Heteroaryl Analogues of AMPA. Synthesis and Quantitative Structure−Activity Relationships

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A number of 3-isoxazolol bioisosteres, **7a**−**i**, of (*S*)-glutamic acid (Glu), in which the methyl group of (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA, **1**) was replaced by different 5-membered heterocyclic rings, were synthesized. Comparative *in vitro* pharmacological studies on this series of AMPA analogues were performed using receptor binding assays (IC_{50} values) and the electrophysiological rat cortical slice model (EC_{50} values). None of these compounds showed detectable affinity for the *N*-methyl-D-aspartic acid subtype of Glu receptors. Some of the compounds were weak inhibitors of [^{3}H]kainic acid binding. The inhibitory effects on [^{3}H]AMPA binding and agonist potencies at AMPA receptors of **7a**−**i** were strictly dependent on the structure, electrostatic potential, and methyl substitution of the heterocyclic 5-substituent. Thus, while **7a** (IC_{50} = 0.094 μM; EC_{50} = 2.3 μM) was approximately equipotent with AMPA (IC_{50} = 0.023 μM; EC_{50} = 5.4 μM), (*RS*)-2-amino-3-[3-hydroxy-5-(1*H*-imidazol-2-yl)isoxazol-4-yl]propionic acid (**7b**) (IC_{50} = 48 μM; EC_{50} = 550 μM) was some 2 orders of magnitude weaker than AMPA, and (*RS*)-2-amino-3-[3-hydroxy-5-(1-methyl-1*H*-imidazol-2-yl)-isoxazol-4-yl]propionic acid (**7c**) (IC_{50} > 100 μM; EC_{50} > 1000 μM) was inactive. Furthermore, (*RS*)-2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-tetrazol-5-yl)isoxazol-4-yl]propionic acid (**7i**) (IC_{50} = 0.030 μM; EC_{50} = 0.92 μM) was more potent than AMPA, whereas its N-1 methyl isomer, (*RS*)-2-amino-3-[3-hydroxy-5-(1-methyl-1*H*-tetrazol-5-yl)isoxazol-4-yl]propionic acid (**7h**) (IC_{50} = 54 μM; EC_{50} > 1000 μM) was inactive as an AMPA agonist. A quantitative structure−activity relationship (QSAR) analysis revealed a positive correlation between receptor affinity, electrostatic potential near the nitrogen atom at the "*ortho*" position of the heterocyclic 5-substituent, and the rotational energy barrier around the bond connecting the two rings. We envisage that a hydrogen bond between the protonated amino group and an *ortho*-positioned heteroatom of the ring substituent at the 5-position stabilize receptor-active conformations of these AMPA analogues.

Introduction

(*S*)-Glutamic acid (Glu, Figure 1), which is the main excitatory neurotransmitter in the central nervous system (CNS), and other excitatory amino acids (EAAs) operate through four different classes of receptors. In addition to the three heterogeneous classes of ionotropic EAA receptors (iGluRs), named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid (KAIN) receptors,[1−4] a heterogeneous class of metabotropic receptors (mGluRs) has been shown to have important functions in neurotransmission processes in the CNS.[5] It is now generally agreed that all subtypes of these receptors are potential targets for therapeutic intervention in a number of CNS diseases.[6,7]

EAA receptors are involved in the mechanisms of long-term potentiation, which is believed to play an important role in learning and memory functions, and the deficits of these functions in Alzheimer patients may, to some extent, be caused by hypoactivity at iGluRs and/or mGluRs in the brain.[8−11] There also is growing evidence of an implication of EAA receptors in schizophrenia.[12,13] As in Alzheimer's disease (AD), the role of these receptors in the etiology and the clinical manifestations of schizophrenia is still very incompletely understood, but there is evidence to suggest that hypoactivity at EAA receptors also is a factor of importance in the latter CNS disorder.[13−15] Thus, in AD as well as schizophrenia compounds capable of activating EAA receptors may have therapeutic interest.[15]

During the past years we have developed a number of agonist and antagonist ligands for pharmacological characterization of subtypes of EAA receptors, notably the AMPA receptors.[16,17] Whereas AMPA (**1**) is a high-affinity and very potent AMPA receptor agonist,[18,19] (*RS*)-2-amino-(3-hydroxyisoxazol-4-yl)propionic acid (demethyl-AMPA, **2**) (Figure 1) is a high-affinity but very low-potency AMPA agonist.[20] (*RS*)-2-Amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid (ATPA, **3**), on the other hand, is relatively weak both as an AMPA agonist and as an inhibitor of [^{3}H]AMPA binding.[20,21] Replacement of the bulky *tert*-butyl group of ATPA (**3**) by a hydroxymethyl group, to give (*RS*)-2-amino-3-[3-hydroxy-5-(hydroxymethyl)isoxazol-4-yl)propionic acid (**4**),[22] or by a phenyl group, to give (*RS*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid (APPA, **5**),[23,24] further reduced AMPA receptor agonist activity. These structure−activity relationships and the recent observation that substitution of a 2-thienyl group for the phenyl group of APPA (**5**) provided the high-affinity

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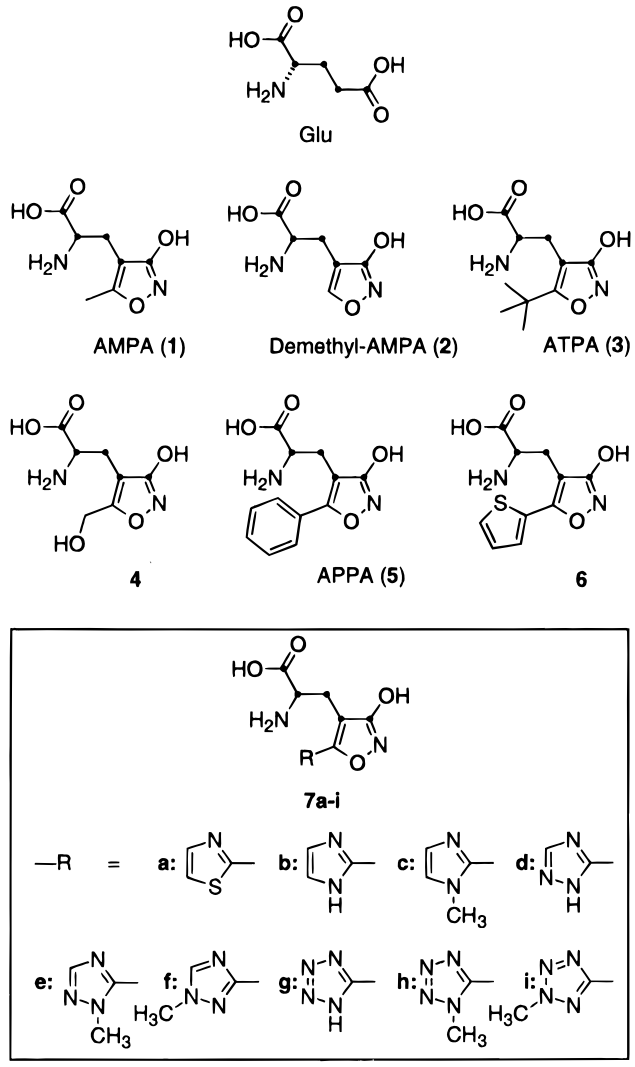


Figure 1. Structures of Glu, AMPA (**1**), demethyl-AMPA (**2**), and a number of 5-substituted AMPA analogues (**3**−**6**). The compounds synthesized (**7a**−**i**) are depicted in the box.

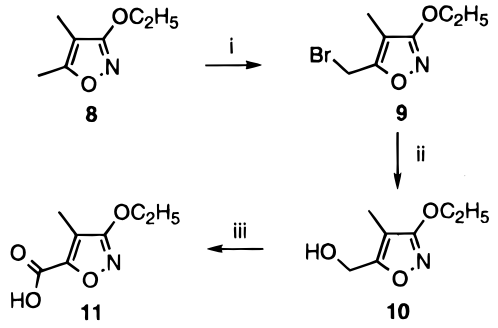
and very potent AMPA agonist (*RS*)-2-amino-3-[3-hydroxy-5-(2-thienyl)isoxazol-4-yl)propionic acid (**6**)[25] emphasized that the pharmacology of AMPA analogues is strongly affected by the structure of the 5-substituent of these compounds.

Mapping of the structural parameters of importance for AMPA agonist−receptor interactions may open up the prospects of designing on a rational basis AMPA receptor ligands showing desired pharmacological profiles. These prospects prompted us to synthesize and pharmacologically characterize the heterocyclic AMPA analogues **7a**−**i** and to perform a quantitative structure−activity relationship (QSAR) analysis.

Results

Chemistry. The bicyclic analogues of AMPA were synthesized as shown in Schemes 1−5. It was envisaged that the different heterocyclic rings at the 5-position of the isoxazole ring could be constructed from a carboxylic acid functionality. Hence, synthesis of the common intermediate **11** was vital to the strategy. Carboxylic acid **11** was synthesized as shown in Scheme 1. In analogy with a published procedure,[26] treatment of dimethyl derivative **8**[27] with bromine gave a 1:1 mixture of starting material **8** and 5-bromomethyl

Scheme 1[a]

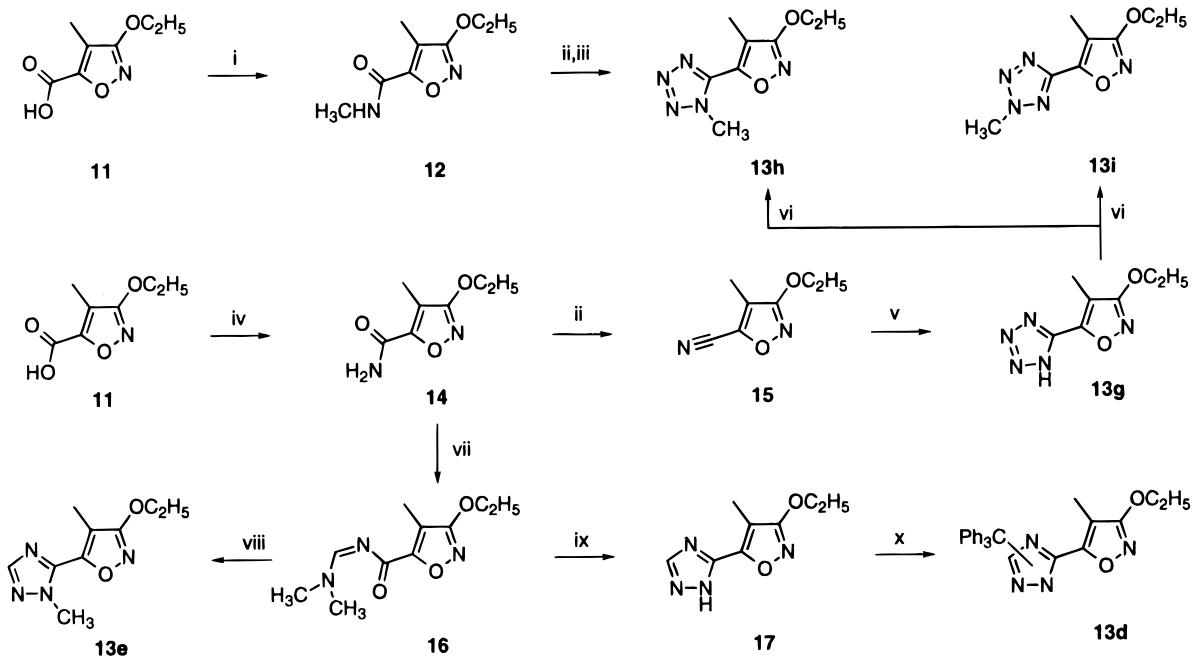


[a] Reagents and conditions: (i) Br_2, CCl_4, room temperature; (ii) H_2O/NMP (2:1), reflux; (iii) CrO_3, H_2SO_4, CH_3COOH, room temperature.

derivative **9**. The crude reaction mixture was subsequently treated with a mixture of water and *N*-methylpyrrolidone (2:1) to furnish a mixture of starting material **8** and alcohol **10**. Alcohol **10** was cleanly oxidized to carboxylic acid **11** by addition of an excess of chromium trioxide in a mixture of acetic acid and sulfuric acid.[28] The synthetic sequences to the AMPA analogues **7a**,**c**−**f**,**h**,**i** were as follows: (1) construction of the heterocyclic 5-substituent from the carboxy group of carboxylic acid **11** (compounds **13a**,**c**−**i**; Schemes 2 and 3); (2) regioselective bromination of the methyl group of 5-heteroaryl-4-methylisoxazoles with *N*-bromosuccinimide (compounds **21a**,**c**−**f**,**h**,**i**; Scheme 4); (3) introduction of the acetamidomalonate group by a Sorensen synthesis (compounds **22a**,**c**−**f**,**h**,**i**; Scheme 4); and (4) complete deprotection in 47% aqueous HBr or 6 M HCl (Scheme 4).

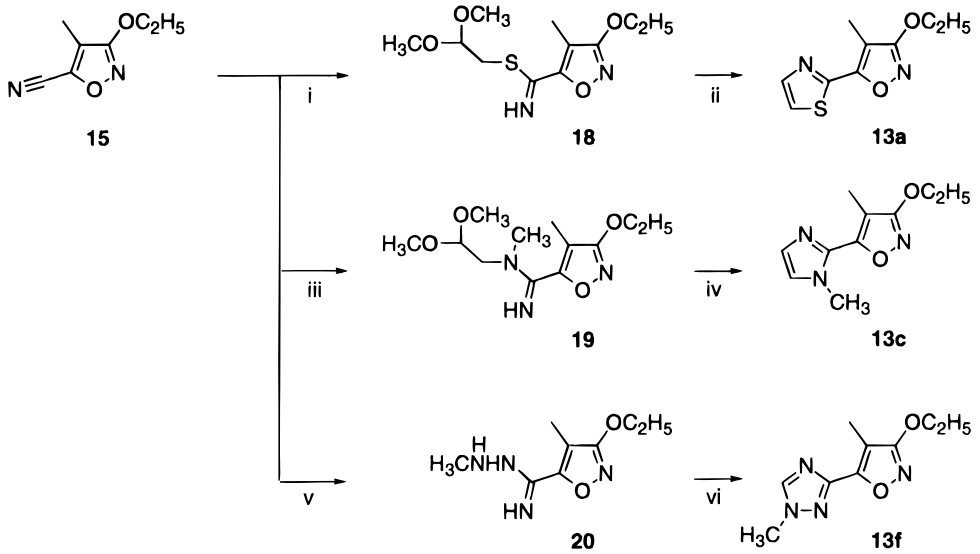
Construction of the heterocyclic 5-substituent, cf. (1) above, is a key transformation and will be discussed in more detail. Unsubstituted tetrazole **13g** and the two methyltetrazoles **13h**,**i** were synthesized as shown in Scheme 2. The amide **14** was obtained from carboxylic acid **11** by addition of the corresponding freshly prepared acyl chloride to 25% aqueous ammonia. Amide **14** was dehydrated with neat phosphoryl chloride to give nitrile **15**, which was then converted into the unsubstituted tetrazole **13g** with triethylammonium azide[29,30] in 1,2-dimethoxyethane. The methyltetrazoles **13h**,**i** were prepared by methylation of the unsubstituted tetrazole **13g** with methyl iodide under basic condition. Thereby, a 1:2 mixture of 1-methyltetrazole **13h**, which has the methyl group "*ortho*" to the isoxazole ring, and its 2-methyl isomer **13i** was obtained. However, the two isomers were not easily separated by either flash chromatography or crystallization, but pure 1-methyltetrazole **13h** was finally obtained after several crystallizations. The 2-methyltetrazole **13i** could only be enriched in the mother liquor to a degree of 80% and was used in the next step without further purification. It may be noted that the bromomethyl derivative of 2-methyltetrazole **13i**, compound **21i** (Scheme 4), could be obtained in pure form after flash chromatography and crystallization. The ratio between the methyltetrazoles **13h**,**i** was not affected by the reaction temperature (methyl iodide in acetone at reflux temperature, room temperature, or −10 °C), the methyl halide (methyl iodide or methyl bromide in acetone at room temperature), or the solvent (methyl bromide in acetone or a 1:1 mixture of water and ethanol at room temperature). 1-Methyltetrazole **13h** was prepared by a regioselective

Scheme 2[a]



[a] Reagents and conditions: (i) $SOCl_2$, reflux; NH_2CH_3, 5 °C; (ii) $POCl_3$, reflux; (iii) NaN_3, DMF, room temperature; (iv) $SOCl_2$, reflux; NH_3/H_2O (25%), 5 °C; (v) NaN_3, TEA·HCl, DME, reflux; (vi) CH_3I, K_2CO_3, acetone, reflux; (vii) $CH(OCH_3)_2N(CH_3)_2$, 120 °C; (viii) NH_2NHCH_3, CH_3COOH, 90 °C; (ix) NH_2NH_2, CH_3COOH, 90 °C; (x) Ph_3CCl, TEA, DMF, room temperature.

Scheme 3[a]

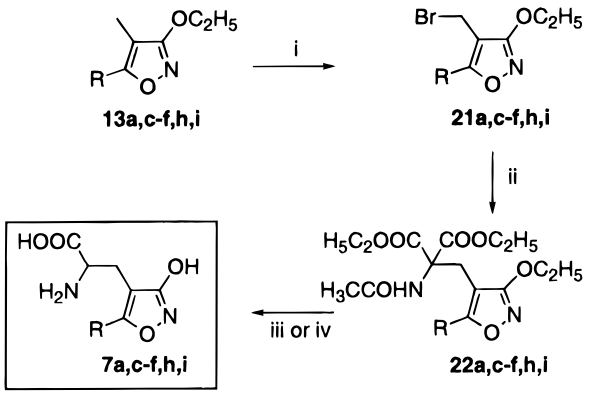


[a] Reagents and conditions: (i) $CH_2(SH)CH(OCH_3)_2$, TEA, −10 °C; (ii) TsOH, toluene, reflux; (iii) $NaOCH_3/CH_3OH$, room temperature; $NH(CH_3)CH_2CH(OCH_3)_2$, CH_3COOH, reflux; (iv) 1 M HCl, reflux; (v) NH_2NHCH_3, C_2H_5OH, 5 °C; (vi) HCOOH, reflux.

synthesis in order to prove the structure of the two methyltetrazoles. *N*-Methylamide **12** was prepared from carboxylic acid **11** by addition of the corresponding freshly prepared acyl chloride to 40% aqueous methylamine. *N*-Methylamide **12** was subsequently treated with phosphoryl chloride to give the imidoyl chloride, which was then added to sodium azide in *N*,*N*-dimethylformamide[31] to furnish 1-methyltetrazole **13h** (Scheme 2). The two methyltetrazoles **13h**,**i** were easily distinguished by ^1H NMR spectroscopy.

Unsubstituted 1,2,4-triazole **17** (1,2,4-triazole is abbriviated triazole in the following) and 2-methyltriazole **13e**, which has the methyl group *ortho* to the isoxazole ring, were synthesized as shown in Scheme 2. Acylamidine **16** was prepared by treatment of amide **14** with *N*,*N*-dimethylformamide dimethyl acetal.[32] Treatment of acylamidine **16** with either hydrazine or methylhydrazine in acetic acid[32] afforded unsubstituted triazole **17** and 2-methyltriazole **13e**, respectively. The structural proof of 2-methyltriazole **13e** is based on the unambiguous synthesis of its N-1 methyl isomer **13f** (Scheme 3). The unsubstituted triazole **17** was protected with a trityl group to avoid complications with the acidic hydrogen of the triazole ring in the following synthesis steps. Treatment of triazole **17** with trityl chloride gave a 7:1 mixture of two isomers, termed trityltriazole **13d**, which were tentatively assigned to 1-trityltriazole (major isomer) and the 2-trityltriazole (minor isomer) in agreement with general observations for alkylation of 3-substituted 1,2,4-triazoles.[33] The mixture of the two isomers was used in the next step without further purification.

Scheme 4[a]



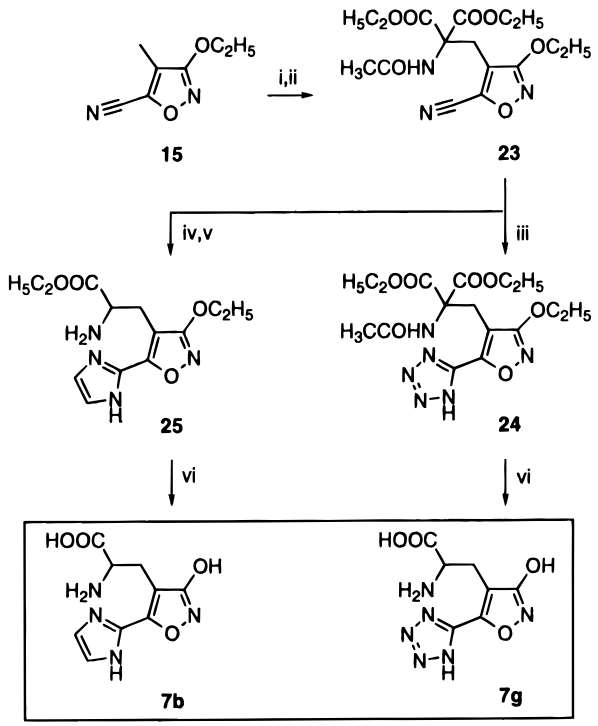
[a] Reagents and conditions: (i) NBS, CCl_4, reflux; (ii) $CH_3CONHCH(COOC_2H_5)_2$, $(CH_3)_3COK$, NMP, room temperature; (iii) 47% HBr(aq), reflux; (iv) 6 M HCl, reflux. Substituents, R, are found in Table 1.

Thiazole **13a** and methylimidazole **13c** were synthesized as shown in Scheme 3. Appropriate nucleophiles were added to nitrile **15**, and the addition products **18**, **19** cyclized in acidic media to give the corresponding cyclization products **13a**,**c**. The 1-methyltriazole **13f** was synthesized as shown in Scheme 3. Addition of methylhydrazine to nitrile **15** in ethanol[34] gave amidrazone **20**. Methylhydrazine has two nucleophilic centers, and addition to the nitrile could theoretically give two isomeric amidrazones. On the basis of ^{1}H NMR spectroscopy it was clear that a single compound was obtained, which was assigned structure **20**. The single proton on the nitrogen atom (CH_3NHN') (δ 5.04) displayed a coupling constant of 4.6 Hz to the protons of the *N*-methyl group (CH_3NHN') (δ 2.74), thus ruling out the structure where the methyl group is attached to nitrogen atom N′. Condensation of **20** with formic acid afforded 1-methyltriazole **13f**, which was easily distinguished from 2-methyltriazole **13e** on the basis of ^{1}H NMR spectroscopy. A difference-NOE experiment was performed on **13f** to further support the regioisomeric proof. Thus, by saturation of the N-1 methyl group of the triazole ring, NOE was observed at the hydrogen atom at the 5-position.

The imidazole and tetrazole analogues **7b**,**g** were synthesized following a slightly different scheme, where the imidazole and tetrazole rings were constructed at a later stage in the syntheses (Scheme 5). Acetamidomalonate derivative **23**, which was derived from nitrile **15** (Scheme 2), was treated with triethylammonium azide[29,30] in 1,2-dimethoxyethane to give tetrazole derivative **24**. Analogously, the nitrile group of acetamidomalonate derivative **23** was converted into the imidazole ring by addition of aminoacetaldehyde dimethyl acetal and subsequent cyclization in 1 M HCl. Thereby, a decarboxylated and partly deprotected imidazole intermediate was obtained, which was then treated with a solution of HCl in ethanol to give ester **25**. Imidazole and tetrazole analogues **7b**,**g** were obtained after complete deprotection in 47% aqueous HBr.

***In Vitro* Pharmacology.** The bicyclic analogues of AMPA were studied in different receptor binding assays in order to determine their affinity for various iGluRs. The ligands [^{3}H]CPG 39653,[35] [^{3}H]AMPA,[36] and [^{3}H]-KAIN[37] were used to determine affinity for NMDA, AMPA, and KAIN receptors, respectively (Table 1). The

Scheme 5[a]



[a] Reagents and conditions: (i) NBS, dibenzoyl peroxide, CCl_4, reflux; (ii) $CH_3CONHCH(COOC_2H_5)_2$, $(CH_3)_3COK$, NMP, room temperature; (iii) NaN_3, TEA·HCl, DME, reflux; (iv) $NaOCH_3$/CH_3OH, room temperature; $NH_2CH_2CH(OCH_3)_2$, CH_3COOH, room temperature; 1 M HCl, reflux; (v) C_2H_5OH/HCl, reflux; (vi) 47% HBr(aq), reflux.

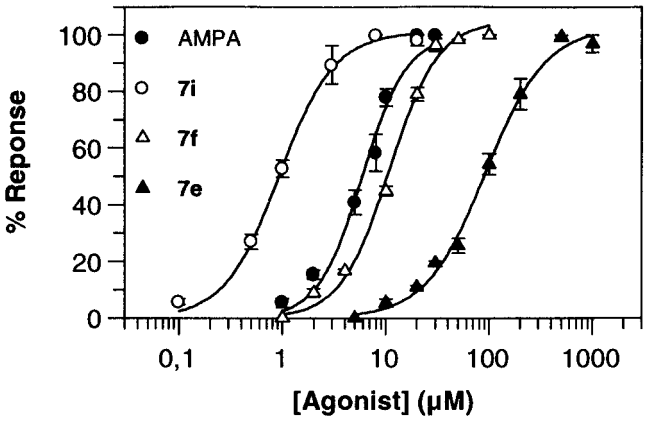
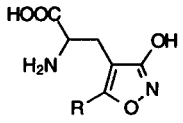


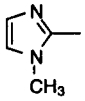
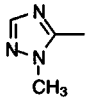
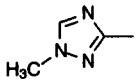
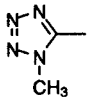
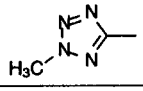
Figure 2. Dose−response curves as determined in the rat cortical slice model for compounds **7e**,**f**,**i** compared to AMPA (**1**). The data are the mean of at least three determinations (see Table 1).

compounds were also studied in the electrophysiological rat cortical slice model[38] (Table 1).

None of the compounds displayed significant affinity for NMDA receptors (IC_{50} > 100 μM), whereas the affinities for AMPA receptors ranged from insignificant affinity to very high affinities comparable with that of AMPA (**1**) (Table 1). The compounds with high affinity for AMPA receptors, except for **7f**, also displayed some affinity for KAIN receptors, but mostly, the compounds showed selectivity for AMPA receptors. Furthermore, it was shown in the rat cortical slice model that the compounds were potent neuronal excitants. Concentration−response curves were constructed for every compound, and it was shown that the compounds were full agonists, as exemplified in Figure 2. The depolarizing

Table 1. Receptor Binding, Electrophysiological Data and pK_a Values[a]



	substituents	receptor binding			electrophysiology[b]	
compd	R	[^{3}H]AMPA	[^{3}H]KAIN	[^{3}H]CPG39653	cortical slice	pK_a values[c]
AMPA (**1**)	H_3C—	0.023	68	>100	5.4 [4.7; 6.5]	
7a		0.094	4.9	>100	2.3 [2.0; 2.5]	< 2 (two); 4.4; 9.8
7b[d]		48	>100	>100	550 [456; 651]	< 3.4; 3.4; 4.9; 9.7
7c[d]		>100	74	>100	>1000	< 3.4; 3.4; 5.3; 9.8
7d		1.5	58	>100	63 [55; 79]	< 2.4; 2.4; 4.6; 8.5; 10.2
7e		5.8	16	>100	93 [84; 111]	< 2.3; 2.3; 4.5; 9.5
7f		0.70	>100	>100	11 [9.89; 12.6]	< 2.3; 2.3; 4.6; 9.5
7g[d]		72	>100	>100	>1000	< 3 (two); 5.0; 9.7
7h[d]		54	>100	>100	>1000	3.7; 5.1; 9.4
7i		0.030	41	>100	0.92 [0.81; 0.98]	2.9; 5.0; 9.6

[a] Binding data are expressed as IC_{50} values in μM and they are the antilog to the log mean of at least two determinations. Two full concentration−inhibition curves were measured by using five concentrations of test drug in triplicate (covering three decades). Standard deviation ratios (SD_R) of each of the assay methods were obtained by calculating the variance of repeated measures of ratios between the first and the second determination for a series of 30 ([^{3}H]AMPA- and [^{3}H]KAIN-binding) and 16 ([^{3}H]CPG39653-binding) drugs. If the ratio was greater than two times the SD_R (95% confidence interval), extra determinations were performed and outliers were discarded. The following SD_R's were calculated: [^{3}H]AMPA-binding, 1.6; [^{3}H]KAIN-binding, 1.7; [^{3}H]CPG39653-binding, 1.3. Electrophysiological data are expressed as EC_{50} values in μM and are the antilog to the log mean of at least three determinations with the minimum and maximum values in brackets. [b] AMPA agonists. [c] See Experimental Section for details. [d] No antagonism at 300 μM toward NMDA (10 μM) and AMPA (5 μM).

effects of these compounds could be antagonized by the competitive non-NMDA receptor antagonist 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) (5 μM), but not by the competitive NMDA receptor antagonist (*RS*)-[3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid (CPP) (5 μM), indicating that the compounds are non-NMDA receptor agonists. Thus, while replacement of the methyl group of AMPA with a thiazole ring, to give compound **7a**, resulted in a compound equipotent with AMPA, the imidazole analogue **7b** was some 2 orders of magnitude weaker than AMPA. The methylimidazole analogue **7c** was inactive. Replacement of the methyl group of AMPA with a 1,2,4-triazole ring or a tetrazole ring containing methyl substituents *ortho* or *meta* to the isoxazole ring, resulted in comparable structure−activity relationships. Thus, whereas the *meta* methyl isomers **7f**,**i** were as potent as AMPA itself, the corresponding *ortho* methyl isomers **7e**,**h** were nearly inactive. The unsubstituted 1,2,4-triazole and tetrazole analogues **7d**,**g** were weak or inactive as AMPA agonists, respectively. The compounds showing no excitatory activity in the slice model were tested for NMDA and non-NMDA receptor antagonism. None of these compounds showed significant antagonism at 300 μM

Table 2. Calculated Parameter Values for the 5-Substituted 4-Methyl-3-isoxazolol Moieties (**13a,b,d−f,h,i**) of the Test Compounds (**7a,b,d−f,h,i**)Used in the QSAR Analysis

compd	$V(r)$ (kcal/mol)	$E(\omega)$ (kcal/mol)	log IC_{50} (μM)
7a	−106.3	2.14	−1.03
7b	−121.8	4.52	1.68
7d	−106.8	4.59	0.18
7e	−103.6	6.73	0.76
7f	−111.5	2.17	−0.15
7h	−94.7	6.94	1.73
7i	−97.0	2.63	−1.52

toward excitation induced by either NMDA (10 μM) or AMPA (5 μM).

QSAR Analysis. QSAR analysis was performed on seven of the AMPA analogues (compounds **7a**,**b**,**d**−**f**,**h**,**i**). The properties of the 5-substituents were described by two parameters: (1) the electrostatic potential ($V(r)$) at a position 2.8 Å in the lonepair direction of the *ortho*-positioned nitrogen atom, and in the plane of the heterocyclic ring; and (2) the energy barrier ($E(\omega)$) for the rotation of the substituted heterocyclic ring by 360°. These torsional drives were performed on the 5-substituted 4-methyl-3-isoxazolol moieties (Table 2). The calculated log IC_{50} values were plotted against the experimental log IC_{50} values (Figure 3) by performing a multiple linear regression, and a r^2 value of 0.91 was obtained.

$$\log IC_{50} = 0.56E(\omega) + 0.06V(r) - 8.00 \qquad (1)$$

$$n = 7, \quad r^2 = 0.91, \quad s = 0.46, \quad F = 20.11$$

The binding affinity of the seven compounds can partly be explained by these two parameters in the QSAR model and by the equation described above. A favorable correlation was obtained with $E(\omega)$, a positive $V(r)$ value, and a negative constant. A large bulky substituent at the 5-position of the isoxazole ring will therefore increase the IC_{50} value, and a more negative electrostatic potential at the *ortho* position of the heterocyclic ring gives a lower IC_{50} value.

Discussion

The 3-isoxazolol amino acid, AMPA (**1**) is a high-affinity, potent, and very selective AMPA receptor agonist,[18,19] and it is generally agreed that the Glu structural element of AMPA (**1**) (Figure 1) is recognized and bound by the AMPA receptor recognition site. On the basis of structure−activity studies on AMPA and a series of AMPA analogues containing alkyl substituents at the 5-position of the ring, we have earlier proposed that the alkyl group of these AMPA agonists may be accommodated by a lipophilic cavity of limited size located at or near the AMPA recognition site.[39,40] Thus, whereas substitution of ethyl[40] or trifluoromethyl[41] groups for the methyl group of AMPA led to AMPA agonists slightly more potent than AMPA, the *tert*-butyl analogue of AMPA, ATPA (**3**) was markedly weaker than AMPA,[20,21] and incorporation of even more bulky groups like 4-heptyl or 2,2-dimethylpropyl groups provided totally inactive compounds.[20] Demethyl-AMPA (**2**), on the other hand, turned out to be a potent inhibitor of [^{3}H]AMPA binding, but showing remarkably low AMPA agonist activity.[20]

On the basis of these structure−activity relationships and the observation that all active alkyl-substituted AMPA analogues were shown to be full agonists, we hypothesized that occupancy of the proposed cavity by an appropriately sized alkyl group contributes to the stabilization of the agonist conformation of the AMPA receptor.[20] We have recently shown that substitution of a 2-thienyl group for the phenyl group of the weak AMPA agonist, APPA (**5**)[23,24] provided the AMPA agonist **6**, approximately equipotent with AMPA.[25] On the other hand, the hydroxymethyl analogue of AMPA, compound **4**, which contains a potential hydrogen bond donor group turned out to be a low-affinity, low-potency, but full AMPA agonist.[22] These observations suggested that AMPA analogues containing appropriately sized 5-substituents producing oriented electrostatic potentials and capable of acting as hydrogen bond acceptors might show potent agonist effects.

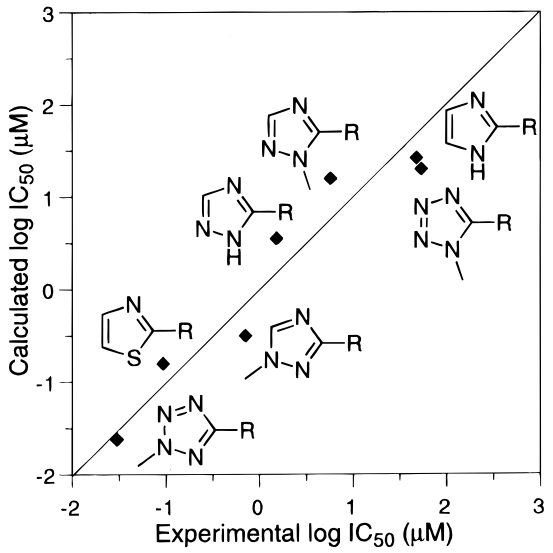


Figure 3. Plot of calculated versus experimental [^{3}H]AMPA binding data (IC_{50} value in μM). The calculated data are derived from eq 1.

This proposal led to the synthesis and pharmacological characterization of the heterocyclic AMPA analogues **7a**−**i**, all of which contain at least one unsubstituted *ortho*-positioned nitrogen atom in the heterocyclic ring substituent (Figure 1). In preparation for a QSAR analysis, we introduced methyl substituents into selected positions of some of these new compounds.

In the QSAR analysis, the electronic and steric effects of the heterocyclic rings located at C-5 of the 3-isoxazolol unit were described by the electrostatic potential near the *ortho*-positioned nitrogen atom and the rotational barrier around the C−C bond connecting the two rings. This analysis revealed a linear relationship between experimental and calculated receptor binding data (IC_{50} values), using multiple linear regression methods (Figure 3).

On the basis of this analysis, we postulate that the receptor-active conformations of this group of compounds are largely determined by *intra*molecular hydrogen bonds between the protonated amino groups and the *ortho*-positioned nitrogen atoms of the heterocyclic ring substituents. We envisage that the geometry of the hydrogen-bonded structures are influenced by the location of the methyl substituents in these rings (compounds **7c**,**e**,**f**,**h**,**i**). Thus, due to steric interactions between the methyl groups at the second *ortho* position of the rings of **7c**,**e**,**h** and the oxygen atom of the

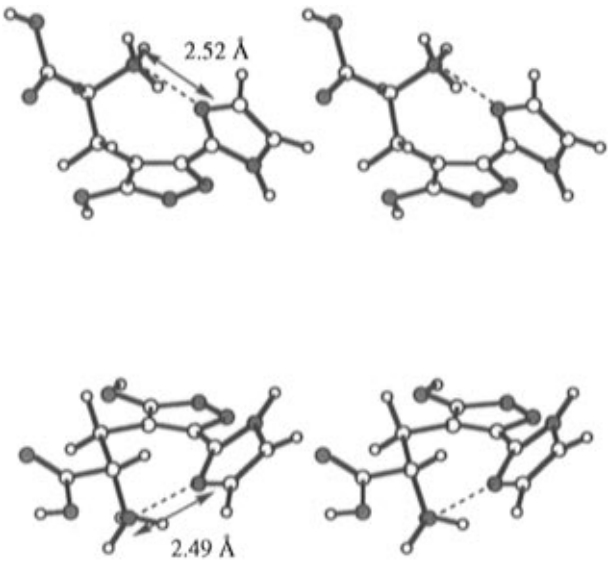
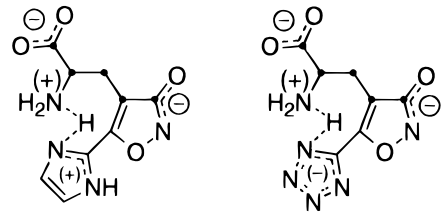


Figure 4. Energy minimized structures of (*S*)-**7b** in stereoviews. Two conformations are represented with plausible hydrogen bonds between the protonated amino group and the imidazole ring.



7b **7g**

Figure 5. Possible "charge-transfer" hydrogen bonds for compounds **7b** and **7g**.

isoxazole ring the ring substituents may be twisted out of the rather planar receptor-active conformations (Figure 4). These interactions are reflected by the relatively high rotational energy barriers calculated for **7e**,**h** (Table 2).

Although **7b** contains an unsubstituted imidazole ring, a rather high IC_{50} value for inhibition of [^{3}H]AMPA binding (Table 1) and a high rotational energy barrier (Table 2) was measured and calculated, respectively, for this compound. The latter value is assumed to reflect the formation of a stable "charge-transfer" hydrogen bond between the protonated amino group and the weakly basic imidazole ring ($pK_a = 3.4$) (Figure 5). The unexpectedly low affinity of **7b** for the AMPA receptor site ($IC_{50} = 48\ \mu M$) may reflect that the imidazole ring carrying a partial positive charge is not optimally accommodated by the proposed lipophilic cavity at this receptor site. Similar effects in addition to infavorable steric effects of the *ortho*-positioned methyl group may explain the inactivity of **7c**.

Compound **7g** containing an unsubstituted tetrazole ring substituent ($pK_a < 3$) also showed unexpectedly weak AMPA receptor affinity ($IC_{50} = 72\ \mu M$) and no detectable AMPA receptor agonist effect (Table 1). Under the test conditions, this negatively charged tetrazole ring may form a "charge-transfer" hydrogen bond, as depicted in Figure 5, and may not interact optimally with the lipophilic AMPA receptor cavity in analogy with the partially charged imidazole ring of **7b**.

The presence of a methyl group at the *meta*-positioned nitrogen atoms of **7f** and **7i** does not significantly influence the rotational energy barriers calculated for these compounds (Table 2). The high AMPA receptor affinities and agonist activities of **7f**,**i**, in particular of **7i**, are consistent with the results of these calculations and seem to indicate that these substituted rings are easily accommodated by the proposed AMPA receptor cavity.

In agreement with earlier findings for alkyl-substituted AMPA analogues,[20,40,41] the compounds under study were either full agonists (Figure 2) or inactive. In no case were partial agonist or antagonist effects observed. The results of this structure−activity analysis may, however, be further developed into principles for the design of partial AMPA receptor agonists, which may have particular therapeutic interest in certain CNS disorders.[17]

Experimental Section

Chemistry. All reactions were performed under an inert atmosphere (nitrogen). Reagents were purchased commercially and used without purification. Melting points were determined in capillary tubes (Büchi 535 apparatus) and are uncorrected. ^{1}H NMR was recorded on a Bruker AC 250 spectrometer at 250 MHz. Unless otherwise stated, chemical shift values (δ) are expressed in ppm relative to TMS. The following abbreviations are used for multiplicity of NMR signals: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, m = multiplet. NMR signals corresponding to acidic protons are omitted. Difference-NOE spectra were obtained by use of standard Bruker software. Mass spectra were obtained on a Quattro MS−MS system from VG Biotech, Fisons Instruments. The MS−MS system was connected to an HP 1050 modular HPLC system. A volume of 20−50 μL of the sample (10 μg/mL) dissolved in a mixture of acetonitrile/water/acetic acid = 250:250:1 (v/v/v) or in a mixture of acetonitril/water/aqueous ammonia (25%) = 25:25:1 (v/v/v) was introduced *via* the autosampler at a flow of 30 μL/min into the electrospray source. Spectra were recorded for all target compounds and for selected key intermediates at standard operating conditions to obtain molecular weight information ($(M + H)^+$) or ($(M - H)^-$). The background was subtracted. Compounds containing the 3-isoxazolol moiety were visualized on TLC plates (Merck silica gel 60 F_{254}) using UV light and a $FeCl_3$ spraying reagent (yellow colors). Compounds containing amino groups were visualized using a ninhydrin spraying reagent. Microanalyses were performed by Lundbeck Analytical Department, and results obtained were within ±0.4% of the theoretical values if not otherwise stated. It shall be mentioned that the relatively high nitrogen content of the target compounds in several cases caused instrumental problems, and higher deviations from the theoretical values than ±0.4% were observed.

(3-Ethoxy-4-methylisoxazol-5-yl)methanol (10). A mixture of 3-ethoxy-4,5-dimethylisoxazole (**8**) (114 g, 0.8 mol), bromine (83 mL, 1.6 mol), and CCl_4 (150 mL) was stirred in the dark at room temperature for 6 days. Water was added, and $NaHSO_3$ was added until the phases were colorless. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic extracts were washed with water and brine and dried ($MgSO_4$), and the solvents were evaporated *in vacuo* to give crude 5-(bromomethyl)-3-ethoxy-4-methylisoxazole (**9**) (according to ^{1}H NMR, a 1:1 mixture of **8** and **9** was obtained). The crude **9** was dissolved in a water/*N*-methylpyrrolidone mixture (2:1, 750 mL) and stirred at 105 °C for 2 days. The mixture was cooled and extracted with ether. The organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was evaporated *in vacuo*. The residue was subjected to flash chromatography (silica gel, eluent: heptane/EtOAc = 3:1 then 1:2) to give crude starting material **8** (45.6 g, 40%) and pure **10** as a colorless solid (50 g, 39%): mp 40−41 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3 H), 1.90 (s, 3 H), 4.30 (q, 2 H), 4.59 (s, 2 H). Anal. ($C_7H_{11}NO_3$) C, H, N.

3-Ethoxy-4-methylisoxazole-5-carboxylic Acid (11). To a solution of **10** (50 g, 0.31 mol) in H_2SO_4/water/AcOH (1:2:7, 400 mL) was added a mixture of CrO_3/H_2O/AcOH (1:1:2, 400 mL), and the resulting mixture was stirred at room temperature for 2 h. Water was added (500 mL), and the aqueous phase was extracted with ether (3 × 1 L). The organic extracts were washed with water and brine and dried ($MgSO_4$), and the solvent was evaporated *in vacuo* to give **11** as a grayish crystalline compound (38 g, 70%): mp 120−122 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3 H), 2.18 (s, 3 H), 4.39 (q, 2 H); MS ($(M - H)^-$) *m/z* 170. Anal. ($C_7H_9NO_4$) C, H, N.

***N*-Methyl-3-ethoxy-4-methylisoxazole-5-carboxamide (12).** A mixture of **11** (5.0 g, 29.2 mmol), thionyl chloride (10 mL), and DMF (1 mL) in CH_2Cl_2 (50 mL) was boiled under reflux for 3.5 h. The solvents were evaporated to dryness *in vacuo*, and the residue dissolved in THF (50 mL) was poured onto an aqueous solution of methylamine (40%) at 0−5 °C. The mixture was allowed to warm to room temperature and stirred for another 30 min. The aqueous phase was extracted with EtOAc (3 × 100 mL), and the organic extracts were washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was crystallized (EtOAc) to give **12** as colorless crystals (3.3 g, 61%): mp 113−115 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3 H), 2.18 (s, 3 H), 2.98 (d, 3 H), 4.34 (q, 2 H), 6.49 (br s, 1 H). Anal. ($C_8H_{12}N_2O_3$) C, H, N.

5-(3-Ethoxy-4-methylisoxazol-5-yl)-1-methyl-1*H*-tetrazole (13h). A mixture of **12** (3.8 g, 20.6 mmol) and phosphoryl chloride (40 mL) was boiled under reflux for 3 h. The solvent was removed *in vacuo*, and the residue dissolved in DMF was added to a suspension of NaN_3 (2.7 g, 41.5 mmol) in DMF (18 mL) at 25−35 °C. The resulting suspension was stirred at room temperature for 1 h and then poured onto water (150 mL). The aqueous phase was extracted with EtOAc (3 × 150 mL), the organic extracts were washed with water and brine and dried ($MgSO_4$), and the solvent was removed *in vacuo*. Flash chromatography (silica gel, eluent: EtOAc/heptane = 1:2) gave crude **13h** (0.6 g, 14%). A small sample was recrystallized (EtOAc) to give colorless crystals: mp 136−138 °C; ^{1}H NMR ($CDCl_3$) δ 1.49 (t, 3 H), 2.31 (s, 3 H), 4.38 (s, 3 H), 4.40 (q, 2 H); MS ($(M + H)^+$) *m/z* 210. Anal. ($C_8H_{11}N_5O_2$) C, H, N. The crude product was used in the next step without further purification.

3-Ethoxy-4-methylisoxazole-5-carboxamide (14). A mixture of **11** (5.0 g, 29 mmol), thionyl chloride (11 mL, 150 mmol), and DMF (0.5 mL) in CH_2Cl_2 (75 mL) was boiled under reflux for 2 h. The solvents were evaporated *in vacuo*, and the residue dissolved in THF (30 mL) was poured onto an aqueous solution of ammonia (25%) at 0−5 °C. The mixture was allowed to warm to room temperature and then stirred for another 1 h. The aqueous phase was extracted with ether (3 × 150 mL), the organic extracts were washed with water and brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give crude **14** (4.5 g, 91%). A small sample was recrystallized (EtOH) to give colorless crystals: mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, 3 H), 2.05 (s, 3 H), 4.29 (q, 2 H), 7.84 (br s, 1 H), 8.09 (br s, 1 H). Anal. ($C_7H_{10}N_2O_3$) C, H, N. The crude product was used in the next step without further purification.

3-Ethoxy-4-methylisoxazole-5-carbonitrile (15). A mixture of **14** (10 g, 59 mmol) and phosphoryl chloride (120 mL) was boiled under reflux for 45 min. The solvent was removed *in vacuo*, and the residue dissolved in CH_2Cl_2 (200 mL) was poured onto an ice/water mixture (200 mL). The two layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 200 mL). The organic extracts were washed with water and brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give crude **15** as a brownish oil (7.9 g, 88%): ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3 H), 2.08 (s, 3 H), 4.37 (q, 2 H). The crude product was used in the next step without further purification.

5-(3-Ethoxy-4-methylisoxazol-5-yl)-1*H*-tetrazole (13g). A suspension of **15** (1.5 g, 9.9 mmol), NaN_3 (0.77 g, 11.8 mmol), and TEA hydrochloride (1.63 g, 11.8 mmol) in 1,2-dimethoxyethane (50 mL) was boiled under reflux for 48 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in water (50 mL). The aqueous phase was washed with EtOAc and the pH adjusted to ca. 1 by addition of 6 M HCl. The aqueous phase was extracted with EtOAc (3 × 75 mL), the organic extracts were washed with water and brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give crude **13g** (1.6 g, 83%). A small sample was recrystallized (EtOH) to give colorless crystals: mp 147−149 °C; ^{1}H NMR ($CDCl_3$) δ 1.52 (t, 3 H), 2.34 (s, 3 H), 4.48 (q, 2 H); MS ($(M + H)^+$) *m/z* 196. Anal. ($C_7H_9N_5O_2$) C, H, N. The crude product was used in the next step without further purification.

5-(3-Ethoxy-4-methylisoxazol-5-yl)-2-methyl-2*H*-tetrazole (13i). A mixture of **13g** (0.64 g, 3.2 mmol) and K_2CO_3 (0.9 g, 6.5 mmol) in acetone was stirred at room temperature for 5 min, and a solution of methyl iodide (0.3 mL, 4.8 mmol) in acetone (5 mL) was added. The resulting mixture was stirred at room temperature for 16 h, and the solvent was removed *in vacuo* (according to ^{1}H NMR, a 2:1 mixture of **13i** and **13h** was obtained). Crude product equivalent to 1.8 g (9.1 mmol) of **13g** was subjected to flash chromatography (silica gel, eluent: EtOAc/heptane = 1:2). The residue was crystallized (EtOAc) to give pure **13h** (for analytical data see elsewhere), and crude **13i** was enriched in the mother liquor (1.0 g, 53%, according to ^{1}H NMR, a 4:1 mixture of **13i** and **13h** was obtained): ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3 H), 2.26 (s, 3 H), 4.40 (q, 2 H), 4.45 (s, 3 H). Crude **13i** was used in the next step without further purification.

***N*-[(Dimethylamino)methylidene]-3-ethoxy-4-methylisoxazole-5-carboxamide (16).** A mixture of **14** (3.5 g, 21 mmol) and DMF dimethyl acetal (15 mL) was stirred at 120 °C for 15 min. After being cooled, pure **16** was collected by filtration as colorless crystals (4.2 g, 91%): mp 115−117 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3 H), 2.21 (s, 3 H), 3.20 (s, 3 H), 3.22 (s, 3 H), 4.36 (q, 2 H), 8.64 (s, 1 H). Anal. ($C_{10}H_{15}N_3O_3$) C, H, N.

3-(3-Ethoxy-4-methylisoxazol-5-yl)-2-methyl-2*H*-1,2,4-triazole (13e). To a solution of methylhydrazine (0.6 mL, 11 mmol) in AcOH (15 mL) was added **16** (2.2 g, 10 mmol). The resulting reaction mixture was stirred at 90 °C for 1 h, and the solvents were evaporated *in vacuo*. Flash chromatography (silica gel, eluent: EtOAc/heptane = 1:2) gave compound **13e** as colorless crystals (1.0 g, 49%): mp 86−87 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (t, 3 H), 2.23 (s, 3 H), 4.20 (s, 3 H), 4.39 (q, 2 H), 7.98 (s, 1 H); MS ($(M + H)^+$) *m/z* 209. Anal. ($C_9H_{12}N_4O_2$) C, H, N.

3-(3-Ethoxy-4-methylisoxazol-5-yl)-1*H*-1,2,4-triazole (17). To a solution of hydrazine hydrate (0.6 mL, 12.4 mmol) in AcOH (15 mL) was added **16** (1.8 g, 8.0 mmol). The resulting reaction mixture was stirred at 90 °C for 15 min and then allowed to cool to room temperature. Pure **17** was collected by filtration (1.2 g, 77%): mp 194−196 °C. Water (40 mL) was added to the filtrate, and the aqueous phase was extracted with EtOAc (3 × 30 mL). The organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to give crude **17** (0.3 g, 20%). Overall yield of **17**: 97%. ^{1}H NMR (DMSO-d_6) δ 1.40 (t, 3 H), 2.15 (s, 3 H), 4.31 (q, 2 H), 8.74 (s, 1 H); MS ($(M + H)^+$) *m/z* 195. Anal. ($C_8H_{10}N_4O_2$) C, H, N. A mixture of the two crops were used in the next step.

3-(3-Ethoxy-4-methylisoxazol-5-yl)-1-trityl-1*H*-1,2,4-triazole (13d). To a mixture of **17** (1.1 g, 5.7 mmol), TEA (2.5 mL, 18 mmol), and DMF (20 mL) was added trityl chloride (1.6 g, 5.7 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 5 h and then poured onto an ice/water mixture (200 mL). The aqueous phase was extracted with ether (3 × 200 mL), and the organic extracts were washed with an aqueous solution of Na_2CO_3 (10%) and brine. The solution was dried (Na_2SO_4) and concentrated *in vacuo* to give crude **13d** (2.5 g, 100%, according to ^{1}H NMR, a 7:1 mixture of two isomers was obtained). A small sample was crystallized (EtOAc) to give a single isomer as colorless crystals: mp 181−183 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3 H), 2.11 (s, 3 H), 4.37 (q, 2 H), 7.08−7.22 (m, 6 H), 7.22−7.42 (m, 9 H), 8.03 (s, 1 H). Anal. ($C_{27}H_{24}N_4O_2$) C, H, N. The crude product was used in the next step without further purification.

***S*-(2,2-Dimethoxyethyl) 3-Ethoxy-4-methylisoxazole-5-thiocarboximidate (18).** A mixture of **15** (2.6 g, 17 mmol), sulfanylacetaldehyde dimethyl acetal[42] (3.6 g, 34 mmol), and

TEA (4.8 mL, 34 mmol) was stirred at (−10 °C) for 48 h. Flash chromatography (silica gel, eluent: EtOAc/heptane = 1:3) afforded crude **18** as a yellow oil (3.6 g, 77%): ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3 H), 2.14 (s, 3 H), 3.40 (t, 2 H), 3.43 (s, 6 H), 4.34 (q, 2 H), 4.58 (br t, 1 H). The crude product was used in the next step without further purification.

3-Ethoxy-4-methyl-5-(thiazol-2-yl)isoxazole (13a). A mixture of **18** (2.5 g, 9 mmol) and *p*-toluenesulfonic acid (catalytic amount) in toluene (75 mL) was boiled under reflux for 3 days. The mixture was concentrated *in vacuo* and subjected to flash chromatography (silica gel, eluent: EtOAc/heptane = 1:5), affording crude **13a** (0.85 g, 45%). A small sample was recrystallized (heptane) to give a yellow crystalline compound: mp 84−86 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3 H), 2.26 (s, 3 H), 4.39 (q, 2 H), 7.47 (d, 1 H), 7.99 (d, 1 H); MS ((M + H)$^+$) *m/z* 211. Anal. ($C_9H_{10}N_2O_2S$) C, H, N. The crude product was used in the next step without further purification.

3-Ethoxy-4-methyl-5-(1-methyl-1*H*-imidazol-2-yl)isoxazole (13c). A mixture of **15** (3.0 g, 19.7 mmol), 5.4 M NaOMe (0.7 mL, 3.8 mmol), and MeOH (60 mL) was stirred at room temperature for 45 min. The mixture was cooled (0−5 °C), and AcOH (2.6 mL, 45.4 mmol) was added. *N*-(Methylamino)-acetaldehyde dimethyl acetal (2.8 mL, 21.8 mmol) was added, and the resulting mixture was boiled under reflux for 6 h. The reaction mixture was reduced *in vacuo*, added a boiling solution of 1 M HCl (150 mL), and boiled under reflux for 4 h. The reaction mixture was cooled, washed with ether, and basified by addition of 24% NaOH. The aqueous phase was extracted with EtOAc (3 × 150 mL), the organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was evaporated *in vacuo*. Flash chromatography (silica gel, eluent: EtOAc/heptane = 4:1) followed by treatment with charcoal, filtration, and concentration *in vacuo* gave pure **13c** as slightly brown crystals (2.5 g, 61%): mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3 H), 2.20 (s, 3 H), 3.93 (s, 3 H), 4.37 (q, 2 H), 6.94 (d, 1 H), 7.19 (d, 1 H); MS ((M + H)$^+$) *m/z* 208. Anal. ($C_{10}H_{13}N_3O_2$) C, H, N.

***N*-Methyl-3-ethoxy-4-methylisoxazole-5-carboxamidrazone (20).** Compound **15** (2.2 g, 14.5 mmol) in EtOH (5 mL) at 5 °C was added methylhydrazine (7 mL, 130 mmol), and the reaction mixture was stirred at room temperature for 45 min. Water (25 mL) was added, and crude **20** was collected by filtration (1.9 g, 66%). A small sample was recrystallized (heptane) to give colorless crystals: mp 117−118 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, 3 H), 1.96 (s, 3 H), 2.74 (d, 3 H, J = 4.6 Hz), 4.24 (q, 2 H), 5.04 (q, 1 H, J = 4.6 Hz), 5.61 (br s, 2 H). Anal. ($C_8H_{14}N_4O_2$) C, H, N. The crude product was used in the next step without further purification.

3-(3-Ethoxy-4-methylisoxazol-5-yl)-1-methyl-1*H*-1,2,4-triazole (13f). A mixture of formic acid (10 mL) and **20** (1.9 g, 9.6 mmol) was boiled under reflux for 1 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in EtOAc (100 mL) and poured onto brine (100 mL) and K_2CO_3 (5 g). The two layers were separated, and the aqueous phase was extracted with EtOAc (2 × 100 mL). The organic extracts were washed with brine and dried ($MgSO_4$), and the solvent was evaporated *in vacuo* to give crude **13f** as colorless crystals (2.0 g, 100%). A small sample was recrystallized (EtOAc) to give colorless crystals: mp 129−131 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3 H), 2.21 (s, 3 H), 4.01 (s, 3 H), 4.38 (q, 2 H), 8.14 (s, 1 H); MS ((M + H)$^+$) *m/z* 209. Anal. ($C_9H_{12}N_4O_2$) C, H, N. The crude product was used in the next step without further purification.

4-(Bromomethyl)-3-ethoxy-5-(thiazol-2-yl)isoxazole (21a). A mixture of **13a** (0.85 g, 4.0 mmol) and NBS (0.8 g, 4.5 mmol) in CCl_4 (70 mL) was boiled under reflux for a total of 64 h. Additional NBS was added after 16 h (0.8 g, 4.5 mmol) and again after 40 h (0.4 g, 2.2 mmol). The reaction mixture was cooled and filtered, and the solvent was evaporated *in vacuo*. Flash chromatography (silica gel, eluent: EtOAc/heptane = 1:6) gave crude **21a** as a yellow oil (0.75 g, 64%): ^{1}H NMR ($CDCl_3$) δ 1.51 (t, 3 H), 4.44 (q, 2 H), 4.77 (s, 2 H), 7.57 (d, 1 H), 8.08 (d, 1 H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(thiazol-2-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22a). A mixture of diethyl acetamidomalonate (0.62 g, 2.9 mmol) and potassium *tert*-butoxide (0.35 g, 3.1 mmol) in *N*-methylpyrrolidone (15 mL) was stirred at room temperature for 30 min. Compound **21a** (0.75 g, 2.6 mmol) in *N*-methylpyrrolidone (5 mL) was added, and the resulting mixture was stirred at room temperature for 2 h. The mixture was poured onto an ice/water mixture (100 mL), and the aqueous phase was extracted with EtOAc (3 × 100 mL). The organic extracts were washed with an aqueous solution of base and brine and dried ($MgSO_4$), and the solvent was removed *in vacuo*. Flash chromatography (silica gel, eluent: heptane/EtOAc = 1:1 then 1:2) followed by crystallization (2-propanol) gave **22a** as slightly yellow crystals (0.63 g, 57%): mp 136−137 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6 H), 1.45 (t, 3 H), 1.80 (s, 3 H), 3.82 (s, 2 H), 4.00−4.29 (m, 4 H), 4.36 (q, 2 H), 6.80 (br s, 1 H), 7.50 (d, 1 H), 7.96 (d, 1 H). Anal. ($C_{18}H_{23}N_3O_7S$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(thiazol-2-yl)isoxazol-4-yl]propionic Acid Hemihydrate (7a). A suspension of **22a** (0.6 g, 1.4 mmol) in 6 M HCl (150 mL) was boiled under reflux for 8 days. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in water (40 mL). The aqueous phase was washed with EtOAc, treated with charcoal, filtered, and concentrated *in vacuo*. The residue was dissolved in water, and the pH was adjusted to *ca*. 3 by addition of 1 M NaOH. The resulting precipitate was stirred at room temperature for 16 h, and **7a** was collected by filtration as colorless crystals (0.26 g, 70%): mp 218−219 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.08 (dd, 1 H), 3.43 (dd, 1 H), 3.76 (dd, 1 H), 7.98 (d, 1 H), 8.08 (d, 1 H); MS ((M − H)$^-$) *m/z* 254. Anal. ($C_9H_9N_3O_4S\cdot 0.5H_2O$) C, H, N.

4-(Bromomethyl)-3-ethoxy-5-(1-methyl-1*H*-imidazol-2-yl)isoxazole (21c). Compound **21c** was prepared from **13c** (1.0 g, 4.8 mmol) by the method described for compound **21a**. Crude product (1.4 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.49 (t, 3 H), 3.98 (s, 3 H), 4.42 (q, 2 H), 4.78 (s, 2 H), 6.98 (d, 1 H), 7.24 (d, 1 H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(1-methyl-1*H*-imidazol-2-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22c). Compound **22c** was prepared from **21c** (1.5 g, 5.2 mmol) by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane = 4:1 then EtOAc) followed by crystallization (2-propanol) gave **22c** as colorless crystals (1.2 g, 54%): mp 112−114 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 6 H), 1.44 (t, 3 H), 1.89 (s, 3 H), 3.58 (s, 2 H), 3.91 (s, 3 H), 3.99−4.26 (m, 4 H), 4.34 (q, 2 H), 6.98 (d, 1 H), 7.14 (d, 1 H), 8.23 (br s, 1 H). Anal. ($C_{19}H_{26}N_4O_7$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(1-methyl-1*H*-imidazol-2-yl)isoxazol-4-yl]propionic Acid Monohydrate (7c). A suspension of **21c** (1.0 g, 2.4 mmol) in 47% HBr(aq) (40 mL) was boiled under reflux for 1.5 h. The solvent was removed *in vacuo*, and the residue was dissolved in water (50 mL). The aqueous phase was washed with EtOAc and reduced *in vacuo* (5 mL). A pH of *ca*. 3 was obtained by addition of 1 M NaOH, and **7c** was collected by filtration as colorless crystals (0.44 g, 69%): mp 234−237 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.96 (dd, 1 H), 3.18 (dd, 1 H), 3.72 (dd, 1 H), 3.85 (s, 3 H), 7.11 (d, 1 H), 7.41 (d, 1 H); MS ((M + H)$^+$) *m/z* 253. Anal. ($C_{10}H_{12}N_4O_4\cdot H_2O$) C, H, N.

3-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-1-trityl-1*H*-1,2,4-triazole (21d). Compound **21d** was prepared from **13d** (2.4 g, 5.5 mmol) by the method described for compound **21a**. Crude product (2.8 g, 100%, according to ^{1}H NMR, a 15:1 mixture of two isomers was obtained): ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3 H), 4.42 (q, 2 H), 4.57 (s, 2 H), 7.11−7.23 (m, 6 H), 7.23−7.44 (m, 9 H), 8.09 (s, 1 H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(1-trityl-1*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22d). Compound **22d** was prepared from **21d** (2.8 g, 5.4 mmol) by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane/TEA = 50:50:2) gave compound **22d** (2.2 g, 62%, according to ^{1}H NMR, a single isomer was obtained): mp 145−149 °C; ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 6 H), 1.43 (t, 3 H), 1.80 (s, 3 H), 3.77 (s, 2 H), 3.93−4.10 (m, 2 H), 4.10−4.28 (m, 2 H), 4.35 (q, 2 H), 6.82 (br s, 1

H), 7.10−7.22 (m, 6 H), 7.29−7.42 (m, 9 H), 8.02 (s, 1 H). Anal. ($C_{36}H_{37}N_5O_7$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(1*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]propionic Acid Monohydrate (7d). A suspension of **22d** (0.7 g, 1.1 mmol) in 47% HBr(aq) (60 mL) was boiled under reflux for 1.5 h. Water (100 mL) was added, and the aqueous phase was washed with ether and CH_2Cl_2 and concentrated *in vacuo*. Water (1 mL) was added, and the pH was adjusted to *ca.* 3 by addition of 1 M NaOH. The precipitate which formed was collected by filtration, resuspended in water (10 mL), and stirred for another 24 h at room temperature. Compound **7d** was collected by filtration as colorless crystals (0.16 g, 58%): mp 193−196 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.01 (dd, 1 H), 3.35 (dd, 1 H), 3.69 (dd, 1 H), 8.68 (s, 1 H); MS ((M + H)$^+$) *m*/*z* 240. Anal. ($C_8H_9N_5O_4\cdot H_2O$) C, H; N: calcd, 27.23; found, 26.67.

3-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-2-methyl-2*H*-1,2,4-triazole (21e). Compound **21e** was prepared from **13e** (1.0 g, 4.8 mmol) by the method described for compound **21a**. Crude product (1.34 g, 97%): ^{1}H NMR ($CDCl_3$) δ 1.52 (t, 3 H), 4.25 (s, 3 H), 4.45 (q, 2 H), 4.73 (s, 2 H), 8.03 (s, 1 H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(2-methyl-2*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22e). Compound **22e** was prepared from **21e** (1.34 g, 4.7 mmol) by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane = 2:1 then 4:1) followed by crystallization (2-propanol) gave compound **22e** (1.35 g, 68%): mp 130−131 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 6 H), 1.45 (t, 3 H), 1.88 (s, 3 H), 3.73 (s, 2 H), 3.94−4.00 (m, 2 H), 4.00−4.27 (m, 2 H), 4.15 (s, 3 H), 4.37 (q, 2 H), 6.91 (br s, 1 H), 7.95 (s, 1 H). Anal. ($C_{18}H_{25}N_5O_7$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(2-methyl-2*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]propionic Acid Monohydrate (7e). A suspension of **22e** (1.1 g, 2.6 mmol) in 47% HBr(aq) (60 mL) was boiled under reflux for 1.5 h. The reaction mixture was treated with charcoal and filtered. Water (90 mL) was added, and the aqueous phase was washed with CH_2Cl_2 and concentrated *in vacuo*. The residue was dissolved in water (5 mL), and pH of the solution was adjusted to *ca.* 3 by addition of 1 M NaOH. The precipitate which formed was collected by filtration, resuspended in water, and stirred at room temperature for another 16 h. Compound **7e** was collected by filtration (0.52 g, 74%): mp 222−223 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.04 (dd, 1 H), 3.24 (dd, 1 H), 3.72 (dd, 1 H), 4.06 (s, 3 H), 8.15 (s, 1 H); MS ((M + H)$^+$) *m*/*z* 254. Anal. ($C_9H_{11}N_5O_4\cdot H_2O$) C, H, N.

3-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-1-methyl-1*H*-1,2,4-triazole (21f). Compound **21f** was prepared from **13f** (2.0 g, 9.6 mmol) by the method described for compound **21a**. Crude product (2.6 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.49 (t, 3 H), 4.05 (s, 3 H), 4.44 (q, 2 H), 4.72 (s, 2 H), 8.17 (s, 1 H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(1-methyl-1*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22f). Compound **22f** was prepared from **21f** (2.6 g, 9.1 mmol) by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane = 5:1 then EtOAc/MeOH = 95:5) followed by crystallization (2-propanol) gave compound **22f** as colorless crystals (2.45 g, 64%): mp 122−123 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 6 H), 1.42 (t, 3 H), 1.74 (s, 3 H), 3.77 (s, 2 H), 4.00 (s, 3 H), 4.05−4.33 (m, 4 H), 4.35 (q, 2 H), 6.70 (br s, 1 H), 8.11 (s, 1 H). Anal. ($C_{18}H_{25}N_5O_7$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(1-methyl-1*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]propionic Acid Hydrate (7f). A suspension of **22f** (2.0 g, 4.7 mmol) in 47% HBr(aq) (70 mL) was boiled under reflux for 2 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in water (70 mL). The aqueous phase was washed with EtOAc, treated with charcoal, filtered, and reduced *in vacuo* (20 mL). A pH of *ca.* 3 was obtained by addition of 1 M NaOH, affording compound **7f** as colorless crystals (1.0 g, 82%): mp 250−252 °C dec; ^{1}H NMR (DMSO-d_6, CF_3COOH) δ 3.11 (dd, 1 H), 3.32 (dd, 1 H), 3.98 (s, 3 H), 4.18 (br s, 1 H), 8.71 (s, 1 H); MS ((M + H)$^+$) *m*/*z* 254. Anal. ($C_9H_{11}N_5O_4\cdot 0.25H_2O$) C, H, N.

5-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-1-methyl-1*H*-tetrazole (21h). Compound **21h** was prepared from **13h** (0.6 g, 2.9 mmol) by the method described for compound **21a**. Crude product (0.8 g, 97%): ^{1}H NMR ($CDCl_3$) δ 1.52 (t, 3 H), 4.41 (s, 3 H), 4.47 (q, 2 H), 4.72 (s, 2 H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(1-methyl-1*H*-tetrazol-5-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22h). Compound **22h** was prepared from **21h** (0.8 g, 2.8 mmol) by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane = 2:1) followed by crystallization (2-propanol) gave **22h** as colorless crystals (0.73 g, 62%): mp 139−141 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6 H), 1.47 (t, 3 H), 1.94 (s, 3 H), 3.80 (s, 2 H), 3.97−4.25 (m, 4 H), 4.33 (s, 3 H), 4.37 (q, 2 H), 6.53 (br s, 1 H). Anal. ($C_{17}H_{24}N_6O_7$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(1-methyl-1*H*-tetrazol-5-yl)isoxazol-4-yl]propionic Acid Dihydrate (7h). A suspension of **22h** (0.65 g, 1.5 mmol) in 47% HBr(aq) (40 mL) was boiled under reflux for 2.5 h. The reaction mixture was concentrated *in vacuo* and the residue dissolved in water (50 mL). The aqueous phase was washed with EtOAc, filtered, and reduced *in vacuo* (3 mL). A pH of *ca.* 3 was obtained by addition of 1 M NaOH, affording **7h** as colorless crystals (0.36 g, 81%): mp 255−257 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.10 (dd, 1 H), 3.31 (dd, 1 H), 3.78 (dd, 1 H), 4.27 (s, 3 H); MS ((M + H)$^+$) *m*/*z* 255. Anal. ($C_8H_{10}N_6O_4\cdot 2H_2O$) C, H, N.

5-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-2-methyl-2*H*-tetrazole (21i). Compound **21i** was prepared from **13i** (1.0 g, 4.8 mmol) by the method described for compound **21a**. Flash chromatography (silica gel, toluene/EtOAc = 15:1) gave crude **21i** (0.34 g, 25%). A small sample was recrystallized (EtOAc) to give colorless crystals: mp 97−99 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (t, 3 H), 4.46 (q, 2 H), 4.49 (s, 3 H), 4.69 (s, 2 H). Anal. ($C_8H_{10}BrN_5O_2$) C, H, N.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(2-methyl-2*H*-tetrazol-5-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (22i). Compound **22i** was prepared from **21i** (0.32 g, 1.1 mmol) by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane = 2:1) followed by crystallization (2-propanol) gave **22i** as colorless crystals (0.33 g, 70%): mp 144−145 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6 H), 1.45 (t, 3 H), 1.78 (s, 3 H), 3.78 (s, 2 H), 4.04−4.33 (m, 4 H), 4.37 (q, 2 H), 4.44 (s, 3 H), 6.52 (br s, 1 H). Anal. ($C_{17}H_{24}N_6O_7$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(2-methyl-2*H*-tetrazol-5-yl)isoxazol-4-yl]propionic Acid Hemihydrate (7i). A suspension of **22i** (0.3 g, 0.7 mmol) in 47% HBr(aq) (40 mL) was boiled under reflux for 2.5 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in water (40 mL). The aqueous phase was washed with EtOAc, filtered, and reduced *in vacuo*. The residue was dissolved in water, and pH of the solution was adjusted to *ca.* 3 by addition of 1 M NaOH. The resulting precipitate was recrystallized (water), affording **7i** as colorless crystals (0.12 g, 64%): mp 236−238 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.06 (dd, 1 H), 3.29 (dd, 1 H), 3.73 (dd, 1 H), 4.47 (s, 3 H); MS ((M + H)$^+$) *m*/*z* 255. Anal. ($C_8H_{10}N_6O_4\cdot 0.5H_2O$) C, H; N: calcd, 31.93; found, 30.89.

Ethyl 2-Acetamido-3-(5-cyano-3-ethoxyisoxazol-4-yl)-2-(ethoxycarbonyl)propionate (23). A mixture of **15** (2.0 g, 13.1 mmol), NBS (2.6 g, 14.5 mmol), and dibenzoyl peroxide (catalytic amount) in CCl_4 (100 mL) was boiled under reflux for 3 days. The reaction mixture was filtered and evaporated to dryness *in vacuo* (3.0 g, 100%, according to ^{1}H NMR, a 4:2:1 mixture of 4-(bromomethyl)-3-ethoxyisoxazole-5-carbonitrile, **15**, and 4-(dibromomethyl)-3-ethoxyisoxazole-5-carbonitrile was obtained). Compound **23** was prepared from the residue by the method described for compound **22a**. Flash chromatography (silica gel, eluent: EtOAc/heptane = 1:3 then 1:2) followed by crystallization (EtOAc/heptane) gave compound **23** as colorless crystals (1.5 g, 55%): mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 6 H), 1.42 (t, 3 H), 2.05 (s, 3 H), 3.58 (s, 2 H), 4.15−4.41 (m, 6 H), 6.61 (br s, 1 H). Anal. ($C_{16}H_{21}N_3O_7$) C, H, N.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(1*H*-tetrazol-5-yl)-isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (24). A suspension of **23** (9.8 g, 26.7 mmol), NaN_3 (3.5 g, 53.4 mmol), and TEA hydrochloride (7.4 g, 53.4 mmol) in 1,2-dimethoxyethane (250 mL) was boiled under reflux for 17 h. The mixture was cooled and poured onto water (200 mL). pH of the aqueous phase was adjusted to *ca.* 1 by addition of 37% HCl(aq), and the aquoeus phase was extracted with ether. The organic extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo*. Flash chromatography (silica gel, eluent: 1,2-dimethoxyethane/1% AcOH) afforded a residue, which was was stirred with acetone. Crude **24** was collected by filtration (6.3 g, 58%). A small sample was recrystallized (2-propanol) to give colorless crystals: mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 1.11 (t, 6 H), 1.37 (t, 3 H), 1.61 (s, 3 H), 3.58 (s, 2 H), 3.85−4.14 (m, 4 H), 4.30 (q, 2 H), 8.19 (br s, 1 H). Anal. ($C_{16}H_{22}N_6O_7$) C, H, N. The crude product was used in the next step without further purification.

(*RS*)-2-Amino-3-[3-hydroxy-5-(1*H*-tetrazol-5-yl)isoxazol-4-yl]propionic Acid Hydrate (7g). A suspension of **24** (1.0 g, 2.5 mmol) in 47% HBr(aq) (15 mL) was boiled under reflux for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue dissolved in water (12 mL). The precipitate which formed was collected by filtration to give **7g** as colorless crystals (0.52 g, 83%): mp 227−230 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.00−3.23 (m, 2 H), 4.23 (t, 1 H); MS (($M + H)^+$) *m/z* 241. Anal. ($C_7H_8N_6O_4 \cdot H_2O$) C, H; N: calcd, 32.56; found, 31.49.

Ethyl (*RS*)-2-Amino-3-[3-ethoxy-5-(1*H*-imidazol-2-yl)-isoxazol-4-yl]propionate (25). A mixture of **23** (1.1 g, 3.0 mmol), 5.4 M NaOMe (0.1 mL, 0.5 mmol), and MeOH (5 mL) was stirred at room temperature for 30 min. The mixture was cooled (0−5 °C) and AcOH added (0.6 mL, 10.5 mmol). Aminoacetaldehyde dimethyl acetal (0.4 mL, 3.7 mmol) was added, and the resulting mixture was stirred at room temperature for 24 h. The solvents were removed *in vacuo*, and the residue was added a boiling solution of 1 M HCl (30 mL) and boiled under reflux for 48 h. The solution was cooled, filtered, and evaporated to dryness *in vacuo*. The residue was dissolved in a saturated solution of HCl in EtOH (110 mL) and boiled under reflux for 4 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in an ice/water mixture, which was basified by careful addition of an aquoeus solution of Na_2CO_3 (5%). The aquoeus phase was extracted with EtOAc (3 × 50 mL), and the organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting residue was crystallized (EtOH) to give crude **25** (0.45 g, 51%). A small sample was recrystallized (EtOH) to give colorless crystals: mp 122−123 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, 3 H), 1.43 (t, 3 H), 2.89 (dd, 1 H), 3.04 (dd, 1 H) 3.85 (dd, 1 H), 4.15−4.31 (m, 2 H), 4.37 (q, 2 H), 7.20 (s, 2 H); MS (($M + H)^+$) *m/z* 295. Anal. ($C_{13}H_{18}N_4O_4$) C, H, N. The crude product was used in the next step without further purification.

(*RS*)-2-Amino-3-[3-hydroxy-5-(1*H*-imidazol-2-yl)isoxazol-4-yl]propionic Acid Hemihydrate (7b). A suspension of **25** (0.44 g, 1.5 mmol) in 47% HBr(aq) (30 mL) was boiled under reflux for 2.5 h. The reaction mixture was concentrated *in vacuo*, and the residue dissolved in water (40 mL). The aqueous phase was washed with EtOAc, filtered and reduced *in vacuo* (1 mL). A pH of *ca.* 3 was obtained by addition of 1 M NaOH, and compound **7b** was collected as colorless crystals (0.24 g, 65%): mp 266−269 °C dec; ^{1}H NMR (DMSO-d_6, CF_3-COOH) δ 3.21 (d, 2 H), 4.24 (br s, 1 H), 7.77 (s, 2 H); MS (($M + H)^+$) *m/z* 239. Anal. ($C_9H_{10}N_4O_4 \cdot 0.5H_2O$) C, H, N.

Radioligand Binding Assays. Affinities for NMDA, AMPA, and KAIN receptors were determined using the ligands [^{3}H]CGP 39653,[35] [^{3}H]AMPA,[36] and [^{3}H]KAIN,[37] respectively. The membrane preparations used in all the receptor binding experiments were prepared according to the method of Ransom and Stec.[43]

***In Vitro* Electrophysiology.** A rat cortical slice model for determination of EAA-evoked depolarizations described by Harrison and Simmonds[38] was used in a slighly modified version. 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 Ag/AgCl pellet electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{2+}-free oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium, and the potential difference between the electrodes was recorded on a chart recorder. Application of agonists were done for 90 s, and for antagonist experiments, the antagonists were applied alone for 90 s followed by coapplication of the agonist and antagonist for another 90 s.

Potentiometric Titrations. The method by Clarke and Cahoon[44] was used in a slightly modified version. A solution of the compound to be titrated was prepared in dilute HCl containing 150 mM NaCl. The molarity and volume of the solution were determined by the solubility of the compound. All compounds were of relative low solubility in water and titrated in volumes up to 100 mL of dilute HCl of molarity down to 5 mM. As titrant was used 0.5 M NaOH. During the titration procedure the sample solution was bubled with nitrogen. Due to the low solubility of the compounds, the titration curve of solvent alone was subtracted from that of the solution. The resulting difference curve was then analyzed by a curve-fitting algorithm developed in Lotus 1-2-3 program (version 5.0 for Windows). The spreadsheet provided a fitted curve to the actual measured points based on the dependent variables. These are the pK_a values, the equivalence volume, and the initial titrant volume. The measured pH during the titration is treated as an independent variable. The method allowed pK_a determination in the pH range from 3 to 10.

Computational Methods. The construction of input structures for the illustrations were done by means of the molecular modeling program MacMimic.[45] The statistical analysis (Multiple Linear Regression) was performed by the use of the Microsoft Excel program (version 5.0) for Macintosh computers. Semiemperical calculations were performed in SPARTAN 4.1.1.[46]

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