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Synthesis and Antifungal Activities of Some Aryl (3-Methyl-Benzofuran-2-yl) Ketoximes

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In this study, some aryl (3-methyl-benzofuran-2-yl) ketoximes and their ethers and esters were synthesised. The structure elucidation of the compounds was performed by IR, ^{1}H-NMR, MASS spectroscopy and elemental analyses. Antifungal activities of the compounds were examined and moderate activity was obtained.

Key words: Aryl (3-methyl-benzofuran-2-yl) ketoximes, Antifungal activity.

INTRODUCTION

Among the antifungal agents, oxiconazole, i.e. carrying both azole and oxime residue, became of interest with its effectiveness against the phytopathogenic fungi and directed studies on oxime residue. Since then, a number of oximes (Raga *et al.*, 1992; Massolini *et al.*, 1993; Papadaki-Valiraki *et al.*, 1993; Massolini *et al.*, 1994; Foye *et al.*, 1995; Massolini *et al.*, 1996; Papakonstantinou-Garoufalias *et al.*, 1998; Tunçbilek *et al.*, 1999; Karakurt *et al.*, 2001; Kaneko *et al.*, 2002; Serrano-Wu *et al.*, 2002) were synthesized and found to be active against fungi. In these works, especially among diaryl ketoximes, it is noteworthy that the activity increased when one of the aryl residues was heteroaryl (Massolini *et al.*, 1993; Massolini *et al.*, 1994; Massolini *et al.*, 1996). Encouraged by the successful results obtained from these works, we had decided to prepare series of diaryl ketoximes, in which one of the aryl residue was replaced with benzofuran in a bioisosteric approach, being inspired by the same rationale and obtained significant antifungal activity, against *Candida albicans*, from our first work (Demirayak *et al.*, 2002). In this study, in continuation of our works on aryl benzofuryl ketoxime series, we aimed to obtain some new aryl (3-methylbenzofuran-2-yl) ketoximes and their ethers, esters followed by test their antifungal activities.

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MATERIALS AND METHODS

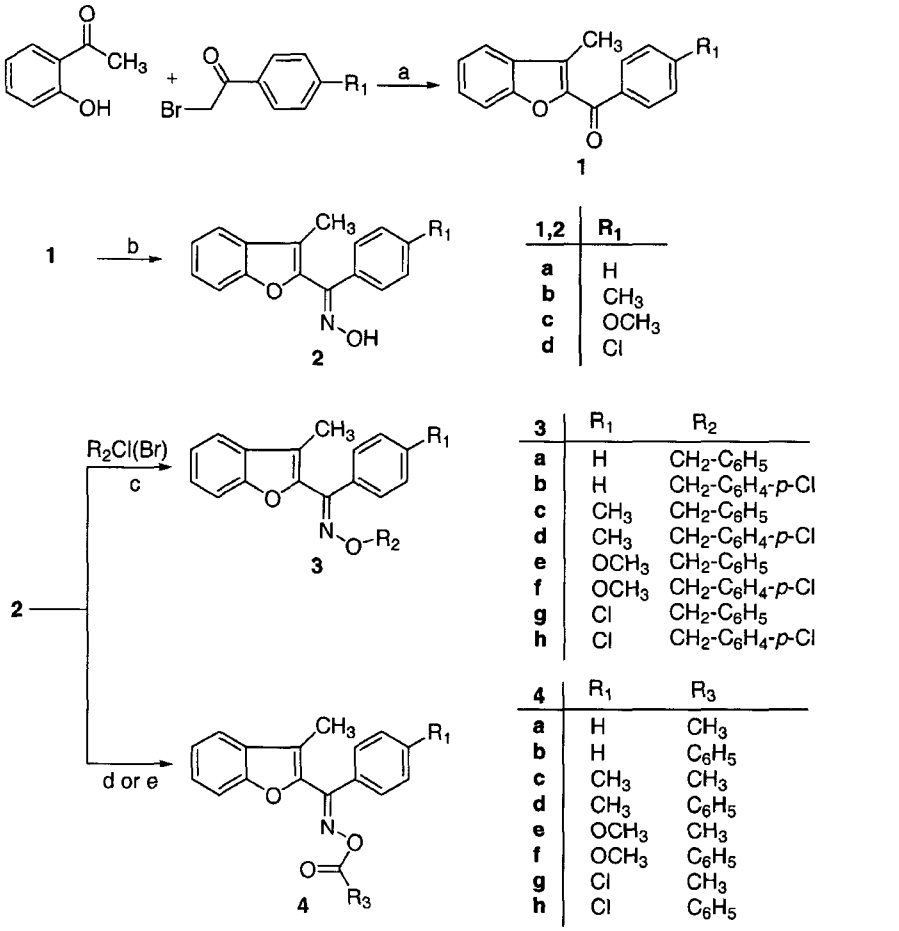
Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected. Spectroscopic data were recorded on the following instrument, IR: schimadzu 435 IR spectrophotometer. ^{1}H-NMR: Bruker DPX 400 NMR spectrometer in DMSO-d_6 using TMS as internal standard. MS: VG Platform Mass spectrometer. Analyses for C, H, N were within 0.4% of the theoretical values. Aryl (3-methyl-benzofuran-2-yl) ketones were prepared according to the literature method (Pestellini *et al.*, 1988).

The reaction sequences depicted in Scheme 1 were followed to obtain the new derivatives. Some characteristics of the compounds were given in Table I.

Aryl (3-methyl-benzofuran-2-yl) ketoximes (2)

The suitable aryl (3-methyl-benzofuran-2-yl) ketone (**1**) (5 mmol), hydroxylamine hydrochloride (7 mmol) and anhydrous sodium acetate (7 mmol) were refluxed in ethanol for 3 h. The reaction mixture was cooled. The crystalline of raw product was filtered and recrystallised from ethanol.

2b IR (KBr) υ_{max} (cm^{-1}) : 3189 (O-H), 1635-1515 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.15(3H, s, CH_3), 2.35 (3H, s, Ar-CH_3), 7.25-7.64 (8H, m, Ar-H), 11.77 (1H, s, O-H), EI-MS: m/z: 266.84 (M+1), 265.32 (M^+), 116.45, 89.17 (100%).



Scheme I. Preparation of compound **1~4**.

2c IR (KBr) υ_{max} (cm^{-1}) : 3176 (O-H), 1630-1524 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.19 (3H, s, CH_3), 3.82 (3H, s, Ar-OCH_3), 7.02 (2H, d, J = 8.84 Hz, Ar-H), 7.28-7.36 (2H, m, Ar-H), 7.42 (2H, d, J = 8.83 Hz, Ar-H) 7.49 (1H, d, J = 7.87 Hz, Ar-H), 7.64 (1H, d, J = 7.69 Hz, Ar-H), 11.60 (1H, s, O-H).

Aryl (3-methyl-benzofuran-2-yl) ketoxime ethers (3)

The suitable aryl (3-methyl-benzofuran-2-yl) ketoxime (**2**) (2 mmol), an appropriate alkylhalide (benzylbromide or 4-chlorobenzylchloride) (2 mmol) and potassium carbonate (2 mmol) were refluxed in acetone for 8 h. The solvent was evaporated and the residue was washed and crystallised from ethanol.

3a R (KBr) υ_{max} (cm^{-1}) : 1630-1512 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.10 (3H, s, CH_3), 5.29 (2H, s, Ar-CH_2-), 7.31-7.44 (12H, m, Ar-H), 7.55 (1H, d, J = 8.08 Hz, Ar-H), 7.70 (1H, d, J = 7.35 Hz, Ar-H).

3d R (KBr) υ_{max} (cm^{-1}) : 1632-1516 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.10 (3H, s, CH_3), 2.30 (3H, s, Ar-CH_3), 5.28 (2H, s, Ar-CH_2-), 7.38-7.50 (10H, m, Ar-H), 7.52-7.54 (1H, m, Ar-H), 7.70-7.72 (1H, m, Ar-H), EI-MS: m/z: 391.62 (M+2), 389.56 (M^+), 124.92 (100%), 89.03, 41.32.

3e IR (KBr) υ_{max} (cm^{-1}) : 1642-1500 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.09 (3H, s, CH_3), 3.81 (3H, s, Ar-OCH_3), 5.23 (2H, s, Ar-CH_2-), 7.03 (2H, d, J = 8.83 Hz, Ar-H), 7.28-7.42 (9H, m, Ar-H), 7.50 (1H, d, J = 8.09 Hz, Ar-H), 7.64 (1H, d, J = 7.19 Hz, Ar-H).

3h IR (KBr) υ_{max} (cm^{-1}) : 1645-1508 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.11 (3H, s, CH_3), 5.29 (2H, s, Ar-CH_2-), 7.42-7.51 (10H, m, Ar-H), 7.53-7.55 (1H, m, Ar-H), 7.69-7.71 (1H, m, Ar-H).

Aryl (3-methyl-benzofuran-2-yl) ketoxime acetates (4a, c, e, g)

The suitable aryl (3-methyl-benzofuran-2-yl) ketoxime (**2**) was refluxed with an excess of acetic anhydride for 1

Table I. Some characteristics of the compounds

Compounds	m.p. (°C)	Yield (%)	Formulae	Mol. Weight
2a	112-4	71	$C_{16}H_{13}NO_2$	251.2
2b	171-2	77	$C_{17}H_{15}NO_2$	265.2
2c	160-3	70	$C_{17}H_{15}NO_3$	281.2
2d	125-6	81	$C_{16}H_{12}ClNO_2$	285.6
3a	113-6	50	$C_{23}H_{19}NO_2$	341.3
3b	85-8	58	$C_{23}H_{18}ClNO_2$	375.8
3c	95-7	47	$C_{24}H_{21}NO_2$	355.3
3d	80-2	58	$C_{24}H_{20}ClNO_2$	389.8
3e	77-9	51	$C_{24}H_{21}NO_3$	371.3
3f	84-6	67	$C_{24}H_{20}ClNO_3$	405.8
3g	98-9	50	$C_{24}H_{18}ClNO_2$	375.8
3h	93-4	53	$C_{23}H_{17}Cl_2NO_2$	410.2
4a	Oily	61	$C_{18}H_{15}NO_3$	293.2
4b	108-11	58	$C_{23}H_{17}NO_3$	355.3
4c	Oily	44	$C_{19}H_{17}NO_3$	307.3
4d	115-6	65	$C_{24}H_{19}NO_3$	369.3
4e	Oily	57	$C_{19}H_{17}NO_4$	323.3
4f	117-9	60	$C_{24}H_{19}NO_4$	385.3
4g	120-2	70	$C_{19}H_{14}ClNO_3$	327.7
4h	121-4	62	$C_{23}H_{16}ClNO_3$	389.7

h. The reaction mixture was poured into water and neutralised with sodium bicarbonate solution. The precipitate formed was filtered and crystallised from ethanol.

4g IR (KBr) υ_{max} (cm^{-1}) : 1645-1495 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.15 (3H, s, CH_3), 2.27 (3H, s, $COCH_3$), 7.36 (1H, t, J = 7.46 Hz, Ar-H), 7.46 (1H, t, J = 7.53 Hz, Ar-H), 7.55 (2H, d, J = 8.53 Hz, Ar-H), 7.59 (1H, d, J = 8.13 Hz, Ar-H), 7.65 (2H, d, J = 8.44 Hz, Ar-H), 7.77 (1H, d, J = 7.77 Hz, Ar-H).

Aryl (3-methyl-benzofuran-2-yl) ketoxime benzoates (4b, d, f, h)

The suitable aryl (3-methyl-benzofuran-2-yl) ketoxime (**2**) (2 mmol) and benzoic anhydride (3 mmol) were refluxed in tetrahydrofurane for 2 h. The solvent was evaporated. The precipitate formed was crystallised from ethanol.

4f IR (KBr) υ_{max} (cm^{-1}) : 1620-1510 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.27 (3H, s, CH_3), 3.86 (3H, s, Ar-OCH_3), 7.14 (2H, d, J = 8.7 Hz, Ar-H), 7.35 (1H, t, J = 7.42 Hz, Ar-H), 7.45 (1H, t, J = 7.72 Hz, Ar-H), 7.51-7.61 (5H, m, Ar-H), 7.68 (1H, t, J = 7.35 Hz, Ar-H), 7.75 (1H, d, J = 7.72 Hz, Ar-H), 7.82 (2H, d, J = 7.42 Hz, Ar-H), EI-MS: m/z : 385.24 (M^+), 263.02, 220.49, 105.15 (100%), 76.92.

4h IR (KBr) υ_{max} (cm^{-1}) : 1628-1516 (C=N, C=C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm) : 2.13 and 2.30 (3H, two s, CH_3), 7.35-7.83 (12H, m, Ar-H), 7.91 (1H, d, J = 7.56 Hz, Ar-H).

Table II. Antifungal activities of the compounds (μg/mL)

Compounds	C.albicans	C.glabrata	C.parapsilosis
2a	4	8	4
2b	4	4	8
2c	4	4	4
2d	4	8	4
3a	>512	>512	>512
3b	256	128	256
3c	>512	>512	>512
3d	16	16	16
3e	>512	>512	>512
3f	128	128	256
3g	8	16	8
3h	>512	>512	>512
4a	8	8	4
4b	256	256	128
4c	128	256	128
4d	64	256	128
4e	8	8	8
4f	>512	>512	>512
4g	16	32	16
4h	>512	>512	>512
O	2	4	2
C	2	4	4
F	4	8	4

O: Oxiconazole, **C**: Clotrimazol, **F**: Fluconazole

ANTIFUNGAL ACTIVITY

All the compounds were evaluated *in vitro* for antifungal activity. Antifungal susceptibility testing was done by using macrobroth dilution test, in accordance with the National Committee for Clinical Laboratory Standards (1999). Results are given as minimal inhibitory concentrations (MIC) in μg/mL in Table II.

Macrobroth Dilution Method: Testing was performed according to the guidelines of NCCLS document M27-A. *Candida* strains were subcultured twice on Sabouraud dextrose agar (Oxoid) plates and were incubated at 35°C for 24 h to ensure optimal growth prior to testing. Stock solutions of compounds were prepared in 100% dimethyl sulfoxide. Stock solutions of the compounds were then diluted with RPMI 1640 medium (with L-glutamine but without bicarbonate; Sigma Chemical Co., St. Louis, Mo.) buffered to pH 7.0 with 0.165 M morpholinopropanesulfonic acid (MOPS; Sigma). The final concentration ranges used were 0.25 to 250 μg/mL for all compounds testing was performed in 96-well round-bottom microtitration plates. Yeast inocula were prepared in sterile water and were

diluted in RPMI 1640 medium to give a final inoculum concentration of approximately 5×10^2 to 2.5×10^3 blastoconidia/mL. The plates were incubated at 35°C, and endpoints were read visually after 48 h. The MIC of the compounds was defined, as the lowest concentration at which there was 80% inhibition of growth compared with the growth for a drug-free control.

RESULTS AND DISCUSSION

Chemistry

Aryl (3-methyl-benzofuran-2-yl) ketoxime derivatives were synthesised as outlined in the scheme. The ketones **1** were obtained in Modified Rap-Störmer Reaction condition (Pestellini *et al.*, 1988). *O*-Alkylketoximes **3** were obtained by reacting oxime derivatives **2** with benzylbromide or 4-chlorobenzylchloride. Acetates or benzoates **4** were prepared by reacting **2** with acetic anhydride or benzoic anhydride respectively. As expected the presence of *E* and *Z* isomers of the oxime derivatives was confirmed by thin layer chromatography and NMR spectra. Thus, in the NMR spectra of some of our compounds, protons of methyl or methoxy groups on aromatic rings resonated in two different groups with corresponding integral values. However, aromatic protons were observed as multiple peaks.

Antifungal activity

Antifungal activity tests were performed by macrobroth dilution method using *Candida albicans, Candida glabrata*, and *Candida parapsilosis* (All are clinical isolates, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey) strains. Three antifungal agents i.e. oxiconazole, clotrimazole and fluconazole were used as control. The MIC values obtained for these control compounds are 2, 2 and 4 µg/mL for *Candida albicans*, 4, 4, 8 µg/mL for *Candida glabrata* and 2, 4, 4 µg/mL for *Candida parapsilosis* respectively. In consideration of the results we may conclude that some of our products have noticeable antifungal activity. Some of our compounds MIC values are determined as 4 or 8 µg/mL which is almost equal to those of the controls. The most significant compounds are appeared to be **2a, 2b, 2c, 2d** with MIC values equivalent to the control compounds, i.e. 4 µg/mL. Highly effective compounds, **2a-d** should be characterized as non-substituted on the oxime residue. As mentioned above, the lowest MIC value obtained from the compound **2a**, which has the simplest structure of its group in analogy with this generalization. However, this relationship could not be observed in oxime esters of acetyl and benzoyl derivatives.

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