Microbiology

Chemotherapy

Chemotherapy 2009;55:15–19
DOI: 10.1159/000166999

Received: March 28, 2008
Accepted after revision: July 18, 2008
Published online: October 31, 2008

Investigation of Antimicrobial Activities of Indole-3-Aldehyde Hydrazide/Hydrazone Derivatives

Gokce Gurkok[a] Nurten Altanlar[b] Sibel Suzen[a]

[a]Department of Pharmaceutical Chemistry and [b]Department of Pharmaceutical Microbiology, Ankara University, Faculty of Pharmacy, Tandogan, Ankara, Turkey

Key Words
Indole · Hydrazone · Hydrazide · Antibacterial activity · Antifungal activity

Abstract
Background: Indoles and hydrazone-type compounds constitute an important class of compounds for new drug development in order to discover an effective compound against multi-drug-resistant microbial infections. ***Methods:*** A series of indole-3-aldehyde and 5-bromoindole-3-aldehyde hydrazide and hydrazones was evaluated for their in vitro antimicrobial activities using the 2-fold serial dilution technique against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*. The minimum inhibitory concentration (MIC) was determined for test compounds and for the reference standards sultamicillin, ampicillin, fluconazole and ciprofloxacin. ***Results:*** Compounds possessed a broad spectrum of activity having MIC values of 6.25–100 mg/ml against the tested microorganisms. Compounds **1a–1j**, in particular, displayed better activity against MSRA and significant activity against *S. aureus* relative to ampicillin. Unexpectedly, indole nicotinic acid hydrazides showed no significant activity while indole anisic acid hydrazides displayed better activity. ***Conclusion:*** The results may be instructive to researchers attempting to gain more understanding of the antimicrobial activity of indole hydrazide/hydrazone-type compounds.

Introduction

The noticeably increasing numbers of multi-drug-resistant microbial infections have become a serious health care problem. In particular, the appearance of multi-drug-resistant strains of Gram-positive bacterial pathogens such as *Staphylococcus aureus* (methicillin-resistant, MRSA) and *Enterococcus* (*Staphylococcus epidermidis* and vancomycin-resistant) is causing serious problems in health care [1–3]. Also there has been a noteworthy increase of systematic fungal infection in humans. Organ transplant and anticancer chemotherapy patients, receiving long-term treatment with antimicrobial drugs, and AIDS patients are immunosuppressed and very defenseless to fungal infections such as candidiasis, cryptococcosis and aspergillosis.

Hydrazone-type compounds containing an azomethine constitute an important class of compounds for new drug development. It is well known that the hydrazone group plays an important role in antimicrobial activity [4–6]. It has been claimed that a number of hydrazide-hydrazone derivatives possess interesting antibacterial-antifungal [7, 8], anticonvulsant [9], anti-inflammatory [10], and antituberculosis activities [11–14].

Electron-rich nitrogen heterocyclic compounds play an important role in diverse biological activities [15]. Indole has been reported to have an inhibitory effect on several fungi [16, 17]. Some indole derivatives show significant antimicrobial activity; examples include 1H-in-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0009–3157/09/0551–0015$26.00/0

Accessible online at:
www.karger.com/che

Prof. Sibel Suzen
Department of Pharmaceutical Chemistry
Ankara University, Faculty of Pharmacy
TR–06100 Tandogan, Ankara (Turkey)
Tel. +90 312 203 3074, Fax +90 312 213 1081, E-Mail sibel@pharmacy.ankara.edu.tr

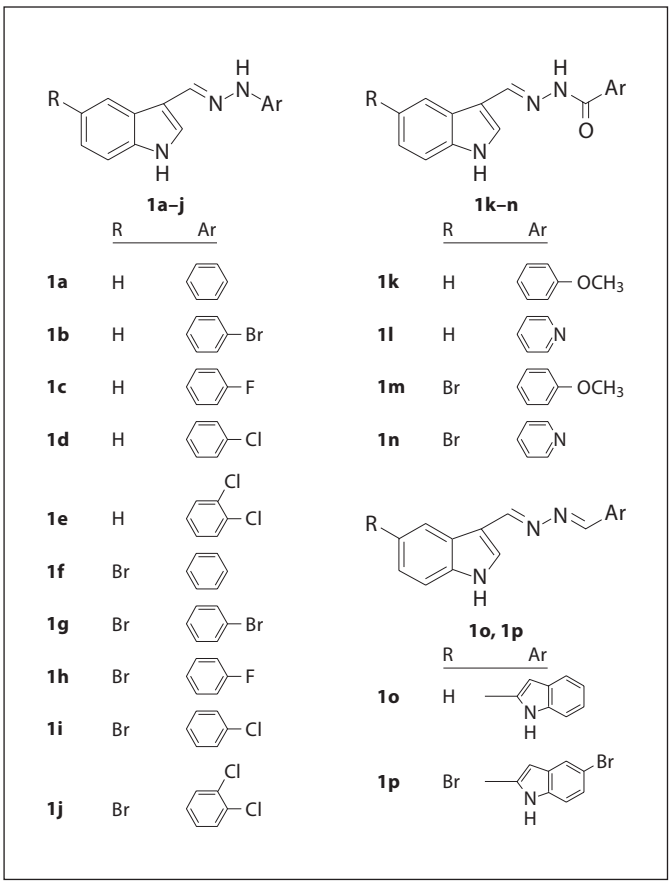

Fig. 1. Indole-3-aldehyde hydrazide/hydrazone derivatives.

dole-2-one derivatives [18], Schiff and Mannich bases of isatin (indole-2,3-dione) [19], its 5-chloro and 5-bromo derivatives [20] and triazole derivatives [21], thiosemicarbazide indole derivatives [22], indolocarbazoles [23], monoindolyl and indolocarbazolyl oxazolones and imidazolones [24] and indolo-benzo-triazine [25]. Recently antimicrobial effects of indole-3-carbinol against various human pathogens and its mode of action regarding antifungal activity against fungal pathogens were reported [26]. A tryptophan-rich antimicrobial peptide indolicidin has 13 amino acids and is highly active against *S. aureus* and *Escherichia coli* [27].

These investigations led to the conception that hydrazide/hydrazone derivatives of indole-3-aldehyde would potentially possess antimicrobial properties. In this paper, antimicrobial properties of sixteen indole hydrazide/hydrazone derivatives were reported (fig. 1). Except for compounds **1a** [28], **1b** [29], **1k** [30], **1l** [31], **1n** [32], and **1o** [33], the synthesis and characterization of the compounds were published in our earlier study [34]. The results of this study may be useful to researchers attempting to gain more understanding of the antimicrobial activity of indole hydrazide/hydrazone-type compounds.

Materials and Methods

Chemistry

Formylation was performed on indole and 5-bromoindole derivatives with $POCl_3$ and N,N-dimethyl formamide to result in indole/5-Br-indole-3-carboxaldehydes that were condensed with appropriate amines, finally to result in indole hydrazide/hydrazone derivatives [34].

In vitro Antimicrobial and Antifungal Activities of Indole Derivatives

In antibacterial and antimycotic assays, the compounds and the standards were dissolved in 12.5% DMSO at concentrations of 200 μg/ml. Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 μg/ml concentrations with Mueller-Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the 2-fold serial dilution technique [35, 36]. A control test was also performed containing inoculated broth supplemented with only ethanol at the same dilutions as those used in our experiments and this was found to be inactive in the culture medium. All the compounds were tested for their in vitro growth inhibitory activity against *Candida albicans* ATCC 10145 as fungus, *S. aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, MRSA standard ATCC 43300, MRSA isolate as Gram-positive bacteria and *E. coli* ATCC 23556 as Gram-negative bacteria. ATCC strains of the microorganisms used in this study were obtained from the culture collection of the Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of the Faculty of Pharmacy of the Ankara University. Sultamicillin, ampicillin, fluconazole and ciprofloxacin were used as control drugs. MIC (mg/ml) values of the antimicrobial activity of the compounds and the control drugs are given in table 1.

Antibacterial and Antifungal Activity Assay

The cultures were obtained from Mueller-Hinton broth (Difco) for all the bacterial strains after 24 h of incubation at 37 ± 1°C. *C. albicans* was maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 ± 1°C. Testing was carried out in Mueller-Hinton broth and Sabouraud dextrose broth (Difco) at pH 7.4 and the 2-fold serial dilution technique was applied. The final inoculum size was 10^5 CFU/ml for the antibacterial assay and 10^4 CFU/ml for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. After incubation for 24 h at 37 ± 1°C for the antibacterial assay and for 48 h at 25 ± 1°C for the antifungal assay, the last tube with no growth of microorganism and/or yeast was recorded to represent the MIC (expressed in mg/ml). Every experiment in the antibacterial and antifungal assays was performed in duplicate.

Table 1. MIC values (μg/ml) of compounds **1a–1p**

	S. aureus	MRSA standard	MRSA isolate	*E. coli*	*B. subtilis*	*C. albicans*
1a	6.25	6.25	6.25	50	25	6.25
1b	6.25	6.25	6.25	50	6.25	6.25
1c	6.25	6.25	6.25	50	6.25	6.25
1d	6.25	6.25	6.25	50	6.25	6.25
1e	6.25	6.25	6.25	50	25	6.25
1f	6.25	6.25	6.25	50	6.25	6.25
1g	6.25	6.25	6.25	50	6.25	6.25
1h	6.25	6.25	6.25	50	12.5	6.25
1i	6.25	6.25	6.25	50	12.5	6.25
1j	6.25	6.25	6.25	50	12.5	3.125
1k	25	25	50	100	25	50
1l	25	100	100	50	50	50
1m	50	50	50	100	50	50
1n	≥100	100	100	100	100	≥100
1o	25	25	25	50	12.5	25
1o	50	50	50	50	25	25
1p	12.5	6.25	6.25	50	25	25
Sultamicillin	0.78	–	–	–	0.78	–
Ampicillin	1.56	12.5	–	–	50	–
Fluconazole	–	–	–	–	–	0.78
Ciprofloxacin	0.19	–	–	0.09	0.09	–

Results and Discussion

The antibacterial activity of compounds against MRSA standard and MRSA isolate showed promising results when it was compared to the control drug ampicillin. Compounds **1a**–**1j** and **1p** with an MIC value of 6.25 μg/ml indicated more potent antimicrobial activity than ampicillin for which the MIC is 12.5 μg/ml. Also **1k**, **1m** and **1o** showed moderate activity against MSRA standard and MSRA isolate.

Against *B. subtilis* with the exception of compound **1n**, all the compounds indicated more potent or similar activity (compound **1l** and **1m**) than that of ampicillin. Compounds **1b**–**1d**, **1f** and **1g** showed moderate activity against *B. subtilis* compared to ampicillin; however, all the compounds showed lower activity than sultamicillin and ciprofloxacin against *B. subtilis*.

Table 1 indicates that all the compounds showed lower antibacterial activity against the screened *S. aureus* than the control drugs. However compounds **1a**–**1j** showed moderate activity with an MIC value of 6.25 μg/ml when it was compared to ampicillin with an MIC value of 1.56 μg/ml. None of the compounds showed any significant activity against *E. coli*.

Among the tested compounds **1j** showed good antifungal activity against *C. albicans* with an MIC value of 3.125 μg/ml which was close to that of fluconazole (MIC 0.78 μg/ml). In previous studies, the indole ring was found not to be a very strong antimicrobial agent in inhibiting the growth of microorganisms but it was found to have a wide antifungal spectrum [37]. This antifungal mode of action of indole derivatives was investigated by Sung et al. [26] by the change in the membrane dynamics that was monitored by using fluorescence changing experiments against *C. albicans*. The results suggest that indole derivatives may exert antifungal activity by disrupting the structure of the cell membrane. Also, the indole derivative CBR-4830 that was discovered through a whole-cell antibacterial screen as a growth inhibitor of efflux-compromised *Pseudomonas aeruginosa* strains [38].

Falla et al. [39] showed that indolicidin is capable of killing Gram-negative bacteria by crossing the outer membrane and causing disruption of the cytoplasmic membrane by channel formation. Lee et al. [40] reported that the fungicidal activity indolicidin involves disruption of the structure of cell membranes, via direct interaction with the lipid bilayers, in a salt-dependent and energy-independent manner.

The ways in which different Schiff base compounds react with bacteria and fungi vary due to the difference in their structures. The biological activity of compounds depends on individual properties including structure, affinity for the target, and survival in the medium of application and biological system and state of the target organism [41, 42]. The structure-activity relationships of these Schiff base derivatives indicated that the aromaticity seemed to be important for the antimicrobial activity. Generally, the activity of compounds was increased with the introduction of halogens. In this study compounds **1b–1j** were found to be the most potent antimicrobial agents, indicating that the halogen atom plays an important role in the antimicrobial activity of Schiff bases. Moreover, indole nicotinic acid hydrazides (**1l–1n**) showed no significant activity while indole anisic acid hydrazides (**1k–1m**) indicated better activity.

In conclusion, the most significant results were obtained against MRSA standard and MRSA isolate with the compounds **1a–1j** and **1p** that showed more potent activity than ampicillin. Evidently, further studies are needed to better understand the efficacy of indole-3-aldehyde and 5-bromoindole-3-aldehyde hydrazide-hydrazones for the development of new antimicrobial agents.

References

1 Poole K: Multidrug resistance in Gram-negative bacteria. Curr Opin Microbiol 2001;4:500–508.
2 Abbanat D, Macielag M, Bush K: Novel antibacterial agents for the treatment of serious Gram-positive infections. Expert Opin Investig Drugs 2003;12:379–399.
3 Goossens H: European status of resistance in nosocomial infections. Chemotherapy 2005;51:177–181.
4 Abdel-Fattah ME, Salem EE, Mahmoud MA: Synthesis and antimicrobial activity of some 3-p-chlorophenoxymethyl-4-phenyl-1,2,4-triazol-5-yl-thio-acetyl hydrazine derivatives. Ind J Heteroc Chem 2000;10:121–128.
5 Yildir I, Perçiner H, Sahin MF, Abbasoglu U: Hydrazones of [(2-benzothiazolylthio)acetyl]hydrazine: synthesis and antimicrobial activity. Arch Pharm 1995;328:547–549.
6 Vittorio F, Ronsisvalle G, Marrazzo A, Blandini G: Synthesis and antimicrobial evaluation of 4-phenyl-3-isoquinolinoyl-hydrazones. Il Farmaco 1995;50:265–272.
7 Vicini P, Zani F, Cozzini P, Doytchinova I: Hydrazones of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigations. Eur J Med Chem 2002;37:553–564.
8 Loncle C, Brunel JM, Vidal N, Dherbomez M, Letourneux Y: Synthesis and antifungal activity of cholesterol-hydrazone derivatives. Eur J Med Chem 2004;39:1067–1071.
9 Sridhar SK, Pandeya SN, Stables JP, Ramesh A: Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives. Eur J Pharm Sci 2002;16:129–132.
10 Todeschini AR, Miranda ALP, Silva KCM, Parrini SC, Barreiro E: Synthesis and evaluation of analgesic, anti-inflammatory and anti-platelet properties of new 2-pridylaryhydrazone derivatives. Eur J Med Chem 1998;33:189–199.
11 Kaymakçıoğlu BK, Rollas S: Synthesis, characterization and evaluation of antituberculosis activity of some hydrazones. Farmaco 2002;57:595–599.
12 Maccari R, Ottanà R, Vigorita MG: In vitro advanced antimycobacterial screening of isoniazid-related hydrazones, hydrazides and cyanoboranes. Part 14. Bioorg Med Chem Lett 2005;15:2509–2513.
13 Rando DG, Sato DN, Siqueira L, Malvezzi A, Leite CQF, Amaral AT, Ferreira EI, Tavares LC: Potential tuberculostatic agents. Topliss application on benzoic acid [(5-nitro-thiophen-2-yl)-methylene]-hydrazide series. Bioorg Med Chem 2002;10:557–560.
14 Suriyati M, Pazilah I, Habibah AW: Effects of isoniazid on viability, cell morphologies and acid fastness properties of *Mycobacterium avium* NCTC 8559 during the growth cycle. Chemotherapy 2007;53:263–266.
15 Suzen S: Bioactive heterocycles V, antioxidant activities of synthetic indole derivatives and possible activity mechanisms; Khan MTH (ed): Topics in Heterocyclic Chemistry. Berlin, Spinger, 2007, vol 11, pp 145–178.
16 Zsolnai T: New fungistatic compounds. VI. Hydrazine derivatives and organic bases or their salts. Pharm 1962;11:995–1016.
17 Koivistoinen P, Risser E, Pohjakallio O: The inhibitory effect of certain indole compounds upon the growth of *Sclerotinia trifoliorum* ERIKSS. Acta Agr Scand 1959;9:403–411.
18 Altintaş H, Ateş O, Uyde-Doğan BS, Alp FI, Kaleli D, Ozdemir O, Birteksöz S, Otük G, Atana D, Uzun M: Synthesis and evaluation of antimicrobial and anticonvulsant activities of some new 3-[2-(5-aryl-1,3,4-oxadiazol-2-yl/4-carbethoxymethylthiazol-2-yl) imino-4-thiazolidinon-5-ylidene]-5-substituted/nonsubstituted 1H-indole-2-ones and investigation of their structure-activity relationships. Arzneimittelforschung 2006;56:239–248.
19 Pandeya SN, Sriram D, Yogeeswari P, Ananthan S: Antituberculous activity of norfloxacin mannich bases with isatin derivatives. Chemotherapy 2001;47:266–269.
20 Pandeya SN, Sriram D, Nath G, De Clercq E: Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin derivatives with 3-amino-2-methylmercapto quinazolin-4(3H)-one. Pharm Acta Helv 1999;74:11–17.
21 Pandeya SN, Sriram D, Nath G, de Clercq E: Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin and its derivatives with triazole. Arzneimittelforschung 2000;50:55–59.
22 Varvaresou A, Tsantili-Kakoulidou A, Siatra-Papastaikoudi T, Tiligada E: Synthesis and biological evaluation of indole containing derivatives of thiosemicarbazide and their cyclic 1,2,4-triazole and 1,3,4-thiadiazole analogs. Arzneimittelforschung 2000;50:48–54.
23 Moreau P, Anizon F, Sancelme M, Prudhomme M, Bailly C, Carrasco C, Ollier M, Sevère D, Riou JF, Fabbro D, Meyer T, Aubertin AM: Syntheses and biological evaluation of indolocarbazoles, analogues of rebeccamycin, modified at the imide heterocycle. J Med Chem 1998;41:1631–1640.
24 Pereira ER, Prudhomme M, Sancelme M, Ollier M, Severe D, Riou JF, Crevel H, Savineau JP, Fabbro D, Meyer T: Synthesis and biological evaluation of monoindolyl and indolocarbazolyl oxazolones and imidazolones. Chem Pharm Bull (Tokyo) 1997;45:733–736.
25 Cirrincione G, Almerico AM, Barraja P, Diana P, Lauria A, Passannanti A, Musiu C, Pani A, Murtas P, Minnei C, Marongiu ME, La Colla P: Derivatives of the new ring system indolo[1,2-c]benzo[1,2,3]triazine with potent antitumor and antimicrobial activity. J Med Chem 1999;42:2561–2568.

26 Sung WS, Lee DG: In vitro antimicrobial activity and the mode of action of indole-3-carbinol against human pathogenic microorganisms. Biol Pharm Bull 2007;30:1865–1869.
27 Selsted ME, Novotny MJ, Morris WL, Tang YQ, Smith W, Cullor JS: Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils. J Biol Chem 1992;267:4292–4295.
28 Kidwai M, Negi N, Gupta SD: Synthesis and antifertility activity of 1,5-diaryl-3 (3′-indolyl)formazans. Chem Pharm Bull (Tokyo) 1994;42:2363–2364.
29 Wiley RH, Clevenger RL: Aldehyde hydrazone derivatives in cancer chemotherapy. J Med Pharm Chem 1962;5:1367–1371.
30 Alemany A, Bernabe PM, Fernandez AE, Lora-Tamayo M, Nieto LO: Potential psychotropic agents. I. Synthesis of 1-(2-indolylcarbonyl)-2-alkylhydrazines, 1-(3-indolylcarbonyl)-2-alkylhydrazines and 1-(3-indolylacetyl)-2-alkylhydrazines, and measurement, in vitro, of their monoamine oxidase inhibitory activity. Bull Soc Chim Fr 1966;8:2486–2497.
31 Popp FDJ: Potential anticonvulsants. VIII. Some hydrazones of indole-3-carboxaldehyde. Heterocycl Chem 1984;21:617–619.
32 Rector DL, Conder GA, Folz SD: Preparation of antihelmintic acylhydrazones, method of use and compositions, 1987. PCT Int Appl 57 pp CODEN: PIXXD2 WO 8706133 A1 19871022 CAN 108:101381 AN 1988:101381 caplus.
33 Buu-Hoi NP, Saint-Ruf G: Thermal decomposition of aromatic aldazines. Applications to preparative chemistry. Bull Soc Chim Fr 1967:955–960.
34 Gurkok G, Coban T, Suzen S: Melatonin analogue new indole hydrazide/hydrazone derivatives with antioxidant behavior: synthesis and discussion on structure activity relationships. J Enzyme Inhib Med Chem, in press.
35 Charles ES, Agrawal VK, Sharma S, Iyer RN: Synthesis of 2,5-disubstituted benzimidazoles as potential antihookworm and antimicrobial agents. Part 1. Eur J Med Chem Chim Ther 1979;14:435–438.
36 Shadomy S, Espinel A: Susceptibility testing with fungi; in Manual of Clinical Microbiology. Washington, American Society of Microbiology, 1980, pp 647–653.
37 Sinha D, Tiwari AK, Singh S, Shukla G, Mishra P, Chandra H, Mishra AK: Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde. Eur J Med Chem 2008;43:160–165.
38 Robertson GT, Doyle TB, Du Q, Duncan L, Mdluli KE, Lynch AS: A novel indole compound that inhibits *Pseudomonas aeruginosa* growth by targeting MreB is a substrate for MexAB-OprM. J Bacteriol 2007;189:6870–6881.
39 Falla TJ, Karunaratne DN, Hancock REW: Mode of action of the antimicrobial peptide indolicidin. J Biol Chem 1996;271:19298–19303.
40 Lee DG, Kim HK, Kim SA, Park Y, Park SC, Jang SH, Hahm KS: Fungicidal effect of indolicidin and its interaction with phospholipid membranes. Biochem Biophys Res Commun 2003;305:305–310.
41 Lei S, Hui-Ming G, Shu-Hua T, Huan-Qiu L, Yong-Chun S, Hai-Liang Z, Ren-Xiang T: Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. Eur J Med Chem 2007;42:558–564.
42 Kosower EM, Miyadera T: Glutathione. 6. Probable mechanism of action of diazene antibiotics. J Med Chem 1972;15:307–312.