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RESEARCH ARTICLE

# Evaluation of Antimicrobial Activities of Some 2(3*H*)-Benzoxazolone Derivatives

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***Evaluation Of Antimicrobial Activities Of Some 2(3H)-Benzoxazolone Derivatives***

***Summary***

*In this study, 12 w-(2-oxo-2-benzoxazoline-3-yl)-N-phenylacetamide and propionamide derivatives bearing substituents with different lipophilic and electronic nature on N-phenyl ring were tested for antimicrobial activity. For this purpose, minimal effective concentrations of the compounds were determined against Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Candida albicans by using microdilution method. The results demonstrated that 3-(2-oxo-2-benzoxazoline-3-yl)-N-(m-tolyl)propionamide derivative was the most active compound in the series against Escherichia coli, Staphylococcus aureus and Enterococcus faecalis.*

***Key Words:*** *Antimicrobial activity, 2(3H)-Benzoxazolone, amide, synthesis.*

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***Bazı 2(3H)-Benzoksazolon Türevlerinin Antimikrobiyal Aktivitelerinin Değerlendirilmesi***

***Özet***

*Bu çalışmada, N-fenil halkasında farklı lipofilik ve elektronik özelliklere sahip sübstitüent taşıyan w-(2-okso-2-benzoksazolin-3-il)-N-fenilasetamit ve propiyonamit türevi oniki adet bileşiğin antimikrobiyal aktiviteleri test edilmiştir. Bileşiklerin minimum inhibitör konsantrasyon (MİK) değerleri, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa ve Candida albicans üzerinde mikrodilüsyon metodu kullanılarak tayin edilmiştir. Sonuçlar, çalışılan bileşikler arasında Escherischia coli, Staphylococcus aureus ve Enterococcus faecalis'e karşı 3-(2-okso-2-benzoksazolin-3-il)-N-(m-tolil)propiyonamit türevinin en aktif bileşik olduğunu göstermiştir.*

***Anahtar kelimeler:*** *Antimikrobiyal aktivite, 2(3H)-Benzoksazolon, Amit, Sentez.*

## INTRODUCTION

Because of the increasing resistance problem, current antimicrobial drug therapy clearly is in need of new chemical entities with enhanced activity profiles. In order to realize those expectations, efforts to develop new antimicrobial drugs directed to novel microbial targets seem to have precedence, in addition to the conventional approaches consisting of derivatization of current agents (1-5).

On the other hand, 2(3*H*)-Benzoxazolone, as one of the most versatile heterocyclic rings, produces diverse compounds with a wide range of biological activities, such as antibacterial-antifungal, analgesic-antiinflammatory, anticonvulsant, dopaminergic, and human immunodeficiency virus (HIV)-1 reverse transcriptase activities (6-18).

The above statements encouraged us to investigate the potential antimicrobial activity of a group of 2-(2-oxo-2-benzoxazoline-3-yl)-*N*-phenylacetamide and propionamide derivatives bearing substituents with different lipophilic and electronic nature on *N*-phenyl ring.

For this purpose, 12 2(3*H*)-Benzoxazolone compounds (Table 1), 11 of which (compounds 1-11) were synthesized in our previous study (19), were evaluated for the first time

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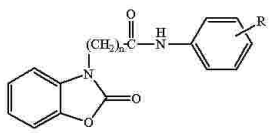
against Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Candida albicans by using microdilution method (20).

**Table 1.** Structures of the tested compounds

| Compound No. | n | R |
|---|---|---|
| 1 | 1 | H |
| 2 | 2 | H |
| 3 | 1 | *o*-CH3 |
| 4 | 1 | *m*-CH3 |
| 5 | 1 | *p*-CH3 |
| 6 | 2 | *m*-CH3 |
| 7 | 2 | *p*-CH3 |
| 8 | 1 | *p*-Cl |
| 9 | 2 | *o*-Cl |
| 10 | 2 | *m*-Cl |
| 11 | 2 | *p*-Cl |
| 12 | 1 | *o*-OCH3 |



## MATERIALS and METHODS

### Chemistry

The melting point was determined on an Electrothermal IA9100 Melting Point apparatus and was uncorrected. The IR spectra of compound 12 were recorded as potassium bromide pellets on a Jasco FT/IR-400 spectrometer. The NMR spectra were recorded on a Varian AS 400 Mercury Plus NMR using DMSO-$d_6$ as the solvent. Chemical shifts were reported in parts per million (d). *J* values were given in Hz. Liquid chromatography and mass spectrometry (LC-MS) spectra (atmospheric pressure ionization with electrospray [API-ES]) were measured on an Agilent 1100 MSD spectrometer. Elemental analysis (C, H, N) was performed with Leco-CHNS 932 and within ± 0.4% of the theoretical values.

The synthesis and detailed analytical data of compounds 1-11 were reported in previous studies (19,21,22). Compound 12 was prepared in two-step synthesis according to the reported procedure (Scheme 1) (23). In the first step, 2-chloroacetyl chloride was reacted with o-methoxyaniline to yield corresponding 2-chloro-*N*-(*o*-methoxyphenyl) acetamide. In the second step, the intermediate was refluxed with 2(3*H*)-Benzoxazolone in acetone to furnish compound 12. 2(3*H*)-Benzoxazolone was prepared by the reaction of *o*-aminophenol with urea as described in the literature (24).

Compound 12 is listed in the literature with the registry number CASRN 440660-79-7, but corresponding scientific reference data are not available. The physical, analytical and spectral data are originally reported in our study.

2-(2-oxo-2-benzoxazoline-3-yl)-*N*-(o-methoxyphenyl) acetamide (12)

Yield 48 %, mp 194 °C. IR (KBr): 3309, 1764, 1679, 1542, 1256, 1033 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 3.80 (3H, s, $OCH_3$, min), 3.87 (3H, s, OCH3), 4.43 (1H, d, *J*=17.6 Hz, $CH_2$, min), 4.57 (1H, d, *J*=17.6 Hz, $CH_2$, min), 4.82 (2H, s, $CH_2$), 6.83-6.91 (1H, m, Ar-H), 6.83-6.91 (1H, m, Ar-H, min), 6.95 (1H, dd, *J*=1.4, 8.0 Hz, Ar-H, min), 7.04-7.30 (5H, m, Ar-H), 7.04-7.30 (5H, m, Ar-H, min), 7.34 (1H, d, *J*= 2.0 Hz, Ar-H, min), 7.36 (1H, d, *J*= 7.8 Hz, Ar-H-Phenyl), 7.46 (1H, td, J= 1.8, 8.0 Hz, Ar-H, min), 7.90 (1H, d, *J*= 7.8 Hz, Ar-H-Phenyl), 9.69 (1H, bs, NH) ppm. API-ES: m/z 299 $[M+H]^+$. Calcd. for $C_{16}H_{14}N_2O_4$: C 64.42, H 4.73, N 9.39. Found: C 64.02, H 4.997, N 9.467.

### Microbiology

Minimum inhibitory concentrations (MICs) of the compounds were determined by the broth microdilution testing method recommended by the Clinical and Laboratory Standards Institute (CLSI) (20). The in vitro antimicrobial activity of the compounds was evaluated against standard strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 90028.

### Determination of Antimicrobial Activities

Antibacterial and antifungal activity assays were performed in Mueller-Hinton broth (Oxoid) and Sabouraud dextrose broth (Oxoid) liquid mediums respectively. Stock solutions of all compounds at 2048 µg/ml concentration were prepared by dissolving in pure DMSO or mixture of 250 µl distilled water + 750 µl DMSO. Serial dilutions of the stock solutions were done from 512 to 0.25 µg/ml in 96-wells plates. 24-hour (h) cultures of microorganisms were diluted to give a final concentration of approximately 106 cfu/ml and added to each well. The plates were incubated at 35 °C for 24 h. After incubation, concentrations of the compounds that prevented visible growth were recorded as the MICs. For quality control of the method, ciprofloxacin was tested as antimicrobial agent in parallel. The procedure was carried out twice.

## RESULTS AND DISCUSSION

### Chemistry

Eleven derivatives (compounds 1-11) of the title compounds were synthesized in our previous study and their structural confirmation was performed by elemental, $^1$H NMR, IR and ESI-MS analysis and already published (19). Compound 12, namely 2-(2-oxo-2-benzoxazoline-3-yl)-*N*-(*o*-methoxyphenyl)acetamide, was prepared by two-step synthesis for this study. The structural confirmation of compound 12 was performed by analytical and spectral data. The result of elemental analysis was within ± 0.4% of the theoretical values. The IR spectrum of compound 12 presented the expected characteristic absorption bands indicating the amide carbonyl (1679 cm$^{-1}$), amide N-H (3330 cm$^{-1}$) and 2(3*H*)-Benzoxazolone carbonyl stretching (1764 cm$^{-1}$) bands (25,26).

Concerning the $^1$H NMR spectrum, an intriguing feature was some unexpected minor signals, which resembled the major ones, indicating the existence of two different rotamers with 0.6/1.2 minor/major ratio. The rotamer formation could well be related to the restricted rotation of amide *N*-CO bond causing the E/Z rotamers (27). This is a well-known situation for our title compounds and has been discussed extensively in our previous study (19). The assessment of the chemical shifts in the $^1$H NMR spectrum demonstrated that the aromatic and aliphatic protons were observed in the expected regions with expected multiplicities confirming the substitution pattern.

Mass spectra (API-ES) of compound 12 provided $[M+H]^+$ ion with 299 m/z value, which is completely consistent with the expected molecular weight.

### Microbiology

The antimicrobial activity of compounds 1-12 were evaluated for the first time against *S. aureus, E. faecalis, E. coli, P. aeruginosa,* and *C. albicans* using the microdilution method. The results were expressed as MICs, which account for the minimum concentration of a compound inhibiting the growth of the tested microorganisms, and results are summarized in Table 2.

The MIC values of the compounds against Gram (-) (*E. coli* and *P. aeruginosa*) and Gram (+) (*S. aureus* and *E. faecalis*) bacteria were within the range 8-512 µg/ml, indicating lower potencies in comparison with the standard drug ciprofloxacin (20). In general, there was no marked difference between the acetamide and propionamide counterparts in terms of antibacterial activity. Concerning Gram (+) antibacterial activity, introducing chlorine atom on the para position of the *N*-phenyl ring increased the activity in both series against S. aureus (see compounds 8 and 11 in Table 2). Similarly, in the propionamide series, a meta or para methyl substitution on the *N*-phenyl ring increased the activity significantly against *S. aureus* and *E. faecalis* (see compounds 6 and 7 in Table 2). In terms of Gram (-) activity, all derivatives except compound 1 had equal antibacterial activity against *P. aeruginosa*. However, compound 6 demonstrated superior activity against *E. coli* in comparison to the other derivatives.

**Table 2.** Minimum Inhibitory Concentration (MIC) values of the title compounds (µg/ml)

| Compound No. | *E. coli* | *P. aeruginosa* | *S. aureus* | *E. faecalis* | *C. albicans* |
|---|---|---|---|---|---|
| 1 | 512 | 512 | 512 | 512 | 256 |
| 2 | 512 | 256 | 256 | 128 | 128 |
| 3 | 512 | 256 | 512 | 128 | 128 |
| 4 | 512 | 256 | 256 | 256 | 256 |
| 5 | 512 | 256 | 512 | 256 | 128 |
| 6 | 8 | 256 | 8 | 32 | 128 |
| 7 | 512 | 256 | 32 | 64 | 128 |
| 8 | 256 | 256 | 64 | 128 | 128 |
| 9 | 512 | 256 | 128 | 256 | 128 |
| 10 | 512 | 256 | 128 | 256 | 128 |
| 11 | 256 | 256 | 64 | 128 | 128 |
| 12 | 256 | 256 | 128 | 256 | 256 |
| Ciprofloxacin | < 0.03 | 0.12 | < 0.03 | 0.25 | - |

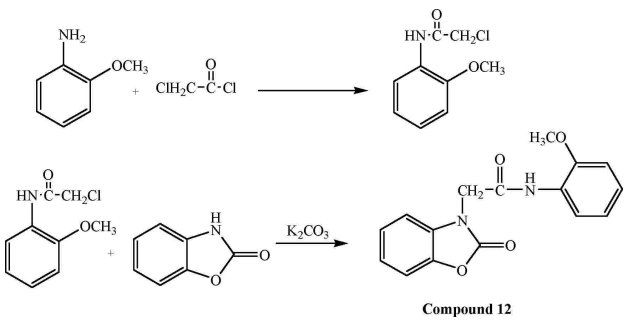



**Figure 1:** The synthesis of compound 12

Concerning the antifungal activity of compounds against *C. albicans*, all derivatives except compounds 1, 4 and 12, which were less potent, showed equal activity with 128 µg/ml MIC value.

In conclusion, compound 6, namely 3-(2-oxo-2-benzoxazoline-3-yl)-*N*-(*m*-tolyl) propionamide, was the

most active derivative in terms of Gram (-) and Gram (+) antibacterial activity in the series studied and can be used as a template for the further structural variations to explore more active 2(3*H*)-Benzoxazolone derivatives with antibacterial activity.

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