Oguzhan Karaosmanoglu1,2, Burcu Butun3,*, Hakan Dal4, Hulya Sivas1 and Kadriye Benkli3

1Department of Biology, Faculty of Science, Eskisehir Technical University University, 26400, Eskisehir, Turkey
2Department of Biology, Kamil Özdağ Science Faculty, Karamanoğlu Mehmetbey University, 70100, Karaman, Turkey
3Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, 34093, Istanbul, Turkey
4Department of Chemistry, Faculty of Science, Eskisehir Technical University University, 26400, Eskisehir, Turkey

Received: 20 November, 2018
Accepted: 07 March, 2019
Published: 08 March, 2019

*Corresponding author: Burcu Butun, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, 34093, Istanbul, Turkey, E-mail: bbutun@bezmialem.edu.tr

Keywords: Antimicrobial activity; Cytotoxicity; Ketoximes; 3-Methyl-benzofuran-2-yl; x-Ray analysis

Introduction

Benzofuran is a heterocyclic compound which formed by a fused benzene and furan ring. Like other heterocyclic structures, benzofuran has several pharmacological effects due to their scaffolds. Their derivatives have attracted attention in last years. They are found in various natural sources or synthesized for different purposes. Moreover compounds that contains benzofuran heterocyclic earned some features such as solubility, salt formation, absorption and bioavailability [1]. They play key role in design and synthesis of new pharmacologically active compounds. Even some medicinal plants earned pharmacological effect due to benzofuran cores. Primarily they have several biological activities such as antitumor, cytotoxic, anticancer [2], antimicrobial [3], antifungal [4], antiproliferative [5], inhibition of angiogenesis [6].

Cancer is the most dangerous life-threatening disease that cause mortality with a big proportion in all over the world [7]. Previous studies claimed that number of cancer cases will increase by 2050 and reach a peak with 16 million, so that it is very important to understand the mechanism of cancer types. They have several complex mechanisms
resistant triazoles derivatives were studied against results to different pathogenic fungi. A series of benzofuran-12]. Antifungal studies on benzofurans also gave satisfactory high antimicrobial activity against nearly all tested organisms and showed benzofuran pyrazol derivatives have example in a study, researchers studied on a series of different benzofuran derivatives which show promising results. For benzofurans. There is many research on antimicrobial potential ways to synthesis bioactive heterocyclic moieties such as

In addition to side effects of the cancer drugs is drug resistance to cancer therapy transience [10]. Unconscious usage of antitumors and antibiotics cause to suppression of the immune system. Infection diseases are increasing with the improvement of mutagenicity due to bacteria’s resistant to drugs also [11]. This leads to the need for new antimicrobial agents that antibiotics do not resist. It is necessary to discover/ design new antimicrobial agents and find practical/economical ways to synthesis bioactive heterocyclic moieties such as benzofurans. There is many research on antimicrobial potential on benzofuran derivatives which show promising results. For example in a study, researchers studied on a series of different bacteria and showed benzofuran pyrazol derivatives have high antimicrobial activity against nearly all tested organisms [12]. Antifungal studies on benzofurans also gave satisfactory results to different pathogenic fungi. A series of benzofuran-triazoles derivatives were studied against fluconazole-resistant Trichophyton rubrum and Cryptococcus neoformans and found as having in vitro antifungal activity [13]. Benzofuran ketoxy analogues were also studied with docking studies as antifungal potency. They found ketoxy moiety and at least one hydrogen bound between enzyme and molecule directly increases the activity [4].

In this study new aryl (3-methyl-benzofuran-2-yl) ketones were synthesized and identified with nuclear magnetic resonance (NMR), infrared spectroscopy (IR), mass spectroscopy (MS) and X-ray analysis. Cytotoxicity and anti-microbial potential of this benzofuran derivatives were investigated.

Materials and Methods

Experimental

Chemistry: Chemicals and solvents were obtained from Sigma–Aldrich and E. Merck (Darmstadt, Germany). The synthetic route of compounds is outlined in Scheme 1. Synthesis of arylketoximes H1-5 (methylbenzofuran-2-yl) ketones were synthesized and identified with suitable 2′-hydroxyacetophenone (5 mmol), 2-bromoacetophenone (5 mmol) and potassium carbonate (6 mmol) were refluxed in acetonitrile for 4 hours. After reflux the reaction was cooled and the solvent was evaporated. The residue was washed with water and crystallized from ethanol [14–16].

**Scheme 1: Synthesis of arylketoximes H1-5 (methylbenzofuran-2-yl) (phenyl) methanone.**

**Scheme 2: Chemical structures of arylketoximes.**

NR stock solution was prepared in sterile distilled water with the concentration of 3, 3 mg/ml and was filtered. At the end of the treatment periods (24 h, 48 h and 72 h) NR working solution with the concentration of % 1 was prepared with DMEM, 250 µl working solution was added to each well. After incubation period which is 3 h at 37 °C 100 µl desorb solution (glacial acetic acid: ethanol: distilled water 1:49:50) was added to each well. After 15 min incubation, 96–well plate was read by ELISA reader (Biotech ELX 808 Ultra microplate reader) at 540 nm wavelength. By that way, the cell viability was determined in terms of absorbance values. Then it was converted to % viability with the following formula:

\[
\% \text{ viability} = \frac{(\text{test–blank})}{(\text{negative control–blank})} \times 100.
\]

Three independent experiments were done by that way.

The MTT assay

F2408 and HepG2 cells were seeded 5,000 cells/well and 10,000 cells/well in 96–well plates respectively. After grown for 24 h, treated with certain concentrations of the H1, H2 and H3 compounds. The stock solutions, 100 mM, were prepared by dissolving the compounds with sterile distilled water. In all the compounds were obtained at 1510–1616 cm⁻¹ region. Ketone’s C=O bands were observed at 1638–1550 regions. All the protons resonated as expected in the NMR spectra. Aliphatic protons resonated in two groups for methyl 2.12 and 2.15, methoxy 3.77 and 3.80 and methylene 5.28 and 5.42 ppm regions, respectively.

\[
\text{H}1 \text{ (3-methylbenzofuran-2-yl) (phenyl) methanone: M.p. 236.30 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1645 (C=O), 1647–1564 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.58 (3H, s, CH}_3\text{), 7.39–7.88 (7H, m, Ar-H), 8.01–8.04 (2H, m, Ar-H). ES-MS: m/z: 237 (M+1).}
\]

\[
\text{H2 (3,5-dimethylbenzofuran-2-yl) (phenyl) methanone: M.p. 350.30 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1643 (C=O), 1600–1552 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.45 (3H, s, CH}_3\text{), 2.94 (3H, s, Ar-CH}_3\text{), 7.37–7.69 (6H, m, Ar-H), 7.96–7.99 (2H, m, Ar-H). ES-MS: m/z: 251 (M+1).}
\]

\[
\text{H3 (5-methoxy-3-methylbenzofuran-2-yl) (phenyl) methanone: M.p. 266.45 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1647 (C=O), 1651–1550 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.45 (3H, s, CH}_3\text{), 3.84 (3H, s, Ar-CH}_3\text{), 7.55–8.23 (8H, m, Ar-H). ES-MS: m/z: 267 (M+1).}
\]

\[
\text{H4 (6-chloro-3-methylbenzofuran-2-yl) (phenyl) methanone: M.p. 270.95 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1645 (C=O), 1648–1565 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.65 (3H, s, CH}_3\text{), 7.49–8.16 (8H, m, Ar-H). ES-MS: m/z: 271 (M+1).}
\]

\[
\text{H5 (5-chloro-3, 6-dimethylbenzofuran-2-yl) (phenyl) methanone: M.p. 330.40 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1641 (C=O), 1638–1550 (C=C).}
\]

For positive control and chloramphenicol for reference negative control. The minimal inhibitory concentration (MIC) values were determined after incubation at 37 °C for 18–24 h. The MIC values were determined as the lowest compound concentration where absence of growth was recorded. Each test was repeated at least twice with triplicate for all microorganisms.

Results

Chemistry

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. \textit{H}-NMR: Bruker DPX 400 NMR spectrometer in DMSO-d₆ using TMS as internal standard. MS: VG Platform Mass spectrometer. Analysis for C, H, N were within 0.4% of the theoretical values.

Structure elucidation

As expected, the presence of the derivatives was confirmed by a thin layer chromatography and NMR spectral data. In the IR spectra C=C and C=N stretching bands, characteristic for all the compounds were obtained at 1510–1616 cm⁻¹ region. Ketone’s C=O bands were observed at 1638–1647 regions. All the protons resonated as expected in the NMR spectra. Aliphatic protons resonated in two groups for methyl 2.12 and 2.15, methoxy 3.77 and 3.80 and methylene 5.28 and 5.42 ppm regions, respectively.

\[
\text{H1 (3-methylbenzofuran-2-yl) (phenyl) methanone: M.p. 236.30 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1645 (C=O), 1647–1564 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.58 (3H, s, CH}_3\text{), 7.39–7.88 (7H, m, Ar-H), 8.01–8.04 (2H, m, Ar-H). ES-MS: m/z: 237 (M+1).}
\]

\[
\text{H2 (3,5-dimethylbenzofuran-2-yl) (phenyl) methanone: M.p. 250.30 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1643 (C=O), 1600–1552 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.45 (3H, s, CH}_3\text{), 2.94 (3H, s, Ar-CH}_3\text{), 7.37–7.69 (6H, m, Ar-H), 7.96–7.99 (2H, m, Ar-H). ES-MS: m/z: 251 (M+1).}
\]

\[
\text{H3 (5-methoxy-3-methylbenzofuran-2-yl) (phenyl) methanone: M.p. 266.45 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1647 (C=O), 1651–1550 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.45 (3H, s, CH}_3\text{), 3.84 (3H, s, Ar-CH}_3\text{), 7.55–8.23 (8H, m, Ar-H). ES-MS: m/z: 267 (M+1).}
\]

\[
\text{H4 (6-chloro-3-methylbenzofuran-2-yl) (phenyl) methanone: M.p. 270.95 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1645 (C=O), 1648–1565 (C=C).}
\]

\[
\text{\textit{H}}-\text{NMR (400 MHz) (DMSO-d}6\text{) } \delta \text{(ppm): 2.65 (3H, s, CH}_3\text{), 7.49–8.16 (8H, m, Ar-H). ES-MS: m/z: 271 (M+1).}
\]

\[
\text{H5 (5-chloro-3, 6-dimethylbenzofuran-2-yl) (phenyl) methanone: M.p. 330.40 °C. IR (KBr) } \ \tilde{\text{\nu}}\text{ max (cm}^-1\text{): 1641 (C=O), 1638–1550 (C=C).}
\]


**1H-NMR (400 MHz) (DMSO-d6) δ (ppm): 2.45 (3H, s, CH₃), 2.54 (3H, s, Ar-CH₃), 7.47–8.09 (7H, m, Ar-H). ES-MS: m/z: 285 (M+1).**

**X-ray crystallography**

The red coloured crystals of the title compound was crystallized from chloroform at room temperature. The crystallographic data are given in table 1 and the selected bond lengths and angles are listed in table 2. Crystallographic data were recorded on a Bruker Kappa APEXII CCD area–detector diffractometer using Mo Kα radiation (λ=0.71073 Å) at T=108(2) K. Absorption correction by multi-scan was applied. Structure solved by direct methods and refined by full–matrix least squares against F² using all data [20]. All non–H atoms were refined anisotropically. The C-bound H–atoms were positioned geometrically with C––H = 0.93 and 0.96 Å for aromatic and methyl H–atoms, respectively, and constrained to ride on their parent atoms, with Uiso (H) = k x Ueq (C), where k = 1.5 for methyl H–atoms and k = 1.2 for aromatic H–atoms.

**Crystal structure**

In the molecule of the title compound (Scheme 3), the bond lengths and angles (Table 2) are generally within normal ranges. The compound contains one benzofuran [A (O1/C2–C9)] and one benzene [B (C11–C16)] rings, where ring A is approximately planar with a maximum deviation of –0.018(2) Å (for atom C8). Its mean plane is oriented with respect to ring at a dihedral angle of 41.22(6)°. Atoms C1, C10 and C17 are 0.0015[24], -0.0237[24] and -0.0346(25) Å away from the benzofuran ring plane, respectively [20,21].

**Biology**

A serial concentration of H1, H2 and H3 compounds was tested against seven standard microorganisms and they were not exhibited antimicrobial activities up to 500 μg/ml concentration whereas chloramphenicol as a positive control inhibited the microbial growth. MTT and NRU cytotoxicity assays showed that H1, H2 and H3 compounds were not cytotoxic on both cell lines as normal fibroblast F2408 cells (Scheme 4) and hepatocarcinoma HepG2 cells (Scheme 5) up to 1000 μM tested. On the other hand, cisplatin [22], which is an anticancer drug used as a positive control [23], showed very strong cytotoxicity on the both cell types (Scheme 6) at 100 μM concentration and for all periods of time tested.

**Table 1: Crystallographic data of compound H2.**

<table>
<thead>
<tr>
<th>Empirical Formula</th>
<th>C₁₇H₁₄O₂</th>
<th>Fw</th>
<th>250.28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal System</td>
<td>Orthorhombic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Space Group</td>
<td>P 2₁,2₁,2₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a (Å)</td>
<td>3.9294 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b (Å)</td>
<td>10.0895 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c (Å)</td>
<td>31.1683 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α (°)</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β (°)</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ (°)</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V (Å³)</td>
<td>1235.69 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ρ (MoKα) (mm⁻³)</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ρ (calc) (g cm⁻³)</td>
<td>1.345</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Reflections Total</td>
<td>7163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Reflections Unique</td>
<td>3030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rₑₑ</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2θₑₑ</td>
<td>54.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₑₑ / Tₑₑ₀</td>
<td>0.969 / 0.985</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Parameters</td>
<td>174</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R [F²&gt;2σ(F²)]</td>
<td>0.052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wR</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: The Selected Bond Lengths (Å) and Angles (deg) of compound H2.**

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1– C5</td>
<td>1.369 (2)</td>
</tr>
<tr>
<td>O1– C9</td>
<td>1.396 (3)</td>
</tr>
<tr>
<td>O2– C10</td>
<td>1.229 (3)</td>
</tr>
<tr>
<td>O1– C5– C6</td>
<td>125.50 (19)</td>
</tr>
<tr>
<td>O1– C5– C4</td>
<td>111.10 (18)</td>
</tr>
<tr>
<td>O1– C9– C10</td>
<td>117.99 (18)</td>
</tr>
<tr>
<td>O1– C9– C8</td>
<td>112.12 (18)</td>
</tr>
<tr>
<td>C5– O1– C9</td>
<td>104.96 (15)</td>
</tr>
<tr>
<td>C9– C10– O2</td>
<td>117.97 (19)</td>
</tr>
<tr>
<td>C11–C10–O2</td>
<td>120.7 (2)</td>
</tr>
<tr>
<td>C9–C10–C11</td>
<td>121.22 (18)</td>
</tr>
</tbody>
</table>

**Scheme 3: X-ray structure of compound H2.**
not exhibited any cytotoxic activity for mammalian cell lines even at higher doses.

It has been reported that some benzofuran (bearing 2-methylimidazole or 2-ethylimidazole ring and substitution of the imidazolyl-3-position with a naphthylacyl or methoxyphenacetyl group) and benzofuran-2-carboxamide derivatives (especially bearing benzo[b] furan, in particular, 2-imidazolynyl substituted compound) were found to have cytotoxic activity in vitro against a panel of human tumor cell lines [24,25,4]. Telvekar et al. [4], have performed a 3-D QSAR analysis and docking studies on synthesized benzofuran ketoxime analogues by Benkli et al. [16], and provided useful information about designing of new benzofuran for their bioactivities such as antifungal agent. They suggested that the hydrogen bonding between the hydroxyl group of ketoxime of benzofuran, hydrophobic interaction between phenyl of benzofuran core, and phenyl ring attached to carbon of ketoxime are important to bind amino acids. Therefore, the reason our molecules being ineffective may be due to the absence of these type of functional groups in the molecules that allows interaction with amino acids or other macromolecules.

In our next studies, different derivatives of our compounds such as oxime esters, acetyl and benzoyl compounds will be synthesized and tested. They are thought to have key role to discover more potent molecules.

Acknowledgements

We thank to Prof. Dr. Mustafa Yamac Eskisehir from Osmangazi University who kindly provided us test microorganisms and facilities in his laboratory. We also thank to Anadolu University Faculty of Pharmacy and Bezmialem Vakuf University Faculty of Pharmacy for their support. We are grateful to Scientific Research Projects Commission of Anadolu University, Eskisehir, Turkey for the funding grant 1507F563 (to H.S and O.K.). The authors gratefully acknowledge a financial support of this work by TUBITAK (the Scientific and Technological Research Council of Turkey), carried out in the frame of a project (Project No:113Z694).
References


Discover a bigger Impact and Visibility of your article with Peertech Publications

Highlights

- Signatory publisher of ORCID
- Signatory Publisher of DORA (San Francisco Declaration on Research Assessment)
- Articles archived in worlds’ renowned service providers such as Portico, CNKI, AGRIS, TDNet, Base (Bielefeld University Library), CrossRef, Scilit, J-Gate etc.
- Journals indexed in ICMJE, SHERPA/RoMEO, Google Scholar etc.
- OAI-PMH (Open Archives Initiative Protocol for Metadata Harvesting)
- Dedicated Editorial Board for every journal
- Accurate and rapid peer-review process
- Increased citations of published articles through promotions
- Reduced timeline for article publication

Submit your articles and experience a new surge in publication services

Copyright: © 2018 Karaosmanoglu O, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.


Peertech journals wishes everlasting success in your every endeavour.