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Synthesis and evaluation of antimicrobial activity of hydrazones derived from 3-oxido-1*H*-imidazole-4-carbohydrazides

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ABSTRACT

In this work we reported the synthesis and evaluation of *in vitro* antimicrobial activities of hydrazones **6** obtained from 3-oxido-1*H*-imidazole-4-carbohydrazides **4**. All new compounds were characterized by spectroscopic methods. Hydrazones **6** were tested for their *in vitro* antimicrobial activity against four Gram-positive and four Gram-negative strains of bacteria as well as one fungal species. Three of the tested compounds appeared to be promising agents against reference strains of *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. They were also tested against twelve clinical isolates of *S. aureus* and their cytotoxic effect on murine fibroblasts and HeLa human tumor cell line was determined.

1. Introduction

Infectious diseases caused by microorganisms are one of the main reasons of death in the world. The search for new antibacterial and antifungal drugs is like never-ending story because of the increasing resistance of microbial pathogens. It is desirable to find drugs with improved potency and wide activity spectrum. Acid hydrazides and the corresponding hydrazones are well known as a class of compounds with diverse biological activities [1–3]. Hydrazides of imidazolecarboxylic acids and their derivatives, e.g., hydrazones, thiosemicarbazides and 1,2,4-triazoles have been also reported to exhibit antibacterial, fungicidal, antiparasitic, anti-inflammatory, antitumor, antinociceptive, anticonvulsant, antihypertensive, and antidepressant properties [4–18]. Moreover, nitroimidazoles such as metronidazole, tinidazole, ornidazole are well known as antibacterial agents [19,20].

The aim of the present study was the synthesis of new hydrazones derived from 3-oxido-1*H*-imidazole-4-carbohydrazides and evaluation of their biological activity.

2. Results and discussion

2.1. Chemistry

In a series of recent reports, methods of synthesis and reactivity of new imidazole derivatives bearing 3-oxido function were described [21–26]. General strategy applied for the construction of the central imidazole ring is based on condensation of the corresponding α-hydroxyiminoketones with methylideneamines. Thus, starting with ethyl 2-hydroxyimino-3-oxobutanoate **1** and corresponding methylideneamines **2a**–**2c** expected ethyl 3-oxidoimidazole-4-carboxy late **3** can be smoothly obtained at room temperature in glacial acetic acid [22] (Scheme 1). Upon treatment with hydrazine hydrate, esters **3** were converted into carbohydrazides **4**, which after isolation and purification were used for reactions with aldehydes and ketones **5** [25].

Reactions of **4** with *p*-nitrobenzaldehyde and 5-nitrofurfural required heating in MeOH solution, whereas analogous reactions with benzaldehyde and other aromatic aldehydes containing electron donating substituent, were carried out at room temperature. In all cases expected hydrazones **6** were formed as single isomers, and isolated as crystalline materials in good to excellent yields (Table 1).

Their structures were confirmed based on spectroscopic data (^{1}H NMR, ^{13}C NMR, IR, HR-MS). For example, the ^{1}H NMR spectrum

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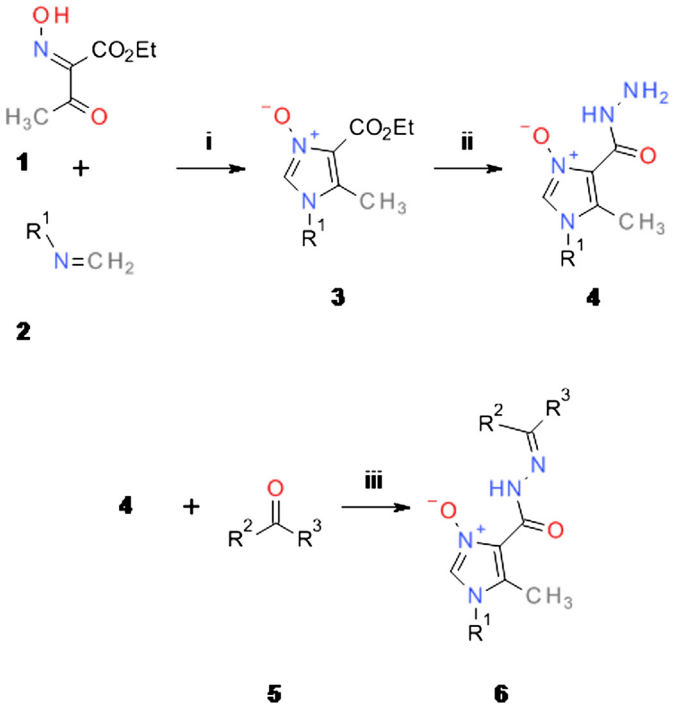

Scheme 1. Reagents and conditions: (i) AcOH, r. t., 16 h; (ii) $NH_2NH_2 \cdot H_2O$, r.t., 16 h; (iii) MeOH, r.t., 16 h or heating, 2 h.

of hydrazone **6i** reveals the presence of two characteristic signals at 8.76 and 8.41 ppm attributed to HC(2) of imidazole and HC=N protons, correspondingly. In the ^{13}C NMR spectra absorption signals of the C=O and C=N groups were found at 156.8 and 136.9 ppm, respectively. In all compounds typical signals of HC(2) protons of imidazole ring appeared between 8.55 and 8.78 ppm.

2.2. Biological activities

The *in vitro* antimicrobial activities of the compounds **6a**–**6i**, at concentrations ranging from 1 to 600 μg/mL, were screened using the microdilution method against four Gram-negative and four Gram-positive reference species of bacteria and one fungal species. The results showed that compounds **6a**–**6h** were inactive against all tested bacteria and yeast (data not shown). Only compound **6i** showed strong antibacterial activity against some Gram-negative and Gram-positive bacteria. Due to the high activity of the tested compound **6i** an attempt was made to potentially improve its antimicrobial effect by introducing some modifications. Thus, on the basis of **6i** two more compounds were prepared, bearing 5-nitrofuryl group, differing from each other by substituent at N(1) atom in the imidazole ring. We selected small alcylic methyl group (**6j** R^1 = Me) and sterically more crowded cyclohexyl group (**6k** R^1 = cyclohexyl). It is worth mentioning that compounds bearing 5-nitrofuryl substituent have limited solubility in DMSO, which was increased by transferring compounds **6i** and **6j** into their hydrochloride salts. Such modification had no influence on their antibacterial activity. Compounds **6j** and **6k** were similarly tested against reference microorganisms and showed positive although slightly lower antibacterial activity than the initial one.

Table 1
Yields and melting points of the new hydrazones **6**.

	R^1	R^2	R^3	Yield [%]	mp [°C]
6a	Bn	H	Ph	92	232–234
6b	Bn	–$(CH_2)_5$–		98	220–222
6c	Bn	Me	Ph	76	222–226
6d	Bn	H	*p*-MeO-C_6H_4	90	221–222
6e	Bn	H	*p*-$(Me)_2N$–C_6H_4	89	252–254
6f	Bn	H	*o*-HO–C_6H_4	89	229–232
6g	Bn	H	Furyl	78	220–221
6h	Bn	H	*p*-NO_2–C_6H_4	97	264–265
6i	Bn	H	5-NO_2–furyl	87	237–240
6j	Me	H	5-NO_2–furyl	84	313–315
6k	cHex	H	5-NO_2–furyl	76	222–225

The *in vitro* results of antibacterial activity of the three compounds **6i**–**6k** are presented in Table 2 as a minimal inhibitory concentration (MIC) and a minimal bactericidal concentration (MBC). Among the Gram-positive species, the most sensitive to all of the active compounds was *Staphylococcus epidermidis*, and in case of **6i** and **6k** (MIC = 4 μg/mL) the results were similar to the control antibiotic vancomycin (MIC = 3 μg/mL). The most effective against both reference *Staphylococcus aureus* strains was compound **6i** (MIC = 11 μg/mL) although its activity was lower than that exhibited by vancomycin and oxacillin, which are the antibiotics commonly used in the therapy of staphylococcal infections. Among three tested compounds, **6i** was also the most effective against *Escherichia coli* reference strain (MIC = 11 μg/mL), which was higher activity than in case of nitrofurantoin but lower then for chloramphenicol (MIC = 16, and 0.25, respectively) – two antibiotics used in a treatment of *E. coli* infections.

Compounds that showed activity in this test were then examined using the disc diffusion method in order to determine growth inhibition zones of susceptible species of pathogens (Table 3). These results confirmed inhibitory activity of the tested compounds with the largest growth inhibition zones obtained for *S. epidermidis*.

Considering good activity of the tested **6i**, **6j**, and **6k** compounds against microorganisms of *Staphylococcus* spp., a set of 12 *S. aureus* clinical strains including the ones isolated from 2 typical sources such as naso-pharynx (carrier state) and ulcers/furuncles (skin and soft tissue infections), but also those from infected bones (invasive infections) was tested against these agents. Similarly to the reference *Staphylococcus* spp. strains clinical isolates displayed high level of susceptibility to the analyzed compounds (MIC ranging from 10 to 100 μg/mL) (Table 4). Compound **6i** again showed the strongest activity, with MIC being equal to 10 μg/mL for all of the tested clinical isolates, which for most of the strains was a better result than in case of vancomycin used as the positive control antibiotic. On the other hand, the second common antibiotic – oxacillin showed up to be the most active in almost all cases (MIC = 0.2–1.5 μg/mL), except for the two strains isolated from bones (*S. aureus* D15, and D17, MIC = 75 μg/mL) which were 7 times more sensitive to **6i** compound.

For all three tested compounds the MBC values were in the similar range, suggesting their bactericidal activity (MBC/MIC ≤ 4) (Table 4).

S. aureus permanently colonizes the epithelium of more than 20% of the population and also is one of the common human pathogens. These bacteria cause wide range of diseases: from acute infections involving staphylococcal toxins and enzymes (e.g. ulcers, furuncles, food poisoning, bacteremia, septic shock) to chronic infections, usually associated with biofilm formation (e.g. infections developed after invasive medical procedures using biomaterial devices, wounds infections, arthritis, osteomyelitis, endocarditis, bronchopulmonary infections in patients with cystic fibrosis) [27,28]. The emergence of multidrug-resistant *S. aureus* strains, including those resistant to β-lactams, glycopeptides, MLS-B (macrolides, lincosamides, streptogramin B), aminoglycosides, tetracyclines, fluoroquinolones, and even linezolid generate significant problem for the treatment of staphylococcal infections [29–31]. Moreover, these bacteria very easily form biofilm, which confers them severe