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Original Article

MICROWAVE SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 5-CHLORO-2(3H)-BENZOXAZOLINONE-3-ACETYL-2-(p-SUBSTITUTED BENZAL)HYDRAZONE AND 5-CHLORO-2(3H)-BENZOXAZOLINONE-3-ACETYL-2-(p-SUBSTITUTED ACETOPHENONE)HYDRAZONE DERIVATIVES

Tijen ÖNKOL[1] Mehtap GÖKÇE[1]*, Ali Ulvi TOSUN[1], Serpil POLAT[2], Mehmet S. SERIN[2], Seda TEZCAN[3]

[1] Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06330, Ankara-TURKEY.
[2] Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Mersin-TURKEY.
[3] Mersin University, Faculty of Medicine, Department of Medicinal Microbiology, Mersin-TURKEY.

Abstract

In the present study seven 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal-hydrazone derivatives and four 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted acetophe-none)hydrazone derivatives were synthesized. Among them, the analytical data of five original compounds were given. In this study, the microwave synthesis method and antimicrobial evaluation of all the compounds were also reported for the first time. The minimum inhibition concentration (MIC) values of the compounds were determined by the Microdilution method using two Gram positive bacteria (Staphylococcus aureus, Bacillus subtilis), two Gram negative bacteria (Pseudomonas aeruginosa, Escherichia coli) and two yeast like fungi (Candida albicans, Candida parapsilosis).

Key Words: *Benzaldehyde hydrazones, Acetophenone hydrazones, Microwave synthesis, Antibacterial activity, Antifungal Activity.*

5-Kloro-2(3H)-Benzoksazolinon-3-Asetil-2-(*p*-Sübstitüe Benzal)Hidrazon ve 5-Kloro-2(3H)-Benzoksazolinon-3-Asetil-2-(*p*-Sübstitüe Asetofenon)Hidrazon Türevlerinin Mikrodalga Sentezi ve Antimikrobiyal Aktivitelerinin Değerlendirilmesi

Sunulan bu çalışmada yedi 5-kloro-2(3H)-benzoksazolinon-3-asetil-2-(p-sübstitüe benzal)hidrazon ve dört 5-kloro-2(3H)-benzoksazolinon-3-asetil-2-(p-sübstiüe asetofenon)hidrazon türevinin sentezi yapılmıştır. Bunların arasında, beş orijinal bileşiğin analiz değerleri verilmiştir.. Bu çalışmada aynı zamanda tüm bileşiklerin mikrodalga sentezi ve antimikrobiyal değerlendirilmesi ilk defa rapor edilmiştir. Bileşiklerin minimum inhibitör konsantrasyonları (MİK) iki Gram pozitif bakteri (Staphylococcus aureus, Bacillus subtilis), iki Gram negatif bakteri (Pseudomonas aeruginosa, Escherichia coli) ve iki maya benzeri fungus üzerinde (Candida albicans, Candida parapsilosis) üzerinde değerlendirilmiştir.

Anahtar Kelimeler: *Benzaldehit hidrazonlar, Asetofenon hidrazonlar, Mikrodalga sentez, Antibakteriyel aktivite, Antifungal aktivite.*

***Correspondence**: Phone: +90 2023226 Fax: 2235218
E-mail: mgokce@gazi.edu.tr

INTRODUCTION

Morbidity and mortality due to enteric bacterial infection remain important health problems worldwide mainly in developing countries and regions such as the Indian sub-continent, part of South America and tropical part of Africa (1,2). Invasive dysentery and diarrhea caused by Escherichia coli are the world's most prevalent and fatal infectious diseases (3, 4). Patients usually show wide range of symptoms such as stomachache, cramps, bloating or tenderness (5). Abscess of the brain is a dreadful complication of E. coli infection (6). Amoxicillin, norfloxacin and ciprofloxacin are the most common drugs used for E. coli infection (7) but are associated with severe side effects. Toxicity and resistance to the drugs also play important role in the treatment failure (8). There is an urgent need to screen new compounds for the development of new antibacterial agents against E. coli infection. Thus, there is still need for the new classes of antimicrobial agents. Benzoxazoles and benzimidazoles, which are the structural isosters of natural nucleotides and interact easily with the biopolymers, constitute an important class of heterocyclic compounds with antitumor, antiviral, antibacterial and antibiotic activities (9-15). Therefore, these have been the aim of many researchers for many years. A benzoxazole derivative, calcimycin is a carboxylic polyether antibiotic from a strain of Streptomyces chartreusis. It was found to be very active against Gram-positive bacteria including some Bacillus and Micrococcus strains (16, 17). Also, it is well known that the hydrazone group plays an important for the antimicrobial activity a number of hydrazone derivatives have been claimed to possess interesting antibacterial and antifungal activities (18-27).

Furthermore, Microwave-Induced Organic Reaction Enhancement (MORE) chemistry has gained popularity as a non-conventional technique for rapid synthesis and many researches have described accelerated organic reactions, and a large number of papers has appeared proving the synthetic utility of MORE chemistry in routine organic synthesis (28-35). Microwave-assisted organic synthesis could help achieve high yields and clean reaction outcomes at short reaction time. Organic solvent free reaction conditions eliminate the toxicity and flammability issues associated with common solvents. Together, solvent free organic syntheses assisted by microwave irradiation have being regarded as environmentally benign methodologies.

Considering above, and earlier reported applications of MORE, we report here microwave synthesis of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal)hydrazone **4** and 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted aceto-phenon)hydrazone **5** derivatives. Although microwave-assisted organic syntheses have been well documented, no example of 5-chloro-2(3H)-benzoxazolinone derivatives synthesized with using microwave irradiation has been reported yet. Except for compound **IVd** all of the **IV** derivatives were synthesized by conventional method in our previous study (36). All of the **V** derivatives and compound **IVd** are new compounds (Table 2).

MATERIALS AND METHODS

Apparatus

Melting points of the compounds were determined on Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the values given are uncorrected.

The IR spectra of the compounds were recorded on a Bruker Vector 22 IR Spectrophotometer (Bruker Analytische Messtechnik, Karlrure, Germany).

The ^{1}H-NMR of the compounds spectra were recorded on a Bruker 400 MHz-NMR Spectrometer (Rheinstetten, Karlrure, Germany) using tetramethylsilane as an internal standard. All the chemical shifts were recorded as δ (ppm) in d_6-DMSO.

Elemental analyses were performed with Leco-932 (C,H,N,S,O-Elemental analyzer, St. Joseph, USA) at Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara-Turkey) and within ± 0.4 % of the theoretical values.

Microwave irradiation synthesis of the compounds were conducted on Milestone MicroSYNTH (Microwave Labstation for synthesis) microwave apparatus.

Chemistry

The fine chemicals and all solvents used in this study were purchased locally from E. Merck (Darmstadt, F. R. Germany) and Aldrich Chemical Co. (Steinheim, Germany).

Synthesis of 5-chloro-2(3H)benzoxazolone **1** was carried out by the reaction of 2-amino-4-chlorophenol with urea (37). The physical and spectral properties of compound **1** were accordance with the literature. Therefore we carried out the next steps of the reaction without any further analysis. 5-Chloro-2(3H)benzoxazolone **1** was reacted with methyl chloroacetate to obtain methyl-(5-chloro-2(3H)benzoxazolone)acetate **2**. 5-chloro-2(3H)-benzoxazolinone-3-acetyl hydrazide **3** was obtained by the reaction of **2** with hydrazine hydrate, the method of synthesis was described in our laboratory (38). The hydrazides thus obtained was reacted with various benzaldehyde and acetophenone derivatives to obtain title compounds **4**, **5.**

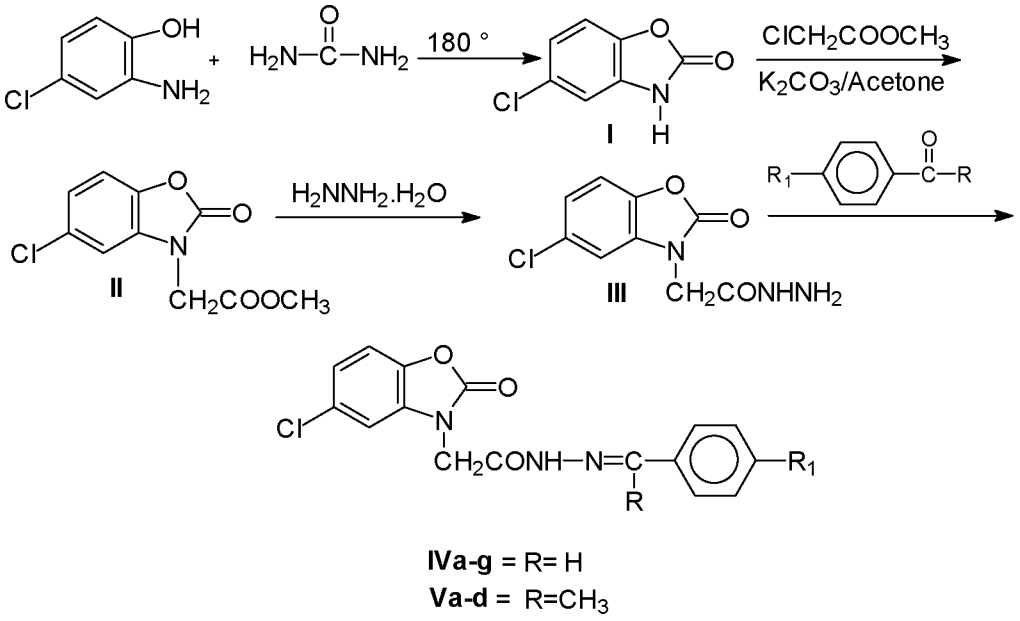
Microwave mediated synthesis of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal)hydrazone (IVa-g) derivatives

A mixture of 5-chloro-2(3H)-benzoxazolinone-3-acetyl hydrazide **3** (0.01 mole 2.41 g) and appropriate benzaldehyde derivatives (0.01 mole) and 2-3 drops glacial acetic acid in ethanol (20 mL) was taken in round bottom flask placed in a microwave oven and irradiated (400 W, 76-78 °C) for 15 min. After completion of reaction (monitored by TLC) the solvent was removed on the rotary and residue recrystallized from ethanol or acetone.

Microwave mediated synthesis of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted acetophenon)hydrazone (Va-d) derivatives

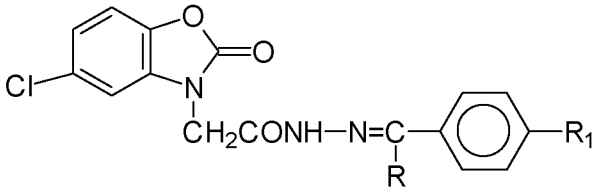
A mixture of 5-chloro-2(3H)-benzoxazolinone-3-acetyl hydrazide **3** (0.01 mole 2.41 g) and appropriate acetophenone derivatives (0.01 mole) and 2-3 drops glacial acetic acid in ethanol (20 mL) was taken in round bottom flask placed in a microwave oven and irradiated (400 W, 76-78 °C) for 15 min. After completion of reaction (monitored by TLC) the solvent was removed on the rotary and residue recrystallized from ethanol or acetone.

The reaction is depicted in Scheme 1. Some physical characteristic of the compounds are given Table 1. Spectral data of the compounds are given in Table 2. Microwave mediated synthesis of all of the **IV** and **V** derivatives are reported for the first time in present study. Compound **IVd** and **Va**, **Vb**, **Vc**, **Vd** are original.



Scheme 1: Synthesis pathway of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal)hydrazone **IV** and 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted acetophenon)hydrazone **V** derivatives.

Table 1 : Some Physical Characteristic of the synthesized compound



Com.	R	R_1	M.p. (C°)	Yield(%)	Formula	Mol. weight
IVa	H	H	256-257	73	$C_{16}H_{12}ClN_3O_3$	329.74
IVb	H	Br	258-259	71	$C_{16}H_{11}BrClN_3O_3$	408.64
IVc	H	Cl	272-273	77	$C_{16}H_{11}Cl_2N_3O_3$	364.19
IVd	H	F	295-297	71	$C_{16}H_{11}ClFN_3O_3$	347.73
IVe	H	CH_3	284-285	81	$C_{17}H_{14}ClN_3O_3$	343.77
IVf	H	OCH_3	262-263	80	$C_{17}H_{14}ClN_3O_4$	359.77
IVg	H	OH	292-294	62	$C_{16}H_{12}ClN_3O_4$	345.74
Va	CH_3	H	191-192	50	$C_{17}H_{14}ClN_3O_3$	343.76
Vb	CH_3	Br	239-240	40	$C_{17}H_{13}BrClN_3O_3$	422.66
Vc	CH_3	Cl	237-238	56	$C_{17}H_{13}Cl_2N_3O_3$	378.21
Vd	CH_3	OH	245-246	48	$C_{17}H_{14}ClN_3O_4$	359.76

Table 2: Spectral data of the compounds

Comp	IR(KBr)cm^{-1}			^{1}H NMR(DMSO-d_6) ppm (δ)
	C=O (amide)	C=O (ring)	N-H	
IVa	1682	1768	3185	11.81 (1H, s, NH), 7.81 and 7.56 (1H, s, s, N=CH), 7.69-6.91 (8H, m, Ar-H), 5.08 and 4.76 (2H, s, s, CH_2).
IVb	1669	1758	3165	11.87 (1H, s, NH), 8.26 and 8.07 (1H, s, s, N=CH), 7.84-7.17 (7H, m, Ar-H), 5.09 and 4.68 (2H, s, s, CH_2).
IVc	1667	1767	3187	11.83 (1H, s, s, NH), 8.64 and 8.07 (1H, s, s, N=CH), 7.91-6.98 (7H, m, Ar-H), 5.09, 4.87 and 4.48(2H, s, s, s, CH_2).
IVd	1668	1770	3183	11.87 (1H, s, s, NH), 8.67 and 8.10 (1H, s, s, N=CH), 7.94-7.05 (7H, m, Ar-H), 5.11, 4.90 and 4.53 (2H, s, s, s, CH_2).
Ive	1673	1774	3181	11.71 (1H, s, s, NH), 8.22 and 8.03 (1H, s, s, N=CH), 7.78-6.96 (7H, m, Ar-H), 5.08 and 4.48 (2H, s, s, CH_2), 2.36 and 2.29 (3H, s, s Ar-CH_3).
IVf	1673	1768	3176	10.15 (1H, s, s, NH), 7.88 and 7.74(1H, s, s, N=CH), 7.65-6.73 (7H, m, Ar-H), 5.06, 4.85 and 4.43(2H, s, s, s, CH_2), 3.86(3H, s, Ar-OCH_3).
IVg	1669	1746	3178	10.12 (1H, s, Ar-OH), 9.31 (1H, s, NH), 7.82 and 7.63 (1H, s, s, N=CH), 7.41-6.92 (7H, m, Ar-H), 4.82 and 4.28 (2H, s, s,CH_2).
Va	1710	1784	3222	10.18 (s, 1H, N<u>H</u>), 6.89-7.50 (m, 8H, phenyl protons), 4.86 (s, 2H, -C<u>H$_2$</u>-CONH=N-), 1.95 (s, 3H, CH_3).
Vb	1706	1779	3219	10.17 (s, 1H, N<u>H</u>), 6.91-7.66 (m, 7H, phenyl protons), 4.86 (s, 2H, -C<u>H$_2$</u>-CONH=N-), 2.24 (s, 3H, CH_3).
Vc	1714	1782	3212	10.18 (s, 1H, N<u>H</u>), 6.89-7.39 (m, 7H, phenyl protons), 4.86 (s, 2H, -C<u>H$_2$</u>-CONH=N-), 2.24 (s, 3H, CH_3),
Vd	1714	1782	3215	10.63 (1H, Ar-OH), (s, 2H, -C<u>H$_2$</u>-CONH=N-), 6.89-7.36 (m, 7H, phenyl protons), 10.18 (s, 1H, N<u>H</u>), 4.86(s, 2H, -C<u>H$_2$</u>-CONH=N-), 2.23 (s, 3H, CH_3).

Antibacterial and Antifungal Activity

Material

The following bacteria were used for antibacterial study: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonans aeruginosa*. The following yeast-like fungi were used for antifungal study: *Candida albicans, Candida parapsilosis.*

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 (10^8cfu/ml). 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 of 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^R microplates which have 96 wells were used for microdilution method. Brinkmann transferpette was used for two fold-dilution of compounds in the wells.

Method

Microdilution method was employed for antibacterial and antifungal activity tests (39). The synthesized compound and the standarts ampicillin trihydrate and fluconazole were dissolved in DMSO at 1000 μg/ml concentration at the beginning.

The solution of each compounds at 500-3.9 μg/ml were prepared in the wells by diluting with the mediums. Suspension of the microorganisms at 10^6cfu/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 and 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 assessed as the minimum inhibitory concentration (MIC) of the compounds. There was no inhibitory activity in the wells containing only DMSO. The microbial growth occurred, and the medium were not contaminated during the tests. The MIC values of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal)hydrazone **IV** and 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted acetophenon)hydrazone **V** derivatives were given in Table 3.

Table 3: The MIC values of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal)hydrazone **IV** and 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted acetophenon)hydrazone **V** derivatives

Comp.	A	B	C	D	E	F
Iva	125	250	250	250	125	125
IVb	62.5	125	125	125	62.5	62.5
IVc	62.5	125	125	125	62.5	62.5
IVd	62.5	125	125	125	62.5	62.5
IVe	125	250	250	250	125	125
IVf	125	250	250	250	125	125
IVg	125	250	250	250	125	125
Va	125	250	250	250	125	125
Vb	62.5	125	125	125	62.5	62.5
Vc	62.5	125	125	125	62.5	62.5
Vd	62.5	125	125	125	62.5	62.5
Amp.	62.5	1.95	62.5	3.9	-	-
Fluc.	-	-	-	-	250	125

A: *Staphylococcus aureus* ATCC 25813 **B:** *Bacillus subtilis* ATCC 6633
C: *Pseudomonas aeruginosa* ATCC 25853 **D:** *Escherichia coli* ATCC 25923
E: *Candida albicans* ATCC 36232 **F:** *Candida parapsilosis* ATCC 22019

RESULTS AND DISCUSSION

We have developed a facile and efficient approach for the synthesis of title compounds. In order to draw a comparison between microwave irradiation and conventional heating for preparation of the 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal)hydrazone **IV** and 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted acetophenon)hydrazone **V** derivatives either conventional heating or microwave irridation have been utilized by us. It is obvious that the microwave irradiation approach for the synthesis of compounds **IV** and **V** derivatives is faster and the yields are higher than conventional heating method (36). Also, this simple and reproducible technique affords the title compounds without formation of undesirable by-products.

As it seen in Table 3 Compounds **IVb**, **IVc**, **IVd**, **Vb**, **Vc** and **Vd**, are effective against Staphylococcus aureus as standard compound ampicillin. The rest of the **IV** and **V** derivatives showed moderate activity towards Gram-positive and Gram-negative bacteria when compared to ampicillin.

Entire derivatives of **IVb**, **IVc**, **IVd**, **Vb**, **Vc** and **Vd** have pronounced antifungal activity and exceeded that of fluconazole, which was used as the reference compound. The rest of the **IV** and **V** derivatives exhibited equal antifunfal activity against Candida parapsilosis with fluconazole. Also **IVb**, **IVc**, **IVd**, **Vb**, **Vc** and **Vd** derivatives have been found two fold active than fluconazole against Candida albicans. The presence of an electronegative substituent on the phenyl ring in derivatives of **IV** and **V** increases antibacterial and antifungal activities. It was reported in literature that a lot of antibacterial and antifungal drugs bearing a halogen substituent on aromatic ring (40-44). These results suggest that compound **IV** and **V** derivatives may be worth studying further in terms of their antifungal activity.

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