Introduction

Benzofuran is a heterocyclic compound which formed by a fused benzene and furan ring. Like other heterocyclic structures, benzofurans have 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...
[8]. For example, in a study two different derivative series of benzofurans were synthesized. They tried to understand relationship between the benzene, hydroxy and methoxy fragments on 4- and 5-positions and antiproliferative activity. They wanted to search the effects of electron donating groups on antiproliferative activity. They discovered the best activity provides by the methoxy group on para position of the benzoyl moiety. Meta position was not ideal for the tubulin polimerization. In addition to the best antiproliferator activity was showed by (5-hydroxy-4-phenylbenzofuran-2-yl) (4-methoxyphenyl) methanone derivative (3d), which was activate sub-micromolar concentrations against Molt/4, CEM and HeLa cancer cell lines [9].

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 design new antimicrobial agents and find practical/economical ways to synthesis bioactive heterocyclic moieties such as benzofurans. There is many research on antimicrobial potential of 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 ketoamine analogues were also studied with docking studies as antifungal potency. They found ketoamine 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 were started with suitable 2′-hydroxyacetophenone (5 mmol), 2-bromoacetophenone (5 mmol) and potassium carbonate (6 mmol) used as starting materials to synthesized Aryl (3-methyl-benzofuran-2-yl) ketones as shown as Scheme 2. They were refluxed in acetonitrile for 4 hours. The reaction was controlled with thin layer chromatography. When the starter materials were run out and the product occurred, the reaction mixture was cooled. The solvent was evaporated and the raw product was filtered. The residue was washed with water and crystallized from ethanol [14–16].

**Biology**

**Cell Culture:** A human hepatocellular carcinoma cell line, HepG2 (ATCC® 77400™) and a rat embryo fibroblast cell line, F2408 (JRCB) were used in the study. The cells were maintained in DMEM supplemented with 10% FBS (Sigma) and 1% penicillin–streptomycin (PAA) at 37 °C under 5% CO2. Cells were harvested and passaged using 0.025 % trypsin/EDTA.

**The neutral red uptake 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 hours, 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 order to obtain 10-fold decreasing concentrations (1000–0.001 μM) of the test compounds proper dilutions were applied with DMEM. A positive control which was treated with any test agent and a negative control in which 2μM cisplatin was maintained. The neutral red uptake (NRU) assay was performed as previously described [17]. The results to different pathogenic fungi were shown as Table 1.
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:

\[
\text{% viability} = \frac{\text{test} - \text{blank}}{\text{negative control} - \text{blank}} \times 100
\]

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 hours, treated with certain concentrations of the H1, H2 and H3 compounds. The stock solutions, 100 mM, were prepared by dissolving the compounds with sterile distillate water. In order to obtain 10-fold decreasing concentrations (1000–0.001 μM) of the test compounds proper dilutions were applied with DMEM. A positive control was established with any test agent and a negative control in which F2408 cells treated with DMEM. A positive control which was treated with any test agent and a negative control in which F2408 cells treated with 10 μM and was maintained. The MTT viability assay was performed as previously described [18], with little changes. The MTT stock solution was prepared in PBS with the concentration of 5 mg/ml and was transferred to 96-well microtitre plates with 100 μM and was maintained. The MTT assay was performed as previously described [18], with little changes. The MTT stock solution was prepared in PBS with the concentration of 5 mg/ml and was filtered. At the end of the treatment periods (24 h, 48 h and 72 h) MTT working solution with the concentration of 1 mg/ml was prepared with the DMEM, 125 μl of working solution was added to each well. After incubation period which is 3 h at 37°C 100 μM DMSO was added to each well. After 15 min incubation, 96–well plate was read by ELISA reader (Biotech ELX 808 Ultramicroplate reader) at 570 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:

\[
\text{% viability} = \frac{\text{test} - \text{blank}}{\text{negative control} - \text{blank}} \times 100
\]

Three independent experiments were done by that way.

Anti-microbial activity

Anti-microbial activity of the compounds was further determined by a thin layer chromatography and NMR spectral data. In the IR spectra C=C and C=N stretching bands, characteristic for ketone’s C=O bands were observed at 1638–1616 cm⁻¹ region. 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.

\[\text{H}1\ (3\text{-methylbenzofuran-2-yl} \text{ (phenyl) methanone: M.p. 236.30 °C. IR (KBr) } \nu_{\text{max}} \text{(cm⁻¹): } 1645 \text{ (C=O), } 1647–1654 \text{ (C=C).} \]

\[\text{H}1\text{-NMR (400 MHz) (DMSO-d}_6\text{) } \delta \text{(ppm): } 2.58 \ (3\text{H, s, CH}_3), \ 7.39–7.88 \ (7\text{H, m, Ar-H}), \ 8.01–8.04 \ (2\text{H, m, Ar-H}). \text{ ES-MS: m/z: } 237 \text{ (M+1).} \]

\[\text{H}2\ (3,5\text{-dimethylbenzofuran-2-yl} \text{ (phenyl) methanone: M.p. 250.30 °C. IR (KBr) } \nu_{\text{max}} \text{(cm⁻¹): } 1643 \text{ (C=O), } 1640–1552 \text{ (C=C).} \]

\[\text{H}2\text{-NMR (400 MHz) (DMSO-d}_6\text{) } \delta \text{(ppm): } 2.45 \ (3\text{H, s, CH}_3), \ 2.54 \ (3\text{H, s, Ar-CH}_3), \ 7.37–7.69 \ (6\text{H, m, Ar-H}), \ 7.96–7.99 \ (2\text{H, m, Ar-H}). \text{ ES-MS: m/z: } 251 \text{ (M+1).} \]

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

\[\text{H}3\text{-NMR (400 MHz) (DMSO-d}_6\text{) } \delta \text{(ppm): } 3.84 \ (3\text{H, s, Ar-OCH}_3), \ 7.55–8.23 \ (8\text{H, m, Ar-H}). \text{ ES-MS: m/z: } 267 \text{ (M+1).} \]

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. ¹H-NMR: Bruker DPX 400 NMR spectrometer in DMSO-d₄, 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 ketone’s C=O bands were observed at 1510–1616 cm⁻¹ region.

\[\text{H}1\ (3\text{-methylbenzofuran-2-yl} \text{ (phenyl) methanone: M.p. 270.95 °C. IR (KBr) } \nu_{\text{max}} \text{(cm⁻¹): } 1645 \text{ (C=O), } 1647–1654 \text{ (C=C).} \]

\[\text{H}1\text{-NMR (400 MHz) (DMSO-d}_6\text{) } \delta \text{(ppm): } 2.65 \ (3\text{H, s, CH}_3), \ 7.55–8.16 \ (8\text{H, m, Ar-H}). \text{ ES-MS: m/z: } 251 \text{ (M+1).} \]

\[\text{H}2\ (3,5\text{-dimethylbenzofuran-2-yl} \text{ (phenyl) methanone: M.p. 266.45 °C. IR (KBr) } \nu_{\text{max}} \text{(cm⁻¹): } 1647 \text{ (C=O), } 1651–1550 \text{ (C=C).} \]

\[\text{H}2\text{-NMR (400 MHz) (DMSO-d}_6\text{) } \delta \text{(ppm): } 3.84 \ (3\text{H, s, Ar-OCH}_3), \ 7.55–8.23 \ (8\text{H, m, Ar-H}). \text{ ES-MS: m/z: } 267 \text{ (M+1).} \]
\textsuperscript{1}H-NMR (400 MHz) (DMSO-d\textsubscript{6}) \( \ddot{\text{a}} \) (ppm): 2.45 (3H, s, CH\textsubscript{3}), 2.54 (3H, s, Ar-CH\textsubscript{3}), 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 K\( \alpha \) radiation (\( \lambda =0.71073 \text{\textsc{\AA}} \)) at T=108(2) K. Absorption correction by multi-scan was applied. Structure was solved by direct methods and refined by full-matrix least squares against \( F^2 \) 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 \text{\textsc{\AA}} for aromatic and methyl H-atoms, respectively, and constrained to ride on their parent atoms, with \( U_{iso}(H) = k \times U_{eq}(C) \), 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) \text{\textsc{\AA}} (for atom C8). Its mean plane is oriented with respect to ring at a dihedral angle of 41.22(6)\(^\circ\). Atoms C1, C10 and C17 are 0.0015[24], -0.0237[24] and -0.0346(25) \text{\textsc{\AA}} 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 \( \mu \text{g/ml} \) 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 \( \mu \text{M} \) 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 \( \mu \text{M} \) concentration and for all periods of time tested.

**Discussion**

In this work, we have synthesized molecules named as H1, H2 and H3, completely purified, elucidated and subjected to X-ray analysis. Further the molecules were tested for their bioactivity using anti–microbial and cytotoxicity assays. As a results of activity tests, H1, H2 and H3 compounds was not able to inhibit the microbial growth even at 500 ug/ml concentration whereas chloramphenicol inhibited the microbial growth. Consistent with the anti–microbial activity, H1, H2 and H3 did
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 methoxyphenacyl 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 been 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.

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