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ANTIMICROBIAL ACTIVITY OF 4-SUBSTITUTED- STYRYL-2-AZETIDINONES

Sema TAN[1], Vildan Adar GÜNER[2,*], Aysun ERGENE[1]

[1] Kırıkkale University, Department of Biology, 71450, Yahşihan-Kırıkkale,TURKEY
[2] Hacettepe University, Department of Chemistry, 06532, Beytepe-Ankara, TURKEY

Abstract

In this study, antimicrobial activity of previously synthesized 1-(Substituted phenyl)-4-(substituted styryl)-2-azetidinones(3a-i) have been examined. All compounds have been tested against Gram(+) and Gram(-) bacteria and yeasts. It was found that activity is not significantly influenced by the substituents on the ring.

Key words: 1-(substitutedphenyl)-4-(substituted styryl)2-azetidinones, *antimicrobial* activity.

4-Substitue-stiril-2-azetidinonlarin Antimikrobiyal Aktivitesi

Bu çalışmada, daha onceden sentezleri yapılan 1-(substitutefenil)-4-(substituestril)-2-azetidinonların (3a-i) antimikrobiyal aktiviteleri incelenmistir. Bütün bilesikler Gram (+) ve Gram (-) bakterilere ve mayaya karşı test edilmistir. Aktivite, halkadaki substituentlerden önemli derecede etkilenmediği bulundu.

Anahtar kelimeler: *1-(substitutefenil)-4-(substitestril)-2-azetidinonlar, antimikrobiyal aktivite.*

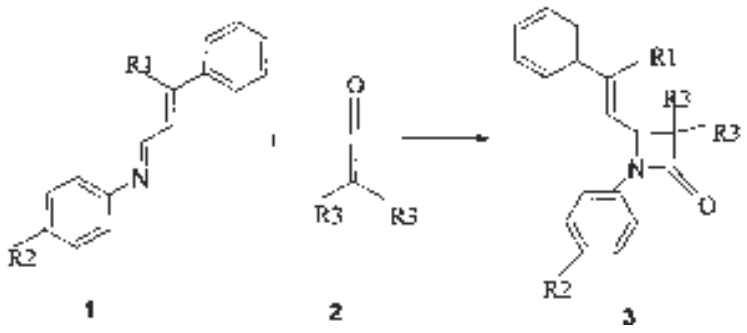
***Corresponding author:** vildan@hacettepe.edu.tr
Fax:+90-312-2992163

INTRODUCTION

Monocyclic β-lactams are an important class of hetero cyclic compounds because of their use in the synthesis of biologically active classical or non-classical β-lactam antibiotics(1-3). Recently anticancer activity of mono- and bicyclic β-lactam systems (4-6) are discovered. Previous studies on 1,4-diarylazetidinones (7) demonstrated the possibility of using the system for designing cytotoxic compounds by employing a variety of aromatic and heterocyclic ring systems in positions 1 and 4.

We have previously reported antibacterial activity of 1,4-diaryl-2-azetidinones (8). Activity against Gram (-) bacteria and fungi has been observed. It was studied that ethylenes in which 2-azetidinone is a heterocyclic system have shown activity (9). Duirno and et al. shown that 1,3,4-triaryl-2-azetidinones have remarkable activity against *Pseudomonas aeruginosa* (10). It was noted that the activity is more significantly influenced by the para substituent at 4-aryl group than at the 1-aryl group. Walsh and et al. studied antifungal and antibacterial activity for 3-acetoxy- and 3-hydroxy-azetidinones (11). Many of compounds showed antifungal and anti bacterial activity

In this study, antibacterial activity of synthesized 1-(Substituted phenyl)-4-(substituted styryl)-2-azetidinones(3a-i) against Gram(+) and Gram(-) bacteria and fungi have been evaluated. All compounds are given in Figure 1. The products of 1-azabutadienes (1a-i) with diphenyl- and dichloroketene, generated in situ from diphenyl acetyl chloride and dichloro acetyl chloride on triethylamine in benzene, respectively, resulted β-lactams(3a-i), were characterized by ^{1}H-NMR, IR- spectra. X-ray analysis of compound 3b (12) and 3i (13) was determined.



Comp.3	R1	R2	R3
a	Ph	H	Ph
b	Ph	OMe	Ph
c	Ph	Cl	Ph
d	Ph	Me	Ph
e	H	H	Ph
f	H	OMe	Ph
g	H	Me	Ph
h	H	Cl	Ph
i	H	OMe	Cl

Figure 1. Cycloaddition reactions of 1-aza-1,3-butadienes(1a-i) with diphenyl- and dichloroketene.

EXPERIMENTAL

Chemistry

We have previously described the chemistry employed in the synthesis and characterization of 1-(Substituted phenyl)-4-(substituted styryl)-2-azetidinones(3a-i) (14).

Microbiological studies

Microorganisms

Compounds (**3a-i**) were subjected to an antimicrobial screening procedure against Gram(+) and Gram(-) strains of *Staphylococcus aureus ATCC 25923*; *Bacillus subtilis ATCC 6633; Escherichia coli ATCC 35218; Pseudomonas aeruginosa ATCC 10145*; *Candida albicans ATCC 90028*, *Candida glabrata ATCC 90030.* The microorganism used in this study was obtained from Hacettepe University.

Medium

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

Equipment

Falcon® 96-well microplates were used for the microdilution method. A Brinkmann transferpette® was used for two fold-dilution of the compounds in the wells.

Method

The microdilution method was employed for antibacterial and antifungal activity tests (15, 16). For the antifungal activity test, 0.1 ml Sabouraud liquid medium and for the antibacterial activity test 0.1 ml Mueller Hinton medium were placed into each well of the microplates. 0.1 ml of the compound solution in %10 DMSO at 1000 μg/ml concentration was added into the first raws of microplates; ampicillin anhydrate and griseofulvin were used as control agents under the same conditions. Double dilutions of the compounds and standard 250, 125, 0.1 μg/ml were made by dispensing the solutions to the remaining wells. 0.1 ml microorganism suspensions, at 10^6 cfμ/ml (colony forming unit/ml) concentration, were inoculated into all the wells. The sealed microplates were incubated at 36°C for 24 and 36 h in the humid chamber. The lowest concentration of the compound that completely inhibits macroscopic growth was controlled and MIC (minimum inhibitory concentration) reported. MIC values of the two derivatives, ampicillin anhydride and griseofulvin as standards and nine compounds have been shown as μg/ml in Table 1.

RESULTS AND DISCUSSION

Compounds 3a-i were evaluated for their in vitro antimicrobial activity against some Gram(+) bacteria and yeast (Table 1). Their antimicrobial activity was determined as MIC values. The activity of the compounds 3a-i, reported in Table 2. The inhibitory effect is found against Gram (-) bacteria. Compounds 3a.3b,3c and 3d were particularly interesting against *Pseudomonas aeruginosa*.

Table 1. Antibacterial and antigungal activity of compounds 3a-i and the standard drugs (MIC/ml)[a]

Compound 3	S.a	B.s	E.coli	P.a	C.a	C.g
a	> 250	> 250	250	125	125	125
b	> 250	> 250	250	125	125	125
c	> 250	> 250	250	125	125	125
d	125	> 250	250	125	125	125
e	125	> 250	250	> 250	125	125
f	250	> 250	250	> 250	125	125
g	> 250	> 250	250	> 250	125	125
h	> 250	> 250	250	> 250	125	125
i	125	> 250	250	> 250	125	125
Ampicillin	0.1	0.1	62.5	62.5	-	-
Greseofulvin	**-**	**-**	**-**	**-**	**62.5**	**62.5**

a Microorganisms selected are as follows: **S.a**, *Staphylococcus aureus ATCC 25923*; **B.s,** *Bacillus subtilis ATCC 6633;* **E.c**, *Escherichia coli ATCC 35218;* **P.a**, *Pseudomonas aeruginosa ATCC 10145*; **C.a**, *Candida albicans ATCC 90028***, C.g**, *Candida glabrata ATCC 90030.*

Compounds 3d, 3e and 3i showed activity against Gram (+) bacteria (*Staphylococcus aureus*). This compounds showed better activity than others against Gram (+) bacteria *Staphylococcus aureus* . No activity against *Bacillus subtilis* was noted.

The reported data suggest that the unexpected activity of all compounds against yeast is not significantly influenced by substituents.

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