Antimicrobial Evaluation of Indole-Containing Hydrazone Derivatives
Hanif Shirinzadeha, Nurten Altanlarb, Nihal Yucelc, Seckin Ozdena, and Sibel Suzen*a

a Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University,
06100, Tandogan, Ankara, Turkey. Fax: +90 312 2131081.
E-mail: sibel@pharmacy.ankara.edu.tr

b Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ankara University,
06100, Tandogan, Ankara, Turkey

c Department of Biology, Faculty of Arts and Sciences, Gazi University, 06500,
Teknik Okullar, Ankara, Turkey

* Author for correspondence and reprint requests

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There has been an increasing importance of drug-resistant pathogens in clinical microbiological and antibacterial research. Indoles and hydrazone-type compounds constitute important classes of compounds in the search for effective agents against multidrug-resistant microbial infections. In this study a series of 1-methylindole-3-carboxaldehyde hydrazone derivatives were evaluated for their in vitro antimicrobial activities using the two-fold serial dilution technique against Staphylococcus aureus, methicillin-resistant S. aureus, methicillin-resistant S. aureus isolate, Escherichia coli, Bacillus subtilis, and Candida albicans. The minimum inhibitory concentration (MIC) of the test compounds and the reference standards sulfamicillin, ampicillin, fluconazole, and ciprofloxacin was determined. All compounds possessed a broad spectrum of activity having MIC values of 6.25 – 100 μg/ml against the tested microorganisms. Aromaticity and disubstitution of the phenyl ring with especially fluorine and chlorine atoms were found to be significant for the antimicrobial activity

Key words: Indole, Hydrazone, Antimicrobial

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE) pose infection risks in most intensive care units. Multidrug-resistant strains of MRSA and VRE have been causing serious problems in health care (Lin and Hayden, 2010). The rising clinical importance of drug-resistant pathogens is a challenge to drug development research. In recent years, many 1H-indole derivatives, including Schiff’s bases, have been reported to exhibit chemotherapeutic properties such as antiviral, antituberculosis, antifungal, and antibacterial activities (Karali et al., 2007; Shirinzadeh et al., 2010; Bektas et al., 2010). Hydrazone-type compounds containing an azomethine group also represent a significant class of compounds for new drug development. The hydrazone group in these molecules plays an essential role for antimicrobial activity (Abdel-Fattah et al., 2000). It has been claimed that a number of hydrazide hydrazone derivatives possess interesting antibacterial-antifungal (Loncle et al., 2004) and antituberculosis activities (Sridhar et al., 2002; Maccari et al., 2005; Suriyati et al., 2007).

In new drug development studies, combination of different pharmacophores in the same molecule may lead to new compounds having higher biological activity. Therefore the combination of indole- and hydrazone-type compounds might provide new effective drugs against multidrug-resistant microbial infections.

1H,10H-Benzo[e]pyrrolo[3,2-g]indole derivatives possess high antimicrobial activity (Samsoniya et al., 2009). 3-(4,5-Bis(4-fluorophenyl)-1H-imidazol-2-yl)-5-bromo-1H-indole was identified as a potent antimicrobial compound with a MIC value of 1 μg/ml against MRSA by Al-Qawasmeh et al. (2010). The condensed pyrazole heterocycles called 3,5-disubstituted-4,5-dihydropyrazol-1-yl-1H-indol-5-yl methanones showed significant antifungal activity (Sarma et al., 2010). Some of the indole isoxazole derivatives were found to have activity against S. aureus and P. aeruginosa (Panda et al., 2009).
It is noteworthy that the indole ring has better antimicrobial activity if it is attached to simple aromatic rings such as phenyl, pyrazole or isoxazole. Indole pyrimidine derivatives were active at 5–10 μg/ml against various bacteria in the cup-plate agar diffusion assay. The presence of a halogen atom at position 4 of the phenyl ring showed good activity against Gram-negative bacteria. The presence of a nitro group or methoxy group at position 4 of the phenyl ring displayed good activity against Gram-positive bacteria (Panda and Chowdary, 2008).

In our earlier study (Gurkok et al., 2009), a series of indole-3-aldehyde and 5-bromoindole-3-aldehyde hydrazides and hydrazones were evaluated for their in vitro antimicrobial activities using the two-fold serial dilution technique against Staphylococcus aureus, MRSA, Escherichia coli, Bacillus subtilis, and Candida albicans. It was found that compounds with a halogenated phenyl ring display better activity against MSRA and significant activity against S. aureus relative to ampicillin. As part of our ongoing study, we have now tested nineteen 1-methylindole-3-carboxaldehyde hydrazone derivatives (Fig. 1) for their antibacterial activity.

### Material and Methods

#### Chemistry

1-Methylindole-3-carboxaldehyde was condensed with the appropriate hydrazine to result in indole hydrazone derivatives which were characterized on the basis of their spectroscopic data in our earlier study (Shirinzadeh et al., 2010).

#### In vitro antimicrobial and antifungal activities of indole derivatives

The tube dilution technique was employed for antibacterial and antifungal activity tests. The synthesized compounds and the standards were dissolved in 12.5% dimethyl sulfoxide (DMSO) at concentrations of 200 μg/ml. Further dilutions of the compounds and standard drugs in the test medium were prepared at the following concentrations: 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/ml with Mueller-Hinton broth (MHB; Difco, Detroit, USA) and Sabouraud dextrose broth (SDB; Difco).

The minimum inhibitory concentrations (MIC) were determined using the two-fold serial dilution technique (Charles et al., 1979; Shadomy and...
The MIC value of a compound is defined as the lowest concentration which completely inhibits visible growth judged by lack of turbidity in the tube.

At the concentrations used, DMSO did not affect microbial growth. All compounds were tested for their in vitro growth inhibitory activity against the fungus C. albicans ATCC 10145, the Gram-positive bacteria S. aureus ATCC 25923, B. subtilis ATCC 6633, MRSA standard ATCC 43300, MRSA isolate, and the Gram-negative bacterium E. coli ATCC 23556. ATCC strains were obtained from the culture collection of the Refik Saydam Health Institution of the Health Ministry, Ankara, Turkey, and kept at the Microbiology Department of the Faculty of Pharmacy Ankara University, Ankara, Turkey. Sultamicillin with MIC values of 0.78 (against S. aureus, B. subtilis) and 25 μg/ml (against E. coli), ampicillin with MIC values of 1.56, 12.5, and 50 μg/ml (against S. aureus, MRSA, B. subtilis), fluconazole with an MIC value of 0.78 μg/ml (against C. albicans) and ciprofloxacin with MIC values of 0.09, 0.09, and 0.09 μg/ml (against S. aureus, E. coli, B. subtilis) were used as control drugs.

The bacterial strains were incubated on Mueller-Hinton agar (MHA; Oxoid, Basingstoke, UK) for 24 h at 37 °C and fungi on Sabouraud dextrose agar (SDA; Difco) for 48 h at 25 °C.

The cell density of each inoculum was adjusted in sterile water of 0.5 Mc Farland standard. Final concentrations of approximately 10⁵ CFU/ml and 10⁸ CFU/ml for the bacteria and fungi, respectively (Biosan Den-1 Mc Farland densitometer; Riga, Latvia), were prepared. The MIC values were determined using the two-fold serial dilution technique. A set of tubes containing only inoculated broth was used as controls. After incubating bacteria for 8–24 h at (37 ± 1) °C and fungi for 2–5 d (25 ± 1) °C, the last tube with no growth of microorganisms was recorded to represent the MIC value expressed in μg/ml. Every assay was performed in duplicate. The values were found to be almost identical and are presented in Table I.

### Results and Discussion

The occurrence of substituted indoles and indolines in antimicrobial compounds has inspired researchers to develop new indole molecules (Suzen et al., 2006, 2007; Das-Evcimen et al., 2009). In the present study indole derivatives of hydrazones were evaluated for their antibacterial and antifungal activities.

The antibacterial activities of the compounds (Fig. 1) against the MRSA standard and MRSA isolate showed promising results compared to the control drug ampicillin. Compound 8 with an MIC value of 6.25 μg/ml indicated more potent antimicrobial activity than ampicillin for which the MIC value was 12.5 μg/ml. Also compound 1 with 12.5 μg/ml was quite potent, and compounds 6, 13, and 16 showed moderate activity against the MRSA standard.

Against B. subtilis, with the exception of compounds 4, 11, 15, 18, and 19, all compounds had an activity more potent than (2 and 5) or similar (1, 3, 6–10, 12–14, 16, 17) to ampicillin. However, all compounds showed lower activity compared to sultamicillin and ciprofloxacin against B. subtilis.

Table I indicates that all compounds had a lower antibacterial activity against the drug-sensitive strain of S. aureus than the control drugs. However, compound 7 showed moderate activity with an MIC value of 6.25 μg/ml compared to ampicillin with an MIC value of 1.56 μg/ml.

None of the compounds showed any significant activity against E. coli. The most active compounds were 1, 5, 6, 7, 13, 14, 15, 17, and 18 with an MIC value of 50 μg/ml.

Among the tested compounds, 1, 7, and 15 showed moderate antifungal activity against C. albicans with an MIC value of 3.125 μg/ml. Although compounds with an indole ring were not found to be very strong antimicrobial agents in many cases, they were found to have a wide antifungal spectrum (Pagniez et al., 2002; Sinha et al., 2008). The indole hydrazone derivatives were not found to have significant antifungal activity.

The indole nicotinic acid derivative 17 showed no significant activity, while the indole anisic acid derivative 18 displayed better activity, especially against C. albicans and the MRSA standard.

The antifungal mode of action of indole derivatives was investigated by Sung and Lee (2007) who monitored the change in the membrane dynamics by fluorescence changing experiments with C. albicans using molecular probes. The results suggested that indole derivatives may exert antifungal activity by disrupting the structure of the cell membrane.

Electron-rich nitrogen heterocyclic compounds play an important role in diverse biological activities (Suzen, 2007). Indole has been reported to...
have an inhibitory effect on several fungi (Koivistoinen et al., 1959).

The structure-activity relationships of the investigated indole hydrazone derivatives revealed that the aromaticity appears to be significant for the antimicrobial activity. Generally, the activity of compounds increased with the introduction of halogen atoms into the phenyl ring. Compounds 5 (2,4-difluoro), 6 (2,5-difluoro), 7 (3,5-difluoro), and 13 (3,5-dichloro), which have two fluorine or two chlorine atoms on the phenyl ring, were found to be the most promising antimicrobial agents. The monohalogenated derivatives 2, 8, 15, and 16 were less active than the dihalogenated compounds. Especially ortho-halogenated compounds were found more active than the others. These results indicate that the halogen atom plays an important role in the antimicrobial activity of the Schiff’s bases tested here.

In the present study compounds combining an indole aldehyde and halogenated phenyl rings were evaluated for their synergistic antimicrobial activity. The results may be instructive to researchers attempting to gain more understanding of the antimicrobial activity of indole hydrazide/hydrazone-type compounds.

### Acknowledgements

This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) Research and Development Grant (109S099).

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### Table I. MIC values (μg/ml) of compounds 1–19.

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* No activity was observed. * Not tested.


