INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

MICROWAVE ASSITED SYNTHESIS AND ANTIMICROBIAL EVALUATION OF MANNICH BASES OF 3-[4-ARYLIDENEAMINO-5-THIOXO-1,2,4-TRIAZOL-3-YL) METHYL]-2(3*H*)-BENZOXAZOLONE DERIVATIVES

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Abstract

In this study, new seven Mannich base of 3-[4-arylideneamino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone derivatives have been synthesized in microwave irradiation. These newly synthesized Mannich bases were characterized by IR, 1H-NMR and elemental analyses. The *in vitro* antimicrobial activity of the compounds was determined against some gram positive, gram negative bacteria, fungi and their drug-resistant isolates in comparison with reference drugs by using microdilution method. Compound **6g** was found to exhibit significant antifungal activity.

Keywords: 1,2,4-triazole,2(3*H*)-benzoxazolone, mannich base, antibacterial activity, antifungal activity.

Introduction

During the past decades, the human population was from affected infectious diseases caused by multidrug-resistant gram-positive and gram-negative pathogen bacteria. Due to this reason, new classes of antibacterial agents has become an urgent need for the treatment of multidrug-resistant infections (Bayrak et al, 2009; Almajan et al, 2010).

In recent years, Mannich bases have gained importance due to their application in pharmaceutical chemistry. They have been found to possess antibacterial, antifungal, anticancer (Ashok et al, 2007; Holla et al, 2002; Almajan et al, 2009), antitubercular (Walczak et al. 2004), cytotoxic (Ivanova et al. 2007), analgesic and anti-inflammatory (Amir &Shikha, 2004) properties. A few Mannich bases derived from 1,2,4-triazoles carrying morpholinyl substituent were biologically active (Sithambaram et al, 2006)

The 1,2,4-triazole and its derivatives were reported to exhibit various pharmacological activities such as antimicrobial (Kaplancıklı et al, 2008; Bayrak et al, 2009), analgesic (Aytac et al, 2009; Haider et al, 2014),anti-inflammatory (Amir &Shikha, 2004; Shehry et al, 2010), antitubercular (Walczak et al, 2004, Kumar et al, 2010), anticancer(Holla et al, 2006) and antioxidant (Barbuceanu et al, 2014) properties.

Also, many 2(3*H*)-benzoxazolone containing compounds have been reported to possess various biological activities such as analgesic-anti-inflammatory (Dogruer et al, 1997; Unlu et al, 2003a; 2003b; Kelekci et al, 2009), anticonvulsant (Ucar et al, 1998), antioxidant (Aichaoui et al, 2009), cytotoxic (Petrov et al, 2008; Ivova et al, 2007), antitubercular (Gulkok et al, 2012) properties.

Hereby we report the synthesis of a new series of Mannich bases containing both 1,2,4-triazoles and 2(3H) benzoxazolone skeletons (**Figure 1**).

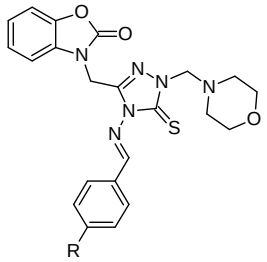


Figure 1.

Experimental

All the chemicals used for the synthesis of the compounds were purchased from Aldrich, Merck AG and Acros Chemicals. Melting points of thecompounds were recorded on an electrothermal-9200 digital melting points apparatus and are uncorrected. The FTIR spectra of the surface layer of grafted membranes were measured with a Perkin-Elmer 400 (USA) ATR attachment (32 scans, wavenumber 4000–650 cm^{-1}) and analyzed using the Spectrum v2.0 software. The 1H-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), in DMSO-d_6. Elemental analysis were performed on Leco 932 CHNS instrument (St. Joseph, MI, USA) and were found within ± 0.4 % of the theoretical values. Microwave irradiation synthesis of the compounds was conducted on Milestone MicroSYNTH (Microwave Labstation for synthesis) microwave apparatus.

2(3H)-benzoxazolone **1**(Eren et al, 2010), ethyl (2(3H)-benzoxazolone-3-yl)acetate **2** (Onkol et al, 2002), 2(3H)-benzoxazolone-3-yl)acetic acid **3**(Onkol et al, 2002) , 3-[(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone **4** (Cicekli et al, 2012),and 3-[(4-arylideneamino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone **5** (Cicekli et al, 2012)were synthesized according to the procedures published in the literature.

General procedure for 3-[(1-morpholinomethyl-4-arylideneamino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone (**6a-g**)

To a suspension of4-arylideneamino-5-(2(3H)-benzoxazolone-3-ylmethyl)-1,2, 4- triazole -3- thione (2mmol) in isopropanole (3 mL), formaldehyde (2 mmol) and morpholine (2mmole) were added. The reaction mixture was placed in microwave oven and irradiated for minutes changing between 15-30 min at 125 °C (300 W). After completion of the reaction by monitoring with TLC, the reaction mixture was kept overnight at room temperature. Then, the precipitate was collected by filtration, dried, and crystallized from appropriate solvent. (Table 1)

Microbiological studies

Micro dilution method

Standard strains of *P. aeruginosa* ATCC 27853, *E.coli* ATCC 35218,*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *C. albicans* ATCC 10231, *C. krusei* ATCC 6258 and clinical isolates of bacteria were included in the study. Resistance in clinical isolates was determined bydisc diffusion method according to the guidelines of clinical and laboratory standards institute (CLSI, 2006). Standard powders of ampicillin, ofloxacine andfluconazole were obtained from the manufacturers. All bacterial isolates were subcultured in Mueller Hinton Agar (MHA; Merck) plates and incubated overnight at 37°C. The solutions of the newly synthesized compounds and standard drugs were prepared and diluted at 2048, 1024, 512,… 0.0625 µg/mL concentrations in the wells of microplates within the liquid media. Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S18 (CLSI, 2008a). The bacterial suspensions used for inoculation were prepared at 10^5 CFU/mL by diluting fresh cultures at MacFarland 0.5 density (10^7 CFU/mL). Suspensions of the bacteria at 10^5 CFU/mL concentration were inoculated to the two-fold diluted solution of the compounds. There were 10^4 CFU/mL bacteria in the wells after inoculations. Mueller Hinton Broth (MHB; Merck) was used for diluting the bacterial suspension and for two-fold dilution of the compound. A 10 µL bacteria inoculum was added to each well of the microdilution trays. The trays were incubated at 37°C and minimum inhibitory concentration (MIC) endpoints were read after 24 h of incubation.

Candida were subcultured in sabouraud dextrose agar (SDA; Merck) plates and incubated at 35 °C for 24-48 h. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine (Sigma) buffered with MOPS (pH 7) (Sigma) and culture suspensions were prepared through the guideline of CLSI M27-A3 (CLSI 2008b).Yeast suspensions were prepared according to McFarland 0.5 density and a working suspension was made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension ($2.5x10^3$ CFU/ml). A 10 µL yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 °C and MIC endpoints were read after 48 h of incubation.

Dimethylsulphoxide (DMSO), PBS, pure microorganisms and pure media were used as control wells. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and MICs were reported.

Result and Discussion

Chemistry

2(3H)-benzoxazolone **1** (Eren et al, 2010), ethyl-(2(3H)-benzoxazolone-3-yl)acetate **2**(Onkol et al, 2002),(2(3H)-benzoxazolone-3-yl)acetic acid **3**(Onkol et al, 2002) **,** 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone **4**(Cicekli et al, 2012) and 3-[(p-substitutedphenylmethylidene]amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)- benzoxazolone **5** (Cicekli et al, 2012) was accomplished according to the previously reported procedures. Mannich bases **6** were synthesized by reacting Schiff bases **5** with morpholine in the presence of formaldehyde and in isopropanole medium in 74-98% yield under microwave irradiation. The mannich bases of 4-arylideneamino-5-(2(3H)-benzoxazolone -3-ylmethyl)-1,2,4-triazole-3-thione were obtained in very short time with high yield. **Scheme 1** illustrates the methods used for the preparation of target compounds. Newly synthesized compounds **6a-g** were characterized by IR, 1H-NMR, and elemental analyses. The data characterization of all the new compounds are summarized in Table **1**and Table **2**

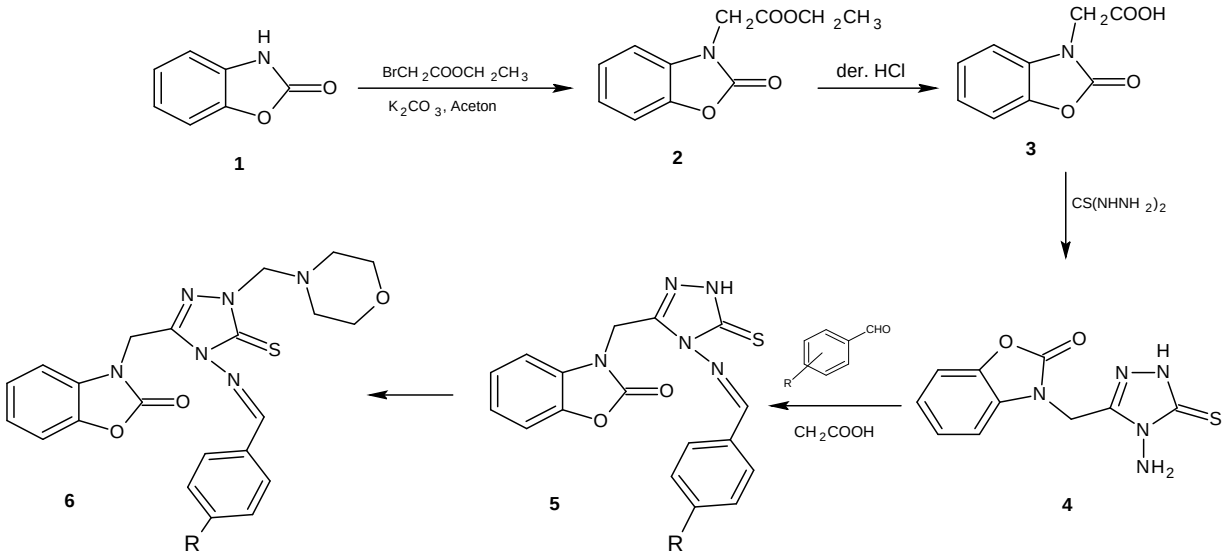
Table 1. Physicochemical data of **6a-g**.

	R	Crys. Sol.	m.p.(^{0}C)	Yield %	Elemental analysis Cal /Found. (%)
6a	H	isopropanol	167-168	79	C: 58.65 / 57.91 H: 4.92 / 4.92 N: 18.65 /18.41 S: 7.12 / 7.15
6b	F	isopropanol	183-184	74	C(56.40%) 56.12 H(4.52%) 4.82 N(17.94%) 17.73 S(6.84%) 6.58
6c	Cl	isopropanol	197-198	94	C(54.49%) 54.14 H(4.36%) 4.47 N(17.33%) 17.35 S(6.61%) 6.64
6d	Br	isopropanol	201-201	95	C(49.91%) 49.75 H(4.00%) 4.13 N(15.87%) 15.91 S(6.06%) 6.09
6e	CH_3	isopropanol	208-209	98	C(59.47%) 59.11 H(5.21%) 5.18 N(18.09%) 18.00 S(6.90%) 6.91
6f	OCH_3	isopropanol	190-191	84	C(57.49%) 57.80 H(5.03%) 5.092 N(17.49%) 17.38 S(6.67%) 6.70
6g	$C(CH_3)_3$	isopropanol	194-195	82	C(61.64%) 61.60 H(5.97%) 5.88 N(16.59%) 16.56 S(6.33%) 6.37

Table 2. IR and ^{1}H-NMR spectral data of the compounds **6a-g**.

Comp.	IR (KBr) cm^{-1}	^{1}H-NMR (ppm, δ)
6a	1765	9.85(1H, s, =CH), 7.80 (2H, d, phenyl -H2,6), 7.59 (1H, t, phenyl H4), 7.56 (2H, t, phenyl-H3,5), 7.33 (1H, d, H7),7.26 (1H, d, H4), 7.17-7.09 (2H, m, H 5,6), 5.30 (2H, s, CH2), 5.03 (2H, s, CH2), 3.50 (4H, t, morpholin-H), 2.61 (4H, t, morpholin-H)
6b	1772	9.86(1H, s, =CH), 7.80 (2H, m, phenyl –H 3,5), 7.41-7.35 (3H, m, phenyl H2,6; H7), 7.29 (1H, d, H4), 7.20-7.13 (2H, m, H 5,6), 5.33 (2H, s, CH2), 5.06 (2H, s, CH2), 3.53 (4H, t, morpholin-H), 2.64 (4H, t, morpholin-H)
6c	1768	9.94(1H, s, =CH), 7.87 (2H, d, phenyl –H 3,5), 7.61 (2H, d, phenyl H2,6), 7.36 (H7, d, H7), 7.29 (1H, d, H4), 7.20-7.12 (2H, m, H 5,6), 5.34 (2H, s, CH2), 5.06 (2H, s, CH2), 3.52 (4H, t, morpholin-H), 2.63 (4H, t, morpholin-H)
6d	1767	9.94(1H, s, =CH), 7.81-7.74 (4H, m, phenyl –H), 7.36 (1H, d, H7), 7.29 (1H, d, H4), 7.20-7.12 (2H, m, H 5,6), 5.34 (2H, s, CH2), 5.06 (2H, s, CH2), 3.52 (4H, t, morpholin-H), 2.63 (4H, t, morpholin-H)
6e	1765	9.80(1H, s, =CH), 7.73 (2H,d, phenyl–H3,5), 7.37-7.33 (3H, m,phenyl-H2,6, H7), 7.28 (1H, d, H4), 7.20-7.14 (2H, m, H 5,6), 5.32 (2H, s, CH2), 5.06 (2H, s, CH2), 3.53 (4H, t, morpholin-H), 2.63 (4H, t, morpholin-H),2.40 (3H, s, CH3)
6f	1767	9.61(1H, s, =CH), 7.77 (2H,d, phenyl –H3,5), 7.33 (1H, d, H7), 7.24 (1H, d, H4), 7.16-7.09 (2H, m, H 5,6), 7.05 (2H, d, phenyl-H2,6), , 5.28 (2H, s, CH2), 5.02 (2H, s, CH2), 3.82 (3H, s, OCH3), 3.50 (4H, t, morpholin-H), 2.60 (4H, t, morpholin-H),
6g	1773	9.79 (1H, s, =CH), 7.78 (2H, d, phenyl–H3,5), 7.56 (2H, d, phenyl-H2,6), 7.36 (1H, d, H7), 7.27 (1H, d, H4), 7.20-7.12 (2H, m, H 5,6), 5.32 (2H, s, CH2), 5.06 (2H, s, CH2), 3.53 (4H, t, morpholin-H), 2.64 (4H, t, morpholin-H), 1.32 (9H, s, CH3)

The IR spectra of compounds **6a-g** showed characteristic bands at 1773-1765 cm-1 C=O, lactone. The 1H-NMR spectra showed of N=CH proton at 9.94- 9.61 ppm. The protons of the morpholine residue appeared as a broad triplet at 3.83 – 3.50 ppm and at 2.64-2.40 ppm in **6a-g**, respectively. The methylene protons of **6a-g** appeared as a singlet at 5.34-5.30 and at 5.06-5.03 ppm, respectively. All the other aromatic and aliphatic protons were observed at the expected regions.



Scheme 1. Synthetic route of the title compounds

Biological Activity

The compounds are tested against gram-positive (*S. aureus*, *E. faecalis* and its isolate) and gram-negative bacterias (*P. aeruginosa*, *E. coli*, and its isolate). The antifungal activities of compounds were evaluated *in vitro* against a yeast-like fungi *C. albicans* and *C.krusei.* The microdilution method was employed for antibacterial and antifungal activity tests.Ampicillin and ofloxacin were used as positive control against bacteria and Fluconazole against fungi. Both antibacterial and antifungal activities and minimum inhibition concentrations (MICs; µg ml-1) value of the compounds were illustrated in **Table3**.The synthesized compounds inhibited growth of the bacteria and fungi with MICs between 16 and 128 µgmL-1.

Table 3. Antibacterial and antifungal activities of the compounds as MIC values.

Comp.	*E.coli* ATCC 25922	*E.coli* isolat (ESBL)	*P. aeruginosa* ATCC 27853	*P.aeruginosa* isolat	*S. aureus* ATCC 29213	*S.aureus* isolat (MRSA)	*E. faecalis* ATCC 29212	*E.faecalis* isolat (VRE)	*C. albicans* ATCC 10231	*C.krusei* ATCC 6258
6a	64	64	64	64	128	128	64	64	32	32
6b	64	64	64	64	128	128	64	64	32	64
6c	64	64	64	64	128	128	64	64	32	64
6d	64	64	64	64	128	128	64	64	32	64
6e	64	64	64	64	128	128	64	64	32	64
6f	64	64	64	64	128	128	64	64	32	64
6g	64	64	64	64	64	64	64	32	16	32
Ampicillin	8	64	-	-	<2	32	<2	4	-	-
Ofloxacine	<2	16	<2	32	<2	<2	<2	<2	-	-
Fluconazole	-	-	-	-	-	-	-	-	1	64

All the synthesized compounds gave slightly inhibitory activity against both gram- positive and gram-negative bacteria with a MIC value of 64 µg mL-1.In contrast, the compounds were less active than ampicillin and ofloxacine.

Apart from compound **6g**, the title compounds showed moderate to good activity against gram-positive including *S. aureus* and its isolate (MIC = 128 µg mL-1).In contrast, the compounds were less active than reference drugs.

With respect to antifungal activity of the synthesized compounds, all compounds evaluated antifungal activity against better than antibacterial activity against. Compounds **6b,6c, 6d, 6e**and **6f** exhibited significant activity against *C. albicans* at 32µg mL-1 and against *C.krusie* at 64 µg mL-1.Compound **6a** found against *C.albicans* as well as *C.krusie* at 32 µg mL-1. Also, Compound **6a**exhibited good inhibition activity against *C.krusie* which is more pronounced than that exhibited by the reference drug.
Compound **6g** showed better activity against *C.albicans* (MIC: 16 µg mL-1) than all the other compounds. Compound **6g**which bearing *tert*-butyl substituent at 4-position on phenyl ring have more potent activity among this Mannich base series towards *C.albicans*.

The compounds **6a–g** carrying a morpholino-methyl moiety exhibited increasing of inhibition against the tested microorganisms. In conclusion, the Mannich bases showed better results when compared to the corresponding Schiff bases(Cicekli et al, 2012).

Conclusion

A series of novel Mannich bases, namely 3-[4-arylideneamino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone were synthesized and characterized by NMR and IR studies. All the newly synthesized compounds were screened for their antibacterial and antifungal activities by the method of minimum inhibitory concentration (MIC). Antimicrobial study reveals that compound **6g**having p-ters-butyl substituent on the phenyl ring exhibited maximum inhibition against microorganism *C. albicans.*

Acknowledgement

This study was supported by a grant from Research Foundation of Gazi University (EF-02/2011-16).

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