Structural Determinants for AMPA Agonist Activity of Aryl or Heteroaryl Substituted AMPA Analogues. Synthesis and Pharmacology

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Summary

We have previously reported the synthesis and pharmacological characterization of analogues of 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA, **1a**), in which the methyl group was replaced by a phenyl group (APPA, **1b**) or heteroaryl groups. While **2b** and its 3-pyridyl analogue 2-amino-3-[3-hydroxy-5-(3-pyridyl)-4-isoxazolyl]propionic acid (3-Py-AMPA, **3**) show very low affinity for AMPA receptors, introduction of heteroaryl substituents containing heteroatom in the 2-position provides potent AMPA receptor agonists. We here report the synthesis and pharmacology of 2-amino-3-(3-hydroxy-5-pyrazinyl-4-isoxazolyl)propionic acid (**7**) (IC_{50} = 1.2 μM; EC_{50} = 11 μM), which is weaker as an AMPA agonist than AMPA (IC_{50} = 0.040 μM; EC_{50} = 3.5 μM) but comparable in potency with 2-Py-AMPA (**4**) (IC_{50} = 0.57 μM; EC_{50} = 7.4 μM), as determined in radioligand binding and electrophysiological experiments, respectively. The AMPA analogues **8a–c**, containing 2-, 3-, or 4-methoxyphenyl substituents, respectively, and the corresponding hydroxyphenyl analogues, **9a–c**, were also synthesized and evaluated pharmacologically. With the exception of 2-amino-3-[3-hydroxy-5-(2-hydroxyphenyl)-4-isoxazolyl]propionic acid (**9a**), which is a very weak AMPA agonist (IC_{50} = 45 μM; EC_{50} = 324 μM), none of these compounds showed detectable effect at AMPA receptors.

Introduction

The excitatory neurotransmitter glutamic acid (Glu) stimulates central neurones by activation of a large number of receptors, subdivided into three heterogeneous classes of ionotropic receptors named *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionic acid (AMPA), and kainic acid receptors[1–3], and a number of subtypes of metabotropic receptors[3,4]. These receptors or perhaps their distinct subtypes may be implicated in certain neurological and psychiatric diseases and are potential therapeutic targets in such diseases[3–6].

There is accumulating evidence of a role of AMPA receptors in the mechanisms associated with cognitive functions[7–9], and enhancement of AMPA receptor functions has been shown to facilitate learning and memory[3,10,11]. Although AMPA receptor agonists may not be therapeutically useful due to potential toxicity, these observations have focused interest on the molecular mechanisms of receptor activation, and thus on the structural basis of AMPA receptor-agonist interactions. Different structural classes of receptor agonists may interact with the AMPA recognition site(s) in a different manner[12], and an important step towards design of AMPA receptor agonists and conversion of such agonists into antagonists on a rational basis is detailed mapping of the structural determinants of AMPA receptor-ligand interactions.

AMPA analogues containing aromatic or heteroaromatic rings in the 5-position of the 3-isoxazolol ring show very distinct structure-activity relationships. 2-Amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionic acid (APPA, **1b**) binds to AMPA receptors with an affinity much lower than that of AMPA (**1a**)[13,14], and its 3-pyridyl analogue **3** is essentially inactive[15]. On the other hand, the 2-pyridyl analogue **4** is a very potent AMPA agonist[15,16], and whereas 2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-5-tetrazolyl)-4-isoxazolyl]propionic acid (2-Me-Tet-AMPA, **5**) is the most potent AMPA agonist so far described, the isomeric compound, 1-Me-Tet-AMPA (**6**) is almost inactive[17,18]. The AMPA analogue with an oxygen-containing heterocyclic ring in the 5-position of the 3-isoxazolol ring, 2-amino-3-[5-(2-furyl)-3-hydroxy-4-isoxazolyl]propionic acid (2-Fu-AMPA, **2**) is only slightly weaker than **5** as an AMPA agonist[15].

The molecular basis for this structure-activity relationship is as yet unclear, but electrostatic as well as steric effects of the 5-substituents of these compounds obviously are important structural determinants of AMPA agonist activity. In order to shed further light on these aspects, we now report the synthesis and pharmacological characterization of compounds **7**, **8a–c**, and **9a–c**.

Chemistry

A key step in the synthetic sequences was the formation of the 3-isoxazolol ring (Scheme 1). The most frequently used route to 3-isoxazolols is cyclization of β-ketoesters with hydroxylamine[19–22]. Thus, β-ketoesters **11a–c**, synthesized from **10a–c** and methyl propionate in a Claisen condensation reaction, were cyclized with hydroxylamine to form the 3-isoxazolol derivatives **12a–c**. In addition to the desired product (41–57% yields), these cyclizations give rise to a major by-product, the isomeric 5-isoxazolone/5-isoxazolol (5–15% yields)[19–22]. The crude reaction products were subjected to column chromatography (CC), and only the desired products **12a–c** were isolated. The structures of these 3-isoxazolols were unequivocally established on the basis of ^{1}H and ^{13}C NMR spectroscopic and thin layer chromatographic (TLC) analyses as described previously[15,17,19–22].

The next step in the synthetic sequences was protection of the hydroxy groups of **12a–c**. In alkylation as well as acyla-

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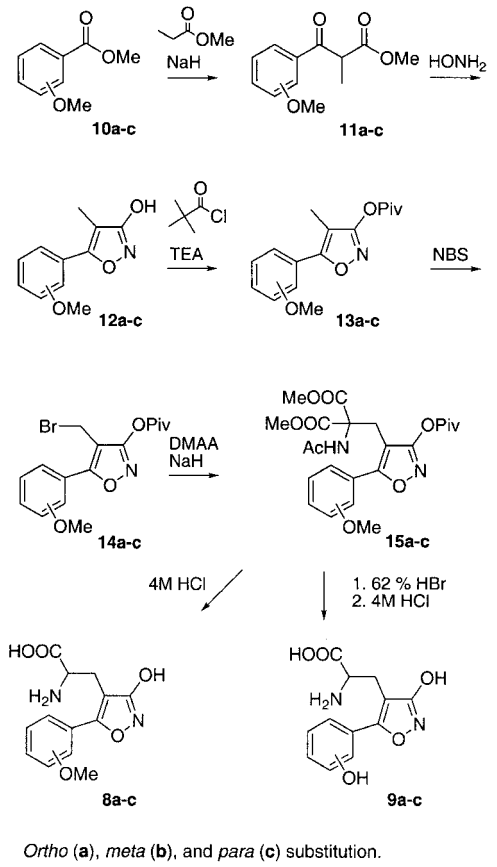
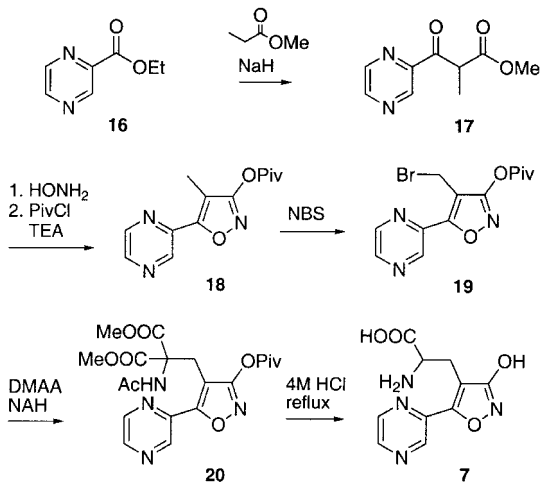


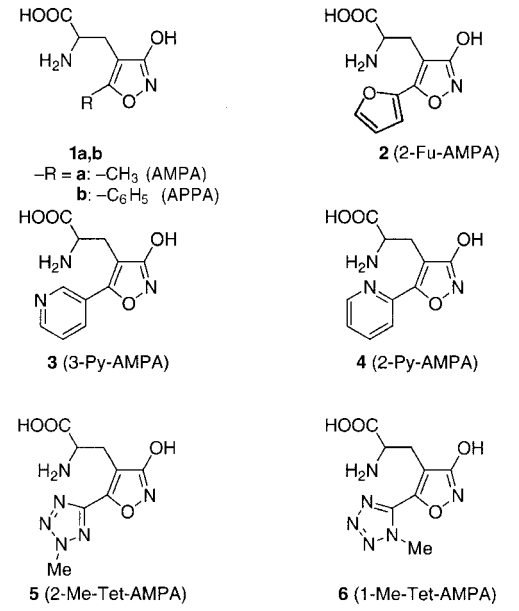
Scheme 1

tion reactions, derivatization normally takes place with both tautomeric forms of the 3-isoxazolol/3-isoxazolone units, resulting in mixtures of *O*- and *N*-protected products[23,24]. In this case, the pivaloyl (Piv) group turned out to be suitable for preferential *O*-acylation (Scheme 1). This acyl group was easily introduced to give very good (**13a,c**) or moderate (**13b**) yields of *O*-protected product. Bromination of **13a–c** was accomplished using NBS in refluxing CCl_4, and the products **14a–c** were obtained in 61–96% yields after CC. Treatment of **14a–c** with dimethyl acetylaminomalonate (DMAA), using sodium hydride in DMF, yielded the protected 3-isoxazolol amino acids **15a–c**. Deprotection using 4 M hydrochloric acid provided the final products **8a–c**, isolated as zwitterions. Complete deprotection of **15a–c** to give the amino acids **9a–c** required treatment with 62% hydrobromic acid in order to cleave the methoxy groups, followed by reflux of the reaction products in 4 M hydrochloric acid in order to fully deprotect the amino acid side chains to give **9a–c**, isolated as zwitterions.

The target compound **7** was synthesized following a similar reaction sequence (Scheme 2), although the presence of the pyrazinyl group generally resulted in markedly lower yields. (3-Hydroxy-4-methyl-5-isoxazolyl)pyrazine, synthesized by treatment of β-ketoester **17** with hydroxylamine could not be isolated by extraction from aqueous solution. Consequently, the evaporated crude reaction product was dissolved in DMF and treated with Piv chloride and triethylamine (TEA) to give **18**. Due to apparent instability, the brominated intermediate **19** could not be purified, and crude **19** was converted into **20**



Scheme 2



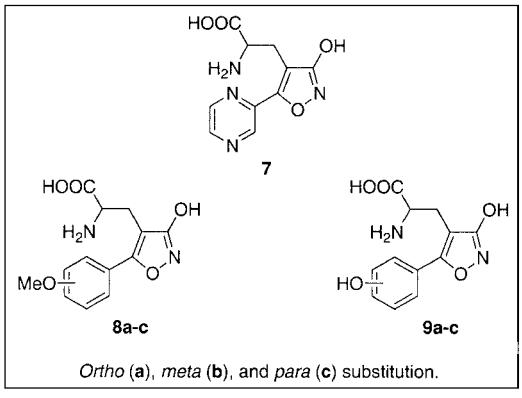
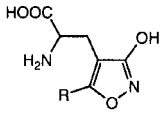


Figure 1

by treatment with DMAA under conditions described above to give **20**, which was deprotected to give **7**. After several unsuccessful attempts to crystallize **7** as a zwitterion or as a halide salt, oily **7** was finally isolated by preparative TLC followed by anion exchange chromatography.

Table 1. Receptor binding and electrophysiological data.



Compound	R	Receptor Binding (IC_{50}, μM) [^{3}H]AMPA	[^{3}H]Kainic acid	[^{3}H]CPP	Electrophysiology (EC_{50}, μM)
1a (AMPA)	Me	0.040[a)]	>100[a)]	100[a)]	3.5[a)]
1b (APPA)	Ph	35[a)]	>100[a)]	>100[a)]	385[a)]
3 (3-Py-AMPA)	3-Pyridyl	>100[b)]	>100[b)]	>100[b)]	>1000[b)]
4 (2-Py-AMPA)	2-Pyridyl	0.57[b)]	>100[b)]	>100[b)]	7.4[b)]
5 (2-Me-Tet-AMPA)	2-Me-5-tetrazolyl	0.030[c)]	41[c)]	>100[c)]	0.92[c)]
6 (1-Me-Tet-AMPA)	1-Me-5-tetrazolyl	54[c)]	>100[c)]	>100[c)]	>1000[c)]
7	Pyrazinyl	1.2±0.4	22[c)]	n.d.	11±1
8a	2-MeO-Ph	>100	>100	>100	>1000
8b	3-MeO-Ph	>100	>100	>100	>1000
8c	4-MeO-Ph	>100	>100	>100	1000
9a	2-HO-Ph	45±9	>100	>100	324±31
9b	3-HO-Ph	>100	>100	>100	>1000
9c	4-HO-Ph	>100	>100	>100	>1000

[a)] Data from Ebert et al. [14]; [b)] Data from Falch et al. [15]; [c)] Data from Bang-Andersen et al. [17]; [d)] Only one determination.

Results and Discussion

The target amino acids **7**, **8a–c**, and **9a–c** were pharmacologically characterized in receptor binding assays using the radioligands [^{3}H]-AMPA[25], [^{3}H]-3-(2-carboxy-4-piperazinyl)propyl-1-phosphonic acid ([^{3}H]-CPP)[26], and [^{3}H]-kainic acid[27] in order to determine the affinity for AMPA, NMDA, and kainic acid receptor sites, respectively. The compounds were tested for agonist or antagonist effects at these receptors using the rat cortical wedge electrophysiological assay system[28].

None of the compounds displayed significant affinity for NMDA or kainic acid receptor sites (IC_{50} > 100μM) (Table 1). Among the compounds **8a–c** and **9a–c**, only **9a**, containing a 2-hydroxyphenyl substituent, showed detectable affinity for AMPA receptor sites (IC_{50} = 45 μM), reflecting a weak AMPA agonist activity (EC_{50} = 324 μM). In contrast, the pyrazinyl-containing AMPA analogue **7** showed potent AMPA receptor affinity (IC_{50} = 1.2 μM) and AMPA agonist activity (EC_{50} = 11 μM) (Table 1). None of the compounds, which were inactive as AMPA agonists showed detectable antagonist effects at AMPA, NMDA, or kainic acid receptors, as demonstrated by the inability of the compounds under study to significantly reduce the depolarizations induced by the standard agonists AMPA (**1a**), NMDA, or kainic acid at the respective receptors.

A number of analogues of the specific AMPA receptor agonist AMPA (**1a**)[29], in which C2–C4 alkyl groups have been substituted for the methyl group of **1a**, have been synthesized and shown to be AMPA agonists comparable in potency with AMPA[30]. Replacement of the methyl group of AMPA (**1a**) by larger alkyl groups abolishes the activity[30].

As exemplified by 2-Py-AMPA (**4**)[15,16], 2-Me-Tet-AMPA (**5**)[17,18], and 2-Fu-AMPA (**2**)[15], substitution of heterocyclic rings containing one or two heteroatoms in 2-position for the phenyl group of the weak AMPA receptor ligand APPA (**1b**)[13,14] has been shown to markedly enhance AMPA agonist activity. On the other hand, the 3-pyridyl analogue, 3-Py-AMPA (**3**) is devoid of affinity for AMPA receptors[15]. These striking structure-activity relationships prompted us to design and synthesize compound **7** as a structural hybrid of **3** and **4**. Interestingly, **7** is slightly weaker than 2-Py-AMPA (**4**) as an AMPA agonist (Table 1), indicating that the presence of a nitrogen atom in the 3-position of the substituent of **7** somehow destabilizes the interaction of the molecule with the AMPA receptor recognition site, in agreement with the observation for 3-Py-AMPA (**3**)[15].

In an attempt to shed light on the structural basis of the pharmacological effects of the presence of ring heteroatoms in the 5-substituted AMPA analogues, the APPA (**1b**) analogues **8a–c** and **9a–c** containing oxygenated phenyl substituents were synthesized and evaluated pharmacologically. With the exception of **9a**, which is a very weak AMPA agonist, all of these compounds were inactive as AMPA receptor agonists or antagonists (Table 1). Thus, although steric effects of the methoxy groups of **8a–c** and the weak acidic character of the phenol groups of **9a–c** may contribute to the very weak effects of these compounds, it may be concluded that the above-mentioned regioselective AMPA agonist-enhancing effects of ring heteroatoms are not mimicked by oxygen substituents.

As shown in Table 1, compound **9a** and APPA (**1b**) are comparable in potency as weak AMPA receptor agonists. Interestingly, a 2-D NOESY spectrum of **9a** showed strong

NOE between the protons of the primary amino group and the H-6 proton of the phenyl ring. Significant NOE was also observed between H-5 of the phenyl ring and the methylene and methine protons of the amino acid side chain of **9a**. These observations and the fact that the NMR spectra showed no signs of hydrogen bonding between the phenol group and the amino acid moiety may reflect the presence of a hydrogen bond between the phenol group and the oxygen atom of the isoxazole ring. It must, however, be emphasized that these NMR spectroscopic experiments were carried out using a solution of **9a** in DMSO. Under these conditions the hydrogen bond situation may be different from that of the compound in aqueous solution.

We postulated that a hydrogen bonding between the charged amino acid unit and the heteroatom in the 2-position of the ring substituents of potent AMPA agonists such as 2-Py-AMPA (**4**) and 2-Me-Tet-AMPA (**5**) stabilizes the amino acid side chain in a receptor-active conformation. This proposal is, however, not supported by the structure-activity analysis of **9a**. An explanation of the structure-activity relationships for AMPA analogues containing ring substituents in the 5-position of the 3-isoxazolol unit may be provided by computational and X-ray crystallographic studies which are in progress.

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Experimental Part

Chemistry. General Methods

Solvents and reagents were purchased from commercial sources and used without further purification unless otherwise stated. Melting points were determined in open capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer. Chemical shifts are given in ppm using TMS or DSS as internal standard. NOESY spectra were obtained on a Bruker AMX400 instrument with mixing time of 500 ms. Elemental analyses were performed by Analytical Research Department, H. Lundbeck A/S, Denmark or by J. Theiner, Microanalytical Laboratory, Institute of Physical Chemistry, University of Vienna, Austria, and results obtained were within ±0.4% of the theoretical values, unless otherwise stated. CC and preparative TLC were performed using silica gel 60 (0.063–0.200 mm) and silica gel 60H (0.045 mm), respectively from Merck. Compounds were visualized on TLC (silica gel 60 F_{254} plates; Merck) using UV light and either a $FeCl_3$ or a $KMnO_4$ spraying reagent. For amino acids, a ninhydrin spraying reagent was used. Ion exchange chromatography of compound **7** was performed using Amberlite IR-120.

*General Procedure for the Preparation of Methyl Methoxybenzoates. Synthesis of Methyl 2-Methoxybenzoate (**10a**)*

Methyl salicylate (11.8 g, 77.8 mmol) was added dropwise to a solution of NaH (55% in mineral oil, 3.74 g, 85.6 mmol) in dry DMF (140 mL) at 0 °C. The reaction mixture was stirred 20 min at 0 °C and methyl iodide (12.1 g, 85.6 mmol) was added slowly. After stirring for 15 h at rt the mixture was concentrated *in vacuo*. Saturated aqueous $NaHCO_3$ was added and the solution extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. CC (EtOAc/toluene 1:9) yielded **10a** as a colorless oil (10.5 g, 81%). ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H), 3.88 (s, 3H), 6.93–6.98 (m, 2H), 7.41–7.47 (m, 1H), 7.60–7.79 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 52.09, 56.06, 112.19, 120.21, 120.34, 131.88, 133.77, 159.39, 167.01.

*Methyl 3-Methoxybenzoate (**10b**)*

Colorless oil; 56.5 g, quantitative yield. ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 3.92 (s, 3H), 7.08–7.65 (m, 4H).

*Methyl 4-Methoxybenzoate (**10c**)*

Colorless oil; 26.4 g, 86%. ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H), 3.89 (s, 3H), 6.91–6.94 (m, 2H), 7.94–8.01 (m, 2H).

*General Procedure for the Preparation of β-Ketoesters. Synthesis of Methyl 2-(2-Methoxybenzoyl)propionate (**11a**)*

To a stirred solution of **10a** (9.78 g, 58.9 mmol) and NaH (60% in mineral oil, 3.06 g, 76.5 mmol) in dry DMF (120 mL), methyl propionate (6.74 g, 76.5 mmol) was added dropwise under N_2. After being stirred at rt for 16 h the reaction mixture was concentrated *in vacuo*, and the residue treated with saturated aqueous $NaHCO_3$ followed by extraction with EtOAc. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. CC (EtOAc/hexane 1:9) yielded **11a** as a colorless oil (8.17 g, 62%). ^{1}H NMR ($CDCl_3$) δ 1.43 (d, 3H, *J* = 7.2 Hz), 3.66 (s, 3H), 3.87 (s, 3H), 4.33 (q, 1H, *J* = 7.2 Hz), 6.94–7.04 (m, 2H), 7.44–7.52 (m, 1H), 7.77–7.82 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 13.52, 52.17, 52.69, 55.35, 111.68, 121.14, 126.77, 131.42, 134.45, 158.77, 172.40, 197.46. Anal. ($C_{12}H_{14}O_4$) C, H.

*Methyl 2-(3-Methoxybenzoyl)propionate (**11b**)*

Colorless oil; 49.3 g, 82%. ^{1}H NMR ($CDCl_3$) δ 1.49 (d, 3H, *J* = 6.9 Hz), 3.69 (s, 3H), 3.86 (s, 3H), 4.40 (q, 1H, *J* = 6.9 Hz), 7.12–7.58 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.96, 48.26, 52.62, 55.55, 112.94, 120.38, 121.37, 129.98, 137.32, 160.24, 171.65, 196.01.

*Methyl 2-(4-Methoxybenzoyl)propionate (**11c**)*

Colorless oil; 9.75 g, 49%. ^{1}H NMR ($CDCl_3$) δ 1.47 (d, 3H, *J* = 7.2 Hz), 3.68 (s, 3H), 3.87 (s, 3H), 4.36 (q, 1H, *J* = 7.2 Hz), 6.93–6.96 (m, 2H), 7.95–7.98 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 13.99, 47.85, 52.57, 55.63, 114.16, 128.91, 131.24, 164.15, 171.86, 194.67. Anal. ($C_{12}H_{14}O_4$) C, H.

*General Procedure for the Preparation of 5-Substituted 4-Methyl-3-isoxazolols. Synthesis of 5-(2-Methoxyphenyl)-4-methyl-3-isoxazolol (**12a**)*

Hydroxylamine hydrochloride (2.50 g, 36.0 mmol) dissolved in MeOH (18 mL) was added to NaOH (1.44 g, 36.0 mmol) dissolved in H_2O (2 mL). The solution was cooled to 0 °C and filtered into a cold (–30 °C) mixture of **11a** (4.00 g, 18.0 mmol) in MeOH (18 mL) and H_2O (2 mL). The reaction mixture was stirred at –30 °C for 3 h, and added portionwise to hot (80 °C) concentrated HCl (40 mL). After stirring for 1.5 h the solution was cooled to rt, concentrated *in vacuo* and the residue redissolved in H_2O (20 mL). The pH was adjusted to 3–4 with solid $NaHCO_3$ and the mixture extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. CC (EtOAc/hexane 1:9) yielded **12a** as a white solid (2.09 g, 57%); mp 167–169 °C. ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 3H), 3.86 (s, 3H), 6.98–7.50 (m, 4H), 9.20 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 6.28, 55.15, 103.12, 111.14, 117.56, 120.33, 130.10, 131.24, 156.65, 163.21, 170.32. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

*5-(3-Methoxyphenyl)-4-methyl-3-isoxazolol (**12b**)*

Solid; 24.2 g, 57%; mp 142–144 °C. ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H), 3.87 (s, 3H), 6.98–7.45 (m, 4H), 10.25 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 6.78, 55.49, 101.57, 111.88, 116.01, 119.15, 129.76, 130.18, 160.09, 164.58, 171.02. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

*5-(4-Methoxyphenyl)-4-methyl-3-isoxazolol (**12c**)*

Solid; 5.75 g, 41%; mp 202–204 °C. ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 3.71 (s, 3H), 5.54 (br s, 1H), 6.83–6.86 (m, 2H), 7.48–7.51 (m, 2H). ^{13}C NMR

($CDCl_3$) δ 6.73, 55.50, 100.03, 114.51, 121.21, 128.30, 161.10, 164.96, 170.80. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

*General Procedure for Pivaloyl Protection of Substituted 3-Isoxazolols. Synthesis of 3-Pivaloyloxy-5-(2-methoxyphenyl)-4-methylisoxazole (**13a**)*

Pivaloyl chloride (9.52 g, 78.9 mmol) was added to compound **12a** (13.5 g, 65.8 mmol) dissolved in CH_2Cl_2 (125 mL) under N_2. TEA (7.99 g, 78.9 mmol) was added dropwise and the reaction mixture was stirred at rt overnight. Quenching with saturated aqueous $NaHCO_3$ was followed by extraction with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. CC (EtOAc/toluene 1:9) yielded **13a** as a colorless oil (17.8 g, 93%). ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.85 (s, 3H), 3.85 (s, 3H), 6.98–7.08 (m, 2H), 7.42–7.52 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 6.85, 26.86, 39.14, 55.31, 106.12, 111.32, 117.29, 120.61, 130.48, 131.76, 156.80, 165.82, 166.35, 174.77. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

*3-Pivaloyloxy-5-(3-methoxyphenyl)-4-methylisoxazole (**13b**)*

Solid; 16.1 g, 50%; mp 84–88 °C. ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.06 (s, 3H), 3.86 (s, 3H), 6.97–7.43 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 7.29, 27.13, 39.45, 55.51, 104.20, 111.94, 116.00, 119.09, 129.69, 130.22, 160.12, 166.88, 167.02, 175.07. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

*3-Pivaloyloxy-5-(4-methoxyphenyl)-4-methylisoxazole (**13c**)*

Solid; 7.35 g, 95%; mp 96–97 °C. ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.04 (s, 3H), 3.87 (s, 3H), 6.98–7.04 (m, 2H), 7.63–7.68 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 7.24, 27.13, 39.43, 55.48, 102.60, 114.53, 121.28, 128.22, 161.04, 166.98, 167.16, 175.10. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

*General Procedure for Bromination of 5-Substituted 4-Methyl-3-isoxazolols. Synthesis of 4-Bromomethyl-3-pivaloyloxy-5-(2-methoxyphenyl)isoxazole (**14a**)*

To a solution of **13a** (17.6 g, 60.8 mmol) in CCl_4 (450 mL) was added NBS (16.2 g, 91.2 mmol) in two equal portions, one portion initially and one after 24 h at reflux. After a total of 3 d at reflux, during which the reaction was followed by ^{1}H NMR, the mixture was allowed to cool to rt, filtered, and concentrated *in vacuo*. The residue was purified by CC (EtOAc/hexane 1:9) to afford **14a** as a yellowish oil (14.9 g, 67%). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.92 (s, 3H), 4.26 (s, 2H), 7.00–7.40 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 20.15, 26.93, 39.28, 55.53, 107.75, 111.45, 116.16, 120.96, 130.53, 132.70, 156.67, 165.53, 166.82, 174.48.

*4-Bromomethyl-3-pivaloyloxy-5-(3-methoxyphenyl)isoxazole (**14b**)*

Yellowish oil; 7.82 g, 61%. ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.88 (s, 3H), 4.32 (s, 2H), 7.10–7.48 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 19.45, 27.11, 39.60, 55.59, 106.31, 112.22, 117.48, 119.65, 128.23, 130.65, 160.33, 165.77, 169.02, 174.64.

*4-Bromomethyl-3-pivaloyloxy-5-(4-methoxyphenyl)isoxazole (**14c**)*

Solid; 8.95 g, 96%; mp 138–140 °C (decomp). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.85 (s, 3H), 4.30 (s, 2H), 7.02–7.10 (m, 2H), 7.70–7.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 20.0, 27.2, 39.8, 55.9, 104.9, 115.0, 119.9, 129.0, 162.0, 164.8, 162.2, 174.3. Anal. ($C_{16}H_{18}NO_4Br$) C, H, N.

*General Procedure for the Preparation of Dimethyl Acetylaminomalonate Derivatives. Synthesis of Methyl 2-Acetylamino-3-[3-pivaloyloxy-5-(2-methoxyphenyl)-4-isoxazolyl]-2-(methoxycarbonyl)propionate (**15a**)*

Dimethyl acetylaminomalonate (7.91 g, 41.8 mmol) dissolved in dry DMF (200 mL) was cooled to 0 °C and NaH (60% in mineral oil, 1.67 g, 41.8 mmol) was added under N_2. After stirring for 20 min, a solution of **14a** (14.0 g, 38 mmol) dissolved in dry DMF (30 mL) was added. The reaction mixture was stirred 2 d at rt and then concentrated *in vacuo*. Saturated aqueous $NaHCO_3$ was added and the solution extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. CC (EtOAc/hexane 1:9) yielded **15a** as a crystalline solid (5.74 g, 32%); mp 170–172 °C. ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.58 (s, 3H), 3.49 (s, 6H), 3.57 (s, 2H), 3.87 (s, 3H), 6.36 (s, 1H), 6.98–7.11 (m, 2H), 7.42–7.58 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 22.29, 25.44, 26.84, 39.28, 53.22, 55.47, 65.37, 104.78, 111.36, 117.64, 120.69, 130.85, 132.08, 156.60, 166.43, 167.40, 167.65, 169.28, 174.94. Anal. ($C_{23}H_{28}N_2O_9$) C, H, N.

*Methyl 2-Acetylamino-3-[3-pivaloyloxy-5-(3-methoxyphenyl)-4-isoxazolyl]-2-(methoxycarbonyl)propionate (**15b**)*

Solid; 3.20 g, 33% (reaction time 16 h); mp 131–133 °C. ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 1.66 (s, 3H), 3.51 (s, 6H), 3.62 (s, 2H), 3.86 (s, 3H), 6.41 (s, 1H), 6.98–7.42 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 22.62, 25.09, 27.07, 39.56, 53.55, 55.57, 65.47, 102.70, 112.41, 117.01, 120.05, 129.69, 130.39, 160.11, 167.13, 167.71, 169.50, 169.87, 175.35. Anal. ($C_{23}H_{28}N_2O_9$) C, H, N.

*Methyl 2-Acetylamino-3-[3-pivaloyloxy-5-(4-methoxyphenyl)-4-isoxazolyl]-2-(methoxycarbonyl)propionate (**15c**)*

Solid; 5.81 g, 50% (reaction time 16 h); mp 183–185 °C. ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H), 1.69 (s, 3H), 3.46 (s, 6H), 3.58 (s, 2H), 3.83 (s, 3H), 6.44 (s, 1H), 6.96–6.99 (m, 2H), 7.56–7.59 (m, 2H). Anal. ($C_{23}H_{28}N_2O_9$) C, H, N.

*General Procedure for the Preparation of α-Amino Acids Containing the 5-Methoxyphenyl Moiety. Synthesis of (RS)-2-Amino-3-[3-hydroxy-5-(2-methoxyphenyl)-4-isoxazolyl]propionic Acid (**8a**)*

Compound **15a** (3.00 g, 6.30 mmol) was dissolved in 4 M aqueous HCl (200 mL) and stirred at reflux for 16 h. The reaction mixture was cooled to rt, concentrated *in vacuo*, and the residue redissolved in H_2O. The solution was washed with EtOAc and pH adjusted to 3–4 with 2 M aqueous NaOH. Upon standing at 5 °C overnight a precipitate was formed and filtered off. The solid was washed with H_2O and dried *in vacuo* to afford **8a** in 71% yield (1.25 g); mp 222–225 °C (decomp). ^{1}H NMR (DMSO) δ 2.43 (dd, 1H, J = 2.4 Hz and 15.6 Hz), 2.72 (dd, 1H, J = 8.7 Hz and 15.6 Hz), 3.53 (dd, 1H, J = 2.4 Hz and 8.7 Hz), 3.78 (s, 3H), 7.01–7.52 (m, 4H), 9.50 (br s, 2H). ^{13}C NMR (DMSO-d_6) δ 25.21, 52.95, 55.68, 104.42, 112.21, 117.36, 120.74, 130.69, 131.99, 156.88, 163.68, 171.08, 171.87. Anal. ($C_{13}H_{14}N_2O_5 \cdot 0.25\ H_2O$) C, H, N.

*(RS)-2-Amino-3-[3-hydroxy-5-(3-methoxyphenyl)-4-isoxazolyl]propionic Acid (**8b**)*

Solid; 355 mg, 64%; mp 215–217 °C (decomp). ^{1}H NMR (DMSO) δ 2.79 (dd, 1H, J = 2.7 Hz and 15.9 Hz), 2.94 (dd, 1H, J = 8.1 Hz and 15.6 Hz), 3.65 (dd, 1H, J = 2.7 Hz and 8.1 Hz), 3.79 (s, 3H), 7.03–7.44 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 25.35, 53.00, 55.65, 103.12, 112.70, 116.01, 119.90, 130.09, 130.68, 159.98, 164.81, 171.85. Anal. ($C_{13}H_{14}N_2O_5 \cdot 0.25\ H_2O$) C, H, N.

*(RS)-2-Amino-3-[3-hydroxy-5-(4-methoxyphenyl)-4-isoxazolyl]propionic Acid (**8c**)*

Solid; 340 mg, 61%; mp > 230 °C (decomp). ^{1}H NMR (DMSO-d_6) δ 2.73 (dd, 1H, J = 2.7 Hz and 15.6 Hz), 2.90 (dd, 1H, J = 8.4 Hz and 15.6 Hz), 3.64 (dd, 1H, J = 2.7 Hz and 8.4 Hz), 3.78 (s, 3H), 7.02–7.05 (m, 2H), 7.53–7.56 (m, 2H). ^{13}C NMR (DMSO) δ 20.94, 48.93, 51.40, 97.43, 110.55, 117.04, 124.87, 156.50, 160.90, 167.45, 167.57. Anal. ($C_{13}H_{14}N_2O_5 \cdot 0.75\ H_2O$) C, H, N.

*General Procedure for the Preparation of -Amino Acids Containing the 5-Hydroxyphenyl Moiety. Synthesis of (RS)-2-Amino-3-[3-hydroxy-5-(2-hydroxyphenyl)-4-isoxazolyl]propionic Acid (**9a**)*

Compound **15a** (950 mg, 1.99 mmol) was dissolved in 62% aqueous HBr (50 mL) and stirred 16 h at 55 °C. The reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was dissolved in aqueous 4 M HCl (75 mL) and stirred under reflux overnight. The solution was cooled to rt, washed with EtOAc, and concentrated *in vacuo*. H_2O was added and pH adjusted to 4 with 2 M aqueous NaOH. Upon standing at 5 °C overnight a precipitate was formed. This solid was filtered off, washed with H_2O and dried *in vacuo* to afford **9a** in 75% yield (395 mg); mp > 220 °C (decomp). ^{1}H NMR (DMSO-d_6) δ 2.52 (dd, 1H, J = 2.1 Hz and 15.6 Hz), 2.74 (dd, 1H, J = 8.4 Hz and 15.6 Hz), 3.55 (dd, 1H, J = 2.1 Hz and 8.4 Hz), 6.83–7.35 (m, 4H), 9.65 (br s, 2H). Anal. ($C_{12}H_{12}N_2O_5 \cdot 0.25\ H_2O$) C, H, N.

*(RS)-2-Amino-3-[3-hydroxy-5-(3-hydroxyphenyl)-4-isoxazolyl]propionic Acid (**9b**)*

Solid, 405 mg, 77%; mp > 230 °C. ^{1}H NMR (DMSO-d_6) δ 2.82 (dd, 1H, J = 3.3 Hz and 15.6 Hz), 2.95 (dd, 1H, J = 7.8 Hz and 15.6 Hz), 3.69 (dd, 1H, J = 3.3 Hz and 7.8 Hz), 6.88–7.35 (m, 4H), 9.90 (br s, 2H). Anal. ($C_{12}H_{12}N_2O_5 \cdot 0.25\ H_2O$) C, H, N.

*(RS)-2-Amino-3-[3-hydroxy-5-(4-hydroxyphenyl)-4-isoxazolyl]propionic Acid (**9c**)*

Solid, 354 mg, 71%; mp > 230 °C. ^{1}H NMR (DMSO-d_6) δ 2.75 (dd, 1H, J = 2.7 Hz and 15.6 Hz), 2.92 (dd, 1H, J = 8.4 Hz and 15.6 Hz), 3.66 (dd, 1H, J = 2.7 Hz and 8.1 Hz), 6.89–6.92 (m, 2H), 7.45–7.48 (m, 2H), 10.1 (br s, 2H). Anal. ($C_{12}H_{12}N_2O_5 \cdot H_2O$) C, H, N.

*Methyl 2-(Pyrazinecarbonyl)propionate (**17**)*

Method as described for **11a**. Yellowish oil; 4.51 g, 42%. ^{1}H NMR ($CDCl_3$) δ 1.52 (d, 3H, J = 6.9 Hz), 3.68 (s, 3H), 4.68 (q, 1H, J = 7.2 Hz), 8.64–8.65 (m, 1H), 8.78–8.79 (m, 1H), 9.28–9.29 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 12.93, 47.21, 52.52, 143.69, 144.51, 146.70, 148.31, 171.81, 197.00. Anal. ($C_9H_{10}N_2O_3$) C, H, N.

*(3-Pivaloyloxy-4-methyl-5-isoxazolyl)pyrazine (**18**)*

Cyclization of β-ketoester **17** with hydroxylamine was performed by the general method described for **12a**. The (3-hydroxy-4-methyl-5-isoxazolyl)pyrazine formed could not be extracted from the aqueous solution, even at varying pH. Instead, the aqueous phase was neutralized with 2 M aqueous NaOH and evaporated to dryness. The remaining solid was dissolved in DMF and reacted with pivaloyl chloride as described in the general procedure, to afford **18** as a white solid (375 mg, 9% from **17**); mp 99–100 °C. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.26 (s, 3H), 8.60–8.61 (m, 1H), 8.66–8.68 (m, 1H), 9.16 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 6.89, 26.86, 39.25, 109.61, 142.41, 143.95, 144.15, 144.47, 162.60, 167.27, 174.65. Anal. ($C_{13}H_{15}N_3O_3$) C, H, N.

*Methyl 2-Acetylamino-3-[3-pivaloyloxy-5-(pyrazinyl)-4-isoxazolyl]-2-(methoxycarbonyl)propionate (**20**)*

The general method described above for **14a** was used for the NBS bromination. However, due to an apparent instability of the product **19**, the reaction mixture was evaporated to dryness *in vacuo* after 2 d and used without further purification in the reaction with DMAA according to the method described for **15a**. The title compound **20** was isolated as a solid in 29% from **18** (303 mg); mp 186–190 °C (decomp). ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.86 (s, 3H), 3.64 (s, 6H), 3.83 (s, 2H), 6.82 (s, 1H), 8.61–8.67 (m, 2H), 9.18–9.19 (m, 1H). Anal. ($C_{20}H_{24}N_4O_8 \cdot 0.5\ H_2O$) C, H, N.

*(RS)-2-Amino-3-[3-hydroxy-5-(pyrazinyl)-4-isoxazolyl]propionic Acid (**7**)*

Reaction conditions as described for **8a**. Oil, 72 mg, 52%. Purified by preparative TLC (EtOAc/CH_3OH 24:1) and subsequent ion exchange chromatography. ^{1}H NMR (D_2O) δ 3.25–3.37 (m, 2H), 4.40–4.55 (m, 1H), 8.56–8.66 (m, 2H), 8.90–8.98 (m, 1H). MS(FAB$^+$) *m/z* 251 ([M+1]$^+$, 9%). HRMS: Calcd for $C_{10}H_{11}N_4O_4$ 251.0780, found 251.0779.

In Vitro Pharmacology

Receptor Binding Assays

Affinity for AMPA receptors was determined using the ligand [^{3}H]AMPA[25]. For determination of NMDA and kainic acid receptor affinities, [^{3}H]CPP[26] and [^{3}H]kainic acid[27], respectively, were used. The membrane preparations used in all receptor binding experiments were prepared according to the method of Ransom and Stec[29].

In Vitro Electropharmacology

A rat cortical wedge preparation for determination of excitatory amino acid-evoked depolarizations described by Harrison and Simmonds[28] was used in a slightly modified version[15]. Wedges (500 μm thick) of rat brain, containing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with an electrode. The cortex was superfused with Mg^{2+}-free Krebs buffer, whereas the corpus callosum part was superfused with Mg^{2+}- and Ca^{2+}-free Krebs buffer, both at 25 °C. The test compounds were added to the cortex superfusion medium, and the induced potential difference between the electrodes was recorded. Agonists were applied for 90 s at each concentration tested. The sensitivity of agonist effects to the AMPA receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulpharmoylbenzo(*f*)quinoxaline (NBQX)[31] (5 μM) was determined at agonist concentrations producing at least 50% of maximal responses. Under these conditions, all of the recorded agonist responses were reversibly reduced by at least 70%. In experiments designed to detect antagonist effects of test compounds at 1 mM concentrations, the compounds were applied alone for 90 s followed by coapplication of the appropriate agonist (5 μM AMPA, 10 μM NMDA or 5 μM kainic acid) and potential antagonist for another 90 s.

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