# Antimicrobial Evaluation of Indole-Containing Hydrazone Derivatives

Hanif Shirinzadeh[a], Nurten Altanlar[b], Nihal Yucel[c], Seckin Ozden[a], 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

Z. Naturforsch. **66 c**, 340–344 (2011); received October 8, 2010/April 15, 2011

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 sultamicillin, ampicillin, fluconazole, and ciprofloxacin was determined. All compounds possessed a broad spectrum of activity having MIC values of 6.25–100 $\mu$g/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

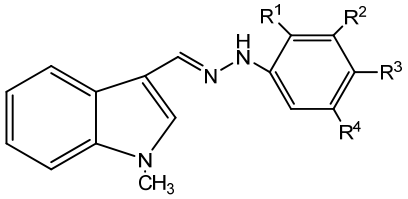
*Key words:* Indole, Hydrazone, Antimicrobial

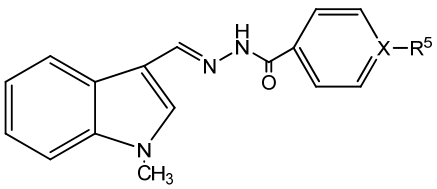
## Introduction

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 1*H*-indole derivatives, including Schiff's bases, have been reported to exhibit chemotherapeutic properties such as antiviral, antituberculosis, antifungal, and antibacterial activities (Karalı *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.

1*H*,10*H*-Benzo[*e*]pyrrolo[3,2-g]indole derivatives possess high antimicrobial activity (Samsoniya *et al.*, 2009). 3-(4,5-Bis(4-fluorophenyl)-1*H*-imidazol-2-yl)-5-bromo-1*H*-indole was identified as a potent antimicrobial compound with a MIC value of 1 $\mu$g/ml against MRSA by Al-Qawasmeh *et al.* (2010). The condensed pyrazole heterocycles called 3,5-disubsituted-4,5-dihydropyrazol-1-yl-1*H*-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).




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **1** | H | H | H | H |
| **2** | H | H | F | H |
| **3** | H | F | H | H |
| **4** | F | H | H | H |
| **5** | F | H | F | H |
| **6** | F | H | H | F |
| **7** | H | F | H | F |
| **8** | Cl | H | H | H |
| **9** | H | Cl | H | H |
| **10** | H | H | Cl | H |
| **11** | Cl | H | H | Cl |
| **12** | H | Cl | Cl | H |
| **13** | H | Cl | H | Cl |
| **14** | H | H | Br | H |
| **15** | H | Br | H | H |
| **16** | Br | H | H | H |

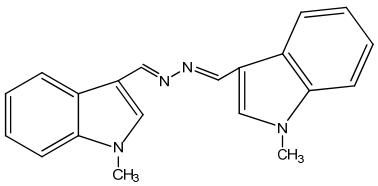
| | X | $R^5$ |
|---|---|---|
| **17** | N | - |
| **18** | C | $OCH_3$ |
| **19** |  | |



Fig. 1. Chemical structures of compounds **1**–**19**.

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 $\mu$g/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 $\mu$g/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 $\mu$g/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

Espinel, 1980). 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 $\mu$g/ml (against *E. coli*), ampicillin with MIC values of 1.56, 12.5, and 50 $\mu$g/ml (against *S. aureus*, MRSA, *B. subtilis*), fluconazole with an MIC value of 0.78 $\mu$g/ml *(against C. albicans*) and ciprofloxacin with MIC values of 019, 0.09, and 0.09 $\mu$g/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 $10^5$ CFU/ml and $10^3$ 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 $\mu$g/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 $\mu$g/ml indicated more potent antimicrobial activity than ampicillin for which the MIC value was 12.5 $\mu$g/ml. Also compound **1** with 12.5 $\mu$g/ml was quite potent, and compounds **6**, **13**, and **16** showed moderate activity against the MSRA 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 $\mu$g/ml compared to ampicillin with an MIC value of 1.56 $\mu$g/ml.

None of the compounds showed any significant activity against *E. coli*. The most active compounds were **1**, **5**, **6**, **7**, **9**, **13**, **14**, **15**, **17**, and **18** with an MIC value of 50 $\mu$g/ml.

Among the tested compounds, **1**, **7**, and **15** showed moderate antifungal activity against *C. albicans* with an MIC value of 3.125 $\mu$g/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

Table I. MIC values ($\mu$g/ml) of compounds **1**–**19**.

| Compound | *S. aureus* | MRSA standard | MRSA isolate | *E. coli* | *B. subtilis* | *C. albicans* |
|---|---|---|---|---|---|---|
| **1** | 100 | 12.5 | 100 | 50 | 50 | 3.125 |
| **2** | 25 | a | 50 | 100 | 25 | 6.25 |
| **3** | 100 | 50 | 100 | 100 | 50 | 6.25 |
| **4** | 100 | 100 | 100 | 100 | 100 | 6.25 |
| **5** | 100 | 100 | 25 | 50 | 25 | 6.25 |
| **6** | 100 | 25 | 100 | 50 | 50 | 6.25 |
| **7** | 6.25 | 100 | 6.25 | 50 | 50 | 3.125 |
| **8** | 100 | 6.25 | 100 | 100 | 50 | 6.25 |
| **9** | 50 | 100 | 50 | 50 | 50 | 6.25 |
| **10** | 100 | 50 | 100 | 100 | 50 | 25 |
| **11** | 50 | 100 | 100 | 100 | 100 | 25 |
| **12** | 100 | a | 50 | 100 | 50 | 12.5 |
| **13** | 25 | 25 | 12.5 | 50 | 50 | 12.5 |
| **14** | 100 | 100 | 50 | 50 | 50 | 6.25 |
| **15** | 50 | 50 | 25 | 50 | 100 | 3.125 |
| **16** | 100 | 25 | 100 | 100 | 50 | 6.25 |
| **17** | 100 | 100 | 50 | 50 | 50 | 12.5 |
| **18** | 100 | 50 | 100 | 50 | 100 | 6.25 |
| **19** | a | 100 | a | 100 | 100 | a |
| Sultamicillin | 0.78 | * | * | 25 | 0.78 | * |
| Ampicillin | 1.56 | 12.5 | * | * | 50 | * |
| Fluconazole | * | * | * | * | * | 0.78 |
| Ciprofloxacin | 0.19 | * | * | 0.09 | 0.09 | * |

[a] No activity was observed. * Not tested.

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).

Abdel-Fattah M. E., Salem E. E., and Mahmoud M. A. (2000), Synthesis and antimicrobial activity of some 3-*p*-chlorophenoxymethyl-4-phenyl-1,2,4-triazol-5-yl-thio-acetyl hydrazine derivatives. Indian J. Heterocycl. Chem. **10**, 121–128.

Al-Qawasmeh R. A., Huesca M., Nedunuri V., Peralta R., Wright J., Lee Y., and Young A. (2010), Potent antimicrobial activity of 3-(4,5-diaryl-1*H*-imidazol-2-yl)-1*H*-indole derivatives against methicillin-resistant *Staphylococcus aureus.* Bioorg. Med. Chem. Lett. **20**, 3518–3520.

Bektas H., Demirbas A., Demirbas N., and Karaoglu S. A. (2010), Synthesis of some new biheterocyclic triazole derivatives and evaluation of their antimicrobial activity. Turk. J. Chem. **34**, 165–180.

Charles E. S., Agrawal V. K., Sharma S., and Iyer R. N. (1979), Synthesis of 2,5-disubstituted benzimidazoles as potential antihookworm and antimicrobial agents. Part 1. Eur. J. Med. Chem. Chim. Ther. **14**, 435–438.

Das-Evcimen N., Yildirim O., and Suzen S. (2009), Relationship between aldose reductase and superoxide dismutase inhibition capacities of indole-based analogs of melatonin derivatives. Arch. Biol. Sci. **61**, 675–681.

Gurkok G., Altanlar N., and Suzen S. (2009), Investigation of antimicrobial activities of indole-3-aldehyde hydrazide/hydrazone derivatives. Chemotherapy **55**, 15–19.

Karalı N., Gursoy A., Kandemirli F., Shvets N., Kaynak F. B., Ozbey S., Kovalishyn V., and Dimogloc A. (2007), Synthesis and structure-antituberculosis activity relationship of 1*H*-indole-2,3-dione derivatives. Bioorg. Med. Chem. **15**, 5888–5904.

Koivistoinen P., Risser E., and Pohjakallio O. (1959), The inhibitory effect of certain indole compounds upon the growth of *Sclerotinia trifoliorum* ERIKSS. Acta Agric. Scand. **9**, 403–411.

Lin M. Y. and Hayden M. K. (2010) Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*: Recognition and prevention in intensive care units. Crit. Care Med. **38**, S335–S344.

Loncle C., Brunel J. M., Vidal N., Dherbomez M., and Letourneux Y. (2004), Synthesis and antifungal activity of cholesterol-hydrazone derivatives. Eur. J. Med. Chem. **39**, 1067–1071.

Maccari R., Ottana R., and Vigorita M. G. (2005), *In vitro* advanced antimycobacterial screening of isoniazid-related hydrazones, hydrazides and cyanoboranes. Part 14. Bioorg. Med. Chem. Lett. **15**, 2509–2513.

Pagniez F., Le Borgne M., Marchand P., Na Y. M., Le Baut G., Robert-Piessard S., and Le Pape P. (2002), *In vitro* activity of a new antifungal azolyl-substituted indole against *Aspergillus fumigates*. J. Enzym Inhib. Med. Chem. **17**, 425–429.

Panda S. S. and Chowdary P. V. R. (2008), Synthesis of novel indolyl-pyrimidine antiinflammatory, antioxidant and antibacterial agents. Indian J. Pharm. Sci. **70**, 208–215.

Panda S. S., Chowdary P. V. R., and Jayashree B. S. (2009), Synthesis, antiinflammatory and antibacterial activity of novel indolyl-isoxazoles. Indian J. Pharm. Sci. **71**, 684–687.

Samsoniya Sh. A., Trapaidze M. V., and Kuprashvili N. A. (2009), Synthesis and antimicrobial activity of 1*H*,10*H*-benzo[*e*]pyrrolo[3,2-g]indole derivatives. Pharm. Chem. J. **43**, 92–94.

Sarma K. N., Subha M. C. S., and Rao K. C. (2010), A facial synthesis and antimicrobial activity of some pyrazole derivatives carrying indole. E-J Chem. **7**, 745–750.

Shadomy S. and Espinel A. (1980), Susceptibility testing with fungi. In: Manual of Clinical Microbiology. American Society of Microbiology, Washington, pp. 647–653.

Shirinzadeh H., Eren B., Gurer-Orhan H., Suzen S., and Ozden S. (2010), Novel indole-based analogs of melatonin: Synthesis and *in vitro* antioxidant activity studies. Molecules **15**, 2187–2202.

Sinha D., Tiwari A. K., Singh S., Shukla G., Mishra P., Chandra H., and Mishra A. K. (2008), Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde. Eur. J. Med. Chem. **43**, 160–165.

Sridhar S. K., Pandeya S. N., Stables J. P., and Ramesh A. (2002), Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives. Eur. J. Pharm. Sci. **16**, 129–132.

Sung W. S. and Lee D. G. (2007), *In vitro* antimicrobial activity and the mode of action of indole-3-carbinol against human pathogenic microorganisms. Biol. Pharm. Bull. **30**, 1865–1869.

Suriyati M., Pazilah I., and Habibah A. W. (2007), Effects of isoniazid on viability, cell morphologies and acid fastness properties of *Mycobacterium avium* NCTC 8559 during the growth cycle. Chemotherapy **53**, 263–266.

Suzen S. (2007), Bioactive heterocycles V: antioxidant activities of synthetic indole derivatives and possible activity mechanisms. In: Topics in Heterocyclic Chemistry, V11 (Khan M. T. H., ed.). Spinger, Berlin, pp. 145–178.

Suzen S., Bozkaya P., Coban T., and Nebioglu D. (2006), Investigation of *in vitro* antioxidant behaviour of some 2-phenylindole derivatives: discussion on possible antioxidant mechanisms and comparison with melatonin. J. Enzym Inhib. Med. Chem. **21**, 405–411.

Suzen S., Das-Evcimen N., Varol P., and Sarikaya M. (2007), Preliminary evaluation of rat kidney aldose reductase inhibitory activity of 2-phenylindole derivatives: affiliation to antioxidant activity. Med. Chem. Res. **16**, 112–118.