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Short Communication

EVALUATION OF ANTIMICROBIAL ACTIVITY OF 2-[(2-NITRO-1-PHENYLALKYL) THIOMETHYL]BENZIMIDAZOLE DERIVATIVES

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Abstract

In this study eight 2-[(2-nitro-1-phenylethyl)thiomethyl]benzimidazole (1a-h) and nine 2-[(2-nitro-1-phenylpropyl)thiomethyl]benzimidazole (2a-i) derivatives were tested for their antibacterial and antifungal activities. The minimum inhibition concentration (MIC) values of the compounds were tested by the microdilution method against Gram- positive, Gram-negative bacteria and yeast like fungi. Compounds (2h) and (2i) were found to be active as ampicillin trihydrate against Staphylococcus aureus and Streptococcus faecalis, ($4.80x10^{-3}$, $4.80x10^{-3}$ and $5.20x10^{-3}$,$5.20x10^{-2}$ and $4.80x10^{-3}$, $4.80x10^{-3}$ μM respectively). Compound (1a) has showed antifungal activity close to Ketoconazole ($1.05x10^{-3}$ and $0.90x10^{-3}$ μM respectively).

Key Words: *Mercaptomethylbenzimidazole, β-nitrostyrene, β-methyl-β-nitrostyrene, antibacterial activity, antifungal activity.*

2-[(2-Nitro-1-fenilalkil)tiyometil]benzimidazol Türevlerinin Antimikrobiyal Aktivitesinin Değerlendirilmesi

Bu çalışmada sekiz 2-[(2-nitro-1-feniletil)tiyometil]benzimidazol (1a-h) ve dokuz 2-[(2-nitro-1-fenilpropil)tiyometil]benzimidazol (2a-i) türevlerinin antifungal ve antibakteriyel aktiviteleri test edilmiştir. Bileşiklerin minimum inhibisyon konsantrasyon değerleri Gram-pozitif, Gram-negatif bakterilere ve maya benzeri mantarlara karşı mikrodilüsyon metodu kullanılarak test edilmiştir. Bileşik (2h) ve (2i) Staphylococcus aureus ve Streptococcus faecalis'e karşı ampisilin trihidrat kadar aktif bulunmuştur. Bileşik (1a) ketokonazole yakın antifungal aktivite göstermiştir.

Anahtar Kelimeler: *Merkaptometilbenzimidazol, β-nitrostiren, β-metil-β-nitrostiren, antibakteriyel aktivite, antifungal aktivite.*

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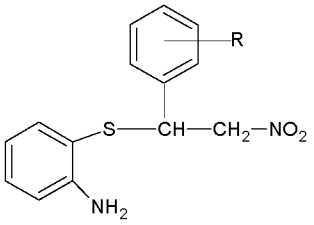
INTRODUCTION

The benzimidazole compounds have been included to be the most important groups of fungicides with systemic activity and are well known for their pronounced ability to control a large number of fungal diseases (1) . Also, a number of certain alkylthio and arylthio benzimidazole derivatives exhibit interesting antimicrobial, antiprotozoal, antimycobacterial and antiparasitic activities (2-6).

Furthermore, in general, the introduction of nitro group to organic compounds enhances its antimicrobial activity (7-11).

In our previous studies, we synthesized 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **(5)** derivatives, which are the Michael type addition products of β-nitrostyrenes with 2-aminothiophenol and investigated their antifungal activities. Some of the **(5)** derivatives had significant antifungal activity against *Candida albicans*, *C. stellatoidea*, *C. parapsilosis*, *C. pseudotropicalis (*12). We also reported synthesis of 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane **(6)** derivatives. It was found that **(6)** derivatives showed significant antibacterial activities against *Streptococcus faecalis* and *S. aureus* (13).



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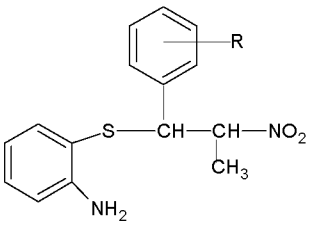

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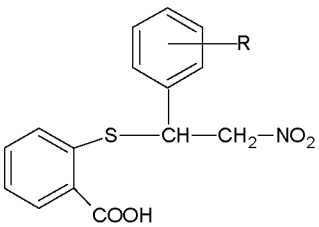
In addition we synthesized 2-[(2-nitro-1-phenylethyl)thio]benzoic acid **(7)** and 2-[(2-nitro-1-phenylpropyl)thio]benzoic acid **(8)** derivatives. These compounds are evaluated against to *Gram-positive* and *Gram-negative* bacteria and yeast-like fungi. The title compounds were also screened by consecutive dilution to explore their toxicity to a Vero cell line. All of the **(7)** derivatives were more active than the standard compound ketoconazole used in the antifungal activity tests (14).



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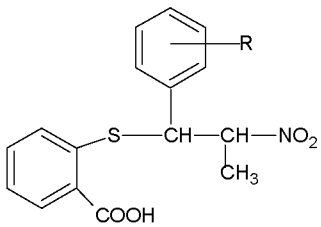

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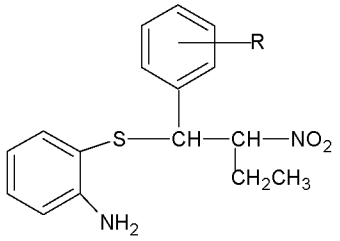
Moreover, 1-[(2-aminophenyl)thio]-1-phenyl-2-nitrobutane **(9)** derivatives were synthesized and their antimicrobial activities were investigated against to *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis*, *Bacillus subtilis*, *S. aureus* and *C. albicans* by the microdilution method. All of the 1-[(2-aminophenyl)thio]-1-phenyl-2-nitrobutane **(9)** derivatives showed varying degrees of inhibition against the tested *Gram-*

positive, Gram-negative bacteria and yeast like fungi. The antiviral activity of the synthesized compounds was also determined. All of them were found to be almost 100-fold more active than standard compound acyclovir (15).



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In our previous studies 2-[(2-nitro-1-phenylethyl)thiomethyl]benzimidazole **(1)** and 2-[(2-nitro-1-phenylpropyl) thiomethyl]benzimidazole **(2)** have been synthesized by the addition of 2-mercaptomethylbenzimidazoles to the active double bond of β-nitrostyrene **(3)** and β-methyl-β-nitrostyrene **(4)** derivatives (16, 17). In the present study, *in vitro* antimicrobial activity of **(1)** and **(2)** derivatives (Table 1) have been evaluated.

Almost all the major classes of antibiotics have encountered resistance in clinical application. The emergence of bacterial resistance to β-lactam antibiotics, macrolides, quinolones and vancomycin is becoming a major worldwide health problem. In particular, antibiotic resistance among *Gram-positive* bacteria is becoming increasingly serious (18-20). In order to overcome these emerging resistance problems, there is an urgent need to discover novel antibacterial agent in structural classes distinct from existing antibiotics.

EXPERIMENTAL

Antibacterial and Antifungal Activity

Material

The following bacteria were used for antibacterial study: *S. aureus* ATCC 25923, *S. faecalis* ATCC 19433, *P. aeruginosa* ATCC 27833, *E. coli* ATCC 25922.

The following yeast-like fungi were used for antifungal study: *C. albicans* ATCC 90528, *C. parapsilosis* 90018.

Inoculation Suspensions

The microorganism suspensions used for inoculation were prepared at 10^6 cfu/ml concentration by diluting of the fresh cultures at McFarland 0.5 density. It was known that there were $5x10^4$ cfu/ml microorganisms in each well after inoculation.

Medium

Mueller Hinton Broth (Oxoid) liquid nutrient medium was used for diluting the microorganism suspension and two fold-dilutions of the compounds. Sabouraud liquid medium (Oxoid) was used for yeast like fungi for the same purpose.

Equipment

Falcon^R microplates which have 96 wells were used for microdilution method. Brinkmann-transferpette^R was used for two fold-dilutions of compounds in the wells.

Method

Microdilution method was employed for antibacterial and antifungal activity test [21]. The synthesized compounds, ampicillin trihydrate and ketoconazole were dissolved in dimethylsulfoxide (DMSO) at 1000 μg/ml concentration at the beginning.

The solution of each compound at 500-3.9 μg/ml was prepared in the wells by diluting with the mediums. Suspension of the microorganisms at 10^6 cfu/ml concentration were inoculated to the two fold–diluted solution of the compounds, consequently the microorganism concentration in each well was approximately $5x10^4$ cfu/ml. DMSO-microorganisms mixture, the pure microorganisms and pure media were used as control wells.

Microplates were covered for contamination of air surface and then incubated at 36°C for 24-48 hours. Wet cotton-wool was placed in the incubation chamber, because it should be kept sufficiently to avoid evaporation. After this period of time, evaluation of the wells was performed. The concentration of the compounds in the wells where no growth was seen were assessed as the minimum inhibitory concentration (MIC) values There was no inhibitory activity in the wells containing only DMSO. The microbial growth occurred, and the medium was not contaminated during the tests. The MIC values of 2-[(2-nitro-1-phenylethyl)-thiomethyl]benzimidazole **(1)** and 2-[(2-nitro-1-phenylpropyl)thiomethyl]benzimidazole **(2)** derivatives and reference compound ketoconazole and ampicillin trihydrate were given as μM in Table 2.

RESULTS AND DISCUSSION

Chemistry

Synthesis and detailed structural analyses of compounds **(1)** and **(2)** were reported in our previous studies [16, 17]. Synthesis pathway of the addition products are given in Figure 1. Yields of synthesized compounds are in range of 70-90% for **(1)** derivatives and 20-80% for **(2)** derivatives. Derivatives of the Compound **(1)** and **(2)** are shown in Table 1.

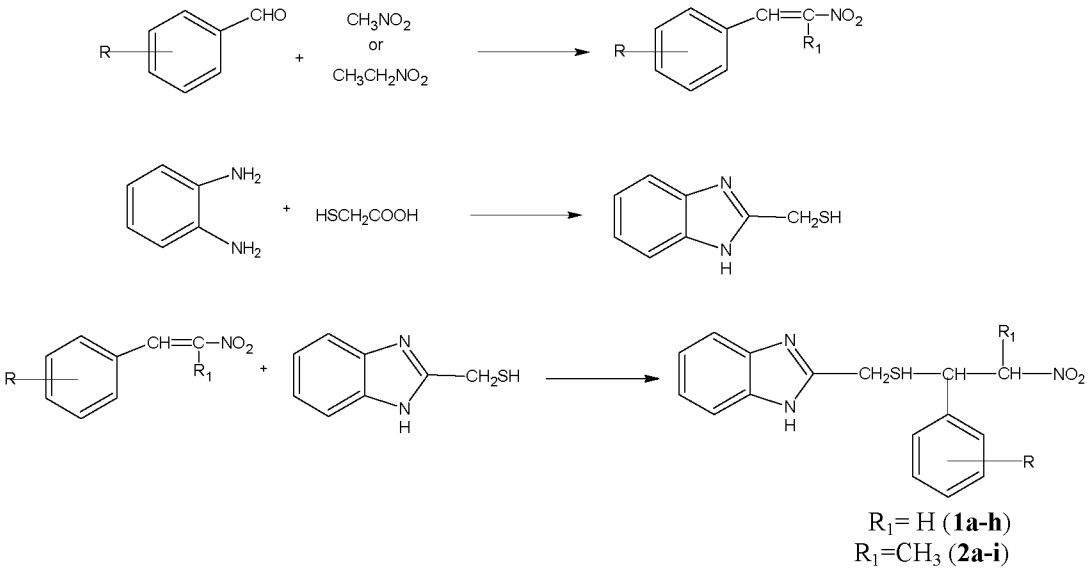
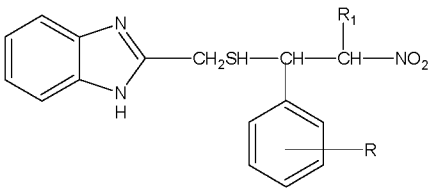


Figure 1. Synthesis of 2-[(2-nitro-1-phenylethyl)thiomethyl]benzimidazole **(1)** and 2-[(2-nitro-1-phenylpropyl)thiomethyl]benzimidazole **(2)** derivatives.

Table 1. Synthesized derivatives of 2-[(2-nitro-1-phenylethyl)thiomethyl]benzimidazole **(1a-h)** and 2-[(2-nitro-1-phenylpropyl)thiomethyl]benzimidazole **(2a-i)**.



Compound	R	R_1
1a	H	H
1b	4-CH_3	H
1c	4-Cl	H
1d	4-OCH_3	H
1e	4-OH, 3-OCH_3	H
1f	4-OC_2H_5	H
1g	4-C_2H_5	H
1h	4-NO_2	H
2a	H	CH_3
2b	4-CH_3	CH_3
2c	4-Cl	CH_3
2d	4-OCH_3	CH_3
2e	4-OH, 3-OCH_3	CH_3
2f	4-OC_2H_5	CH_3
2g	4-NO_2	CH_3
2h	4-Br	CH_3
2i	4-$N(CH_3)_2$	CH_3

Antibacterial and Antifungal Activity

Compounds **(1a-h)** and **(2a-i)** were tested for their antibacterial and antifungal activities against various strains by the microdilution method (19). For the determination of antibacterial activity *S. aureus* ATCC 25923, *S. faecalis* ATCC 19433, *P. aeruginosa* ATCC 27833 and *E. coli* ATCC 25922 strains were utilized. All the compounds were also tested *in vitro* for their antifungal activity against *C. albicans* ATCC 90528 and *C. parapsilosis* ATCC 90018 strains. Ampicillin trihydrate and ketoconazole were used as standard comparison materials. The MIC values of 2-[(2-nitro-1-phenylethyl)thiomethyl]benzimidazole **(1a-h)** and 2-[(2-nitro-1-phenyl propyl)thiomethyl]benzimidazole **(2a-i)** derivatives are given as μM in Table 2.

Table 2. The MIC values (μM) of 2-[(2-nitro-1-phenylethyl)thiomethyl]benzimidazole **(1a-h)** and 2-[(2-nitro-1-phenylpropyl)thiomethyl]benzimidazole **(2a-i)** derivatives.

Compound	A	B	C	D	E	F
1a	$1.68x10^{-2}$	$1.68x10^{-2}$	$3.35x10^{-2}$	$3.35x10^{-2}$	$1.05x10^{-3}$	$1.05x10^{-3}$
1b	$1.09x10^{-2}$	$1.09x10^{-2}$	$2.19x10^{-2}$	$2.19x10^{-2}$	$6.84x10^{-3}$	$6.84x10^{-3}$
1c	$2.72x10^{-2}$	$2.72x10^{-2}$	$2.72x10^{-2}$	$2.72x10^{-2}$	$2.12x10^{-3}$	$2.12x10^{-3}$
1d	$3.14x10^{-2}$	$3.14x10^{-2}$	$2.51x10^{-1}$	$2.51x10^{-1}$	$3.14x10^{-3}$	$3.14x10^{-3}$
1e	$3.06x10^{-2}$	$3.06x10^{-2}$	$3.06x10^{-2}$	$3.06x10^{-2}$	$9.56x10^{-3}$	$9.56x10^{-3}$
1f	$3.49x10^{-2}$	$3.49x10^{-2}$	$2.79x10^{-1}$	$2.79x10^{-1}$	$3.49x10^{-3}$	$3.49x10^{-3}$
1g	$1.40x10^{-2}$	$1.40x10^{-2}$	$2.79x10^{-1}$	$2.79x10^{-1}$	$8.70x10^{-3}$	$8.70x10^{-3}$
1h	$1.30x10^{-2}$	$1.30x10^{-2}$	$5.18x10^{-1}$	$5.18x10^{-1}$	$6.06x10^{-3}$	$6.06x10^{-3}$
2a	$1.19x10^{-2}$	$1.19x10^{-2}$	$1.90x10^{-1}$	$1.90x10^{-1}$	$4.77x10^{-2}$	$4.77x10^{-2}$
2b	$2.28x10^{-2}$	$2.28x10^{-2}$	$1.83x10^{-1}$	$1.83x10^{-1}$	$2.28x10^{-2}$	$2.28x10^{-2}$
2c	$2.15x10^{-2}$	$2.15x10^{-2}$	$1.72x10^{-1}$	$1.72x10^{-1}$	$8.63x10^{-2}$	$8.63x10^{-2}$
2d	$1.09x10^{-2}$	$1.09x10^{-2}$	$1.74x10^{-1}$	$1.74x10^{-1}$	$2.18x10^{-2}$	$2.18x10^{-2}$
2e	$2.08x10^{-2}$	$2.08x10^{-2}$	$1.67x10^{-1}$	$1.67x10^{-1}$	$8.36x10^{-2}$	$8.36x10^{-2}$
2f	$2.09x10^{-2}$	$2.09x10^{-2}$	$1.68x10^{-1}$	$1.68x10^{-1}$	$8.41x10^{-2}$	$8.41x10^{-2}$
2g	$2.09x10^{-2}$	$2.09x10^{-2}$	$1.68x10^{-1}$	$1.68x10^{-1}$	$4.19x10^{-2}$	$4.19x10^{-2}$
2h	$4.80x10^{-3}$	$4.80x10^{-2}$	$1.53x10^{-1}$	$7.49x10^{-2}$	$1.91x10^{-2}$	$1.91x10^{-2}$
2i	$5.20x10^{-3}$	$5.20x10^{-2}$	$1.68x10^{-1}$	$8.43x10^{-2}$	$2.10x10^{-2}$	$2.10x10^{-2}$
Ampicillin	$4.80x10^{-3}$	$4.80x10^{-3}$	$1.93x10^{-2}$	$9.70x10^{-3}$	-	-
Ketoconazole	-	-	-	-	$0.90x10^{-3}$	$0.90x10^{-3}$

A: *Staphylococcus aureus* ATCC 25923
B: *Streptococcus faecalis* ATCC 19433
C: *Pseudomonas aeruginosa* ATCC 27833
D: *Escherichia coli* ATCC 25922
E: *Candida albicans* ATCC 90528
F: *Candida parapsilosis* ATCC 90018

In general, the antibacterial activity of synthesized compounds against *S. aureus* and *S. faecalis* as *Gram-positive* bacteria and *P. aeruginosa* and *E. coli* as *Gram-negative* bacteria showed lower potencies than the standard drug ampicillin, except compound **(2h)** and **(2i)**. It was found that compound **(2)** derivatives were more active than compound **(1)** derivatives. Introduction of methyl group to the side chain resulted in increased antimicrobial activity of compounds **(2a-i)**. Compound **(1)** derivatives showed more antifungal activity almost 10 fold than compound **(2)** derivatives.

As it can be seen in Table 2 **(1a)**, **(1b)**, **(1g)** and **(1h)** are more effective against *Gram-positive* bacteria than Gram-*negative* bacteria compared to standard drug ampicillin.

Among the synthesized compound **(2)** derivatives, **(2h)** and **(2i)** were found to be the most active derivatives against *S. aureus*. The rest of the compound **(1)** and **(2)** derivatives showed only moderate activity towards *Gram-positive* and *Gram-negative* bacteria when compared to standard drug ampicillin.

The antifungal activity of synthesized compounds against *C. albicans* and *C. parapsilosis* showed lower potencies than the standard drug ketoconazole, except compound **(1a)**. Compound **(1a)** exhibited significant antifungal activity with MIC value of $1.05X10^{-3}$ μM, which was comparable with ketoconazole. It is well known that the antifungal drug

ketoconazole is used clinically to treat or suppress various infections (22, 23). This result suggests that compound **(1a)** may be worth further study in terms of their antifungal activity. We will continue our investigations to determine *in vivo* antifungal activity of compound **(1a)** and *in vivo* antibacterial activity of compound **(2h)** and **(2i)**.

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