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Prof. Dr. Feyyaz ONUR
Ankara Üniversitesi, Eczacılık Fakültesi, Analitik Kimya Anabilim Dalı,
06100 Tandoğan - ANKARA, *e-mail:* onur@pharmacy.ankara.edu.tr
Tel: (0312) 212 68 05 , *Fax :* (0312) 213 10 81

Editör Yardımcıları:
- Prof. Dr. Gülbin ÖZÇELİKAY *e-mail:* gozcelik@pharmacy.ankara.edu.tr
- Prof. Dr. İlkay YILDIZ *e-mail:* oren@pharmacy.ankara.edu.tr

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Editorial correspondence:
Prof. Dr. Feyyaz ONUR
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry,
06100 Tandogan - ANKARA, TURKEY, *e-mail:* onur@pharmacy.ankara.edu.tr
Tel: + 90312 2126805, *Fax:+ 90* 312 213 10 81

Editorial assistants:
- Prof. Dr. Gülbin ÖZCELİKAY *e-mail:* gozcelik@pharmacy.ankara.edu.tr
- Prof. Dr. İlkay YILDIZ *e-mail:* oren@pharmacy.ankara.edu.tr

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ANTIMICROBIAL ACTIVITY OF SOME THIADIAZOLYL- AND TRIAZOLYLBENZIMIDAZOLES

BAZI TİYADAZOUL- VE TRİAZOLİLBENZİMİDAZOL TÜREVLERİNİN ANTİMİKROBİYAL AKTİVİTELERİ

Canan KUŞ[1] Gülgün AYHAN-KILCIGİL[1*], Nurten ALTANLAR[2]

[1] Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Tandoğan - Ankara .TURKEY

[2] Ankara University, Faculty of Pharmacy, Department of Microbiology, 06100 Tandoğan-Ankara.TURKEY

ABSTRACT

In this study, thirty nine benzimidazole derivatives namely 1 -[(substituted thiocarbamoylhydrazine carbonyl)methyl\-2-phenyl-lH-benz.imidazoles ***la-13a,*** *N-/ (2 -phenylbenzimidazol-1 -yl methyl)-(1,3,4)-thiadiazoIe-2-yl/-substituted phenyl amines* ***lb-13b,*** *and 5-(2-phenyl benz.imidaz.ol-1-yl-methyl)-4-substituted phenyl-4H-1,2,4-triazole-3-thiones* ***lc-13c*** *were screened for their antimicrobial activities. Minimum Inhibitory Concentration (MIC) values of the compounds were determined by the tube dilution method using Staphylococcus aureus and Bacillus subtilis as Gram positive, Escherichia coli as Gram negative bacteria and Candida albicans, Candida krusei and Candida parapsilosis as yeast-like fungi. All of the compounds were inactive against S. aureus, C. krusei and C. parapsilosis Compounds 5b, 9a and* ***13a*** *(12.5 fig/ml) showed good inhibitory activity against C. albicans.*

Keywords: *Thiosemicarbazides, thiadiaz.olylbenz.imidaz.oles, triaz.olylbenzimidaz.oles, antimicrobial activity*

ÖZET

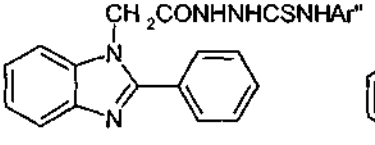
Bu calışmada, I -/(substitute tiyokarbamoilhidrazinkarbonil)metil/-2-fenil- lH-benzimidazol, ***la-13a,*** *N-[(2-fenilbenz.imidazol-1 -il metil)-/1,3,4/-tiyadiazol-2-ilj-substitue fenil arnin,* ***lb-13b,*** *ve 5-(2-fenil benz.imidaz.ol-1 -il- metil)-4-sübstitüe fenil-4H-l,2,4-triaz.oI-3-tiyon,* ***lc-13c,*** *türevi 39 adet bileşiğin antimikrobiyal aktiviteleri incelenmiştir. Bileşiklerin Minimum Inhibitor Konsantrasyon (MIK) değerleri Gram pozitif bakteriler olarak Staphylococcus aureus ve Bacillus subtilis, , Gram negatif bakteri olarak Escherichia coli ve maya-benzeri funguslar olarak da Candida albicans, Candida krusei ve Candida parapsilosis 'e karşı tüp dilüsyon metodu kullandarak saptanmıştır. Bileşiklerin hepsi S. aureus, C. krusei ve C. parapsilosis'e karşı etkisiz bulunmuşlardır. 5b, 9a ve* ***13a*** *bileşikleri C. albicans'a karşı 12.5 /ug/ml MIC değeriyle iyi antifungal aktivite göstermişlerdir.*

Anahtar kelimeler: Tiyosemikarbazitler, tiyadiazolilbenzimidazoller, triazolilbenzimidazoller, antimikrobiyal aktivite

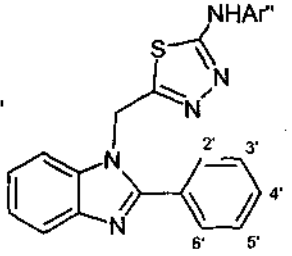
INTRODUCTION

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. In addition, primary and opportunistic fungal infections continue to increased number of immunocomprimised patients such as AIDS, cancer and organ transplantation. It is well known that benzimidazoles exhibit antimicrobial [1-6], antitubercular [7], antitumor [8-9] and anthelmintic [10], antiallergic [11-14], antioxidant [15] activities. It has been reported that thiadiazoles possess anti-inflammatory [16] and antimicrobial[6,17-18] activities. In addition, the triazoles display anti-inflammatory [16], antimicrobial [6,17-18], antiviral [19] and antioxidant [20] activities. Regarding this facts and continuation of our research on antimicrobial benzimidazoles, we report the antimicrobial testing thiosemicarbazides and their corresponding cyclized triazole and thiadiazole derivatives of benzimidazole.

Ar": Phenyl, 4-tolyl, 3-tolyl, 2-tolyl, 4-fluorophenyl, 3-fluorophenyl, 2-fluorophenyl, 4-chlorophenyl, 3-chlorophenyl, 2-chlorophenyl, 4-bromophenyl, 3-bromophenyl, 2-bromophenyl



1a-13a



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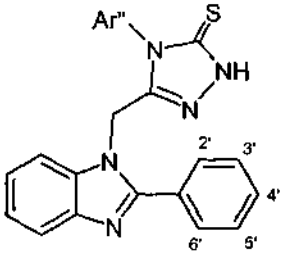

1c-13c

MATERIAL AND METHODS

The *in vitro* antimicrobial activity of the compounds was tested by the tube dilution technique [21]. Since the compounds have a poorly water-solubility each of the test compounds and standards ampicillin trihydrate, miconazole and fluconazole was dissolved in 12.5 % DMSO, at concentrations of 100 ug/ml, further dilutions of the compounds and standards in the test medium were prepared at the required quantities of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 ug/ml concentrations. The final inoculum size was 10^5 CFU/ml. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no antimicrobial activity against any of the test microorganism.

All the compounds were tested for their *in vitro* growth inhibitory activity against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 as Gram positive and *Escherichia coli* ATCC 25922 as Gram negative bacteria and *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 as fungi.

Antibacterial Activity Assay

The cultures were obtained in Mueller-Hinton Broth (Difco) for all the bacteria after 18-24 h of incubation at 37 ± 1 °C. Testing was carried out in Mueller-Hinton Broth at pH 7.4 and two-fold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 18-24 h at 37 ± 1 °C, the last tube with no growth of microorganism was recorded to represent MIC expressed in ug/ml.

Antifungal Activity Assay

The yeasts were maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1 °C. Testing was performed in Sabouraud Dextrose Broth at pH 7.4 and the two-fold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25 ± 1 °C, the last tube with no growth of yeast was recorded to represent MIC expressed in ug/ml.

RESULTS AND DISCUSSION

The synthesis and structural elucidation of the compounds were published in our previous study [22].

All of the compounds were evaluated for antimicrobial activity against in vitro *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Candida albicans, Candida krusei* and *Candida parapsilosis.* None of the compounds were active against S. *aureus, C. parapsilosis* and *C. krusei.* MIC values of the compounds and the standards are presented in Table 1.

Table 1. shows the results of *in vitro* activity determination by a tube dilution method [21] against *E. coli, B. subtilis* and *C. albicans.* As can be seen from Table the antifungal activity results of all compounds were found better than that of their antibacterial activity results. Antimicrobial activity of the tested compounds against *E.coli* and *Bsubtilis* exhibited rather lower potency than the standart drug ampicillin with the 50 / >50 MIC values. Among the compounds **la-13a,** it was observed that compounds 9a and **13a** which bearing 3-fluoro and 2-bromophenyl moieth as Ar" substituents, respectively, showed good inhibitor activity against *C.albicans* (12.5 ug/ml) which close to fluconazole (6.25 ug/ml). For the compounds **lb-13b,** compound 5b was the most active compound with the 12.5 ug/ml MIC value. Compounds 8c, 9b, **10a, 10b, 10c, 13b** and **13c** demonstrated some marginal activity (25 ug/ml) against C. *albicans.*

Table 1. The *in vitro* antifungal activity of the compounds **la-13a, lb-13b, lc-13c** (MIC, ug/ml)

NO	Ar"	**a**	**b**	**c**
la	Phenyl	>50	>50	>50
lb	Phenyl	>50	>50	>50
lc	Phenyl	>50	50	>50
2a	4-tolyl	>50	50	>50
2b	4-tolyl	50	>50	>50
2c	4-tolyl	>50	50	>50
3a	3-tolyl	>50	>50	>50
3b	3-tolyl	>50	>50	>50
3c	3-tolyl	>50	>50	>50
4a	2-tolyl	>50	>50	>50
4b	2-tolyl	>50	>50	>50
4c	2-tolyl	>50	>50	>50
5a	4-fluorophenyl	50	>50	>50
5b	4-fluorophenyl	50	50	12.5
5c	4-fluorophenyl	50	>50	>50
6a	3-fluorophenyl	>50	>50	>50
6b	3-fluorophenyl	>50	>50	>50
6c	3-fluorophenyl	>50	>50	>50
7a	2-fluorophenyl	50	50	>50
7b	2-fluorophenyl	>50	>50	>50
7c	2-fluorophenyl	>50	>50	>50

NO	Ar"	**a**	**b**	**c**
8a	4-chlorophenyl	>50	>50	>50
8b	4-chlorophenyl	>50	>50	>50
8c	4-chlorophenyl	>50	>50	25
9a	3-chlorophenyl	50	>50	12.5
9b	3-chlorophenyl	>50	>50	25
9c	3-chlorophenyl	>50	>50	>50
10a	2-chlorophenyl	50	>50	25
10b	2-chlorophenyl	50	>50	25
10c	2-chlorophenyl	50	>50	25
11a	4-bromophenyl	>50	>50	>50
11b	4-bromophenyl	>50	>50	>50
11c	4-bromophenyl	50	>50	>50
12a	3-bromophenyl	>50	>50	>50
12b	3-bromophenyl	>50	>50	>50
12c	3-bromophenyl	50	>50	>50
13a	2-bromophenyl	>50	>50	12.5
13b	2-bromophenyl	50	>50	25
13c	2-bromophenyl	>50	>50	25
A		3.125	1.625	NT
M		NT	NT	3.125
F		NT	NT	6.25

a: *E. coli* b: *B. subtilis* c: *C. albicans* A: ampicillin M: miconazole F: Fluconazole

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