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Short Communication

4,5-Dihydroisoxazoles Testing of antimicrobial activity

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Abstract

In the 1,3-dipolar cycloaddition of aliphatic nitrile to selected olefins, a series of 4,5-dihydroisoxazoles substituted with various functional groups, ester, carbonyl and ethereal, was obtained. These compounds were investigated for antimicrobial activity on chosen strains. © 2000 Elsevier Science S.A. All rights reserved.

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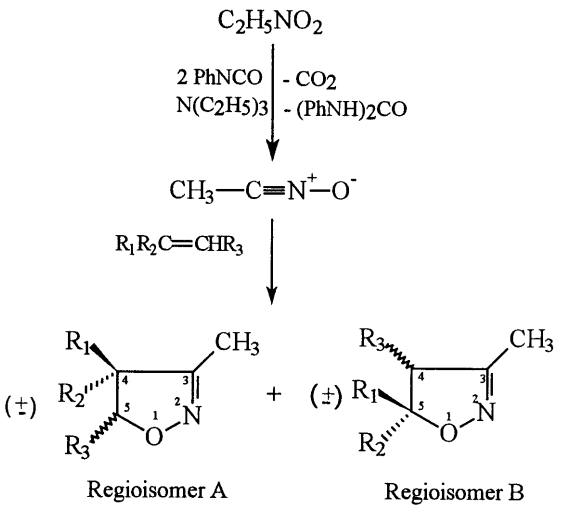
1. Introduction

In relation to literature reports leading to the addition of nitrile oxides to olefins for the synthesis of natural products and biologically active compounds [1–4] it seemed interesting to study selected 4,5-dihydroisoxazoles with various substituents at the heterocyclic ring (ester, carbonyl, ether or halide group) for antimicrobial activity.

2. Chemistry

The 1,3-dipolar cycloaddition of nitrile oxides to olefins is the most useful method of obtaining 4,5-dihydroisoxazoles. Due to the accessibility of the starting materials, aromatic nitrile oxides are generated from the derivatives of hydroxamic acids, and aliphatic nitrile oxides from primary nitro compounds. The addition proceeds mildly and in good yield. The cyclization to an unsaturated 2-isoxazoline ring yields two regioisomers [5], and each stereoselective route yields, however, one pair of stereoisomers, i.e. four products (Scheme 1).

Experimental studies concerning 1,3-dipolar cycloaddition confirmed that monosubstituted olefins exhibit high regioselectivity and yield 5-substituted derivatives, for both electron acceptor (e.g. –COOH) and electron donor (–OH) substituents. In the case of 1,1-disubstituted olefins, high regioselectivity is also observed; 5,5-disubstituted products are then formed. The mixture of



Scheme 1.

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Table 1
Tested compounds

Number	R_1	R_2	R_3	Ratio of regioisomers A:B (%)
1	CO_2Me	H	*p*-NO_2–Ph	56:44
2	CO_2Ph–Me-*p*	H	Ph	10:90
3	CO_2Me	H	*p*-Me–Ph	50:50
4	CO_2MC	Ph	Ph	100:0
5	CO_2Et	Ph	Ph	100:0
6	CO_2Ph	Ph	Ph	100:0
7	CO_2Et	*p*-Cl–Ph	H	100:0
8	CO_2Et	*p*-Me–Ph	H	100:0
9	CO_2Et	*p*-NO_2–Ph	H	100:0
10	H	H	*p*-Cl–Ph	0:100
11	COPh	H	Ph	78:22
12	COMe	H	Ph	43:57
13	H	H	CH_2–O–Ph	0:100
14	H	H	CH_2–O–Ph–Br-*p*	0:100 [10]
15	H	H	CH_2–O–Ph–Bu^t-*p*	0:100
16	COPh	H	*o*-CH_3O–Ph	26:74
17	H	H	CH_2–O–Ph–COMe	0:100

regioisomers is usually obtained from 1,2-disubstituted alkenes [6,7].

The 1,3-dipolar cycloaddition maintains the alkene configuration, which means that using a *trans* isomer for the reaction the product will have substituents on opposite sides of the ring plane, and using a *cis* product the cycloaddition will have substituents on the *same* side. Such a result is a consequence that the reaction of 1,3-dipolar cycloaddition is a pericyclic reaction.

A number of 4,5-dihydroisoxazoles from aliphatic nitrile oxides were obtained and studied for antimicrobial activity. These compounds are the derivatives of *trans* cinnamic acid [8], atropic acid [9], *trans* α,β-unsaturated ketones and phenol allyl ethers [10] (Table 1).

3. Results and discussion

We tested 17 2-isoxazoline derivatives for their antibacterial and antifungal properties. Tetracycline (1 mM) and miconazole (1 mM) were used to compare the inhibition of growth in bacteria and fungi respectively. The inhibition zones were as follows: 6 mm, 10 mm, for *Escherichia coli* and *Staphylococcus aureus* treated with tetracycline, and 8 mm for *Candida albicans* treated with miconazole.

None of the studied compounds inhibited the tested strains at a concentration of 10 mM, with the exception of compound **14**, which slightly inhibited growth of *C. albicans* (3.0 mm inhibition zone). Compounds **3**, **4**, **5**, **6**, **10** and **12** were also tested at higher concentrations (15–20 mM). No inhibition of bacteria growth was obtained, but we found slight inhibition of growth of *C. albicans* by compounds **3**, **10**, and **12**; inhibition zones were 3.5, 5.0, and 3.5 mm at the concentrations 20, 20, and 15 mM, respectively.

Different inhibitory activity against *C. albicans* was found also for regioisomers of compound **3**. The inhibition zone of regioisomer **3a** was 5.0 mm, whereas that of regioisomer **3b** was 3.0 mm; the inhibition zone of the mixture of regioisomers **3a** and **3b** was 3.5 mm.

These preliminary results may suggest that the structure of regioisomers, i.e. location of the functional group at carbon 4 or 5 and/or configuration of these carbons, could affect the antimicrobial activity of isoxazoline derivatives. However, we cannot exclude that the lack of antimicrobial activity of the majority of studied compounds is caused by low accessibility of these compounds to the microorganisms resulting from the limited solubility in the solvent used for studies, rather than by the actual lack of inhibition activity.

The antifungal activities of tested compounds **3**, **10**, **12** and **14** were lower than that of miconazole, but these results encourage us to seek more effective derivatives and to study the mechanisms of yeast growth perturbation, especially depending on singular regioisomers or enantiomers.

4. Materials and methods

The following microbial strains with different cell wall structure were chosen: bacteria Gram (+) *S. aureus* ATTC 6538, bacteria Gram (−) *E. coli* ATTC 8739, fungi (yeast strain) *C. albicans* ATTC 10231. Tetracycline hydrochloride (Pharmaceutical Works, Polfa, Warsaw) and miconazole base (Fisher Chemicals A6) were used as reference antibiotics.

The cylinder-plate method [11] was used in preliminary tests for antimicrobial activity (dimensions of cylinders: ϕ in 6 mm, ϕ out 8 mm, height 7 mm).

Compounds were dissolved in 10% DMF. Each solution to be tested (100 μl) was placed in a cylinder, put on Muller–Hinton agar plates, inoculated with one of the test strains. The plates with bacteria were incubated at 37°C for 18 h and with fungi at 30°C for 24 h. Inhibition of growth was measured as a halo around the cylinder containing the test compound.

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[10] 5-[*p*-Br-benzyloxy]-3-methyl-4,5-dihydroisoxazole **14**: yellow crystal (after crystallization from benzene:heptane 3:7), m.p. 105–107°C. ^{1}H NMR (200 MHz, $CDCl_3$): 2.04 (t, 3H, CH_3), 3.05 (m, 2H, C-4), 4.12 (m, 2H, CH_2), 4.96 (m, 1H, C-5), 6.94–6.99 (m, 2H, *o*-Ar), 8.17–8.22 (m, 2H, *p*-Ar). IR (KBr/cm^{-1}): 1598, 1248, 1032.
[11] FPV, vol. 1, 1990, p. 95.