FABAD J. Pharm. Sci., 26, 169-172, 2001

RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

Evaluation of Antimicrobial Activities of Some 2-Benzoxazolinone Derivatives

Meral ÖZALP*, Ünsal ÇALIŞ**°, Nesrin GÖKHAN**, Hakkı ERDOĞAN**

Evaluation of Antimicrobial Activities of Some 2-Benzoxazolinone Derivatives

Summary : *In this study, six 3-methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinones and their reduction products were screened for their antibacterial and antifungal activities. The minimum inhibition concentration (MIC) values of the compounds were determined by the broth microdilution method using two Gram positive (*Staphylococcus aureus *ATCC 25923,* Enterococcus faecalis *ATCC 29212), two Gram negative (*Escherichia coli *ATCC 25922,* Pseudomonas aeruginosa *ATCC 27853) bacteria and yeast-like fungi (*Candida albicans *ATCC 90028,* Candida krusei *ATCC 6258,* Candida parapsilosis *ATCC 22019). None of the compounds were found to be active against both Gram positive and Gram negative bacteria. 3-Methyl-6-(1-hydroxy-2-morpholinopropyl)-2-benzoxazolinone* ***(Compound III)*** *which possesses remarkable activity against* Candida krusei *was found to be the most active compound in this series.*

Key Words: *2-Benzoxazolinone, antimicrobial activity, antibacterial and antifungal activities, in vitro studies.*

Bazı 2-Benzoksazolinon Türevlerinin Antimikrobial Aktivitelerinin Değerlendirilmesi

Özet : *Bu çalışmada, altı tane 3-metil-6-(2-sübstitüe aminopropanoil)-2-benzoksazolinon ve redüksiyon ürünlerinin antibakteriyal ve antifungal aktiviteleri incelenmiştir. Bileşiklerin minimum inhibisyon konsantrasyonları (MİK) ikisi Gram pozitif (*Staphylococcus aureus *ATCC 25923,* Enterococcus faecalis *ATCC 29212) ve ikisi Gram negatif (*Escherichia coli *ATCC 25922,* Pseudomonas aeruginosa *ATCC 27853) olmak üzere dört çeşit bakteri ve maya benzeri funguslar (*Candida albicans *ATCC 90028,* Candida krusei *ATCC 6258,* Candida parapsilosis *ATCC 22019) kullanılarak mikrodilüsyon yöntemi ile tayin edilmiştir. Bileşiklerin hiçbiri Gram pozitif ve Gram negatif bakterilere karşı etkili bulunmamıştır.* Candida krusei*'ye karşı önemli bir aktivite gösteren 3-metil-6-(1-hidroksi-2-morfolinopropil)-2-benzoksazolinon* ***(Bileşik III)*** *seride en aktif bileşik olarak bulunmuştur.*

Anahtar kelimeler : *2-Benzoksazolinon, antimikrobiyal aktivite, antibakteriyal ve antifungal aktivite, in vitro çalışma.*

Received : 30.5.2001
Revised : 5.10.2001
Accepted : 10.10.2001

INTRODUCTION

Part of our research concerns the synthesis of 3-,6-disubstituted-2-benzoxazolinone derivatives and the evaluation of their analgesic-antiinflammatory and antimicrobial properties. In a previous paper we described the synthesis, structural elucidation and antinociceptive activity of 3-methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinone and 3-methyl-6-(1-hydroxy-2-substituted aminopropyl) - 2 - benzoxazolinone derivatives[1] (Fig. 1).

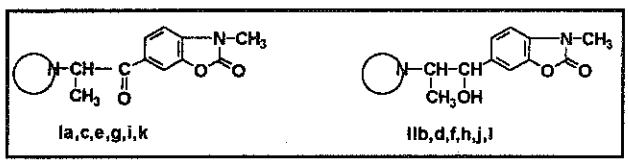


Figure 1 : Structure of the tested compounds Ia-III

2-Benzoxazolinone derivatives are one of the much studied groups. To date, over 1000 different analogues have been synthesized. This means that vari-

* Hacettepe University, Faculty of Pharmacy, Pharmaceutical Microbiology Department, 06100 Ankara - TURKEY
** Hacettepe University, Faculty of Pharmacy, Pharmaceutical Chemistry Department, 06100 Ankara - TURKEY
° Correspondence

ous side chains can be altered and the resulting analogues can be tested for different activities. It has been reported that there are many reports containing accounts of various effects of 2-benzoxazolinone so far[2-11] and some of them are related to antimicrobial effects of the ring system[2-5].

The discovery that 2-benzoxazolinone derivatives have promising effects against *E.coli* has caused new investigations on this type of compound for antibacterial activity[12]. Then Virtanen et al.[13] established the other derivatives exhibiting inhibitory activity against some other bacteria. It is also known that halogen-substituted 2-benzoxazolinones have well expressed fungicide properties[2].

In view of these observations, it was thought worthwhile to evaluate the antimicrobial activity of some previously synthesized 3-methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinone and 3-methyl-6-(1-hydroxy-2-substituted aminopropyl) - 2 - benzoxazolinone derivatives.

EXPERIMENTAL PART

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method following the procedures recommended by the National Committee for Clinical Laboratory Standards[14,15]. Two Gram positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212) and two Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacteria were used as quality control strains. For testing antifungal activities of the compounds, these reference strains were tested: *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019.

Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA) was used when examining bacterial strains. For Candida species, RPMI-1640 medium with L-glutamin, buffered with MOPS (ICN, FLOW; Aurora, OH, USA) was used. The inoculum densities were $5x10^5$ cfu/ml and $0.5\text{-}2.5x10^3$ cfu/ml for bacteria and fungi, respectively. The compounds under test were dissolved in 100 % dimethylsulfoxide and the final two fold concentrations were prepared from 512 g/ml to 0.5 g/ml. Amikacine and fluconazole were used as standard drugs for bacteria and fungi, respectively. Two fold dilutions were prepared from 64 g/ml to 0.0625 g/ml for each of these antibiotics. MICs were determined after incubation for 24 h at 35°C for bacteria and 48 h at 35°C for fungus. Minimum inhibitory concentrations were defined as the lowest concentrations of the antimicrobial agents that inhibited visible growth of the microorganisms.

RESULTS AND DISCUSSION

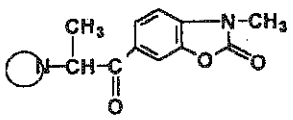
The synthesis and structural elucidation of the compounds 3-methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinone and their reduction derivatives (3-methyl-6-(1-hydroxy-2-substituted aminopropyl)-2-benzoxazolinone) were published in our previous paper[1] (Fig. 1).

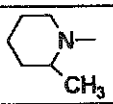
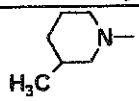
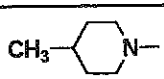
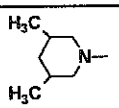
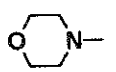
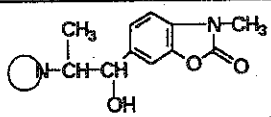
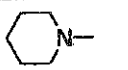
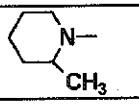
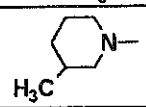
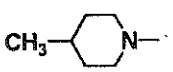
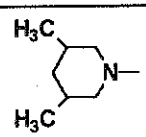
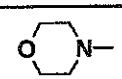
Compounds Ia-Ik and IIb-III were tested for antibacterial and antifungal activities against various strains by the microdilution method. For the determination of antibacterial activity, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were employed. All the compounds were also tested for in vitro antifungal activity against *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019.

The antibacterial activity results of the compounds Ia-III against Gram-positive and Gram-negative bacteria are shown in the Table 1. According to the values, antibacterial activities of the compounds are not close to that of Amikacine which is used as control agent. None of the tested compounds was found to be active. However, compound Ig, Ik, and III were found to have a comparable activity, having MIC values of 128 g/ml, to amikacine against *E.faecalis*.

The results of screening for antifungal activity of Ia-III are reported in the Table 1. Examination of the data in the Table revealed that compound III was the most active compound against *C.krusei*. In view of antimicrobial activity results, we assume that compound III (3-methyl-6-(1 - hydroxy - 2 - morpho-

Table 1 : Antibacterial and antifungal activity results of the tested compounds (MIC µg/ml)



Com. No		Bacteria				Fungi		
		A	B	C	D	E	F	G
Ia		128	256	256	256	128	128	64
Ic		128	256	256	256	64	128	64
Ie		128	256	256	256	128	128	128
Ig		256	128	128	256	128	128	64
Ii		256	256	256	256	128	128	128
Ik		256	128	256	128	128	128	64
								
IIb		256	256	256	128	128	128	64
IId		256	256	256	128	64	128	128
IIf		256	256	256	128	64	128	128
IIh		256	256	256	256	64	128	128
IIj		512	256	256	256	128	128	128
III		128	128	256	256	128	64	128
Amikacine		4	64	0.25	0.25	-	-	-
Fluconazol		-	-	-	-	0.25	32	1

A: *S.aureus*, B: *faecalis*, C: *E.coli*, D: *P.aeruginosa*, E: *C.albicans*, F: *C.krusei*, G: *C.parapsilosis*.

linopropyl)-2-benzoxazolinone) is the most active compound in the series.

Finally, this study suggests that antifungal activities of 2-benzoxazolinone derivatives are more effective than their antibacterial activities.

REFERENCES

1. Çalış Ü, Gökhan N, Erdoğan H. synthesis of some novel 3-methyl-6-(2-substituted propanoyl/propyl)-2-benzoxazolinone derivatives and anti-nociceptive activity, *IL Farmaco*, 56, 719-729, 2001.
2. Varma RS, Nobles LW. Synthesis and antibacterial activity of certain 3-substituted benzoxazolinones, *J. Pharm. Sci.*, 57(1), 39-44, 1968.
3. Gupta AKS, Garg M, Chandra U. Synthesis of new mannich bases derived from substituted benzimidazole, benzoxazol-2-one, benzoxazol-2-thione, oxadiazol-2-thiones and their biological activities; *J. Indian Chem. Soc.*, 56(12), 1230-1232, 1979.
4. Shibata T, Sugimura Y. Synthetic studies of 1--methylcarbapenem antibiotics, *J. Antibiot.*, 42 (3), 374-381, 1989.
5. Kalcheva V, Mincheva Z, Andreeva P. Synthesis and in vitro activity of new cephalosporin derivatives containing a benzoxazolone ring, *Arzneim.-Forsch.*, 40(9), 1030-1034, 1990.
6. Rodgers JD, Johnson BL, Wang H, Greenberg RA, Erickson-Viitanen S, Klabe RM, Cordova BC, Rayner MM, Lam GN, Chang CH. Potent cyclic urea HIV protease inhibitors with benzofused heterocycles as P2/P2' groups; *Bioorg. Med. Chem. Let.*, 6(24), 2919-2924, 1996.
7. Abdelaal SM, Kong SB, Bauer L. Synthesis of 1-[3-Methyl-2(3H) - benzoxazolon or 6-y]-4-{4-[cis-2-(2,4-dichlorophenyl) - 2 - (1H-imidazol-1-ylmethyl) - 1,3 - dioxolan-4-yl]methyleneoxyphenyl} piperazines, *J. Heterocyclic Chem.*, 29, 1069-1076, 1992.
8. Von der Saal W, Hölck JP, Kampe W, Mertens A, Müller-Beckmann B. Nonsteroidal cardiotonics. 2. The inotropic activity of linear, tricyclic 5-6-5 fused heterocycles; *J. Med. Chem.*, 32, 1481-1491, 1989.
9. Bonte JP, Piancastelli MC, Lesieur I, Lamar JC, Beaughard M, Dureng G. Amino ketone and amino alcohol derivatives of benzoxazolinone: synthesis, adrenergic and antihypertensive properties; *Eur. J. Med. Chem.*, 25, 361-368, 1990.
10. Bagley JR, Thomas SA., Rudo FG, Spencer HK, Doorley BM, Ossipov MH, Jerussi TP, Benvenga MJ, Spaulding T. New 1-(heterocyclyalkyl)-4-(propionanilido)-4-piperidinyl methyl ester and methylene methyl ether analgesics; *J .Med.Chem.*, 34, 827-841, 1991.
11. Gökhan N, Erdoğan H, Durlu T, Demirdamar R. Novel antiinflammatory analgesics: 3-(2-/4-pyridylethyl)-benzoxazolinone and oxazolo [4,5-b]pyridin-2-one derivatives, *Arch. Pharm.*, 332 (2), 43-49, 1999.
12. Lespagnol A, Vincent M, Lespagnol C. Etude de la benzoxazolone de certains de ses derives, *Bull. Soc. Pharm. Lille*, 35, 10-17, 1953.
13. Virtanen AI, Hietalo PK, Wahlroos O. An antifungal factor in maize and wheat plants, *Suomen Kemistil*, 29B, 143, 1956.
14. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *Approved Standard.* M7-A4, Viallanova, PA, 1997.
15. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: *Approved Standard.* M27-A, Viallanova, PA, 1997.