Regioisomeric 5(3)-Aminomethyl-3(5)-phenylisoxazoles: Synthesis, Spectroscopic Discrimination, and Muscarinic Activity[+)]

Gerd Dannhardt* [a)], Günter Lambrecht [b)], Stefan Laufer [a)], Ernst Mutschler [b)], and Johannes Schweiger [a)]

[a)] Johannes Gutenberg-Universität, Fachbereich Chemie und Pharmazie, Institut für Pharmazie, D-55099 Mainz, Germany

[b)] Johann Wolfgang Goethe-Universität, Pharmakologisches Institut für Naturwissenschaftler, Biozentrum Niederursel, Marie-Curie-Str. 9, Gebäude 260, D-60439 Frankfurt/M., Germany

Received December 8, 1994

The regioselective synthesis of isomeric 5(3)-aminomethyl-3(5)-phenyl isoxazoles using different methods is described. Spectroscopic data, especially mass spectrometric fragmentation, were used to identify and characterize the regioisomers. The muscarinic activity of these isoxazoles was assayed on isolated guinea-pig ileum and atria as well as on isolated rabbit vas deferens.

Regioisomere 5(3)-Aminomethyl-3(5)-phenyl-isoxazole: Synthese, spektroskopische Unterscheidung und muskarinische Aktivität

Es werden verschiedene Verfahren zur regioselektiven Darstellung von 5(3)-Aminomethyl-3(5)-phenyl-isoxazolen beschrieben, die anhand ihrer spektroskopischen Daten, insbesondere der massenspektroskopischen Fragmentierung, zuverlässig unterschieden werden können. Am isolierten Meerschweinchenileum bzw.-atrium sowie am isolierten Vas deferens des Kaninchens wird die muskarinische Aktivität der phenylsubstituierten Aminomethylisoxazole geprüft.

Animal behaviour and clinical studies have both suggested that loss of cholinergic function may be one of the causes of disturbed learning and memory in patients with senile dementia of the *Alzheimer* type. For this reason ligands for the muscarinic cholinergic receptors capable of enhancing central cholinergic transmission have been extensively investigated [1-6].

In previous reports we have described the synthesis of a series of aminoalkyl and aminoheteroalkyl isoxazoles using a new ANSARO (addition of the nucleophile, spiro annulation, ring opening [7-9]) transformation of pyrrolidine and imidazolidine enaminones [7,8] as well as the pharmacological testing of their cardiovascular activities [9]. The aminomethyl isoxazoles have structural similarities to muscimol acting on $GABA_A$ receptors and to 5-methylfurtrethonium (= 2-trimethylammoniummethyl-5-methylfuran) as a potent muscarinic receptor agonist. In common with these compounds, the regioisomeric isoxazoles synthesized have an methylammonium side chain at a definite distance from a negatively charged moiety, here represented by the oximino unit. On the basis of these structural similarities the isoxazole derivatives were assayed on muscarinic receptor subtypes to determine structural demands for receptor–ligand recognition, affinity, and selectivity.

Chemistry

[3+2] Dipolar cyclo-addition reactions of nitrile oxides and alkynyl esters often yield a mixture of regioisomeric 4(5)-isoxazole carboxylic acid derivatives which are difficult to separate [10]. To avoid these problems a regioselective method was developed using methyl 2,4-dioxo-4-phenyl butyrate (**1**) as the starting material. Addition of hydroxylamine hydrochloride to the highly enolized 2,4-diketo ester **1** at pH 5 gave a mixture of methyl 5-phenylisoxazole-3-carboxylate (**4**) as the major product and a small amount of the 4-phenyl-4-hydroximino-2-oxo-butyric acid derivative (**3**) (= 4-oxime). Addition of the 2,4-diketo ester **1** to a solution of hydroxylamine at pH 7 and 0 °C yielded a 10:1 mixture of both oximes **2** and **3** which were separated by cc. The characteristic EI induced mass spectrometric (MS) fragmentation enabled us to distinguish both regioisomers. The benzoyl cation is seen with 100% rel. int. for the 2-oxime **2**. In contrast, fragmentation between carbon atoms 1 and 2 gave rise to the base peak of the 4-oxime **3**; no benzoyl cation was detected. ^{1}H-NMR investigations demonstrate a solvent-dependent equilibrium of different enolized *E/Z* isomers of the 2-oxime [11]. In contrast, the 4-oxime **3** resonates independently of the solvent forming a seven-membered chelate in the *E* configuration (data based on detailed NOE experiments [12]).

To increase the yield of 4-oxime **3** we focussed on a regiospecific synthesis. The deprotonation of acetophenone oxime with NaH followed by addition of *p*-methoxybenzyl chloride provided the *O*-protected oxime **7** in 90% yield. Treatment of **7** with *n*-butyl lithium and diethyl oxalate gave

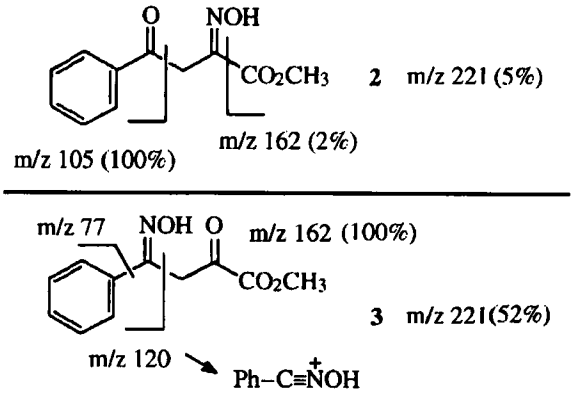
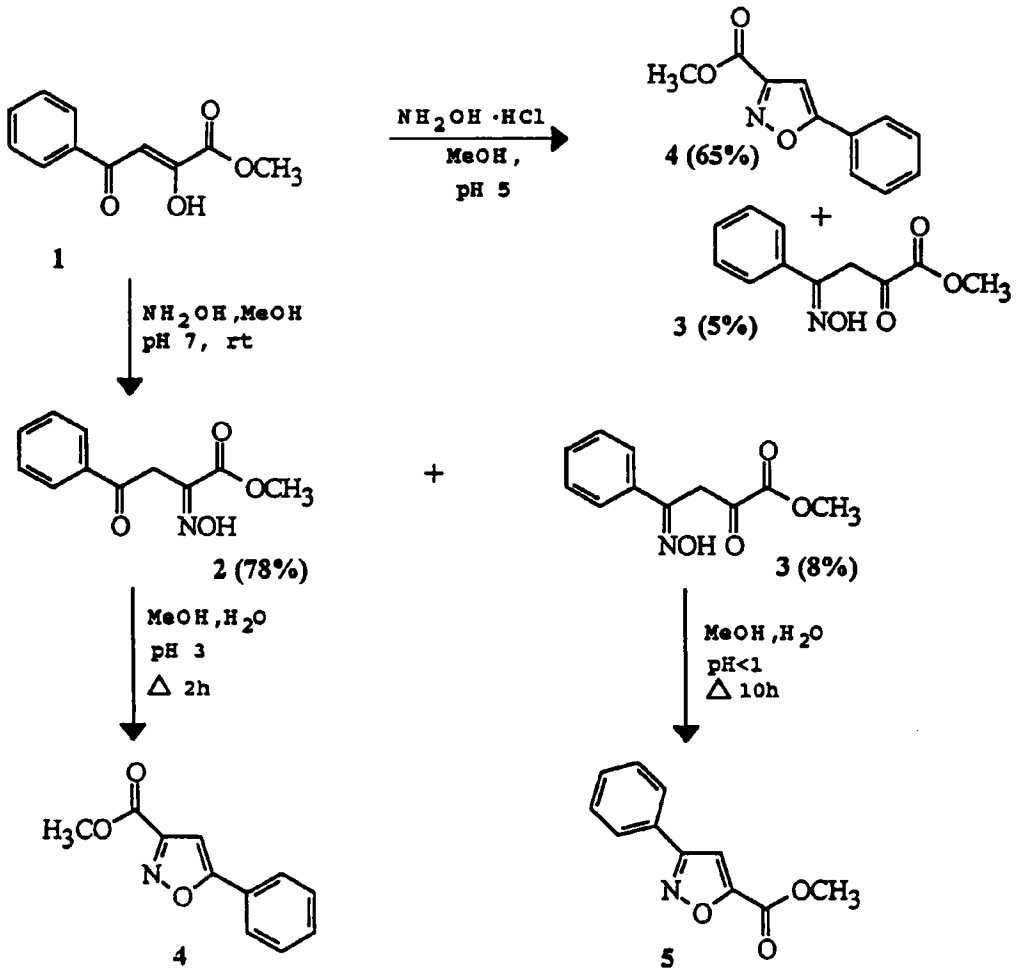


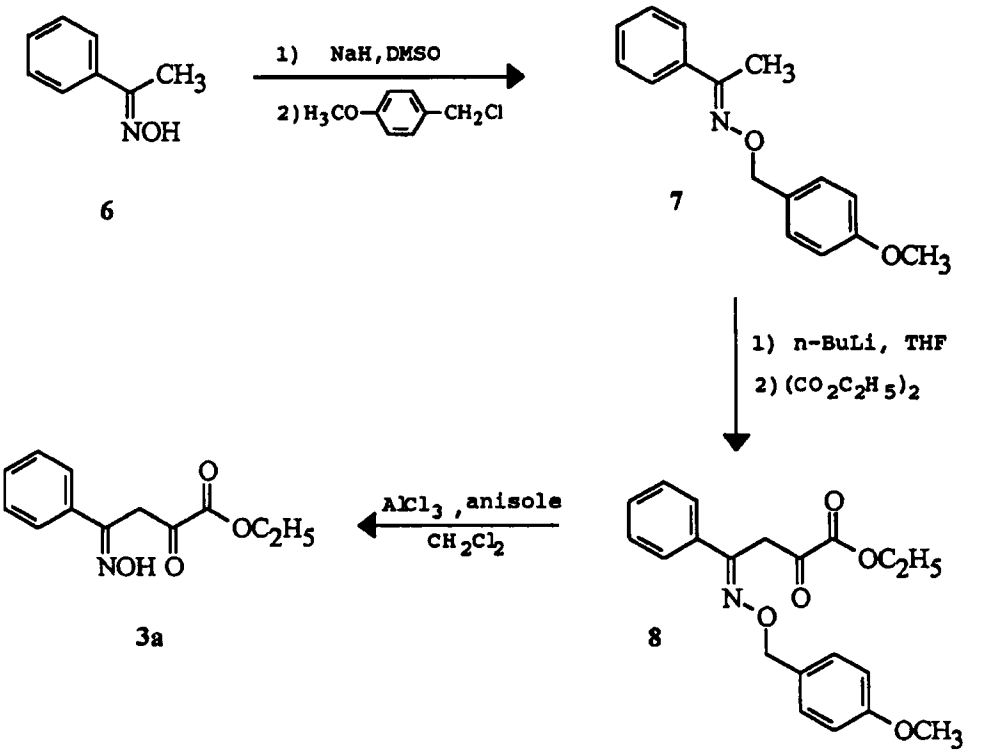
Fig. 1: Mass spectroscopic differentiation of the regioisomeric oximes **2** and **3**

[+)] Dedicated to Prof. Dr. H. Möhrle, Düsseldorf, on the occasion of his 65th birthday.

  0365-6233/95/0505-0437 $ 5.00 + .25/0



Scheme 1



Scheme 2

the corresponding ester **8**. According to the lit. the protecting group was eliminated using a mixture of $AlCl_3$/anisole [13] which is superior to the Ce(IV) method [14]. The overall yield of **3a** in the regiospecific manner is > 30%.

To find the optimum for cyclizing the regioisomeric oximes **2** and **3**, the reaction conditions were varied. In refluxing methanol at pH 3 ring closure of **2** was complete within 2 h, yielding the 5-phenyl-3-isoxazole carboxylic acid ester **4**. On the other hand much more drastic conditions were necessary (10 h, pH 1) to cyclize the 4-oxime **3**; this is explained by the significantly different entropies of both oximes [15]. Compared with the 1,3-dipolar cyclo-addition procedure the methods described gave some lower yields but nevertheless made a regioselective synthesis available using easily accessible starting materials.

^{1}H-NMR and MS data were analyzed for structural assignment of the isoxazole derivatives. According to the lit. [16] and our own investigations [17] the benzoyl and phenylazirine cation, respectively, were used as key fragments in the MS spectra as depicted in Fig. 2. Additionally the chemical shifts of the vinylic protons (7.27 and 6.94 ppm) and a significant bathochromic effect in the 5-phenyl isoxazole series indicate the regiochemistry (Table 1). For analytical HPLC separation of the regioisomers **4** and **5**, an RP-18 column and buffered CH_3CN (pH = 3.5) were used.

The reaction sequence for the synthesis of amides and the corresponding aminomethyl compounds is outlined in Scheme 3. The amides **10** and **11** were formed in good yield and were characterized by their mass spectrometric fragmentation patterns using the same key fragments as discussed for the carboxylic acid derivatives. After reduction using 4 equivalents of BH_3•$(CH_3)_2S$ and the addition of aqueous HCl to destroy the BH_3–amine complex, followed by NaOH to make the solution alkaline, the free bases **12** and **13** were isolated. Alkylation of the aminomethyl isoxazoles with methyl iodide or trimethyl oxonium tetrafluoroborate gave rise to the target derivatives for pharmacological testing.

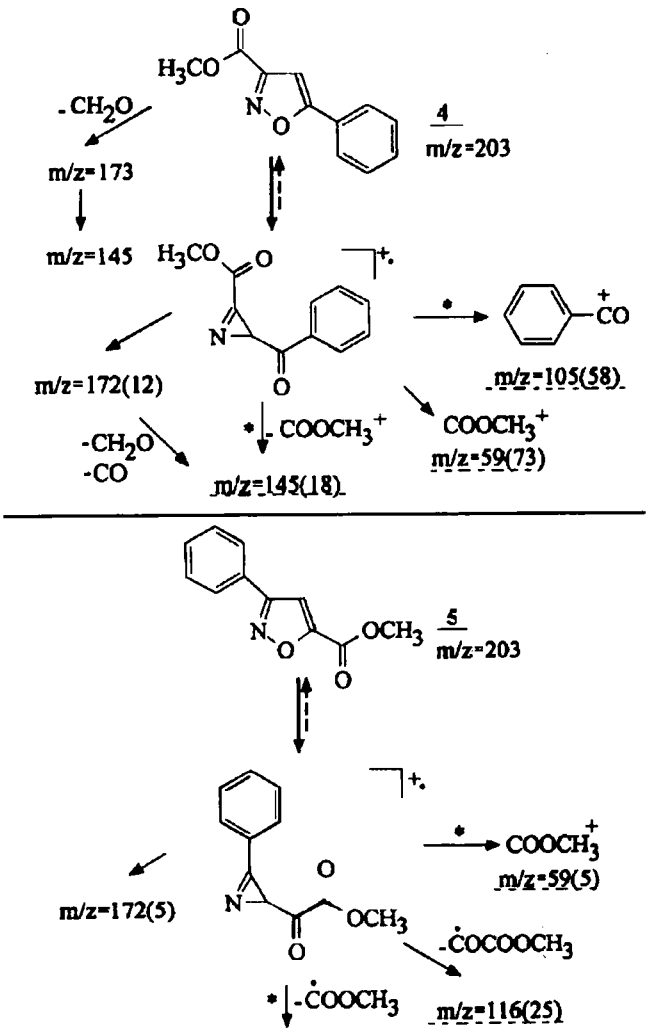
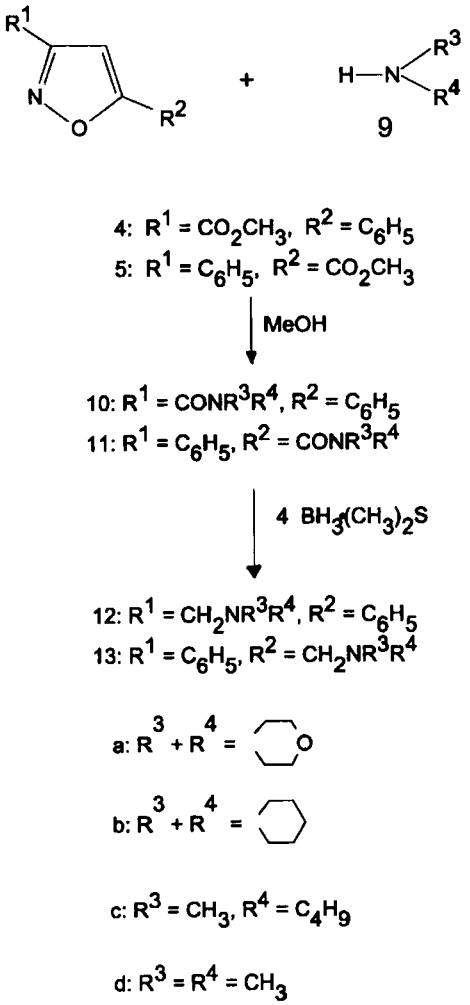


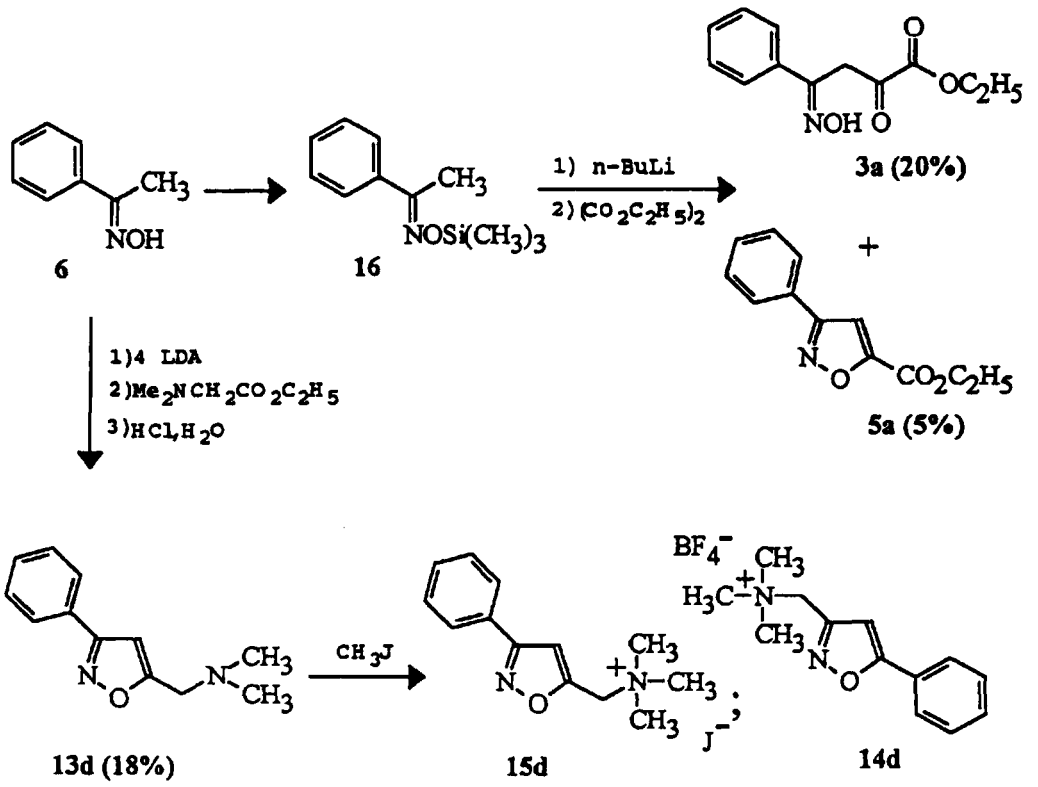
Fig. 2: Mass spectroscopic differentiation of the regioisomeric isoxazoles **4** and **5***. * For azirine formation see ref. [16,17]

Table 1. Spectroscopic data used to discriminate between the 3(5)-phenyl-5(3)-isoxazole carboxylic esters **4** and **5**.

Method	cmpd. 5	cmpd. 4
^{1}H-NMR (Vinyl-H)	7.27 ppm	6.94 ppm
UV (MeOH)	225 nm	271 nm
MS (rel. int.)	59 (5), 144 (100)	59 (100), 105 (58), 145 (18)



Scheme 3



Scheme 4

To optimize the synthesis of 5-aminomethyl-3-phenyl isoxazole (**13**) and to save one step, *i.e.* the regiospecific synthesis of the oxime **3a**, we re-investigated the reaction of methyl 2,4-dioxo-4-phenyl butyrate (**1**) with hydroxylamine under basic conditions, but isolated the 5-phenyl-3-isoxazole carboxylic ester (**4**) only, and not the 3-phenyl regioisomer **5** as stated in the lit. [18]. We now describe the results of our studies employing acetophenone oxime (**6**) as the starting material. First the *O*-silylated oxime **16** was prepared using hexamethyldisilazane. This derivative was treated with an equimolar amount of n-BuLi forming the carbanion which is quenched by addition of diethyl oxalate. After usual work-up and cc the 4-phenyl-4-hydroximino-2-oxo butyric acid ester (**3**) and the corresponding 3-phenyl-5-isoxazole carboxylic ester (**5**) were isolated in 20% and 5% yield, respectively. Carbonylation of *C,O*-dilithium salts of oximes is known to be much more efficient in principle for the synthesis of 3,5 disubstituted isoxazoles [19]. This method is applicable for acetophenone oxime (**6**) and the dimethylglycine ester if a 4-fold molar excess of LDA is used. In a regiospecific manner 3-phenyl-5-dimethylaminomethyl isoxazole (**13d**) was prepared in 20% yield by a one-pot synthesis with commercially available products. Quaternization of **12d** with trimethyl oxonium tetrafluoroborate and of **13d** with methyl iodide gave rise to **14d** and **15d**, respectively.

Pharmacology

The new compounds were investigated for their antimuscarinic activity using the rabbit vas deferens (M1 receptor subtype), the guinea-pig atria (M2), and the guinea-pig ileum (M3) as functional models [20]. The furan derivatives furtrethonium and 5-methylfurtrethonium were used as reference drugs. The antimuscarinic properties of the regioisomeric phenyl substituted 3- and 5-aminomethyl isoxazoles are listed in Table 3. In contrast to furtrethonium [= 2-trimethylammoniummethylfuran; pD_2 = 5.77 ± 0.04 (*M1*), 6.00 ± 0.06 (M2), 6.38 ± 0.02 (M3)] and 5-methylfurtrethonium [= 2-trimethylammoniummethyl-5-methylfuran; pD_2 = 6.89 ± 0.08 (M1), 6.46 ± 0.04 (M2), 7.62 ± 0.05 (M3)], which are agonists at all three muscarinic receptors, the isoxazoles were only weak antagonists, *i.e.* the affinities of all these substances were rather low. Comparing the affinities of the compounds tested at M1 receptors, the highest pA_2 value (5.09) was obtained for 5-phenyl-3-trimethylammoniummethylisoxazole (**14d**). At M2 receptors all pA_2 values were close to 5, the regioisomeric piperidinomethyl isoxazoles **12b** and **13b** displaying equal affinity. At M3 receptors the 5-phenyl-3-morpholinomethylisoxazole (**12a**) was about 10 times more potent than the regioisomeric derivative **13a**. In addition, 3-phenyl-5-trimethylammoniumisoxazole (**15d**) had the highest affinity at this receptor subtype.

Summarizing, the regioisomers in the series of 3- and 5-aminomethyl isoxazoles bearing one additional phenyl group, show low affinities to the muscarinic receptor subtypes without significant subtype selectivity. In contrast, the corresponding furan derivatives 5-methylfurtrethonium and furtrethonium are agonists at all the three muscarinic receptor subtypes. Further investigations are still in progress.

We are grateful to the Fonds der Chemischen Industrie, Frankfurt/Main for financial support.

Table 2. Preparative and analytical data of the regioisomeric isoxazole carboxamides **10** and **11** and the aminomethyl derivatives **12** and **13**.

	formula (m_r)	mp. [°C]	yield [%]
10a	$C_{14}H_{14}N_2O_3$ (258.3)	161	73
10b	$C_{15}H_{16}N_2O_2$ (256.3)	120–121	88
10c	$C_{15}H_{18}N_2O_2$ (258.3)	49–51	80
10d	$C_{12}H_{12}N_2O_2$ (216.2)	107	94
11a	$C_{14}H_{14}N_2O_3$ (258.3)	146	67
11b	$C_{15}H_{16}N_2O_2$ (256.3)	89–91	93
11c	$C_{15}H_{18}N_2O_2$ (258.3)	53–54	73
11d	$C_{12}H_{12}N_2O_2$ (216.2)	114–116	64
12a	$C_{14}H_{16}N_2O_2$ (244.3)	83–85 (91 [21])	63
12a•HCl		218–220 (217–219 [21])	
12b	$C_{15}H_{18}N_2O$ (242.3)	67	88
12b•HCl		225–226 (225–227 [21])	
12c	$C_{15}H_{20}N_2O$ (244.3)	oil	78
12c•HCl		125–126	
12d	$C_{12}H_{14}N_2O$(202.3)	oil	46
12d•HCl		218–220 (223–225 [21])	
13a	$C_{14}H_{16}N_2O_2$ (244.3)	41 (42–46 [21])	59
13a•HCl		207 (205–207 [21])	
13b	$C_{15}H_{18}N_2O$ (242.3)	oil	79
13b•HCl		230–231 (225–226 [21])	
13c	$C_{15}H_{20}N_2O$ (244.3)	oil	81
13c•HCl		106–107	
13d	$C_{12}H_{14}N_2O$(202.3)	oil	68

Table 3. pA_2 Values of the compounds investigated at M1, M2, and M3 receptors. Data are means ± S.E.M. (n = 4–6)

compound	pA_2-M1	pA_2-M2	pA_2-M3
12a•HCl	*	*	4.91 ± 0.10
13a•HCl	*	*	4.06 ± 0.07
12b•HCl	4.88 ± 0.08	4.92 ± 0.07	4.70 ± 0.30
13b•HCl	4.33 ± 0.04	4.92 ± 0.04	4.80 ± 0.04
12c•HCl	*	*	4.86 ± 0.02
13c•HCl	*	*	4.65 ± 0.03
12d•HCl	3.90 ± 0.19	4.36 ± 0.02	4.38 ± 0.13
14d	5.09 ± 0.07	4.67 ± 0.02	4.95 ± 0.15
15d	4.79 ± 0.10	4.80 ± 0.03	5.20 ± 0.20

* No muscarinic activity

Experimental Part

Melting points: Büchi SMP/20 apparatus, uncorrected.– Except where otherwise stated, infrared spectra were measured in KBr, Perkin-Elmer Model 299 Spectrometer.– ^{1}H-NMR spectra: $CDCl_3$, Bruker AC 300 MHz spectrometer (TMS as internal standard, chemical shift in δ ppm).– All structural assignments were consistent with IR, NMR, and MS data.– Silica gel plates (Merck F_{254}) and silica gel 60 (Merck, 63–200 mesh) or Al_2O_3 90 (Merck, neutral, activity 1) were used for analytical and flash chromatography, respectively.– All C, H, N analyses were within ± 0.4% of the theoretical values.– Representative examples are described.

Methyl 4-phenyl-2,4-dioxobutyrate (**1**): [22]
Methyl 5-phenyl-3-isoxazolecarboxylate (**4**)

To a sodium acetate-buffered solution of hydroxylamine hydrochloride (5 mmol) an equimolar amount of methyl 4-phenyl-2,4-dioxobutyrate was added at pH 5, After heating under reflux for 1.5 h the mixture was acidified with 1.5 M H_2SO_4 to pH 3 and heated for a further 2 h. After cooling **4** precipitated and was separated by filtration. A second yield of **4** was recovered from the residue left by evaporation and extraction with CH_2Cl_2. Separation from the oxime **3** (5% yield) and purification by cc (SiO_2; CH_2Cl_2/Et_2O 9:1, R_F=0.9) afforded **4**, 65% yield, mp. 87 °C (86–88 °C: [23]).– $C_{11}H_9NO_3$ (203.2).– IR: 3140; 1730 (C=O); 1615 (C=N); 1595; 1570; 1450; 1250 cm^{-1}.– ^{1}H-NMR: 4.01 (s, 3H, CH_3), 6.94 (s, 1H, vinyl-H), 7.46–7.51 (m, 3H aromat.), 7.83–7.98 (m, 2H aromat.).– UV: 271 nm (MeOH).– MS: m/z = 203 ($M^{+\bullet}$, 100), 172 (12), 145 (18), 105 (58), 77 (47), 59 (73)

Methyl 4-phenyl-4-oxo-2-hydroximinobutyrate (**2**) *and methyl 4-phenyl-2-oxo-4-hydroximino butyrate* (**3**)

The neutralized aqueous solution of 10.2 mmol hydroxylamine hydrochloride was allowed to react with 10 mmol **1** at room temp, for 1 h. Removal of the solvent left a suspension which was extracted with CH_2Cl_2. The org. extracts were dried, and solvent was removed *in vacuo*. The crude product was subjected to cc (SiO_2;, CH_2Cl_2/Et_2O 9:1, R_F = 0.3) to yield **2**, 78% yield, mp. 88 °C.– $C_{11}H_{11}NO_4$ (221.2).– IR (KBr): 3130 (br., OH); 1735 (C=O); 1690 (aryl-C=O); 1600 (C=N); 1440; 1320; 1300; 1200; 1125; 1010 cm^{-1}.– ^{1}H-NMR: 3.88 (s, 3H, CH_3), 2.80– 5.20 (m, br, 2H, CH_2), 7.45–7.70 (m, 5H aromat.), see ref. [11].– $CDCl_3$/D_2O: 3.33–3.27 (d, 0.75H, AB, J = 18.6Hz), 3.5–3.48 (d, 0.75H, AB, J = 18.6Hz), 3.86 (d, 3H), 4.25 (s, exchangeable, 0.5H), 4.35 (s, 2H), 7.98–7.38 (m, 5H, aromat.), 9.79 (s, br., D_2O exchangeable 0.5H).– MS: 221 ($M^{+\bullet}$, 5), 162 (2), 135 (12), 120 (6), 105 (100), 103 (8), 77 (58), 59 (20).

The 4-oxime **3** was separated by cc as described above, R_F=0.5, 8% yield.– $C_{11}H_{11}NO_4$ (221.2).– IR: 3200 (br, OH); 2960; 1740 (C=O); 1605 (C=N); 1580; 1450; 1400; 1350; 1300; 1220; 1140; 1080; 1005 cm^{-1}.– ^{1}H-NMR: 3.45 (d, 1H, AB, J = 17.5Hz), 3.9 (s, 3H, CH_3), 4.0 (d, 1H, AB, J = 17.5Hz), 4.6 (s, 1H exchangeable, OH), 7.38–7.45 (m, 3H aromat.), 7.66–7.7 (m, 2H aromat.), see ref. [12].– MS: 221 ($M^{+\bullet}$, 52), 162 (100), 144 (12), 134 (5), 120 (40), 103 (10), 77 (36), 59 (5).

Methyl 5-phenyl-3-isoxazolecarboxylate (**4**)

CH_3OH/H_2O solution of 5 mmol **2** buffered with NaOAc/CH_3CO_2H was adjusted to pH 3 and refluxed for 2 h. Work-up as described above gave 85% yield **4**.

Methyl 3-phenyl-5-isoxazolecarboxylate (**5**)

A solution of the 4-oxime **3** (5 mmol) was refluxed for 10 h at pH 1 to yield **5** as a white crystalline solid, mp. 106 °C, 50% yield, using the work-up procedure outlined for **4**.– $C_{11}H_9NO_3$ (203.2).– IR: 3140; 3000; 2960; 1730 (C=O); 1600 (C=N); 1580; 1450; 1410; 1330; 1310; 1290; 1230; 1150; 1070; 1010; 960; 930; 850; 810; 780; 700; 695.– ^{1}H-NMR: 4.01 (s, 3H, CH_3), 7.27 (s, 1H, vinyl-H), 7.47–7.51 (m, 3H aromat.), 7.81–7.85 (m, 2H, aromat.).– MS: 203 ($M^{+\bullet}$, 42), 172 (3), 144 (100), 116 (25), 103 (4), 89 (18), 77 (35), 59 (5).– UV: 225 nm (MeOH).

O-(4-Methoxybenzyl)acetophenone oxime (**7**)

To a suspension of 500 mg NaH in 50 ml anhydrous DMSO under a N_2, 20 mmol acetophenone oxime **6** in 20 ml DMSO were added. After 1 h 21 mmol 4-methoxybenzyl chloride were added dropwise, and the mixture was stirred vigorously at room temp. for 2 h. The reaction was quenched with 70 ml ice-cold water and extracted with CH_2Cl_2 (4 × 30 ml). The org. extracts were dried and the solvent removed *in vacuo* to afford crude **7**. After cc (SiO_2; CH_2Cl_2, R_F = 0.85) 90% yield, mp. 72 °C.– $C_{16}H_{17}NO_2$ (225.3).– IR 3080; 3020; 2940; 2850; 1620 (C=N); 1590; 1520; 1450; 1380; 1330; 1310; 1260; 1225; 1120; 1040; 1020; 995; 950; 930; 840; 820; 760; 700; 650 cm^{-1}.– ^{1}H-NMR 2.24 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 5.17 (s, 2H, CH_2), 6.88–6.91 (m, 2H aromat.), 7.32–7.38 (m, 5H aromat.), 7.62–7.66 (m, 2H, aromat.).– MS: m/z = 255 ($M^{+\bullet}$, 1.5), 135 (0.9), 121 (100), 91 (2.5), 77 (1 1).

Ethyl 4-phenyl-4-(4-methoxybenzyloxyimino)-2-oxo-butyrate (**8**)

To a solution of 8.6 mmol **7** in 20 ml anhydrous THF an equimolar amount of n-BuLi was added under N_2. 8.8 mmol diethyl oxalate in 12 ml THF were added dropwise, and the reaction was quenched after 2 h using 3 ml H_2O. Evaporation to dryness and extraction with ether (4 × 20 ml) left a residue which was purified by cc (CH_2Cl_2; SiO_2, R_F = 0.6), 45% yield, yellow oil.– $C_{20}H_{21}NO_5$ (355.4).– IR 3060; 2960; 2940; 2880; 2840; 1730 (C=O, ester); 1640 (C=C); 1615 (C=N); 1590; 1520; 1470; 1450; 1370; 1250; 1175; 1130; 1105; 1020; 930; 890; 820; 760; 740; 700 cm^{-1}.– ^{1}H-NMR (90MHz): 1.3 (t, 3H, J = 7.1 Hz, CH_3), 3.85 (s, 3H, OCH_3), 4.1 (s, 2H, CH_2), 4.1–4.3 (q, 2H, J = 7.1 Hz), 5.15 (s, 2H, CH_2), 6.8– 6.9 (m, 2H aromat.), 7.2–7.8 (m, 7H aromat.).– MS: 355 ($M^{+\bullet}$, 3), 282 (5), 167 (4), 149 (5), 135 (3), 121 (100), 103 (2), 91 (2.5), 77 (4).

Ethyl 4-phenyl-4-hydroximino-2-oxobutyrate (**3a**)

To a mixture of 2.5 mmol $AlCl_3$, 7 ml anisole, and 0.5 ml CH_2Cl_2 a solution of 1 mmol **8** in 4 ml anisole was added at -20 °C. After 20 min the solution was treated with 800 mg $NaHCO_3$ in 40 ml H_2O and then extracted with CH_2Cl_2. Evaporation to dryness and cc (SiO_2; CH_2Cl_2/Et_2O 9:1) yielded **3a**, mp. 137–139 °C, 72% yield.– 10% yield was obtained using the Ce(IV) method [14].– $C_{12}H_{13}NO_4$ (235.2).– IR: 3260 (br., OH); 3100; 3010; 1740 (C=O); 1600 (C=N); 1570; 1450; 1400; 1360; 1290; 1250; 1210; 1130; 1070; 1010; 990; 910; 860; 820; 750; 700 cm^{-1}.– ^{1}H-NMR: 1.36 (t, 3H, J = 7.0Hz, CH_3), 3.44 (d, 1H, AB, J = 17.5Hz, C*H*H), 3.98 (d, 1H, AB, J = 17.5Hz, CH*H*), 4.36 (q, 2H, J = 7.0Hz), 4.57 (s, 1H, exchangeable, OH), 7.4–7.46 (m, 3H aromat.), 7.67–7.72 (m, 2H aromat.).– MS: m/z = 235 ($M^{+\bullet}$, 1 4), 162 (100), 144 (10), 120 (82), 103 (19), 95 (18), 91 (14), 77 (100).– UV: 255 nm (MeOH).

General procedure for isoxazole carboxamides

The carboxylic ester was added in portions with stirring to an excess of the amine, and the solution was stirred for 4–18 h (TLC control). The mixture was evaporated and the resulting residue purified by cc. For yields and analytical data *cf.* Table 2.

General procedure for aminomethyl isoxazoles

To a solution of the amide in anhydrous THF neat boron-dimethylsulfide (4-fold excess according to the amide) was added under N_2 within 10 min under vigorous stirring. The mixture was refluxed for 6 h; after cooling to room temp. methanol was carefully added and the solution was left overnight. The mixture was evaporated to dryness, acidified with dil. HCl and refluxed for 1 h. The resulting solution was cooled to 0 °C, adjusted to pH 9-10 using a solution of NaOH and solid K_2CO_3, and then extracted with ether. The combined org. extracts were dried, filtered, and concentrated *in vacuo*. The residue was purified by cc on silica gel. For spectroscopic data and yields *cf.* Table 2.

O-(Trimethylsilyl)acetophenone oxime (**16**) *and ethyl 3-phenyl-5-isoxazole carboxylate* (**5a**)

0.1 mol Oxime **6** and 0.055 mol hexamethyldisilazane together with 4 drops trimethylchlorosilane were refluxed at 75 °C for 2 h. After cooling the white precipitate was filtered off, and the residue was extracted with CH_2Cl_2. The filtrate and the CH_2Cl_2 layer were combined and evaporated to dryness. The remaining liquid was purified by distillation (0.1 torr, 76–78 °C), 79% yield.– **16**: IR: 3070; 2970; 2905; 1610 (C=N); 1570; 1500; 1450; 1370; 1310; 1255; 1000; 930; 890; 850; 820; 765; 700 cm^{-1}.– ^{1}H-NMR: 0.27 (s, 9H, CH_3), 2.27 (s, 3H, CH_3), 7.35–7.39 (m, 3H aromat.), 7.66–7.69 (m, 2H aromat.).– MS: m/z = 207 ($M^{+\bullet}$, 20), 192 (26), 151 (6), 119 (10), 118 (100), 103 (6), 89 (4), 77 (38).

To 20 mmol **16** in 80 ml THF, 20 mmol n-BuLi were added at 0 °C within 8 min. 20 mmol diethyl oxalate in 30 ml THF were added dropwise after 30 min. The mixture was warmed to room temp. after an additional 2 h and kept for a further 12 h. The solution was treated with 2 ml H_2O and after addition of 20 ml H_2O extracted with CH_2Cl_2. Removal of the solvent and distillation of unreacted educt left a residue which was washed with ether, yielding the hydroximino derivative **3** (20% yield). The ether layer was evaporated to dryness and the resulting residue subjected to cc (SiO_2; petroleum ether–ethyl acetate 1:1) to yield the isoxazolo derivative **5a**, mp. 47 °C (5% yield).– ^{1}H-NMR: (360 MHz): 1.38 (t, J = 7Hz, 3H, CH_3, ester), 4.30 (q, 2H, CH_2, ester), 7.25 (s, 1H, vinyl-H), 7.45–7.51 (m, 3H aromat.), 7.82–7.87 (m, 2H aromat.).

5-N,N-Dimethylaminomethyl-3-phenyl isoxazole (**13d**)

To a solution of 44 mmol LDA in 20 ml anhydrous THF, 10 mmol acetophenone oxime **6** in 30 ml THF were added at 0 °C under N_2. After 45 min 11 mmol ethyl *N,N*-dimethylglycinate in 15 ml THF were added dropwise. The reaction was quenched after 30 min by 100 ml 3N HCl, and the resulting solution was refluxed for 1 h. The cooled solution was made alkaline with 20% aqueous $NaHCO_3$ and extracted with Et_2O. The residue obtained after evaporation of the combined org. layers was subjected to cc (Al_2O_3; activity 2, CH_2Cl_2), to yield a pale yellow oil, 18% yield.– $C_{12}H_{14}N_2O$ (202.3).– IR (NaCl): 3130; 3060; 2980; 2860; 2820; 2780; 1610 (C=N); 1580; 1470; 1440; 1405; 1360; 1300; 1260; 1170; 1140; 1100; 1080; 1040; 1000; 950; 910; 850; 800; 770; 690 cm^{-1}.– ^{1}H-NMR: 2.35 (s, 6H, CH_3), 3.68 (s, 2H, CH_2), 6.5 (s, 1H, vinyl-H), 7.43–7.48 (m, 3H aromat.), 7.79–7.83 (m, 2H aromat.).– MS: m/z = 202 ($M^{+\bullet}$, 52), 159 (8), 144 (6), 117 (6), 103 (7), 98 (10), 82 (12), 77 (13), 71 (14), 58 (100).

General procedure for the quaternization of aminomethyl isoxazoles

To a solution of 1 mmol aminomethyl isoxazole in 2 ml anhydrous ethanol, 1.5 mmol CH3I were added with stirring at room temp. The reaction was complete within 20 min, and the white precipitates were filtered off and washed with anhydrous ether. The products were purified by recrystallization from ether/ethanol.

5-Phenyl-3-(N,N,N-trimethylammoniummethyl)isoxazole tetrafluoroborate (**14d**)

$C_{13}H_{17}N_2O^+BF_4^-$ (304.1); mp. 189–190 °C.– ^{1}H-NMR ([D6]DMSO): 3.17 (s, 9H, N-CH_3), 5.57 (s, 2H, CH_2), 7.29 (s, 1H, vinyl-H), 7.56–7.61 (m, 3H aromat.), 7.92–7.95 (m, 2H aromat.).

3-Phenyl-5-(N,N,N-trimethylammoniummethyl)isoxazole iodide (**15d**)

$C_{13}H_{17}NO_2^+I^-$ (346.3); mp. 188–190 °C.– ^{1}H-NMR ([D6]DMSO): 3.18 (s, 9H, N-CH_3), 4.91 (s, 2H, CH_2), 7.49 (s, 1H, vinyl-H), 7.55–7.57 (m, 3H aromat.), 7.93–7.96 (m, 2H aromat.).

For details of the *pharmacological tests* see [20].

Agonistic and antagonistic effects of the compounds tested were expressed as pD_2 and pA_2 values, respectively.

References and Notes

1 H.A. Ensinger, H.N. Doods, A.R. Immel-Sehr, F.J. Kuhn, G. Lambrecht, K.D. Mendla, R.E. Müller, E. Mutschler, A. Sagada, G. Walther, R. Hammer, *Life Sci.* **1993**, *52*, 473–480.

2 A. Fisher, R. Brandeis, I. Karton, Z. Pittel, D. Gurwitz, R. Haring, M. Sapir, A. Levy, E. Heldmann, *J. Pharmacol. Exp. Ther.* **1991**, *257*, 392–403.

3 S.B. Freedman, G.R. Dawson, L.L. Iversen, R. Baker, R.J. Hargreaves, *Life Sci.* **1993**, *52*, 489–495.

4 G. Lambrecht, U. Moser, U. Grimm, O. Pfaff, U. Hermanni, C. Hildebrandt, M. Waelbroeck, J. Christophe, E. Mutschler, *Life Sci.* **1993**, *52*, 481–488.

5 R.D. Schwarz, L. Coughenour, R.E. Davis, D.T. Dudley, W.H. Moos, M.R. Pavia, H. Tecle, *Cholinergic Basis for Alzheimer Therapy* (Ed.: R.E. Becker and E. Giacobini), Birkhäuser, Boston, **1991**, p. 347–353.

6 F. Wanibuchi, T. Konishi, M. Harada, M. Terai, K. Hidaka T. Tamura, S. Tsukamoto, S. Usuda, *Eur. J. Pharmacol.* **1990**, *187*, 479– 486.

7 G. Dannhardt, A. Grobe, S. Gußmann, R. Obergrusberger, K. Ziereis, *Arch. Pharm. (Weinheim)* **1988**, *321*, 163–166.

8 G. Dannhardt, S. Laufer, *Synthesis* **1989**, 12–15.

9 G. Dannhardt, P. Dominiak, S. Laufer, *Arzneim.-Forsch.* **1993**, *43*, 441–444.

10 M. Christl, R. Huisgen, *Chem. Ber.* **1973**, *106*, 3345–3367.

11 Except the CH_3 singlet at 3.88 ppm all other signals were broadened using $CDCl_3$. Adding D_2O, two CH_3 singlets (3.80; 3.90 ppm) were detected; the CH_2 group gave rise to a singlet at 4.4 ppm and an AB-pattern at 3.30 ppm with J = 18.5 Hz in accordance with at least two isomers.

12 For details of the spectrum see Experimental Part. The OH signal is shifted 4 ppm to lower field in DMSO relative to $CDCl_3$ indicating an H-bridge. The intensity of only one CH_2 proton signal is increased during the NOE experiments saturating the OH resonance and *vice versa*; no effects could be seen for *ortho* protons of the benzene ring. These results agree with the *E* configuration of **3** forming a seven-membered chelate.

13 H. Ona, S. Uyeo, K. Motokawa, T. Yoshida, *Chem. Pharm. Bull. Jpn.* **1985**, *33*, 4346–4360.

14 R. Zibuck, N.J. Liverton, A.B. Smith, *J. Am. Chem. Soc.* **1986**, *108*, 2451–2453.

15 T. Uchimoto, T. Umeda, T. Kowada, H. Sanzaki, *Nippon Kagaku Kaishi* **1977**, 466–469; *Chem. Abstr.* **1977**, *87*, 52389.

16 J.H. Bowie, R.K.M.R. Kallury, R.G. Cooks, *Austr. J. Chem.* **1969**, *22*, 563–575.

17 G. Dannhardt, I. Obergrusberger, *Chemiker Ztg.* **1989**, *113*, 109–113.

18 I. Lapkin, Y.S. Andreichikov, *Zhur. Org. Khim.* **1966**, *2*, 2075–2078; *Chem. Abstr.* **1967**, *66*, 75947k.

19 A.A. Jarrar, A.Q. Hussein, A.S. Madi, *J. Heterocycl. Chem.* **1990**, *27*, 275–280.

20 G. Lambrecht, R. Feifel, U. Moser, J. Aasen, M. Waelbroeck, J. Christoph, E. Mutschler, *Eur. J. Pharmacol.* **1988**, *155*, 167–170.

21 H. Kano, J. Adachi, R. Kido, H. Hiroso, *J. Med. Chem.* **1967**, *10*, 411–418.

22 M. Freri, *Gazz. Chim. Ital.* **1939**, *68*, 612–618.

23 E.R.H. Jones, T.Y. Shen, J. Withing, *J. Chem. Soc.* **1950**, 236–241.

[Ph314]