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# Regioisomeric 3-, 4- and 5-aminomethyl isoxazoles: synthesis and muscarinic activity*

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**Summary** — A series of 3-, 4- and 5-aminomethyl isoxazoles and isoxazoles with one or two additional methyl groups at the heterocycle were synthesized in order to investigate the structural requirements, *ie* heterocyclic moiety, regiochemistry and length of an aminoalkyl unit, for muscarinic activity. This was assayed on isolated rabbit vas deferens ($M_1$ receptor subtype) and isolated guinea-pig atrium ($M_2$ receptor subtype) and ileum ($M_3$ receptor subtype). The isoxazoles tested are one to three orders of magnitude less active than furane or oxadiazole derivatives, having similar structural characteristics except for the heterocycle. Thus, the differences in molecular point charges and charge distribution contribute to the muscarinic activity of these compounds more than small differences in molecular shape and conformational energies.

**regioisomeric aminomethyl isoxazole / synthesis / muscarinic activity / structure–activity relationship**

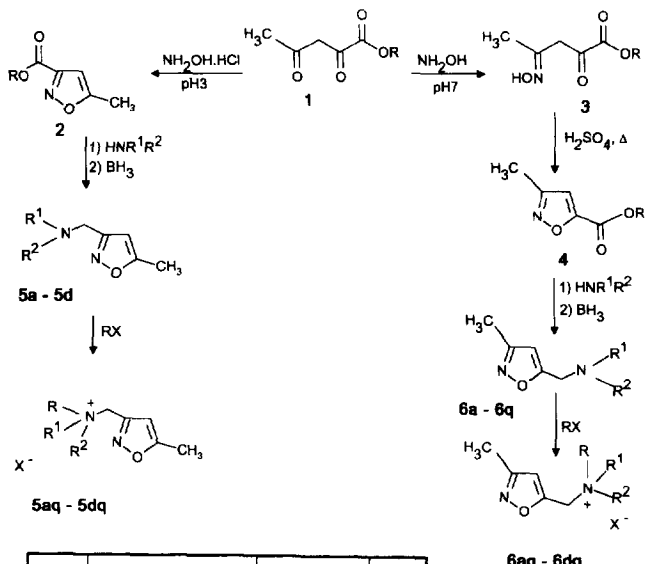
## Introduction

The elucidation of five cloned muscarinic receptor subtypes $m_1$–$m_5$, four of which are pharmacologically defined ($M_1$–$M_4$), provided the opportunity to develop novel compounds for therapeutic intervention [1–3]. Antagonists at the $M_1$ and $M_2$ muscarinic receptors are used for the treatment of peptic ulcers or gastritis [4] and bradyarrhythmia [5], respectively. $M_3$ antagonists are useful for the therapy of gastrointestinal spasms [6, 7], and it has been suggested that lipophilic $M_2$ antagonists and $M_1$ agonists, which can penetrate the blood–brain barrier, could improve disturbed learning and memory in patients with neuro-degenerative disorders [8]. In the search for selective and discriminating ligands we have studied the aminomethyl isoxazoles, which have structural similarities to muscimol, acting on $GABA_A$ receptors, and furtrethonium (2-trimethylammonium methyl furane), a potent muscarinic receptor agonist [9]. In the quinuclidine series, oxadiazoles, oxazoles and 3-methyl isoxazole can function as bioisosteric replacements for the ester moiety [10] found in several muscarinic ligands. Just like these compounds, the isoxazoles tested have an ammonium function at a definite distance from an oxygen atom. This approach is consistent with Ing's rule [11, 12] for muscarine agonists, including the bioisosterism of the isoxazole or the oxime ether moiety and the ester group shown recently [13, 14], as well as the replacement of the metabolically labile ester by more stable groups.

## Chemistry

The preparation of 3- and 5-isoxazole carboxylic esters is depicted in scheme 1. Depending on the conditions, the acetyl pyruvate **1** reacts regioselectively with hydroxylamine hydrochloride to yield the isoxazole **2** and the oxime **3**, which is cyclized by heating with concentrated sulphuric acid to the regioisomer **4**. According to Uchimoto [15], entropy determines the ring closure **3** → **4**, whereas the reaction enthalpy has the same order of magnitude for both cyclizations. In tune with the structure, an AB coupling system for the methylene protons of **3** is proved in the NMR spectra, indicating a preferred conformation with restricted rotation of the molecule favoured by H-bonding between the hydroxyl and the carbonyl group. NMR and MS data were used to

*Dedicated to Prof Dr Dr W Schunack, FU Berlin, on the occasion of his 60th birthday.




| | $R^1$ $R^2$ | R | X |
|---|---|---|---|
| a |  | $CH_3$ | I |
| b |  | $CH_3$ | I |
| c |  | $CH_3$ | I |
| d | $CH_3$ $CH_3$ | $CH_3$ | I |

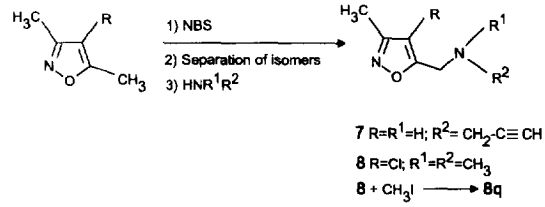
**Scheme 1.**

distinguish the regioisomers **2** and **4**. According to the literature [16] and our own investigations [17, 18], the relative intensities of the benzoyl and azirine cation as key fragments were applied for structural elucidation. The chemical shifts of the vinylic protons also indicate the regiochemistry (table I). The aminomethyl derivatives were prepared by treating **2** and **4** with amines followed by $BH_3$·DMS reduction. Quaternization of **5a–d** and **6a–d** with methyl iodide produced the corresponding salts **5aq–dq/6aq–dq** used for pharmacological testing.

A mixture of mono and dibromo isomers was obtained using *N*-bromosuccinimide and 3,5-dimethyl isoxazole (scheme 2). After column chromatographic separation, the isolated 5-bromomethyl derivative was reacted with propargyl amine to yield **7**. The same procedure was applied to synthesize the 5-dimethylamino isoxazole **8**, which was transferred to the quaternary salt **8q**.

**Table I.** Spectroscopic data to discriminate 5(3)-methyl-3(5)-isoxazole carboxylic esters **2** and **4**.

| *Method* | *2* | *4* |
|---|---|---|
| NMR | | |
| δ (Vinyl-H) | 6.4 ppm | 6.8 ppm |
| $^4J$-coupling | 0.8 Hz | – |
| MS *m/z* (rel int) | 82 (28%) | 82 (100%) |
| | 59 (100%) | 59 (5%) |
| | 43 (30%) | 54 (17%) |

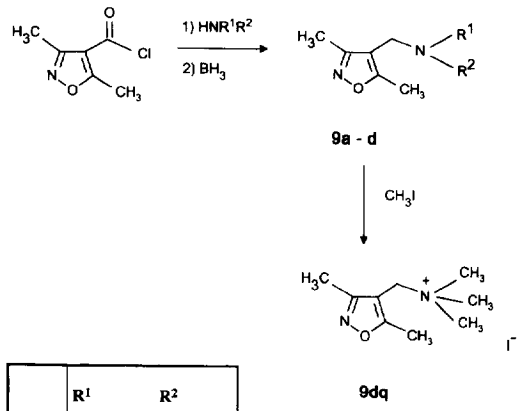



**Scheme 2.**

The 4-amino-methyl isoxazoles **9a–d** were available analogously to 3- and 5-substituted derivatives (scheme 3) using the corresponding isoxazole carboxylic chlorides.

The close structural analogy to furtrethonium induced us to prepare regioisomeric 3-, 4- and 5-aminomethyl isoxazoles without additional substituents. According to the literature [19], the reaction between trichloroacetylchloride, ethyl vinyl ether and hydroxylamine yielded the 5-isoxazole carboxylic acid **10**, which was transferred to the trimethylammonium isoxazole iodide **13** using the methods described above (table II). The $^3J$ coupling (1.8 Hz) of the protons at C-3 and C-4 together with the chemical shifts (8.1–8.9 ppm and 6.7–7.1 ppm, respectively) characterized these compounds as 5-substituted isoxazoles.

A mixture of regioisomeric 3- and 5-chloromethyl isoxazoles **14** and **15** (94%/6%, HPLC) was obtained using ethyne, chloroacetyl chloride and hydroxylamine. The 5-isomer was not mentioned in the literature [20] as a side product of this reaction and therefore it was synthesized independently (**10** → **17** → **15**). All the data obtained agree with the given structures. Repeated recrystallization of the quaternary ammonium salts made the pure 3-derivative **18** available. A diamagnetic shift of about 0.5 ppm for the proton at C-4 distinguishes the 3-regioisomer from the 5-regioisomer; the protons at C-5 (3-isomer) and at C-3 (5-isomer) resonate close together (8.35 *versus* 8.38 ppm).




| | $R^1$ | $R^2$ |
|---|---|---|
| a | | |
| b | $CH_3$ | n-$C_4H_9$ |
| c | $CH_3$ | $CH_2C_6H_5$ |
| d | $CH_3$ | $CH_3$ |

**Scheme 3.**

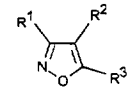
A 7:3 ratio of *Z*- to *E*-4-isoxazole carbaldehyde oxime **19** was obtained with the method of Wudl [21, 22]. After elimination of water to the corresponding carbonitrile **20**, reduction of this compound using different reagents always led to waste products, mostly formed by ring opening. The best and only way to get the 4-aminomethyl isoxazole **21** was to reduce the mixture of aldoximes with aluminium amalgam [23]. The $NH_2$ group resonates at 3.4 ppm, the methylene protons at 4.3 ppm and the singlets of the protons at C-3 and C-5 at 8.6 and 8.85 ppm, respectively, in the NMR spectra. This sequence enabled us to synthesize the 4-aminomethyl isoxazole **21** for the first time. The Leukart–Wallach reaction of **21** yielded the tertiary amine **22** which was methylated to give the quaternary salt **23** (table II).

Lengthening the aminomethyl side chain at position 5 of the heterocycle was achieved using 3,5-dimethyl isoxazole as the starting material. The sequences shown produced 3-methyl-5-aminoethyl and 3-methyl-5-aminopropyl isoxazoles **25** and **28**, respectively.

## Pharmacology

The pharmacological testing was performed according to literature [24]. For details see *Experimental protocols*.

**Table II.** Structures of compounds **10–29**.



| *Compound* | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **10** | H | H | $CO_2H$ |
| **11** | H | H | $CON(CH_3)_2$ |
| **12** | H | H | $CH_2N(CH_3)_2$ |
| **13** | H | H | $CH_2N^+(CH_3)_3I^-$ |
| **14** | $CH_2Cl$ | H | H |
| **15** | H | H | $CH_2Cl$ |
| **16** | $CH_2N^+(CH_3)_2$ | H | H |
| **17** | H | H | $CO_2C_2H_5$ |
| **18** | $CH_2N(CH_3)_3I^-$ | H | H |
| **19** | H | CH=NOH | H |
| **20** | H | CN | H |
| **21** | H | $CH_2NH_2$ | H |
| **22** | H | $CH_2N(CH_3)_2$ | H |
| **23** | H | $CH_2N^+(CH_3)_3I^-$ | H |
| **24** | $CH_3$ | H | $CH_2CO_2C_2H_5$ |
| **25** | $CH_3$ | H | $(CH_3)_2N(CH_3)_2$ |
| **26** | $CH_3$ | H | $(CH_2)_2N^+(CH_3)_3I^-$ |
| **27** | $CH_3$ | H | $(CH_2)_3OH$ |
| **28** | $CH_3$ | H | $(CH_2)_3N(CH_3)_2$ |
| **29** | $CH_3$ | H | $(CH_2)_3N^+(CH_3)_3I^-$ |

## Results and discussion

Regioisomeric 3- and 5-ammonium methyl isoxazoles show agonistic activity in nearly all functional muscarine receptor models tested, demonstrating the essential role of the quaternary ammonium function. Compound **6dq** proved to act as a full agonist at $M_2$- and $M_3$-receptors in GPA and GPI, respectively, the potencies and the intrinsic activities at these receptors being higher than at $M_1$-receptors in RVD (table III). For the regioisomer **5dq** a partial agonism at the $M_2$-receptors was observed. Compared with furtrethonium, all derivatives tested are generally between 1 and 2 orders of magnitude less active.

Compounds with a protonated piperidine moiety (**5b**, **6b**) as the 'headgroup' showed an antagonistic activity in all functional receptor models except for **6b** at $M_3$-receptors. The 3-piperidinium derivative **5b** was a more potent antagonist than the 5-regioisomer **6b**. The morpholinomethyl isoxazoles **5a/6a** were antagonists at $M_3$ and, in contrast, partial agonists at the $M_1$ subtype. After quaternization of **6a** → **6aq** partial agonism at $M_2$ and decreased antagonistic activity at $M_3$-receptors were observed. The piperidinium salt **6bq** was found to have a tenfold higher antimuscari-

nic potency at $M_2$-receptors compared with the base **6b** and additionally an antagonistic activity in the $M_3$ model. The 5-propargylaminomethyl isoxazole hydrochloride **7** proved to be a weak partial agonist/antagonist at $M_1$-receptors.

An additional chlorine at position C-4 of the isoxazole (**8q**) lowered the muscarinic activity compared with **6dq**, which is identical in all other positions. The partial agonism found for **8q** at $M_1$-receptors could not be antagonized by pirenzepine, *ie* it was not due to a muscarinic action.

The quaternary salt **9dq** of the 4-aminomethyl isomer with two methyl groups at C-3 and C-5 of the isoxazole was less potent than the 3- and 5-substituted derivatives (compare **5dq**, **6dq** and **9dq**) with only one methyl group. Partial agonism at $M_1$-receptors is found for the morpholino compound **9a**, with an intrinsic activity of 0.8; the tertiary amines **9b** and **9c** were weak antagonists at the $M_2$- and $M_3$-receptors. Thus, all the regioisomeric 3-, 4- and 5- aminomethyl isoxazoles, bearing one or two additional methyl groups, showed medium or low activity at all muscarinic receptor subtypes tested without significant subtype selectivity. For agonistic effects a trimethylammonium methyl group at position C-5 was most favourable; on the other hand, piperidino- and piperazino methyl substituents at C-3 led to antagonists. In contrast, the regioisomers with a morpholino moiety were agonists at the $M_1$- and $M_2$-receptors, and antagonists at the $M_3$-receptor.

The muscarinic receptors are members of the family of G-protein-coupled receptors consisting of seven hydrophobic transmembrane helices and alternating intracellular and extracellular loops. Detailed information of the actual structure of the receptor protein would contribute significantly to the understanding of structure–activity data. By combining Schulman's pharmacophore model [25] with homology-based modelling of the three-dimensional structure of the receptor protein, Hacksell and Nordvall [26] proposed an $M_1$-receptor model. This model suggests the presence of several hydrophobic interactions between the ligands and the receptor protein revealing the possibility of two hydrogen bond interactions of Thr 192 to the ether oxygen and of Asn 382 to the carbonyl oxygen of acetylcholine. Fanelli [27] applied the same methods as described by Hacksell and Nordvall and his model is based on a heuristic direct QSAR approach with good linear correlations between theoretical interaction descriptors and the biological activity of the ligands. Dougherty and Stauffer [28] have built a completely synthetic receptor providing an overall hydrophobic binding site, but a stabilizing interaction between the quaternary ammonium group of acetylcholine and the electron-rich π-systems of the aromatic amino acids, which is similar to that of the enzyme acetylcholinesterase [29].

In order to ascertain whether the different activities of the aminomethyl furanes (furtrethonium, furmethide) and the aminomethyl isoxazole derivatives can be explained in terms of influence on the conformation and/or on the electronic distribution, a conformational analysis and a study of the atomic point charges were carried out (see *Experimental protocols*). A geometry optimization was performed for compound **13** with respect to the N-O-C-C ($\tau_1$), O-C-C-N ($\tau_2$) and C-C-$N^+$-C ($\tau_3$) torsion angles (fig 1). The dihedral angles $\tau_2$ and $\tau_3$ involved were allowed to rotate with 30° increments and a conjugate gradient minimization was used [30]. The lowest conformational relative energies were found for $\tau_1 = 180°$, $\tau_2 = 94°$ and $\tau_3 = 60°$, remaining practically identical for furtrethonium calculated in this way and in agreement with investigations concerning the bioactive conformation of acetylcholine [31] at muscarinic receptors. The distance between the exocyclic nitrogen and the oxygen is 33.7 nm. Additionally, 3-methoxy-5-tri-

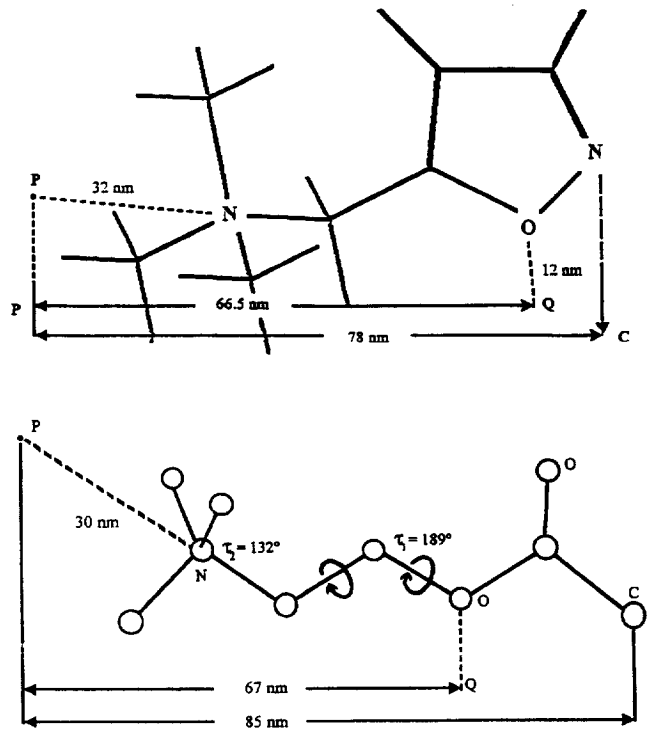



**Fig 1.** Comparison of the preferred conformation of isoxazole **13** and acetylcholine, including the distances of the pharmacophore model proposed by Schulman [27] and Tollenaere [25].

methylammoniummethyl-4,5-dihydroisoxazole with some higher flexibility showed the same degree of muscarinic activity [32] as our compounds. These results indicate that conformational relative energies of furane and isoxazole do not account for the different muscarinic activity found (fig 2).

In figures 3–5 the atomic charges calculated with the semiempirical quantum chemistry program

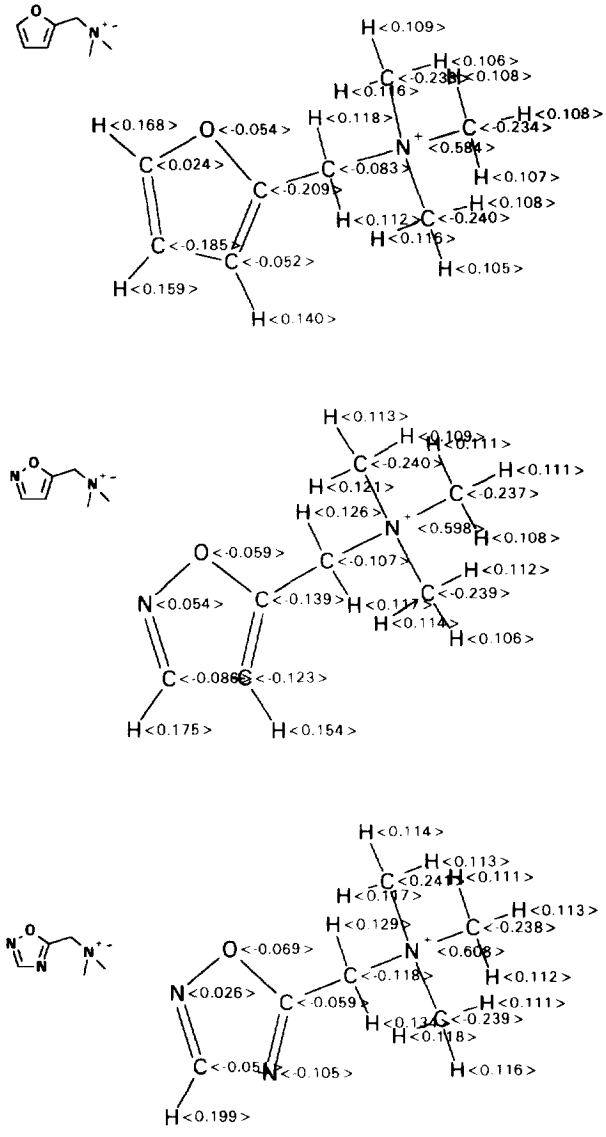



**Fig 2.** Atomic point charges of furtrethonium, isoxazole **13** and the corresponding oxadiazole. Charges from MOPAC PM3 calculation (Mopac Version 6.0)

MNDO-PM3 [30] are given for furtrethonium and oxadiazole (fig 3), for isoxazole **13** and oxadiazole (fig 4) for acetylcholine and oxadiazole (fig 5). Only small differences in partial charges of the trimethylammonium methylene group were calculated for furtrethonium, isoxazole **13**, and the analogous oxadiazole. However, the differences in charge found for the heterocyclic moiety of the three compounds might give rise to differences in polar interactions either in a polar solvent or with some polar amino acid sidechains of the hypothetical binding site of acetylcholine. This was corroborated by calculations with the DELPHI program. Grid points were calculated for all three ammonium ions for an acetylcholine conformer in aqueous solvent on the basis of the coulombic forces. The latice equipotential surfaces were computed for all four cations at the potential of +1 kcal/mol/e and –0.1 kcal/mol/e (fig 3–5). The surfaces were compared visually by superimposition with the potential surface of the strongest agonist oxadiazole (deep blue). All four cations possess a strong positive potential surrounding the whole trimethylammonium group, but the furtrethonium ion also possesses positive electrostatic fields around all three hydrogen atoms of the furane ring (fig 3, red). The space beyond the plane of the furane ring is characterized by a weak negative electrostatic potential (magenta).

The electrostatic potential surface surrounding the trimethylammonium group of isoxazole **13** maps exactly that of oxadiazole (fig 4). However, due to the fact that the carbon atom in position 5 of the furane ring was replaced by nitrogen in the isoxazole **13**, the potential surface of the heterocyclic moiety of the isoxazole **13** differs clearly (light green).

Oxadiazole, which is a more potent agonist than furtrethonium, possesses two nitrogen atoms in positions 3 and 5 related to furtrethonium. Besides the ammonium group, it bears only small areas of lower positive potential at position 3 of the oxadiazole ring, but the negative potential areas of the furane (fig 3, magenta), isoxazole (fig 4, yellow) and acetylcholine (fig 5, yellow) are also diminished so that the electrostatic potential surface is more flat than in the furane.

The most negative point charge in the oxadiazole is localized at nitrogen atom N-4, and in furtrethonium at the oxygen atom of the furane ring; these two atoms are potential H-bond acceptor sites. Both furtrethonium and oxadiazole have a positively charged H-atom in the neighbourhood of these negatively charged positions, when both quaternary ammonium N-atoms are kept at the same point and N-4 of the oxadiazole and oxygen of the furane are superimposed. This positively charged H-atom may additionally interact with the receptor protein.

A similar dipolar interaction of the isoxazole derivative is not possible when the molecules are super-

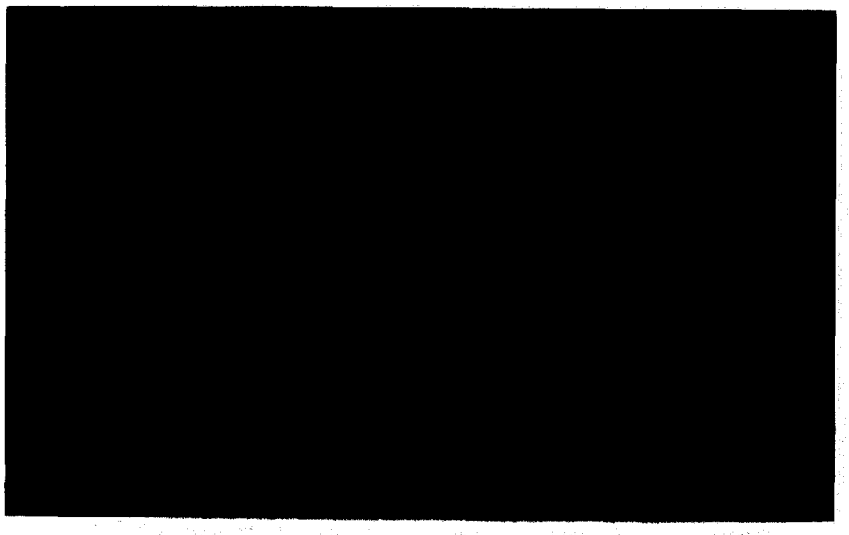


**Fig 3.** Charges of furtrethonium and oxadiazole, superimposition of potential surfaces (DELPHI, furane: red = 1.0 kcal/mol/e, magenta = –0.1 kcal/mol/e; oxadiazole: blue = 1.0 kcal/mol/e).

**Fig 4.** Charges of isoxazole and oxadiazole, superimposition of potential surfaces (DELPHI, isoxazole: green = 1.0 kcal/mol/e, yellow = –0.1 kcal/mol/e; oxadiazole: blue = 1.0 kcal/mol/e).

**Fig 5.** Charges of acetylcholine and oxadiazole, superimposition of potential surfaces (DELPHI, acetylcholine: green = 1.0 kcal/mol/e, yellow = –0.1 kcal/mol/e; oxadiazole: blue = 1.0 kcal/mol/e).

**Table III.** *In vitro* muscarinic agonist/antagonist activity of the compounds investigated at $M_1$, $M_2$ and $M_3$ receptors.

| *Compound* | *$M_1$RVD* | | | *$M_2$GPA* | | | *$M_3$GPI* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *$pD_2$* | *ia* | *$pA_2$* | *$pD_2$* | *ia* | *$pA_2$* | *$pD_2$* | *ia* | *$pA_2$* |
| **5a** | 3.65 ± 0.59 | 0.77 | | 3.83 ± 0.11 | 0.70 | | | | 4.94 ± 0.09 |
| **5b** | | | 4.40 ± 0.11 | | | 4.58 ± 0.05 | | | 4.04 ± 0.03 |
| **5c** | | | 3.84 ± 0.27 | | | | | | |
| **5d** | | | | 4.33 ± 0.12 | 0.30 | | | | 4.03 ± 0.28 |
| **5dq** | 4.15 ± 0.20 | 1.0 | | 4.25 ± 0.01 | 0.70 | | 4.20 ± 0.09 | 0.98 | |
| **6a** | 4.27 ± 0.43 | 0.50 | | | | | | | 4.95 ± 0.20 |
| **6aq** | | | | 4.90 ± 0.32 | 0.48 | | | | 4.53 ± 0.04 |
| **6b** | | | 4.07 ± 0.12 | | | 3.84 ± 0.12 | | | |
| **6bq** | 3.69 ± 0.61 | | | | | 4.73 ± 0.30 | | | 4.79 ± 0.11 |
| **6c** | | | | 3.72 ± 0.04 | 0.62 | | | | |
| **6d** | | | 5.09 ± 0.03 | 3.86 ± 0.05 | 0.86 | | 3.76 ± 0.16 | 0.93 | |
| **6dq** | 4.00 ± 0.21 | 0.89 | | 5.03 ± 0.05 | 1.00 | | 4.76 ± 0.12 | 1.00 | |
| **7-HCl** | 3.94 ± 0.31 | 0.45 | 4.74 ± 0.17 | | | | | | |
| **8q** | 4.03 ± 0.32 | 0.45 | | | 0.36 | | 3.81 ± 0.08 | 0.59 | |
| **9a** | 3.93 ± 0.32 | 0.80 | | | | | | | |
| **9b** | | | | | | 3.70 ± 0.11 | | | 4.11 ± 0.03 |
| **9c** | | | | | | 4.10 ± 0.09 | | | 4.17 ± 0.04 |
| **9dq** | | | | 3.91 ± 0.02 | 1.0 | | 2.97 ± 0.08 | 1.0 | |
| Furtrethonium | 5.77 ± 0.04 | 1.0 | | 6.00 ± 0.06 | 1.0 | | 6.38 ± 0.02 | 1.0 | |
| **13** | 4.78 ± 0.04 | 1.0 | | 5.26 ± 0.03 | 1.0 | | 5.47 ± 0.04 | 1.0 | |
| **18** | 4.42 ± 0.06 | 0.88 | | 4.90 ± 0.05 | 1.0 | | 4.75 ± 0.07 | 1.0 | |
| **23** | 4.61 ± 0.13 | 0.9 | | 4.90 ± 0.12 | 1.0 | | 4.80 ± 0.15 | 1.0 | |
| **26** | 3.99 ± 0.32 | 0.3 | 4.68 ± 0.16 | 4.05 ± 0.2 | 0.57 | | 4.37 ± 0.28 | 0.59 | |
| **29** | 3.62 ± 0.40 | 0.79 | 4.67 ± 0.08 | 4.95 ± 0.09 | 0.73 | | 3.81 ± 0.05 | 0.76 | |

Data are means ± SEM ($n$ = 4–6).

imposed in the same way, because nitrogen atom N-2 occupies this ring position. In addition the dipolar interactions of **13** could be disturbed by electrostatic repulsions of negatively charged atoms at the binding site of the muscarinic receptor and nitrogen atom N-2.

Comparing the electrostatic potential surfaces or the coulombic energy surfaces of these molecules, we must realize that these differences may correlate even better with the differences in binding capabilities than do the conformational energy differences.

Based on *ab initio* INDO calculations, Schulman [25] proposed a muscarinic pharmacophore using acetylcholine as the ligand with an optimal drug–receptor interaction, if the distance between the cationic headgroup of the drug and the anionic receptor site is 30 nm, and 12 nm between the oxygen and the receptor. The positions P and Q marked in figure 1 represent binding areas of the activated receptor protein. The highest activity for muscarinic agonists was predicted for P-Q = 67 nm and P-C = 85 nm. It was found that the isoxazoles studied have P-Q = 66.5 nm and P-C = 78 nm (distance between oximino nitrogen and receptor binding site). Likewise, the distance between the cationic nitrogen and the oxygen of acetylcholine (32 nm, isoxazoles 33.7 nm) and the torsion angles $\tau_1$ and $\tau_2$ (acetylcholine $\tau_1$ = 189°, $\tau_2$ = 132°) agree with our data of conformational optimized isoxazoles. The lower activity of the full agonists tested may be due to the decreased P-C distance in the isoxazoles.

Oxadiazoles with an additional negatively charged nitrogen at the heterocycle and the same aminomethyl side chain as furtrethonium and the isoxazoles described are two or three orders of magnitude more active muscarinic agonists [33, 34], supporting the hypothesis of three distinct hydrogen bonding interactions between drug and receptor protein [35]. Interactions of the cationic headgroup and asparaginic acid and the hydrogen bonding of two serine residues and the nitrogen atoms of the heterocyclic moiety

are being considered [35, 36]. A similar model for receptor–drug interactions is under consideration for 5-methyl furmethide [36].

Ing's rule [11, 12] with the hypothesis of a five atom chain at the quaternary ammonium function was proposed after the increment in potency obtained by the transformation of furtrethonium to 5-methyl furtrethonium. For optimum activity in muscarinic drugs a 50 nm distance between the cationic nitrogen and the negatively charged oxygen has been reported [37]. In contrast to these ideas, the lengthening of the side chain at **6dq** to a five atom unit at the corresponding aminomethyl derivative **26** decreased the muscarinic activity. With the exception of the $M_2$-receptor, this effect is more pronounced when testing the aminopropyl derivative **29**, but with this compound the highest degree of receptor selectivity ($M_2 > M_3 > M_1$) for agonistic activity was observed compared with all other isoxazole compounds tested. Summarizing the data and keeping the limitation of the different models in mind, *ie* flexibilities of the receptor protein and the ligand are not taken into account, it should be noted that these simple alignments can only have qualitative value and are not intended to be a sophisticated representation of the pharmacophore necessary for muscarinic activity. However, they could support the hypothesis that besides a suitable distance of the cationic headgroup and negatively charged oxygen, together with conformational flexibility of the side chain, the drug molecule should have at least three positions capable of polar interactions with the active sites of the receptor protein. These sites are responsible for significant differences of muscarinic activity found in the series of aminomethyl isoxazoles, furanes structurally related to furtrethonium [38] and oxadiazoles. Further investigations of this phenomenon are ongoing.

## Experimental protocols

*Molecular modelling*

Calculations were made on a SG Iris 4D 310 using program package Mopac (version 6.0) included in INSIGHT II (version 2.30), conformational energies were calculated in the DISCOVER program (version 2.9) using the rotor command. Electrostatic potentials were calculated from atomic point charges in the DELPHI program, all programs from BIOSYM Technologies Inc, San Diego, CA.

*Pharmacology*

*Functional studies*
In all functional preparations, control experiments established that there was no consistent sensitivity change to the agonists used over the normal experiment duration.

*Rabbit vas deferens*
Vasa deferentia from male New Zealand white rabbits were removed, dissected free of connective tissues and divided into five to six segments of about 1.0–1.5 cm length. Each tissue was set up in a 6 ml organ bath containing a modified Krebs buffer. The resting tension was adjusted to 500 mg and twitch contractions were elicited by electrical field stimulation (0.05 Hz, 40 V, 0.5 ms) *via* platinum electrodes. The neurogenic responses were measured isometrically with a force-displacement transducer and recorded on a Rikadenki multichannel recorder. Antagonist affinities for prejunctional $M_1$ were obtained from cumulative concentration–response curves to the $M_1$-selective agonist 4-(4-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium iodide (4-Cl-McN-A-343) for inhibition of neurogenic twitch contractions in the absence and presence of antagonists.

*Guinea-pig left atria and ileum*
The organs required were removed and set up in 6 ml organ baths, under 250 (left atria divided in two) and 500 mg tension (ileum), in Tyrode solution.

Left atria were electrically paced (2 Hz, 3 ms, 5 V) by means of platinum electrodes, and negative inotropic effects to cumulative addition of the selective muscarinic agonist arecaidine propargyl ester (APE) were measured as changes in isometric tension and recorded as with the rabbit isolated vas deferens.

Strips of ileal longitudinal muscle were prepared for isotonic contractions in response to cumulative administration of APE or histamine. Responses to the agonists were recorded isotonically using a force-displacement transducer connected to a Hellige amplifier and a Rikadenki multichannel recorder.

*Organ bath solutions*
The bath fluid was maintained at 31°C and aerated with 95% $O_2$ (pH = 7.4)/5% $CO_2$.

The Tyrode solution used in the experiments on guinea-pig atria and ileum was of the following composition (mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaH_2PO_4$ 0.42, $NaHCO_3$ 11.9 and (+)-glucose 5.6.

The modified Krebs buffer used in the experiments with the rabbit vas deferens consisted of (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.0, $MgSO_4$ 0.6, $KH_2PO_4$ 1.2, $NaHCO_3$ 25.0 and (+)-glucose 11.1. Yohimbine (1.0 μM) was included to block $\alpha_2$-adrenoceptors.

*Antagonists affinities*
The tissues were allowed to equilibrate for 30 min. Then, concentration–response curves to the agonists were constructed in the absence (control) and in the presence of at least three concentrations (log interval = 0.48) of antagonists, allowing 30–60 min equilibration time. Preliminary experiments indicated that these intervals were sufficient for equilibration of the antagonist concentration used. Each concentration of antagonist was tested 3–5 times and the ratios of agonist molar $EC_{50}$ values obtained in the presence and absence of antagonists were calculated. The $EC_{50}$ values were determined by fitting the data to non-linear iterative curve fitting procedure. For the assessment of antagonist affinity, Schild plots were made using linear regression by the method of least squares, to estimate $PA_2$ values and the slope of the regression lines. In a second approach, $pA_2$ values were determined from Schild plots in which the slopes of the regression lines had been constrained to 1.00. This is more consistent with the competitive theory which connects $pA_2$ with $-\log K_B$, since the slopes obtained did not differ significantly from unity.

For further details of the pharmacological screening see reference [24].

*Chemistry*

Melting points were determined on a Büchi SMP/20 apparatus and are uncorrected. All C, N, O analyses were within ±0.4%

of the theoretical values; $^1$H-NMR measurements were obtained on a Bruker AC 300 MHz spectrometer; mass spectra were recorded with an MAT 212/SS 188 spectrometer; IR spectra were obtained on a Perkin-Elmer Model 299 spectrometer. All structural assignments were consistent with IR, NMR and MS data.

3,5-Dimethylisoxazole was synthesized according to reference [39]. The donation of 3,5-dimethylisoxazole-4-yl carboxylic chloride by Kalle-Albert, Wiesbaden, is gratefully acknowledged. The physicochemical and spectroscopic data of the isolated and characterized intermediate carboxamides (schemes 1 and 3) are available on request.

*Methyl-5-methyl-3-isoxazole carboxylate* **2**
To a sodium acetate buffered solution of hydroxylamine hydrochloride (5 mmol) (pH 5), was added methyl-2,4-dioxopentanoate (5 mmol). After heating under reflux for 2 h the mixture was acidified with 1.5 M $H_2SO_4$ (pH 1). Heating was continued for a further 3 h. After cooling, the methylene chloride extracts of the reaction mixture were concentrated *in vacuo* to give the pure **2**.

Yield 58%, mp 93°C (94°C [44]), IR: 1720 (C=O), 1595 (C=N) cm$^{-1}$; NMR: δ (ppm) = 2.5 (d, 3H, $J$ = 0.9 Hz), 3.97 (s, 3H), 6.2 (d, 1H, $J$ = 0.9 Hz); MS: $m/z$ = 141 ($M^+$, 45), 127 (4), 110 (26), 82 (46), 59 (100), 54 (20).

*Ethyl-4-hydroximino-2-oxopentanoate* **3**
The synthesis followed the description of reference [40]. The resulting crude product was purified by column chromatography on silica gel (cyclohexane/ethylacetate 1:1, $R_f$ = 0.5).

Yield 35%, mp 75–76°C (77°C), IR: 3260 (br, OH), 1760 (C=O), 1640 (C=N) cm$^{-1}$; NMR: δ (ppm) = 1.32–1.39 (t, 3H), 2.07 (s, 3H), 2.95 (d, 1H, AB, $J$ = 18 Hz), 3.6 (d, 1H, AB, 18 Hz), 4.28–4.38 (q, 2H), 4.55 (s, 1H, OH); MS: $m/z$ = 173 ($M^+$, 4), 156 (2), 131 (4), 127 (8), 100 (100), 82 (8), 72 (6), 58 (63), 56 (16).

*Ethyl-3-methyl-5-isoxazole carboxylate* **4**
Concentrated sulfuric acid (4.1 mmol) was added to 8.2 mmol of **3** dissolved in ethanol. The solution was heated for 6 h at 80°C. After workup the resulting residue was purified by column chromatography on silica gel (cyclohexane/ethylacetate 2:1, $R_f$ = 0.8).

Yield 84%; IR: 1740, 1600 cm$^{-1}$; NMR: δ (ppm) = 1.3– 1.45 (t, 3H), 2.35 (s, 3H), 4.3–4.55 (q, 2H), 6.8 (s, 1H); MS: $m/z$ = 155 ($M^+$, 12), 127 (25), 110 (56), 82 (100), 71 (10), 69 (19), 55 (39), 54 (44), 44 (28).

*General procedure for isoxazole carboxamides*
The carboxylic ester was added portionwise under stirring to an excess of the amine and the solution stirred for 4–18 h (TLC control). The mixture was evaporated and the resulting residue purified by column chromatography. Yield 65–90%.

*General procedure for aminomethyl isoxazoles*
To a solution of the amide in anhydrous tetrahydrofuran, neat borane-dimethylsulfide (fourfold excess to the amide) was added under an $N_2$-atmosphere within 10 min under vigorous stirring. The mixture was refluxed for 6 h. After cooling to room temperature, methanol was carefully added and the solution kept overnight. The mixture was evaporated, acidified with diluted HCl and refluxed for 1 h. After cooling to 0°C, the solution was adjusted to pH 9–10 using a solution of NaOH and solid $K_2CO_3$ and then extracted with ether. The combined organic extracts were dried, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel.

*5-Methyl-3-(N-morpholinomethyl) isoxazole* **5a**. $SiO_2$/MeOH, $R_f$ = 0.6. Yield 77%; IR: 1610 (C=N) cm$^{-1}$; NMR: δ (ppm) = 2.41 (d, 3H, $J$ = 0.7 Hz), 2.48–2.51 (m, 4H), 3.55 (s, 2H), 3.70–3.73 (m, 4H), 6.01 (s, 1H, $J$ = 0.7 Hz); MS: $m/z$ = 182 ($M^+$, 13), 167 (28), 151 (6), 140 (10), 137 (16), 124 (20), 109 (22), 97 (84), 86 (100), 82 (22), 70 (12), 68 (10), 56 (31), 54 (20). **5a-HCl**: mp 179–180°C (182–186°C [41]).

*5-Methyl-3-(N-piperidinomethyl) isoxazole* **5b**. $SiO_2$/$Et_2O$, $R_f$ = 0.4. Yield 74%; IR: 1610 (C=N) cm$^{-1}$; NMR: δ (ppm) = 1.3–1.45 (m, 2H), 1.54–1.61 (m, 6H), 2.40–2.43 (m, 7H, $J$ = 0.85 Hz), 3.51 (s, 2H), 6.01 (d, 1H, $J$ = 0.85 Hz); MS: $m/z$ = 180 ($M^+$, 10), 179 (22), 165 (16), 151 (6), 124 (12), 109 (12), 97 (44), 84 (100), 69 (30), 55 (26), 43 (53). **5b-HCl**: mp 194–196°C (188–190°C [41]).

*3-Methyl-5-(N-piperidinomethyl) isoxazole* **6b**. $SiO_2$/$Et_2O$, $R_f$ = 0.5. Yield 85%; IR: 1610 (C=N) cm$^{-1}$; NMR: δ (ppm) = 1.37–1.46 (m, 2H), 1.56–1.64 (m, 4H), 2.29 (s, 3H), 2.40–2.50 (m, 4H), 3.61 (s, 2H, $CH_2$), 6.00 (s, 1H); MS: $m/z$ = 179 ($M^+$, 100), 165 (4), 138 (12), 111 (12), 96 (76), 84 (34), 68 (13), 55 (24), 42 (36), 32 (66). **6b-HCl**: mp 195–197°C (188–190°C [41]).

*5-Methyl-3-(N-piperazinomethyl) isoxazole* **5c**. The residue of the concentrated ether extracts was used without further purification. Yield 61%; mp 105–107°C; IR: 3440 (N-H), 1600 (C=N) cm$^{-1}$; NMR: δ (ppm) = 2.40–2.47 (m, 7H), 2.75–2.95 (m, 4H), 3.50 (s, 2H), 3.58 (s, 1H), 6.01 (s, 1H); MS: $m/z$ = 181 ($M^+$, 8), 166 (6), 139 (100), 125 (12), 109 (12), 97 (100), 85 (45), 70 (12), 56 (68). **5c-HCl**: mp 174–176°C.

*3-Methyl-5-(N-piperazinomethyl) isoxazole* **6c**. The isolation of the product followed the procedure above. Yield 54%; IR: 3300 (br, N-H), 1610 (C=N) cm$^{-1}$; NMR: δ (ppm) = 2.29 (s, 3H), 2.48–2.52 (m, 5H), 2.90–2.95 (m, 4H), 3.67 (s, 2H), 6.01 (s, 1H); MS: $m/z$ = 181 ($M^+$, 20), 166 (4), 139 (100), 125 (14), 110 (10), 96 (60), 85 (38), 68 (26), 56 (62). **6c-HCl**: mp 171–173°C.

*5-Methyl-3-(N,N-dimethylaminomethyl) isoxazole* **5d**. $SiO_2$/MeOH, $R_f$ = 0.5. Yield 94%; IR: 1610 (C=N) cm$^{-1}$; NMR: δ (ppm) = 2.27 (s, 6H), 2.41 (d, 3H, $J$ = 0.7 Hz), 3.48 (s, 2H), 6.00 (s, 1H, $J$ = 0.7 Hz); MS: $m/z$ = 140 ($M^+$, 4), 139 (6), 97 (27), 82 (10), 71 (12), 57 (38), 44 (100), 43 (50). **5d-HCl**: mp 155–156°C.

*3-Methyl-5-(N,N-dimethylaminomethyl) isoxazole* **6d**. $SiO_2$/MeOH, $R_f$ = 0.6. Yield 72%; IR: 1610 (C=N) cm$^{-1}$; NMR: δ (ppm) = 2.28 (s, 3H), 2.3 (s, 6H), 3.58 (s, 2H), 6.01 (s, 1H); MS: $m/z$ = 140 ($M^+$, 90), 96 (25), 82 (16), 68 (8), 58 (100), 42 (30).

*General procedure for the quaternization of aminomethyl isoxazoles*
To a solution of the aminomethyl isoxazole (1 mmol in 2 ml anhydrous ethanol), 1.5 mmol iodomethane was added under stirring at room temperature. The reaction was complete within 20 min. The white precipitates were filtered and washed with dried ether. The product was recrystallized from ether/ethanol.

*5-Methyl-3-(N,N,N-trimethylammoniummethyl) isoxazole iodide* **5dq**. Yield 89%; mp 117–118°C; NMR: (DMSO-$d_6$): δ (ppm) = 2.48 (d, 3H, $J$ = 0.9 Hz), 3.11 (s, 9H), 4.65 (s, 2H), 6.52 (s, 1H); MS: $m/z$ = 282 ($M^+$, 0.7), 223 (20), 142 (88), 139 (24), 127 (24), 96 (94), 82 (14), 58 (100).

*3-Methyl-5-(N,N,N-trimethylammoniummethyl) isoxazole iodide* ***6dq***. Yield 76%; mp 119–121°C; NMR: (DMSO-$d_6$): δ (ppm) = 2.3 (s, 3H), 3.1 (s, 9H), 4.77 (s, 2H), 6.78 (s, 1H).

*3-Methyl-5-(N-methylmorpholinomethyl) isoxazole iodide* ***6aq***. Yield 74%; mp 155–157°C (EtOH/ethyl acetate); NMR: (DMSO-$d_6$): δ (ppm) = 2.3 (s, 3H), 3.16 (s, 3H), 3.49–3.57 (m, 4H), 3.92–4.03 (m, 4H), 4.98 (s, 2H), 6.84 (s, 1H).

*3-Methyl-5-(N-methylpiperidinomethyl) isoxazole iodide* ***6bq***. Yield 87%; mp 108–110°C (EtOH/$Et_2O$); NMR: (DMSO-$d_6$): δ (ppm) = 1.50–1.62 (m, 2H), 1.78–1.94 (m, 4H), 2.30 (s, 3H), 3.02 (s, 3H), 3.36–3.38 (m, 4H), 4.85 (s, 2H), 6.81 (s, 1H).

*3-Methyl-5-bromomethyl isoxazole*
To a solution of 20 mmol 3,5-dimethyl-isoxazole in 40 ml carbon tetrachloride, was added 200 mg peroxobenzoic acid. The suspension was heated at 80°C and then 20 mmol *N*-bromosuccinimide was added portionwise within 2 h. The mixture was refluxed for 3 h. After cooling to room temperature the resulting succinimide was filtered off and the solution concentrated *in vacuo*. The product was purified by column chromatography on silica gel with toluene ($R_f$ = 0.2).

Yield 36%; IR: 3330, 1610, 1450 $cm^{-1}$; NMR: δ (ppm) = 2.3 (s, 3H), 4.4 (s, 2H), 6.1 (s, 1H); MS: *m/z* = 177 ($M^+$, 6), 175 (6), 96 (72), 82 (7), 54 (10), 40 (14), 32 (100). The side products were as follows.

*3,5-Di(bromomethyl) isoxazole*. $R_f$ = 0.6, toluene. Yield 2%; NMR: δ (ppm) = 4.4 (s, 2H), 4.45 (s, 2H), 6.43 (s, 1H); MS: *m/z* = 255 ($M^+$, 12), 253 (8), 176 (95), 174 (100), 144 (6), 130 (5), 123 (6), 105 (6), 95 (32), 93 (18), 65 (31), 53 (9).

*3-Methyl-5-dibromomethyl isoxazole*. $R_f$ = 0.4, toluene. Yield 22%; NMR: (90 MHz): δ (ppm) = 2.2 (s, 3H), 6.2 (s, 1H), 6.4 (s, 1H); MS: *m/z* = 255 ($M^+$, 3), 174 (58), 146 (4), 122 (4), 105 (8), 96 (10), 82 (8), 67 (10), 42 (28), 39 (22), 32 (100).

*3-Methyl-5-(N-morpholinomethyl) isoxazole* ***6a***
At 0°C 2 mmol of the 5-bromomethyl isoxazole was added dropwise to a solution of 3 ml morpholine (freshly distilled) in 3 ml methanol. The suspension was stirred for 1 h. The volatile products were distilled off, and **6a** was isolated and purified by column chromatography on alumina oxide (Merck, activity 2, methylene chloride, $R_f$ = 0.7).

Yield 62%; IR: 3130, 1610, 1450 $cm^{-1}$; NMR: δ (ppm) = 2.3 (s, 3H), 2.50–2.54 (m, 4H), 3.64 (s, 2H), 3.71–3.74 (m, 4H), 6.03 (s, 1H); MS: *m/z* = 182 ($M^+$, 38), 153 (4), 136 (10), 110 (10), 100 (16), 96 (100), 86 (60), 83 (26), 56 (48). **6a-HCl**: mp 196–197°C.

*3-Methyl-5-(N-propargylaminomethyl) isoxazole* ***7***
5-Bromomethyl isoxazole (2 mmol) was added dropwise to a cooled mixture of 3 ml anhydrous ether, 3 mmol propargyl amine and 2 mmol potassium carbonate. The suspension was stirred for 12 h. After concentration *in vacuo*, the product was purified by column chromatography on silica gel (ethylacetate, $R_f$ = 0.6).

Yield 48%; NMR: δ (ppm) = 2.08 (s, 1H), 2.28 (s, 1H), 2.29 (s, 3H), 3.48 (s, 2H), 4.11 (s, 2H), 6.05 (s, 1H); MS: *m/z* = 150 ($M^+$, 16), 149 (18), 133 (6), 121 (32), 111 (8), 109 (14), 96 (40), 80 (100), 68 (84), 54 (64). **7-HCl**: mp 181–182°C.

*3-Methyl-4-chloro-5-(N,N-dimethylaminomethyl) isoxazole* ***8***
3-Methyl-4-chloro-5-bromomethyl isoxazole, synthesized according to reference [42], was added to a 1:1 mixture of dimethylamine in water (40%) and methanol. After reaction and usual workup, the product was purified by column chromatography (MeOH, $R_f$ = 0.7).

Yield 72%; IR: 2980, 1620, 1450 $cm^{-1}$; NMR: δ (ppm) = 2.29 (s, 3H), 2.32 (s, 6H), 3.64 (s, 2H); MS: *m/z* = 174 ($M^+$, 24), 173 (10), 157 (4), 139 (8), 130 (16), 109 (4), 96 (4), 70 (4), 58 (100), 44 (22), 42 (28), 40 (15).

*3-Methyl-4-chloro-5-(N,N,N-trimethylammoniummethyl) isoxazole iodide* ***8q***. The quaternization was carried out according to the general procedure. Yield 81%; mp: 145–146°C; NMR: (DMSO-$d_6$): δ (ppm) = 2.32 (s, 3H), 3.17 (s, 9H), 4.85 (s, 2H).

*Compounds* ***9a–c, 9dq***
The synthesis followed the general procedure for isoxazole carboxamides and aminomethyl isoxazoles.

*3,5-Dimethyl-4-(N-morpholinomethyl) isoxazole* ***9a***. IR: 2960, 1640 (C=N), 1450 $cm^{-1}$; NMR: δ (ppm) = 2.26 (s, 3H), 2.31 (s, 3H), 2.36–2.39 (m, 4H), 3.21 (s, 2H), 3.66–3.69 (m, 4H); MS: *m/z* = 196 ($M^+$, 24), 158 (14), 151 (5), 142 (6), 125 (6), 110 (35), 100 (82), 89 (26), 86 (30), 71 (100), 68 (44), 55 (86). **9a-HCl**: mp 231–233°C.

*3,5-Dimethyl-4-(N-methyl-N-n-butylaminomethyl) isoxazole* ***9b***. NMR: δ (ppm) = 0.87–0.92 (t, 3H), 1.25–1.35 (m, 2H), 1.41–1.48 (m, 2H), 2.09 (s, 3H), 2.25 (s, 3H), 2.22–2.32 (m, 2H), 2.33 (s, 3H), 3.16 (s, 2H); MS: *m/z* = 196 ($M^+$, 24), 158 (12), 125 (5), 110 (34), 100 (82), 89 (26), 86 (28), 73 (58), 71 (100), 68 (42), 55 (85). **9b-HCl**: mp 156–158°C.

*3,5-Dimethyl-4-(N-methyl-N-benzylaminomethyl) isoxazole* ***9c***. IR: 3060, 1640 (C=N), 1490, 1450 $cm^{-1}$; NMR: δ (ppm) = 2.11 (s, 3H), 2.23 (s, 3H), 2.34 (s, 3H), 3.22 (s, 2H), 7.24–7.35 (m, 5H); MS: *m/z* = 230 ($M^+$, 100), 229 (14), 215 (4), 186 (4), 153 (18), 139 (20), 120 (33), 110 (58), 91 (50), 77 (6), 68 (52), 65 (15), 55 (6). **9c-HCl**: mp 149–151°C.

*3,5-Dimethyl-4-(N,N-dimethylaminomethyl) isoxazole* ***9d***. IR: 2980, 1640 (C=N), 1450 $cm^{-1}$; NMR: δ (ppm) = 2.18 (s, 6H), 2.5 (s, 3H), 2.34 (s, 3H), 3.13 (s, 2H); MS: *m/z* = 154 ($M^+$, 30), 110 (36), 98 (4), 80 (5), 68 (100), 58 (36).

*3,5-Dimethyl-4-(N,N,N-trimethylammoniummethyl) isoxazole iodide* ***9dq***. mp 230°C (dec); NMR ($D_2O$): δ (ppm) = 2.33 (s, 3H), 2.52 (s, 3H), 3.13 (s, 9H), 4.39 (s, 2H).

*5-Isoxazole carboxylic acid* ***10***
The synthesis was carried out according to reference [19].

*N,N-Dimethyl-5-isoxazole carboxamide* ***11***
The synthesis was carried out using the general procedure for isoxazole carboxamides.

*5-(N,N-Dimethylamino) isoxazole* ***12***
The synthesis followed the general procedure for aminomethyl isoxazoles. $SiO_2$/MeOH, $R_f$ = 0.6, oil. Yield 48%; IR: 1600 (C=N) $cm^{-1}$; $^1$H-NMR: 2.87 (s, 6H), 4.45 (s, 2H), 6.95 (d, 1H, $^3J$ = 1.3 Hz, H-4), 8.38 (d, 1H, $^3J$ = 1.3 Hz, vinyl-H-3); MS: *m/z* = 126 ($M^+$, 56), 98 (5), 82 (30), 71 (8), 68 (6), 58 (100), 44 (32), 42 (50), 36 (82).

*5-(N,N,N-Trimethylammoniummethyl) isoxazole iodide* ***13***
The synthesis was carried out using general procedure for the quaternization. Yield 76%; mp 171–173°C (dec.); $^1$H-NMR (DMSO-$d_6$): 3.13 (s, 9H), 4.87 (s, 2H), 6.94 (d, 1H, $^3J$ = 1.7 Hz, H-4), 8.79 (d, 1H, $^3J$ = 1.7 Hz, H-3).

*3-Chloromethyl isoxazole* ***14***
The synthesis was carried out according to reference [20]. Yield 38%; $^1$H-NMR 4.64 (s, 2H), 6.41 (d, 1H, $J$ = 1.73 Hz, H4), 8.32 (d, 1H, $J$ = 1.73 Hz, H-5); MS: $m/z$ = 118 ($M^+$, 56), 116 (100), 88 (6), 82 (65), 68 (15), 61(18), 51 (97).

*5-Chloromethyl isoxazole* ***15***
To 10 mmol of 5-hydroxymethyl isoxazole, obtained by $BH_3$·DMS reduction of the ethyl ester of **10** in 10 ml $CHCl_3$, 10 mmol pyridine and 15 mmol $SOCl_2$ were added successively. The solution was heated under reflux for 1 h and, after cooling, water was added cautiously to the mixture. The separated organic layers were concentrated *in vacuo* and the product purified by distillation (17 mmHg, 97–98°C).

Yield 85%, colourless oil; $^1$H-NMR: 4.65 (s, 2H), 6.34 (d, 1H, $J$ = 1.73 Hz, H-4), 8.24 (d, 1H, $J$ = 1.73 Hz, H-3); MS: $m/z$ = 117 ($M^+$, 38), 82 (100), 68 (65), 40 (82).

*3-(N,N-Dimethylaminomethyl) isoxazole* ***16***
Yield 62%, pale yellow oil; IR (NaCl): 1680 (C=C), 1570 (C=N), cm$^{-1}$; $^1$H-NMR: 2.28 (s, 6H), 3.58 (s, 2H), 6.46 (d, 1H, $J$ = 1.6 Hz, H-4), 8.37 (d, 1H, $J$ = 1.6 Hz, H-5); MS: $m/z$ = 126 ($M^+$, 32), 125 (44), 96 (12), 83 (52), 69 (16), 58 (100), 54 (38).

*3-(N,N,N-Trimethylammonium) isoxazole iodide* ***18***
Yield 84%; mp 202–204°C (dec); $^1$H-NMR (DMSO-$d_6$): 3.13 (s, 9H), 4.75 (s, 2H), 6.89 (d, 1H, $J$ = 1.8 Hz, H-4), 9.18 (d, 1H, $J$ = 1.81 Hz, H-3).

*E/Z-4-Isoxazole carbaldoxime* ***19***
This was synthesized according to references [21, 22]. The *E*- and *Z*-oxime were separated by column chromotography, $SiO_2$, diisopropyl ether. *E*-oxime: $R_f$ = 0.75. Yield 19.8%; mp 104–106°C, IR: 3500–2500 (br, OH), 1595 (C=N) cm$^{-1}$; $^1$H-NMR: 7.79 (s, 1H, CH=N), 8.09 (s, 1H, OH), 8.57 (s, 1H, H-3), 8.61 (s, 1H, H-5). *Z*-Oxime: $R_f$ = 0.5. Yield 46%; mp 97.5–100°C, IR: 3500–2500 (br, OH), 1590 (C=N) cm$^{-1}$; $^1$H-NMR: 7.42 (s, 1H, CH=N), 8.61 (s, 1H, H-3), 9.06 (s, 1H, OH), 9.17 (s, 1H, H-5).

*4-Isoxazole carbonitrile* ***20***
For details of synthesis see reference [21]. All the data agree with those in the literature.

*4-Aminomethyl isoxazole* ***21***
To the mixture of 7 mmol *E/Z*-**19**, 50 ml anhydrous ether and 2.5 g aluminium amalgam [23], 4 ml water was added dropwise. A vigorous reaction started and after stirring for 1.5 h another 1 g of the amalgam was added. The reaction mixture was stirred at room temperature for 2 h, then the amalgam filtered off and washed with ether. The combined organic layers were concentrated, and the resulting colourless liquid transferred to the corresponding hydrochloride using the method described.

Yield 45% (free base), mp (hydrochloride): 170°C; $^1$H-NMR (base, acetone-$d_6$): 3.39 (s, 2H, $NH_2$), 4.32 (s, 2H), 8.42 (s, 1H, H-3), 8.60 (s, 1H, H-5); $^1$H-NMR (hydrochloride $D_2O$): 4.19 (s, 2H), 8.60 (s, 1H, H-3), 8.85 (s, 1H, H-5).

*4-(N,N-Dimethylaminomethyl) isoxazole* ***22***
The Leukart–Wallach reaction was used to convert **21**·**HCl** to **22**. Yield 73%, pale yellow oil; $^1$H-NMR: 2.23 (s, 6H), 3.36 (s, 2H), 8.27 (s, 1H, H-3), 8.33 (s, 1H, H-5); MS: 126 ($M^+$, 48), 125 (56), 109 (8), 97 (14), 82 (18), 70 (10), 58 (100), 52 (12).

*4-(N,N,N-Trimethylammoniummethyl) isoxazole iodide* ***23***
Yield 74%, mp 155–157%; $^1$H-NMR (DMSO-$d_6$): 3.03 (s, 9H), 4.49 (s, 2H), 8.84 (s, 1H, H-3), 9.28 (s, 1H, H-5).

*3-Methyl-5-(N,N,-dimethylaminoethyl) isoxazole* ***25***
The corresponding acid and ester were synthesized according to reference [43], the amide and the amine as shown above. After purification by column chromatography ($SiO_2$, MeOH, $R_f$ = 0.45) a pale yellow oil was obtained.

Yield 48%; $^1$H-NMR: 2.32 (s, 3H), 2.36 (s, 6H), 2.68 (t, 2H), 2.93 (t, 2H), 5.89 (s, 1H); MS: $m/z$ = 154 ($M^+$, 12), 110 (6), 108 (8), 82 (20), 70 (13), 68 (12), 58 (100), 56 (22), 42 (88), 41 (24), 39 (16).

*3-Methyl-5-(N,N,N-trimethylammoniumethyl) isoxazole iodide* ***26***
Yield 72%; mp 218–220°C (dec.); $^1$H-NMR (DMSO-$d_6$): 2.22 (s, 3H), 3.12 (s, 9H), 3.3 (t, 2H), 3.66 (t, 2H), 6.28 (s, 1H).

*5-(3-Hydroxypropyl)-3-methyl isoxazole* ***27***
To 20 mmol 3,5-dimethylisoxazole in 20 ml anhydrous THF under $N_2$, 20 mmol *n*-hexyl-Li was added dropwise while maintaining the temperature at –70°C. Anhydrous ether, previously saturated with ethylene oxide was then added and the reaction controlled by TLC. The mixture was stirred for about 1 h, and then carefully quenched with aqueous $NH_4Cl$ (40 ml) and extracted with ether. The organic layer was dried, evaporated *in vacuo* and the residue purified by column chromatography ($SiO_2$, ether) to yield 58% of an colourless oil. IR: 3400 (br, OH), 1610 (C=N) cm$^{-1}$; $^1$H-NMR: 1.88–1.98 (m, 2H), 2.05 (s, 1H, OH), 2.24 (s, 3H), 2.65–2.85 (t, 2H), 3.66–3.72 (m, 2H), 5.85 (s, 1H); MS: $m/z$ = 141 ($M^+$, 8), 123 (82), 110 (18), 97 (72), 82 (84), 69 (43), 55 (100), 41 (63), 31 (48).

*3-Methyl-5-(N,N-dimethylaminopropyl) isoxazole* ***28***
Isoxazole **28** was synthesized from **27** using the methods described above.

Yield 67%, oil; IR: 1605 (C=N) cm$^{-1}$; $^1$H-NMR: 1.81–1.88 (m, 2H), 2.20 (s, 3H), 2.25 (s, 6H), 2.71–2.76 (t, 2H), 2.88–2.93 (t, 2H), 5.83 (s, 1H); MS: $m/z$ = 168 ($M^+$, 5), 97 (14), 82 (10), 58 (100), 40 (22).

*3-Methyl-5-(N,N-dimethylaminopropyl) isoxazole iodide* ***29***
Yield 89%, mp 229–230°C (dec); $^1$H-NMR (DMSO-$d_6$): 2.01–2.11 (m, 2H), 2.2 (s, 3H), 2.75–2.80 (t, 2H, N), 3.07 (s, 9H), 3.31–3.37 (t, 2H, N), 6.22 (s, 1H).

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