NEW HETEROCYCLIC OXIME ETHERS OF 1-(BENZOFURAN-2-YL)ETHAN-1-ONE AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract: In this study, some O-benzyl (benzofuran-2-yl)ethan-1-one ether oximes were synthesized starting from 2-acetylbenzofuran. The structure elucidation of the compounds was performed by IR, 1H-NMR and 13C-NMR spectra. Antimicrobial activities of the compounds were examined and notable activity was observed.

Keywords: O-benzyl (benzofuran-2-yl)ethan-1-one ether oximes; antimicrobial activity, X-ray analysis

The increase in fungal infections have recently emerged as a growing threat to human health. Candida infections are adverse in their appearance (1). The increase in fungal infections and the resistance gained to the currently used drugs in recent years directed the studies on obtaining new antifungal drugs (2). The studies on imidazole and triazole structured antifungal drugs were observed. (3, 4). After discovery of oxiconazole 1, both azole and ether oximes became of interest. Since then, a number of oximes were synthesized and found to be active against fungi (5-10).

It was proved that the activity of compounds increased when one of the aryl residues was heteroaryl (11). Free oximes and their ethers showed higher activities. When the aryl residue was replaced with benzofuran in a bioisosteric approach, significant antifungal activity was observed (12). In this study, we aimed to obtain compounds derived from oxiconazole, oxime-containing scaffolds. We supposed that if benzofuran is a ring and substituted O-benzyl group is in ether oximes, it causes higher activity of such compounds.

Benzofuran is a unique scaffold that is associated with several biological activities. The broad spectrum antifungal (13, 14) and antibacterial activity (15, 16) of these compounds could lead to a new series of antimicrobials. Highly effective compounds were obtained due to aryl benzofuryl ketoximes (17).

EXPERIMENTAL

Chemistry

All reagents were commercially available or synthesized following the procedures described in

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the literature. All NMR spectra were recorded on a Bruker Avance III 700 MHz and Bruker Avance III 400 MHz spectrometer, using CDCl₃ as solvent, with TMS as an internal standard. The IR spectra were recorded on Shimadzu FTIR-8400 S spectrometer.

Melting points were determined using an Electrothermal 9100 digital melting point apparatus and were uncorrected.

(E)-1-(benzo[b]furan-2-yl)ethanone O-(4-chlorobenzyl) oxime (5)

Yield 67%, white needles m.p. 108°C, IR (KBr, cm⁻¹): 1615 (C=O). ¹H NMR (400 MHz, CDCl₃, δ, ppm): 2.30 (s, 3H, CH₃), 5.30 (s, 2H, CH₂), 7.01 (d, J = 0.8 Hz, 1H, CH), 7.25 (dd, J = 7.6 Hz, J = 0.8 Hz, 1H, CH), 7.35 (dt, J = 7.6 Hz, J = 1.6 Hz, 1H, CH), 7.36-7.40 (m, 4H, 4◊CH), 7.56 (dd, J = 0.8 Hz, J = 8.4 Hz, 1H, CH), 7.60 (dd, J = 0.8 Hz, J = 8.4 Hz, 1H, CH). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 12.92 (CH₃), 75.88 (CH₂), 104.05 (CH), 112.25 (CH), 121.62 (CH), 122.87 (C), 123.89 (CH₂), 128.88 (CH), 128.20 (C), 130.05 (2◊CH), 131.98 (2◊CH), 136.84 (C), 147.52 (C), 151.20 (C), 155.19 (C).

(E)-1-(benzo[b]furan-2-yl)ethanone O-(2,4-dichlorobenzyl) oxime (6)

Yield 70%, white needles m.p. 113-114°C, IR (KBr, cm⁻¹): 1612 (C=O). ¹H NMR (700 MHz, CDCl₃, δ, ppm): 2.36 (s, 3H, CH₃), 5.42 (s, 2H, CH₂), 7.05 (d, J = 0.7 Hz, 1H, CH), 7.27 (dd, J = 0.7 Hz, 1H, CH), 7.30 (dd, J = 2.1 Hz, J = 7.7 Hz, 1H, CH), 7.36 (dt, J = 1.4 Hz, 7.0 Hz, 1H, CH), 7.45 (dd, J = 2.1 Hz, J = 2.8 Hz, 2H, 2◊CH), 7.58 (dt, J = 0.7 Hz, J = 8.4 Hz, 1H, CH), 7.61 (dt, J = 0.7 Hz, J = 8.4 Hz, 1H, CH). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 12.29 (CH₃), 73.27 (CH₂), 107.13 (CH), 111.74 (CH), 121.40 (CH), 123.22 (CH), 125.73 (CH), 127.08 (CH), 128.26 (CH), 130.36 (CH), 133.95 (C), 134.15 (C), 148.36 (C), 151.55 (C), 155.25 (C).

(E)-1-(benzo[b]furan-2-yl)ethanone O-(4-bromobenzyl) oxime (7)

Yield 79%, white solid, m.p. 110-111°C, IR (KBr, cm⁻¹): 1616 (C=O). ¹H NMR (700 MHz, CDCl₃, δ, ppm): 2.36 (s, 3H, CH₃), 5.44 (s, 2H, CH₂), 7.04 (d, J = 0.7 Hz, 1H, CH), 7.26 (dd, J = 0.7 Hz, J = 7.0 Hz, 1H, CH), 7.36 (dt, J = 1.4 Hz, J = 7.0 Hz, 1H, CH), 7.55 (dd, J = 7.0 Hz, 1H, CH), 7.59 (spin system AA’), J = 0.7 Hz, J = 9.1 Hz, 2H, 2◊CH), 7.61 (dd, J = 7.7 Hz, 1H, CH), 8.26 (spin system BB’), J = 2.1 Hz, J = 9.1 Hz, 2H, 2◊CH). ¹³C NMR (176 MHz, CDCl₃, δ, ppm): 12.35 (CH₃), 75.22 (CH₂), 107.37 (CH), 111.73 (CH), 121.45 (CH), 123.28 (CH), 123.69 (2◊CH), 125.85 (CH), 127.82 (C), 128.10 (2◊CH), 145.21 (C), 147.57 (C), 148.58 (C), 151.35 (C), 155.25 (C).
CDCl₃, δ, ppm): 2.30 (s, 3H, CH₃), 5.35 (s, 2H, CH₂), 7.02 (d, J = 0.8 Hz, 1H, CH), 7.25 (dd, J = 0.8 Hz, J = 4.0 Hz, 1H, CH), 7.33 (d, J = 1.6 Hz, 1H, CH), 7.35-7.39 (m, 2H), 7.57 (dd, J = 0.8 Hz, J = 2.0 Hz, J = 8.8 Hz, 1H, CH), 7.61 (dd, J = 0.8 Hz, J = 2.0 Hz, J = 8.8 Hz, 1H, CH), 151.80 (C), 155.23 (C), 159.72 (C), 162.19 (C). 19F NMR (100 MHz, CDCl₃, δ, ppm): -39.31 (dd, J = 0.8 Hz, J = 1.6 Hz, 1H, CH), -42.17 (ddd, J = 0.8 Hz, J = 2.0 Hz, J = 7.2 Hz, 1H, CH). 13C NMR (376 MHz, CDCl₃, δ, ppm): 13.48 (s, 3F, CF₃). 1F NMR (376 MHz, CF₃COOH, δ, ppm): -42.17 (ddd, J = 0.8 Hz, J = 2.0 Hz, J = 7.2 Hz, 1H, CH). 1H NMR (400 MHz, CDCl₃, δ, ppm): 2.25 (s, 3H, CH₃), 5.43 (s, 2H, CH₂), 6.95 (dd, J = 1.2 Hz, J = 7.2 Hz, 2H, 2×CH), 7.00 (d, J = 0.8 Hz, 1H, CH), 7.25 (dd, J = 0.8 Hz, J = 7.2 Hz, 1H, CH), 7.31-7.37 (m, 2H, 2×CH), 7.58 (dd, J = 0.8 Hz, J = 7.2 Hz, 2H, 2×CH). 13C NMR (100 MHz, CDCl₃, δ, ppm): 12.04 (CH₃), 64.11 (CH₂), 106.71 (CH), 111.18 (C), 111.43 (C), 111.73 (CH), 112.92 (C), 121.34 (CH), 121.33 (CH), 125.54 (CH), 127.97 (CH), 130.54 (CH), 148.03 (CH), 151.80 (C), 155.21 (C), 160.99 (C), 163.49 (C). 19F NMR (376 MHz, CDCl₃, δ, ppm): -38.18 (t, J = 6.8 Hz, 2F, 2×CF₂)

Crystal structure determination of 10

Crystalline, colorless block crystal (ethanol) of 0.40 × 0.22 × 0.13 mm was used to record 16216 (MoKα radiation, θmax = 29.09°) intensities on an Agilent Xcalibur A diffractometer. Intensity data collection employed the ω-scan mode with “Enhance (Mo) X-ray Source”. The data were corrected for Lorentz and polarization effects. Data reduction and analysis were carried out with the CrysAlis PRO program (19). The 3506 total unique reflections (R(int) = 0.023) were used for further calculations.

Structure solution and refinement: The structure was solved by the direct methods using the program SHELXS-97 (20) and refinement was done against F² for all data using SHELXL-97 (20). The positions of the H atoms were positioned geometrically and were refined using a riding model, with C–H = 0.98 Å (CH₃), 0.97 Å (CH₂), 0.93 Å (C–CH), and Uiso(H) = 1.2Ueq(C) or 1.5Ueq(C) for methyl H atoms. The methyl group was refined as a rigid group, which was allowed to rotate. The final refinement converged with R = 0.0492 (for 2399 data with I > 2σ(I)), wR = 0.1369 (on F² for all data), and S = 1.048 (on F² for all data). The largest difference peak and hole were 0.294 and -0.218 eÅ³. The molecular illustration was drawn using ORTEP-3 for Windows (21). Software used to prepare material for publication was WINGX (21) and PLATON (22).

The supplementary crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ (UK), phone: (+44) 1223/336 408, fax: (+44) 1223/336 033, e-mail: deposit@ccdc.cam.ac.uk, World Wide. Web:http://www.ccdc.cam.ac.uk (deposition No. CCDC 975847).
Antibacterial and antifungal activity

The antimicrobial activities of the compounds were determined using the broth microdilution reference method (23) in standard 96-well polystyrene plates (Kartell). The tested microorganisms were Gram-negative Escherichia coli ATCC 25922 and Gram-positive Staphylococcus aureus ATCC 25923 bacteria, the yeasts Candida albicans and Malessezia pachydermatis CBS7925. The study was carried out using microdilution method with the following dilutions of the tested compounds from 400 to 6.25 µg/mL for Malessezia (24) and from 512 to 0.125 µg/mL for bacteria and Candida.

The bacterial strains were cultivated in Luria-Bertani (LB) broth and the yeast in Sabouraud dextrose broth (SDB). The tested compounds were dissolved in dimethyl sulfoxide (DMSO); diluted tenfold with culture broth to a concentration of 1.024 mg/mL, and then serially diluted in the appropriate medium. The wells were inoculated with the tested strains to a final concentration of 10⁴ CFU/mL. The control sample included inoculated growth medium without the compound. In all the tests, DMSO was used as the control; DMSO had no effect on the microorganisms in the concentrations studied (up to 2.5%). Ampicillin (Polfa Tarchomin SA) and itraconazole (Janssen-Cilag International NV) were used as antibiotic reference for the bacteria and yeast, respectively. The plates were incubated at 37°C for 24 h for the bacteria, 48 h for Candida and 72 h for Malessezia. The microbial growth rate was measured as an optical density at 550 nm (OD₅₅₀). The tests were performed in triplicate for each concentration.

The minimum inhibitory concentrations (MIC) were defined as the lowest concentration of the compounds at which no visible growth of the tested microorganism occurred.

RESULTS AND DISCUSSION

Chemistry

The final products (4-11) were synthesized as outlined in Scheme 1. Ketone 2 was prepared from salicylic aldehyde with chloroacetone (25). The (E)-oxime 3 was prepared from ketone 2 and recrystallized from ethanol.

Oxime 3 was reacted with appropriate substituted benzyl bromides with high yields. The oxime ethers can be prepared in a reaction with oxime and sodium (26) or sodium hydride (27).

As expected, the presence of E and Z isomers of the oxime derivatives was observed in the raw products. All the final products were crystallized from ethanol. Thus, no isomers Z were observed in the final products; in the NMR spectra aliphatic pro-
tons were not resonated in two different groups with corresponding integral values. We have prepared a few fluorinated products 8-11, supposedly being highly effective. Structural features of synthesized heterocyclic oxime ethers of 1-(benzofuran-2-yl)ethan-1-one were confirmed by X-ray crystallographic analysis of exemplified compound 10.

Crystallographic data

The molecular structure of compound 10 and the atom-labelling scheme is illustrated in Figure 1.

The nine-membered benzofuran system is planar with an r.m.s. deviation of 0.0083 Å and is in $E$ configuration with respect to the 2-fluorobenzyloxy moiety [torsion angle C2-C10-N12-O13: -178.87 (11)°]. Simultaneously, conjugated system of double bonds C2 = C3 and C10 = N12 has $s$-trans conformation [torsion angle C3-C2-C10-N12: 179.62 (16)°].

The interatomic distance C10 = N12 takes the value of 1.286(2) Å and confirms the occurrence of the double bond between these atoms.

Angular orientation of the 2-fluorobenzyloxy fragment in the molecule reveal three torsional angles C10-N12-O13-C14, N12-O13-C14-C15 and O13-C14-C15-C16 of -174.93(13), 74.23(16) and

Table 1. Antibacterial and antifungal activities of the compounds 4-11 and the standard drugs used in the study (MIC µg/mL).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Staphylococcus aureus</th>
<th>MIC [µg/mL]</th>
<th>Escherichia coli</th>
<th>Candida albicans</th>
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<tr>
<td>4</td>
<td>&gt; 512</td>
<td>512</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&gt; 512</td>
<td>512</td>
<td>512</td>
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<tr>
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<td>&gt; 512</td>
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<td>512</td>
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</tr>
<tr>
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<td>-</td>
<td>2</td>
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</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>8</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 1. Reaction sequence for the synthesis of ether oximes 4-11
66.49(19)\(^{\circ}\), respectively. The first one indicates that the C10-N12 and O13-C14 bonds are antiperiplanar to each other while the second and the third torsional angles both reveal mutual synclinal orientation of the bonds N12-C15 and C14-C15 or O13-C14 and C15-C16. The phenyl ring of the 2-fluorobenzylxylo moiety forms a dihedral angle of 80.03(5)\(^{\circ}\) with the planar benzofuran system.

**Antimicrobial activity**

The antimicrobial activity of the synthesized compounds (4-11) was evaluated in vitro against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *C. albicans*, and *M. pachydermatis*. The results of the evaluation of their minimal inhibitory concentration values are presented in Table 1. Two standard drugs ampicillin and itraconazole were used as controls; the MIC values obtained for these compounds are 8 and 2 \(\mu g/mL\) respectively.

The growth of Gram-positive reference strain *S. aureus* was inhibited by compounds 6, 7, 8, 10 and 11 at the concentration of 250 \(\mu g/mL\). A twofold lower dose (128 \(\mu g/mL\)) was required in the case of derivative 9, carrying a 4-trifluoromethylbenzyl substituent, to inhibit the growth of *S. aureus*. Of all the tested substances, only compound 8 (4-bromo-2-fluorobenzyl group) was active against *E. coli* (MIC 256 \(\mu g/mL\)). Derivatives 10 and 11, with 2-fluorine and 2,6-difluorine groups, showed moderate activity against *C. albicans*; MIC values of 250 \(\mu g/mL\), but were less active than the standard antifungal drug, itraconazole.

The other derivatives examined, i.e., oxime ethers 4 and 5, were inactive against all the tested microorganisms (MIC \(\geq 512 \mu g/mL\)).

Evaluation of the antifungal activity of the tested oxime ethers against *M. pachydermatis* showed a slight inhibitory effect at the concentration of 400 \(\mu g/mL\). The growth of the tested *Malessezia* strains was half less intense as against the positive control - an inoculum of the fungus in Sabouraud medium without the compounds. All the tested compounds were ineffective at lower concentrations (200-6.25 \(\mu g/mL\)).

Alper-Hayta et al. (16) showed that 2-substituted phenyl/benzyl)-5-[(2-benzofuryl)carboxamido]-benzoxazole derivatives possessed a broad spectrum of activity against Gram-positive and Gram-negative bacteria as well as *Candida* (MIC range between 15.625-500 \(\mu g/mL\)). Similar results were obtained by other authors; the compounds of cyclobutane substituted benzofuran class were able to inhibit the growth of *C. albicans* and *S. aureus* at the concentration from 2.5 to 0.039 mg/mL (15). The dinaphtho[2,1-b]furan-2-yl-methanone compounds and their oxime derivatives showed weak antimicrobial activity against bacteria and *Candida* (128-512 \(\mu g/mL\)) (28).

We have described the synthesis and antibacterial and antifungal activity of new benzofuran-containing oximes. The results obtained showed that these compounds exhibited relatively weak antimicrobial potency against the tested microorganisms. The most promising activity was detected in derivatives 8–11 containing fluorine residues.

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**Conflicts of interests**

The Authors have declared that there is no conflict of interests.

**REFERENCES**


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