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Novel Naphthyridine-Hydrazone Derivatives and their Antimicrobial Activity

Yeni Naftiridin Hidrazon Türevleri ve Anti-Mikrobiyal Aktiviteleri

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ABSTRACT

Purpose: The synthesis of ten new naphthyridine-hydrazone derivatives and subsequent evaluation of their antimicrobial activities were aimed in this present work.

Material and Methods: The structure of the compounds was elucidated by 1H-NMR and MS spectral data. Additionally the compounds were evaluated for their antimicrobial activity against a panel of pathogenic bacteria and fungus strains. The panel of microorganisms Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Listeria monocytogenes and Candida albicans were tested in vitro using a microdilution method.

Results: All compounds showed significant antimicrobial activity against S. typhimurium and C. albicans when compared with reference agents.

Conclusion: All synthesized compounds had significant antifungal activity when compared with ketoconazole.

Key Words: Naphthyridine, Quinolone, Hydrazone, Antimicrobial Activity

ÖZET

Amaç: Bu çalışmada, on yeni Naftiridin-Hidrazon türevinin sentezi ve antimikrobiyal aktivitelerinin değerlendirilmesi amaçlanmıştır.

Materyal ve Metod: Sentezlenen bileşiklerin yapıları 1H-NMR ve MS spectral verileri ile aydınlatılmıştır. Ayrıca bileşiklerin antimikrobiyal aktiviteleri, patojen bakteri ve funguslar üzerinde araştırılmıştır. Patojen mikroorganizmalar olan Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Listeria monocytogenes ve Candida albicans, mikrodilüsyon yöntemi ile test edilmişlerdir.

Bulgular: Tüm bileşikler referans ajanlarla kıyaslandığında, S. typhimurium'a ve C. albicans'a karşı kayda değer antimikrobiyal aktivite göstermişlerdir.

Sonuç: Tüm bileşikler ketoconazole ile kıyaslandığında, kayda değer antifungal aktivite göstermişlerdir.

Anahtar Kelimeler: Naftiridin, Kinolon, Hidrazon, Antimikrobiyal Aktivite

INTRODUCTION

Deaths from acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria and tuberculosis account for more than 85% of the mortality from infection worldwide. Resistance from first-line drugs in most of the pathogens causing

these diseases ranges from zero to almost 100%. In some instances resistance to second- and third-line agents is seriously compromising treatment outcome. In addıtıon the significant global burden of resistant hospital-acquired infections, the emerging problems of antiviral resistance and the increasing problems of drug resistance in the neglected parasitic diseases ın poor and marginalized populations. Although there has been a unyielding increase in resistance to antimicrobial agents between important bacterial pathogens throughout the world, it is well known that the number of new antimicrobial agents being brought to the market had a consistent decline in the past decades. The deficiency of interest by pharmaceutical industry in antibacterial agents is related to number of factors, including many generic antimicrobial agents currently on the market that still have varying degrees of usefulness and that are considered first lines of therapy by many public health authorities[1,2]. The worldwide rise of antimicrobial resistance, combined with the rapid rate of microbial evolution, and the slower development of novel antibiotics, accents the crucial need for novel therapeutics. Development of new antimicrobial or antipathogenic agents that act upon new microbial targets is a necessity[3].

Quinolones have become a major class of synthetic antibacterial agents, which are under extensive clinical development. They have an attraction because of their extremely potent activity, rapid bactericidal effects, and low incidence of resistance development. As a naphthyridine derivative Nalidixic acid (NAL) was the first quinolone-derived agent demonstrating antibacterial activity as well as useful clinical parameters[4,5]. It inhibits DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase, resulting in rapid bacterial death[6]. It is particularly effective against gram-negative bacteria[7].

Hydrazone is another functional group that is present in many chemotherapeutics agents as the bioactive part of the molecule and it has been an important class of compound for new drug design studies[8-10].

The development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles[11].

In the quest for biologically more potent antimicrobial agents and to increase the molecular diversity and analyze the structure-activity relationships, we envisioned to design and synthesize the Naphthyridine based hydrazone derivatives.

MATERIAL and METHOD

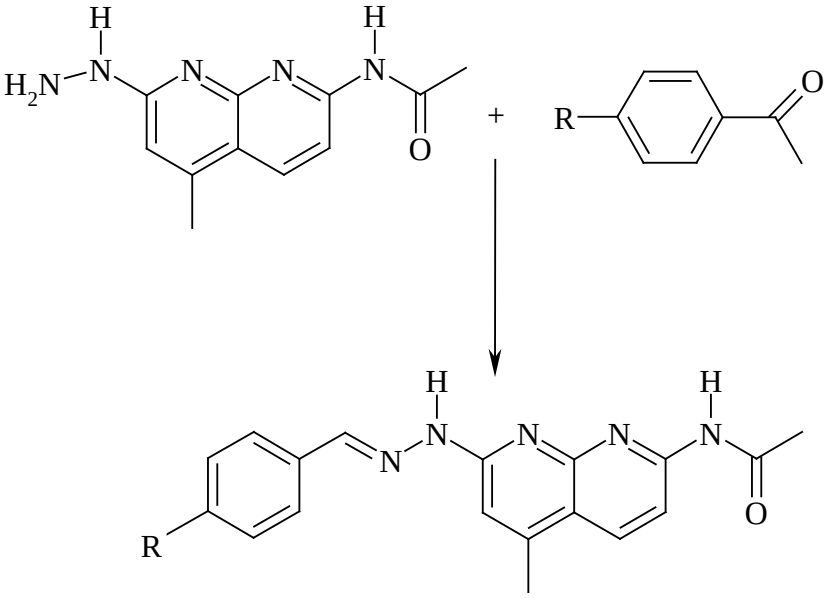
All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough-UK), given in Table 1. The purity of the compounds was routinely checked by silica gel thin layer chromatography (TLC) plates (60 G, Merck, Darmstadt-Germany). Spectroscopic data was recorded with the following instruments: ^{1}H-NMR: Bruker 250 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) in DMSO-d6 using TMS as internal standard; and FAB-MS: VG Quattro Mass spectrometer (Agilent, Minnesota, USA).

Chemistry

*Preparation of N-Benzylidene-N'-(5-methyl-2-acetamido[1,8]naphthyridin-7-yl)hydrazine derivatives (**1-10**).*

Equimolar quantities of 2-acetamido-5-methyl-7-hydrazino[1,8]naphthyridine (30 mmol) and appropriate benzaldehydes in 25 ml of absolute ethanol were refluxed for 3-5 h. The resulting solid was filtered and recrystallized from ethanol. Some characteristics of the synthesized compounds are shown in Table 1. Analytical and spectral data (^{1}H-NMR, MS-FAB) confirmed the structures of the new compounds.

N-Benzylidene-N'-(5-methyl-2-acetamido[1,8]naphthyridin-7-yl)hydrazone (**1**): ^{1}H-NMR (250 MHz) (DMSO-*d*6) δ (ppm): 2.27 (3H, s, CH_3), 2.67 (3H, s, CH_3), 6.00-6.58 (3H, m, aromatic protons), 7.12-7.90 (5H, m, aromatic protons), 8.07 (1H, s, N=CH), 11.22 (1H, s, NH), 12.31 (1H, s, NH).
MS (FAB); *m*/*z*: 320 [M + 1]



Compounds **1-10**

Scheme 1. The general synthetic reaction

Table 1. Some characteristics of the compounds

Compound	R	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
1	H	44	282	$C_{18}H_{17}N_5O$	319,36
2	p-F	53	287	$C_{18}H_{16}FN_5O$	337,35
3	p-Cl	60	283	$C_{18}H_{16}ClN_5O$	353,81
4	p-Br	65	266	$C_{18}H_{16}BrN_5O$	398,26
5	p-NO_2	67	275	$C_{18}H_{16}N_6O_3$	364,36
6	p-CN	74	289	$C_{19}H_{16}N_6O$	344,37
7	p-OH	33	319	$C_{18}H_{17}N_5O_2$	335,36
8	p-CH_3	44	291	$C_{19}H_{19}N_5O$	333,39
9	p-$CH(CH_3)_2$	40	256	$C_{21}H_{23}N_5O$	361,44
10	p-$N(CH_3)_2$	54	264	$C_{20}H_{22}N_6O$	362,43

Microbiology

The antimicrobial activities of the compounds were tested using the microbroth dilution method[12]. Tested microorganism strains were *M. luteus* (NRLL B-4375), *B. subtilis* (NRS-744), *S. typhimirium* (NRRL B-4420), *S. aureus* (NRRL B-767), *E. coli* (ATCC-25922), *L. monocytogenes* (ATCC-7644), *C. albicans* (ATCC-22019). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in

dimethylsulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro–test tubes that were transferred to 96-well microtiter plates. Overnight-grown bacterial and *C. albicans* suspensions in double-strength Mueller–Hinton broth were standardized to 10^8 CFU/mL using McFarland No: 0.5 standard solutions. Hundred microliter of each microorganism suspension was then added into the wells. The last well-chain without a microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37 °C for 18-24 h, antimicrobial activity was detected by spraying of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of compounds that inhibited visible growth, as indicated by the TTC staining. Streptomycin was used as standard antibacterial agent, whereas ketoconazole was used as an antifungal agent. Observed data on the antimicrobial activity of the compounds and control drugs are given in Table 2.

Table 2. Antimicrobial Activities of Compounds (*µ*g/mL)

Compound	A	B	C	D	E	F	G
1	125	125	125	125	125	125	250
2	125	125	125	125	125	125	250
3	250	250	250	125	125	250	250
4	250	250	250	250	125	250	250
5	250	250	250	250	125	250	250
6	250	250	250	250	250	500	250
7	250	250	250	250	250	250	250
8	125	250	250	250	250	250	250
9	125	250	250	250	250	250	250
10	125	250	250	250	125	250	250
Ref. 1	31.25	7.81	31.25	125	15.625	15.625	-
Ref. 2	-	-	-	-	-	-	250

Ref. 1: Streptomycin; **Ref. 2**: Ketoconazole.

A: *S. aureus* (NRRL B-767), **B**: *L. monocytogenes* (ATCC-7644), **C**: *E. coli* (ATCC-25922), **D**: *S. typhimirium* (NRRL B-4420), **E**: *M. luteus* (NRLL B-4375), **F**: *B. subtilis* (NRS-744), **G**: *C. albicans* (ATCC-22019)

RESULTS

+The target compounds were prepared by a three-step reaction as shown Scheme 1. Various aldehydes were reacted with hydrazine in ethanol with catalytic amount of glacial acetic acid to yield the titled compounds (1-10), respectively. The yields of individual compounds in this series ranged from 33% to 74%. In the ^{1}H-NMR spectra of the compounds, all of the aromatic and aliphatic protons were recorded at estimated areas. The mass spectra of the compound showed [M+1] peaks, in agreement with their molecular weight.

Antimicrobial activity of the synthesized compounds against *S. aureus (NRRL B-767), L. monocytogenes (ATCC-7644), E. coli (ATCC-25922), S. typhimirium (NRRL B-4420), M. luteus (NRLL B-4375), B. subtilis (NRS-744), C. albicans (ATCC-22019)* was evaluated by a microdilution

assay. Minimum inhibitory concentrations (MIC) of the tested compounds were reported by comparing with standard drugs such as Streptomycin, and ketoconazole. The MIC values of the compounds and control drugs are summarized in Table 2.

DISCUSSION

All compounds possessed a broad spectrum of activity having MIC values of 125-250 µg/mL against the tested microorganisms, whereas standard drugs MIC's were in between 7.81-250 µg/mL. The most important part of the results was those which are obtained from antifungal activity screening. All compounds were effective against *C. albicans* when compared with ketoconazole; all compounds showed similar activity with 250 µg/mL to reference agent. On the other hand, similar results obtained from *S. typhimirium*. Compounds 1, 2, and 3 showed similar activity with 125 µg/mL when compared with Streptomycin.

CONCLUSION

In this work we report the original synthesis, structural elucidation and antimicrobial activities of ten new naphthyridine-hydrazone derivatives[1-10]. The structure proposed to the synthesized compound[1] are fully supported by spectroscopic data. Additionally, all synthesized compounds were evaluated for their antimicrobial efficacy for the first time. When all compounds were showed significant antifungal activity some of the compounds 1, 2, and 3 which include halogen substituent showed significant antibacterial activity. This outcome confirms that halogen substituent on phenyl may have a considerable influence on antibacterial activity.

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