig. 2). The distances measured (Table 2) show that *N*-benzylation of Ibo increases the distance between the amino nitrogen and the C3 carbon slightly, whereas the distance between the carboxylic acid carbon and C3 is virtually unchanged. All distances for Ibo and **1a-c** in this pharmacophore model are significantly different from the corresponding distances determined for AMAA.

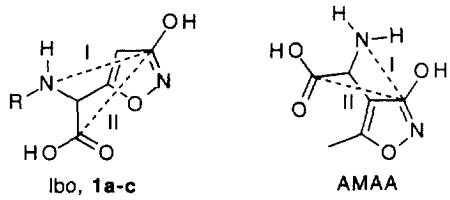


Figure 2 Illustration of measured pharmacophore distances (I and II)

Table 2 Atom distances (Å) according to Fig.2

	$N(NH_2)$ - C3 (I)	C(COOH) - C3 (II)
Ibo	4.29	4.35
1a	4.33	4.35
1b	4.30	4.32
1c	4.60	4.30
AMAA	3.57	3.66

Discussion

In this study derivatives of Ibo, *N*-methyl-, *N*-ethyl- and *N*-benzyl-Ibo (**1a-c**), have been prepared from a common starting material (**2**) and tested for activity at ionotropic and metabotropic EAA receptors. The derivatives were synthesized and tested as racemic mixtures. Ibo itself is stereochemically labile due to a highly activated proton at the chiral center,[19] and the same lability may be expected for the *N*-alkylated derivatives.

Thus, no attempts were made to obtain the pure enantiomers. In contrast to Ibo the three derivatives showed no activity at metabotropic receptors, indicating that *N*-alkylation is not tolerated for metabotropic ligands, at least at the metabotropic receptors represented by mGluR1 and mGluR2 (group I and II).

N-Methyl (**1a**) and *N*-ethyl-Ibo (**1b**) were shown to be agonists at NMDA receptors in the *in vitro* electrophysiological model. The activity was considerably lower compared to Ibo, EC_{50} values being approximately 15 and 30 times higher, respectively, than the EC_{50} for Ibo (Table 1). This fall in activity and the inactivity of *N*-benzyl-Ibo show that *N*-alkylation of Ibo is unfavorable for the activity at NMDA receptors. This is in contrast to the potent activity elicited by the standard agonist NMDA itself, which is also an *N*-methylated compound. In order to investigate whether this fall in activity can be explained by a change in the preferred conformation of the *N*-alkylated derivatives compared to Ibo or due to the increase in steric bulk, molecular mechanics calculations were performed. The MM2 calculations showed good correlations for bond lengths, valency angles and torsion angles of the isoxazole moiety compared to similar data obtained from an X-ray crystallographic analysis of Br-HIBO. Comparison of low energy conformations of Ibo and **1a-c** showed very small changes in the preferred conformations (Table 2). Thus, the fall in activity by *N*-alkylation does not seem to be explained by a change in conformation. A more suitable explanation seems to be the increase in steric bulk, which may not be allowed for Ibo derivatives.

The conformations of Ibo and **1a-c** were also compared to the potent NMDA agonist, AMAA, and the pharmacophore distances were found to be significantly different (Table 2). AMAA and Ibo are fairly rigid molecules, and their low structural flexibilities are primarily determined by rotation around the single bond joining the α-amino acid moiety and the isoxazole nucleus. The pharmacophore distances determined for the low energy conformations (Table 2) does not seem to represent a common pharmacophore for Ibo and AMAA at the NMDA receptors, thus indicating a difference in binding mode for these molecules.

In conclusion the two *N*-alkylated Ibo derivatives, *N*-methyl-Ibo (**1a**) and *N*-ethyl-Ibo (**1b**) only show activity at NMDA receptors, in contrast to Ibo itself, which also shows potent activity at metabotropic receptors. Thus, *N*-alkylation does not seem to be allowed for metabotropic ligands and the extra steric bulk introduced by the alkyl groups seems to explain the loss of activity at NMDA receptors.

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14. General procedure for the synthesis of *N*-alkylated Ibo derivatives (**1a-c**): Ethyl 2-bromo-2-(3-benzenesulfonyloxy-5-isoxazolyl)acetate[13] (**2**) (3.6 g; 9 mmol) was dissolved in a mixture of conc HCl (15 ml) and glacial acetic acid (29 ml) and stirred at 27-28 °C for 55 h. The reaction mixture was evaporated to give a brown oil, which was treated with an aqueous solution of the corresponding amine (100 ml, 23 %) and stirred at 27-28 °C for 48 h. The solvent was evaporated, water (25 ml) was added and the mixture extracted with ether. The aqueous phase was treated with activated charcoal, concentrated to a small volume and then purified by ion-exchange chromatography (Amberlite IR-120, H^+-form). Elution with water gave after evaporation of relevant fractions **1a**, **1b** or **1c**. *N*-Methylibotenic acid (**1a**): yield 50 %, mp 165 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 2.40 (3H, s), 4.42 (1H, s), 6.01 (1H, s). Analysis ($C_6H_8N_2O_4$) C: calcd 41.86, found 41.89; H: calcd 4.68, found 4.42; N: calcd 16.27, found 16.21. *N*-Ethylibotenic acid (**1b**): yield 49 %, mp 167 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.24 (3H, t), 3.04 (2H, q), 4.75 (1H, s), 5.91 (1H, s). Analysis ($C_7H_{10}N_2O_4$) C: calcd 45.16, found 45.17; H: calcd 5.41, found 5.27; N: calcd 15.05, found 14.95. *N*-Benzylibotenic acid (**1c**): yield 30 %, mp 170-171 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 3.83 (2H, s), 4.35 (1H, s), 6.01 (1H, s), 7.25-7.42 (5H, m), 11.3 (1H, broad s). Analysis ($C_{12}H_{12}N_2O_4$, $2H_2O$) C: calcd 51.07, found 51.00; H: calcd 5.67, found 5.69; N: calcd 9.98, found 9.88.
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