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Short communication

Synthesis and antimicrobial activity of some novel derivatives of benzofuran: part 1. Synthesis and antimicrobial activity of (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime derivatives

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Abstract

The reaction of salicylaldehyde with 1-phenyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane **(1)** and potassium carbonate was used to prepare (benzofuran-2-yl)(3-methyl-3-phenylcyclobutyl) methanone **(2)** for the starting reagent purposes. (benzofuran-2-yl)(3-phenyl-3-methyl cyclobutyl) ketoxime **(3)** was synthesized from the reaction of the compound **(2)** with hidroxylamine. New derivatives of (benzofuran-2-yl)(3-phenyl-3-methyl cyclobutyl) ketoxime **(3)** such as, *O*-glycidylketoxime **(4)** and *O*-phenylacylketoxime **(5a–c)** were obtained very high yields. Alkyl, allyl and aryl substituted *N*-oxime ethers **(6a–e)** were obtained from the reaction compound **3** and various halogen contained compounds. The syntheses of the compounds **(7a–f)** were carried out from the reaction of the compound **(4)** and different amines such as, isopropyl amine, natrium azide, morpholine and piperazine. All of the synthesized compounds were tested for antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi, Shigella flexneri, Proteus mirabilis* ATCC 14153 and *Candida albicans* ATCC 10231. Among the synthesized compounds (benzofuran-2-yl)(3-phenyl-3-methyl cyclobutyl)-*O*-[2-hydroxy-3-(*N*-methylpiperazino)] propylketoxime **(7d)** was found the most active derivative against *S. aureus* ATCC 6538. The compounds **2, 5b, 6b, 6c, 7b** and **7f** showed very strong and the same antimicrobial effect against *C. albicans* ATCC 10231. Similarly (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-*O*-benzylketoxime **6a** showed good antimicrobial effect against *C. albicans* ATCC 10231. None of the other compounds exhibited activity against the other test microorganisms.

Keywords: Benzofuran; Cyclobutane; Ketoxime; Antimicrobial activity

1. Introduction

Widespread interest in the chemistry of benzofurans in a large number of natural products has attracted due to their biological activities and their potential applications as pharmacological agents. Several benzofuran ring systems bearing various substituents at the C-2 position are widely distributed in nature. There are well known natural products having related benzofuran ring structures, which are particularly isolated from *Machilus glaucescens, Ophryosporus charua, Ophryosporus lorentzii, Krameria ramosissima*, and *Zanthoxylum ailanthoidol* [1]. The most recognized benzofurans are *ailanthoidol, amiodarone* and *bufuralol* compounds (Scheme 1). *Ailanthoidol*, a neolignan with a 2-arylbenzofuran skeleton, was isolated from the Chinese herbal medicine *Zanthoxylum ailanthoides*. It has been reported that neolignans and lignans possess a variety of biological activities such as anticancer, antiviral, immunosuppressive, antioxidant, antifungal and antifeedant activities [2]. *Amiodarone* is a highly effective antiarrhythmic agent with class III activity according to the classification of Vaughan-Williams. It is used in the

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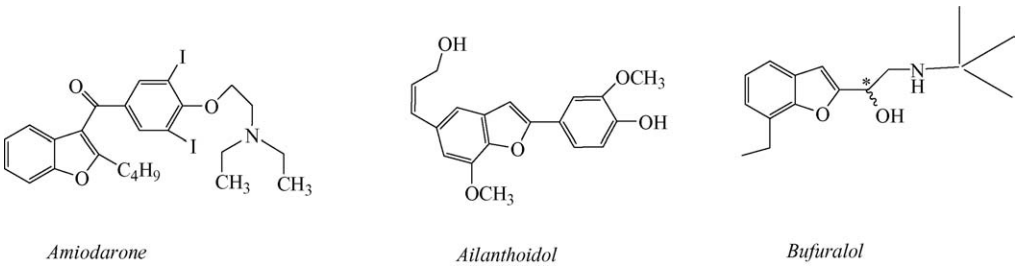


Scheme 1. Benzofuran containing some biological molecules.

treatment and prophylaxis of both ventricular and supraventricular arrhythmias, in particular in patients with heart insuffucuency, because it has no significant negative inotropic effect [3]. *Bufuralol* is a nonselective β-adrenoceptor antagonist developed by Hoffman-La Roche. *Bufuralol* is a chiral molecule having an asymmetric carbon in its ethanol amine side chain, yielding the enantiomer 1′*R-Bufuralol* and 1′*S-Bufuralol*, and the β-adrenoceptor blocking activity resides mainly in 1′*S-Bufuralol*. This compound is a good substrate of cytochrome P450 (CYP) and undergoes enantioselective and regioselective oxidations in liver [4].

The incidence of fungal infection has increased significantly in the past 25 years. The growing number of immunocompromised patients as a result of cancer chemotherapy, organ transplantation, and HIV infection are the major factors contributing to this incidence. Since *Candida albicans* (*C. albicans*), and *Aspergillus fumigatus* (*A. fumigatus*) are the main causative fungi of the systemic mycosis, antifungal drugs for treating patients of deep mycosis should have a broad antifungal spectrum including at least these microorganisms. Currently, only four classes of antifungal drugs, polyene macrolides (*amphotericin* B), azoles (*fluconazole, miconazole, itraconazole* and *voriconazole*), *flucytosine*, and *candins* (*caspofungin acetate and micafungin*), are available for treatment of systemic mycoses. Unfortunately, none of them is ideal in terms of efficacy, antifungal spectrum or safety. Although *amphotericin B* is efficacious against both *candidiasis* and *aspergillosis*, it shows severe renal toxicity [5].

In this study, as the starting material, *1-phenyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane* **(1)** was synthesized according to the cited reference [6]. *(Benzofuran-2-yl)(3-methyl-3-phenylcyclobutyl) methanone* **(2)** was obtained by reaction of salicylaldehyde with *1-phenyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane* **(1)** in presence of potassium carbonate. *(Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime* **(3)** was obtained from the reaction of the compound **(2)** with hidroxylamine hydrochloride ($NH_2OH{\cdot}HCl$). By using the compound **3**, its derivatives such as, **4, 5a–c, 6a–e** and **7a–f** were synthesized in good yields.

This article reports the first synthesis of compounds **3, 4, 5a–c, 6a–e** and **7a–f.** So far, antimicrobial activity of cyclobutane substituted benzofuran derivatives has not been investigated.

2. Chemistry

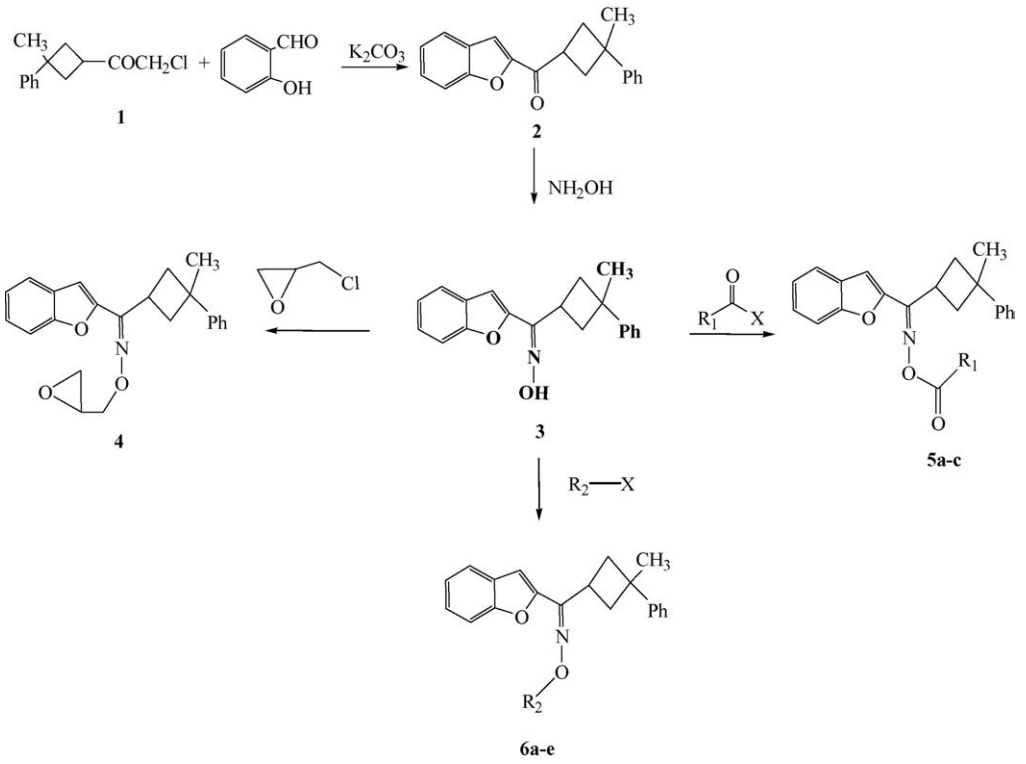
(Benzofuran-2-yl)(3-methyl-3-phenylcyclobutyl) methanone **(2)** was prepared from the reaction of the *1-phenyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane* **(1)** and salicylaldehyde with K_2CO_3 in acetone/acetonitrile solvent mixture. Structure of this compound was characterized by using FT-IR, -NMR (^1H and ^{13}C) and X-ray data [7–9].

In order to improve yields of compound **2**, many reactions were performed with various bases (NaH, KOH, Na_2CO_3 and K_2CO_3, etc.) and the solvents (acetonitrile, acetone, ethanol, benzene and THF, etc.) at various temperatures. When K_2CO_3 base and acetonitrile/acetone solvents were used for synthesis of the compound **2**, maximum yield was obtained. The most relevant temperatures were determined as boiling points of the solvents [8,9].

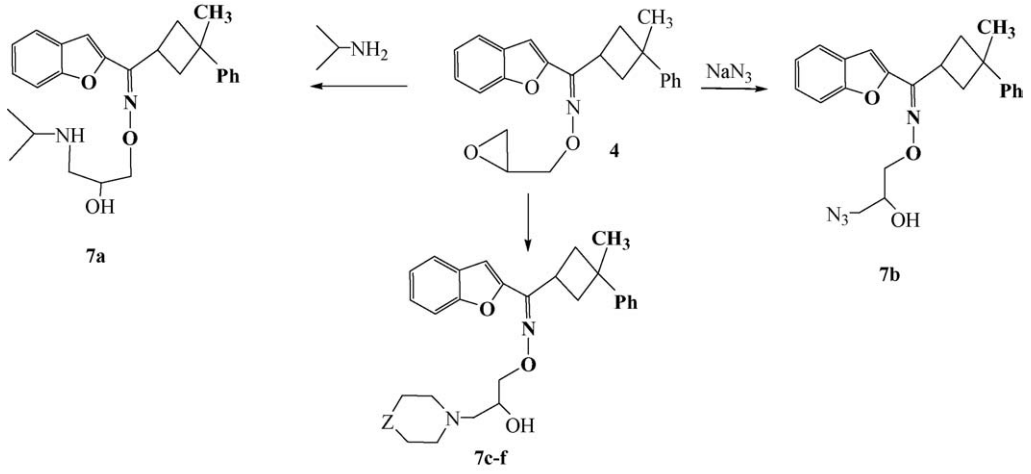
The syntheses of compounds **2**, **3, 4, 5a–c, 6a–e** and **7a–f** were presented in Schemes 2 and 3. *(Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime* **(3)** was synthesized by reflux of *(*benzofuran-*2-yl)(3-methyl-3-phenylcyclobutyl) methanone* **(2)** with hydroxylamine hydrochloride and natrium acetate in ethanol. *(*benzofuran-*2-yl)(3-phenyl-3-methylcyclobutyl)-O-glycidyl ketoxime* **4** was obtained from the reaction of *(*benzofuran-*2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime* **(3)** and epichlorohydrine and potassium hydroxide in acetone. Compound **5a–e** was obtained by reflux of *(*benzofuran-*2-yl) (3-phenyl-3-methylcyclobutyl) ketoxime* **(3)** with various acyl chlorides in acetone. The products **5a**, **b** was obtained, after the reaction mixture neutralizing with 5% ammonia solutions. In order to synthesize **5c**, acetic anhydride was used instead of acyl chloride. Sodium bicarbonate was used for neutralizing the reaction mixture in this reaction. Compounds **6a–e** were synthesized by the reaction of compound **3** and different alkyl halogens and potassium carbonate in acetone.

Compounds **7a–f** were synthesized by nucleophilic substitution reaction of *(*benzofuran-*2-yl)-(3-phenyl-3-methylcyclobutyl)-O-glycidylketoxime* **(4)** and different amines such as, isopropyl amine, piperidine, morpholine, piperazine and sodium azide.

3, 4, 5a–c, 6a–e and **7a–f** were first time synthesized and characterized in this present study. Melting points and yields of obtained compounds are presented in Table 2. The synthesized all compounds were tested in vitro for their antimicrobial activity.



Scheme 2. Synthesis of *(*benzofuran-*2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime*(**3**) derivatives.



7c : Z=CH_2 **7d :** Z= -N-CH_3 **7e :** Z= -N-C_6H_5 **7f :** Z=O

Scheme 3. Synthesis of (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-*O*-gylcidylketoxime derivatives (**7a–f**).

3. Result and discussion

We have synthesized novel compounds of cyclobutane substituted benzofuran class in this work. It was expected that synthesized compounds should have two different isomers in the *cis* and *trans* configurations, due to the methyl and phenyl groups on the cyclobutane ring. In fact, the starting material, *3-phenyl-3-methyl-1-(2-chloro-1-oxoethyl) cyclobutane* itself is an 85:15 mixture of *cis* and *trans* isomeric structures. Unfortunately, only *cis* isomer was isolated. It might be as a result of the crystallization technique. CH proton belonging to cyclobutane ring was observed at δ 3.9 ppm integrating for one proton as pentet. This signal was attributed to cis isomer for all of synthesized compounds. This is appropriate with cited references [6,8,9].

In the IR spectra of *(*benzofuran-*2-yl)(3-methyl-3-phenylcyclobutyl) methanone* **(2)** showed C=O absorption (stretching), which is adjacent to benzofuran ring, at 1689 cm^{-1} and the signals belonging to furan ring absorption were observed at 1562 and 1257 cm^{-1}. In ^{13}C-NMR spectra of this compound the signal belonging to C=O appeared at δ 194 ppm.

In the IR spectra of *(*benzofuran-*2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime* **(3)** display ed broad OH absorption peak between 3276 and 3185 cm^{-1} and N–O (stretching) absorption peak at 985 cm^{-1}. In ^{1}H-NMR spectra of compound **3** showed at δ 12 ppm (N–OH controlled by changing with D_2O). In ^{13}C-NMR spectra this compound C=N signal appeared δ 135 ppm while the signal belonging to C=O (δ 195 ppm) disappeared. As is known, oximes can be found in two different isomeric structures namely, the *syn* and *anti* configurations [10,11]. It was expected that compound **3** shows two different isomeric structures. Unfortunately, only *E*-isomer was isolated. It might be as a result of the crystallization technique. δ 12 ppm (^{1}H-NMR) and δ 135 ppm (^{13}C-NMR) signals were attributed to *E*-isomer of compound **3** of N–OH and C=N, respectively.

In the IR spectra of compound **4, 5a–c** and **6a–e** displayed no signal belonging to OH group; instead, =N–O–C stretching appeared at 1040 cm^{-1} for compound **4**. In the IR spectra of compound **5a–c**, C=O absorption of ester was displayed at 1447, 1756 and 1774 cm^{-1}, respectively. In ^{1}H- and ^{13}C-NMR spectra of compound **4** the signal belonging to 2,3-epoxipropane are appropriate with cited reference for similar to compounds [12]. In the ^{1}H-NMR spectra compounds **5a–c**, the signal belonging to CH_2, which is between phenyl and ester groups, were observed at 3.35 ppm as singlet. CH_3 protons were appeared at δ 3.2 ppm, as singlet for **5c** compound. In ^{13} C-NMR of compounds **5a–c**, C=O signals appeared δ 158, 170 and 170 ppm, respectively.

In IR spectra of compounds **6c–e** were observed C=O absorption at 1702 cm^{-1} for **6c**, $=CH_2$ plane out of plane bending at 990–919 cm^{-1} for **6d** and C=O absorption at 1753 cm^{-1}. ^{1}H-NMR spectra of compounds **6a–e** were observed at 5.40 ppm (CH_2CO for **6a**), 4.1 (OCH_3 for **6b**), 5.41 (CH_2CO for **6c**), 4.7–6.1 (allylic protons for **6d**) and 4.3 and 1.3 (OCH_2 and OCH_3 for **6e**, respectively).

In IR spectra of compounds **7a–f** were appeared broad OH absorption (stretching) at 3115 and 3448 cm^{-1}. The IR spectra **7b** compound showed additional peak at 2099 cm^{-1} due to absorption N_3. In the ^{1}H-NMR spectra of compounds **7a–f** signals belonging to CH_2CH (OH) CH_2 are suitable with cited reference [12,13]. In the ^{1}H-NMR spectra of compound **7f** showed no signal belonging to OH group due to effect of solvent.

3.1. Biological evaluation

In order to investigate the relationships between antimicrobial activity and structure, we have designed and synthesized novel compounds of cyclobutane substituted benzofuran class. The minimum inhibitory concentration (MIC) of the synthesized compounds was determined against the bacteria and the yeast *C. albicans* using a standard broth dilution technique. All the MIC results are presented in Table 1. The obtained data reported that compounds were able to inhibit the growth of the selected micro-organisms in vitro showing MIC values between 2, 5 and 0.039 mg ml^{-1}.

Table 1
Antimicrobial activity results (MIC values in mg ml^{-1}) of synthesized compounds

Sample	S.a.	C.a.
2	–	0.625
5b	–	0.625
6a	–	2.5
6b	–	0.625
6c	–	0.625
7d	0.039	–
7b	–	0.625
7f	–	0.625

S.a.: *S. aureus* ATCC 6538, C.a.: *C. albicans* ATCC 10231.

Among the synthesized compounds *(*benzofuran-*2-yl)(3-phenyl-3-methylcyclobutyl)-O-[2-hydroxy-3-(N-methylpiperazino)] propylketoxime* **7d** was found the most active derivative at an MIC value of 0.039 mg ml^{-1} against *S. aureus.* Chemically similar compounds (**7c**, **7e** and **7f**) did not at all show antimicrobial activity for resembling test microorganisms as *S. epidermidis.* The compounds **2, 5b, 6b, 6c**, **7b** and **7f** showed the same antimicrobial effect against *C. albicans* (0.625 mg ml^{-1}). Similarly *(*benzofuran-*2-yl)(3-phenyl-3-methylcyclobutyl)-O-benzyl ketoxime* **6a** showed good antimicrobial effect against *C. albicans* (2.5 mg ml^{-1}). Also, none of the other compounds exhibited activity against test microorganisms.

4. Experimental

4.1. Chemistry

All chemicals were reagent grade as received from commercial sources (Sigma–Aldrich and Fluka). *1-Phenyl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane* **(1)** [6] and *(*benzofuran-*2-yl)(3-methyl-3-phenylcyclobutyl) methanone* **(2)** were prepared following literature procedures [8,14–16]. Melting points (uncorrected) were determined with a Gallenkamp apparatus. The IR spectra were measured with Mattson 1000 FT-IR spectrophotometer (potassium bromide disks). The ^{1}H-NMR spectra were recorded on a Varian-Gemini 200 MHz spectrometer and are reported in ppm (δ) relative to tetramethylsilane (TMS) as the internal standard and ^{13}C-NMR (50.34 MHz) is referenced to deuterochloroform ($CDCl_3$). Elemental analyses were determined on a LECO CHNSO-932 auto elemental analysis apparatus.

4.1.1. Synthesis of (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime *(3)*

A mixture of the compound **2** (10 mmol, 2.9 g), $NH_2OH{\cdot}HCl$ (14 mmol, 0.973 g), $Na^{+}CH_3COO^{-}$ (14 mmol, 1.148 g) and ethanol/water (100:50 ml) were placed one-necked flask, which was refluxed for 12 h. The reaction mixture was cooled, and then water was added drop wise. The solid was filtrated off, dried and the compound **3** was crystallized from ethanol. The following product was obtained.

Table 2
Melting points, yields (%) and crystallization solvents of synthesized compounds

	R_1	R_2	m.p. (°C)	Yield (%)	Solvent
3	–	–	200–201	94	ethanol
4	–	–	159–160	87	ethanol
5a	Ph–	–	119–120	77	ethanol
5b	Ph–CH_2–	–	144–145	82	ethanol
5c	CH_3–	–	134–135	89	ethanol
6a	–	Ph–CH_2–	166–168	89	ethanol
6b	–	CH_3–	132–133	77	ethanol
6c	–	Ph–C(O)CH_2–	154–155	72	ethanol
6d	–	CH_2=$CHCH_2$–	140–142	85	ethanol
6e	–	$C_2H_5OC(O)CH_2$–	155–156	91	ethanol
7a	–	–	119–120	63	ethanol
7b	–	–	94–95	91	ethanol
7c	–	–	170–171	79	acetone
7d	–	–	143–145	89	acetone
7e			96–97	65	ethanol
7f			103–104	63	ethanol

*4.1.1.1. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime **(3)**.* IR (KBr) (υ, cm^{-1}), 3276–3185 (OH stretching), 1450 (C=N stretching) 1257 (C–O–C benzofuran stretching), 985 (N–O stretching); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.4–2.6 (m, 4H, cyclobuta ne CH_2), 3.9 (p, 1H, cyclobutane CH_2), 7.0–7.7 (m, 10H, aromatic protons), 11.82 (s, 1H, N–OH); ^{13}C-NMR (DMSO-d_6) *δ*: 32.20, 40.20, 40.40, 40.70, 40.90, 113.00, 114.10 , 123.90, 124.00, 126.20, 126.90 (2C), 127.70 (2C), 129.90 (2C), 148.50, 149.00, 153.70, 154.50. Anal. Calc. for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27; N, 4.59. Found: C, 78.69; H, 6.29; N, 5.61.

*4.1.2. Synthesis of (benzofuran-2-yl)-(3-phenyl-3-methylcyclobutyl)-O-glycidylketoxime **(4)***

The compound **3** (1 mmol, 0.305 g) and dry acetone (150 ml) were placed in a 250 ml two-necked flask with a reflux condenser. 3 mmol powder KOH (0.168 g) was added and stirred for 4 h at room temperature. To the mixture, epichlorohydrine (3.18 mmol, 0.25 ml, d = 1.18 gcm^{-3}) was added drop wise and refluxed for 8 h. The solvent was then removed under reduced pressure, the residue extracted with *d*iethyl ether and the ether phase dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the compound **3** was crystallized from ethanol. The following product was obtained.

*4.1.2.1. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-glycidylketoxime **(4)**.* IR (KBr) (υ, cm^{-1}), 2976–2862 (aliphatic C–H stretching), 1493 (C=N stretching), 1253 (epoxide ring symmetric bending), 1098 (C–ON stretching), 940–917 (epoxide ring asymmetric bending); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.7 (s, 3H, CH_3), 2.5–2.9 (m, 4H, cyclobutane CH_2), 2.9 (m, 2H, epoxide ring CH_2) 3.4 (m, 1H, epoxide ring CH) 3.9 (p, 1H, cyclobutane CH), 4.3 (dd, 2H, N–O–CH_2) 7.2–7.8 (m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 33.40, 40.58 (2C), 41.23, 41.53, 46.90, 52.20, 77.70, 113.60, 116.60, 124.30, 126.80 (2C), 127.30 (2C), 128.10, 128.30, 130.20 (2C), 148.40, 151.39, 154.50, 155.50. Anal. Calc. for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.48; H, 6.45; N, 3.91.

*4.1.3. A general method for the syntheses of compounds **5a–c***

The compound **3** (1 mmol, 0.305 g) and THF (40 ml) were placed in a 250 ml one-necked flask with a reflux condenser. 1.1 mmol acyloyl chloride was added and refluxed for 16 h. After the reaction mixture was cooled, it was neutralized with NH_3 solution. Occurred precipitate was filtrated off and washed with water. Compounds **5a–c** was crystallized from ethanol. The following product was obtained.

*4.1.3.1. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-benzoylketoxime **(5a)**.* IR (KBr) (υ, cm^{-1}), 1747 (C=O stretching), 1605 (C=C stretching), 1483 (C=N stretching) 1249 (ester C–O stretching), 998 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.7 (s, 3H, CH_3), 2.6–2.8 (m, 4H, cyclobutane CH_2), 3.9 (p, 1H, cyclobutane CH), 7.1–8.1 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 32.20, 33.40 40.50 (2C), 41.40, 113.70, 117.80, 124.50, 125.80, 126.70, 127.40, 128.10 (2C), 130.10, 130.20 (2C), 130.70 (2C), 131.20, 131.80 (2C), 135.30, 147.50, 153.70, 154.10, 156.00, 158.00. Anal. Calc. for $C_{27}H_{23}NO_3$: C, 79.20; H, 5.66; N, 3.42. Found: C, 79.22; H, 5.69; N, 3.49.

*4.1.3.2. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-phenylacetylketoxime **(5b)**.* IR (KBr) (υ, cm^{-1}), 1756 (C=O stretching), 1597 (C=C stretching), 1493 (C=N stretching), 1235 (ester C–O stretching), 1007 (N–O stretching), ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.5–2.8 (m, 4H, cyclobutane CH_2), 3.7–4.0 (m, 3H, cyclobutane CH and ester CH_2), 7.1–7.8 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 32.30, 34.00, 40.30 (2C), 41.40, 42.70, 113.60, 117.80, 124.50, 126.70 (2C), 127.30, 128.40 (2C), 128.70, 130.20 (2C), 131.70 (3C), 135.20, 148.00, 153.70, 155.80,

158.80, 170.00. Anal. Calc. for $C_{28}H_{25}NO_3$: C, 79.41; H, 5.95; N, 3.31. Found: C, 79.44; H, 5.91; N, 3.36.

*4.1.3.3. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-acetylketoxime **(5c)***. IR (KBr) (υ, cm^{-1}), 1774 (C=O stretching), 1604 (C=C stretching), 1492 (C=N stretching), 1246 (ester C–O stretching), 980 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.3 (s, 3H, CH_3) 2.6–2.8 (m, 4H, cyclobutane CH_2), 3.9 (p, 1H, cyclobutane CH), 7.1–7.7 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 21.80, 32.30, 34.10, 40.50 (2C), 41.40, 113.70, 117.70, 124.50, 125.50, 126.70, 127.40, 128.00 (2C), 128.70, 130.20 (2C), 147.20, 151.10, 153.70, 157.40, 170.00. Anal. Calc. for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 76.10; H, 6.12; N, 4.08.

*4.1.4. A general method for the syntheses of compounds **6a–e***

K_2CO_3 (0.276 g) and different alkyl halogens (2 mmol) was added to a 50 ml solution of the compound **3** (1.0 mmol, 0.305 g) in acetone while stirring at room temperature. The reaction mixture was refluxed for 12 h. After the solvent was removed by using rotary evaporator, the residue extracted with *d*iethyl ether and ether phase dried over $MgSO_4$. After filtration and removal of the solvent by under reduced pressure, the compounds **(6a–e)** were crystallized from ethanol. The following products were obtained.

*4.1.4.1. (Benzofuran-2-yl) (3-phenyl-3-methylcyclobutyl)-O-benzylketoxime **(6a)***. IR (KBr) (υ, cm^{-1}); 1610 (C=C stretching), 1492 (C=N stretching), 1117 (ether C–O stretching), 1009 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.4–2.7 (m, 4H, cyclobuta ne CH_2), 3.9 (p, 1H, cyclobutane CH), 5.3 (s, 2H, Ph–CH_2), 6.9–7.6 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 32.00, 33.30, 40.90 (2C), 41.70, 78.80, 104.40, 113.20, 115.30, 124.40, 126.70, 127.10, 129.60 (2C), 130.00 (2C), 130.20 (6C), 130.40, 139.40, 148.80, 150.10, 154.50, 155.50. Anal. Calc. for $C_{27}H_{25}NO_2$: C, 82.00; H, 6.37; N, 3.54. Found: C, 82.03; H, 6.38; N, 3.49.

*4.1.4.2. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-methylketoxime **(6b)***. IR (KBr) (υ, cm^{-1}); 1598 (C=C stretching), 1492 (C=N stretching), 1137 (ether C–O stretching), 992 (N–O stretching), ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.5–2.8 (m, 4H, cyclobutane CH_2), 3.9 (p, 1H, cyclobutane CH), 4.1 (s, 3H, OCH_3), 7.1–7.8 (m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) δ: 32.40, 33.30, 40.40 (2C), 41.40, 64.50, 115.40, 124.10, 125.00, 125.10, 127.20, 127.80 (2C), 128.00, 128.10, 130.10, 130.30, 148.60, 150.40, 154.40, 155.40. Anal. Calc. for $C_{21}H_{21}NO_2$: C, 78.97; H, 6.63; N, 4.39. Found: C, 78.94; H, 6.59; N, 4.42.

*4.1.4.3. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-phenylacylketoxime **(6c)***. IR (KBr) (υ, cm^{-1}); 1703 (C=O stretching), 1598 (C=C stretching), 1491 (C=N stretching), 1070 (ether C-O stretching), 1006 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.4–2.7 (m, 4H, cyclobutane CH_2), 3.9 (p, 1H, cyclobutane CH), 5.4 (s, 2H, O–CH_2–C=0), 7.1–7.9 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 32.30, 33.20, 40.40 (2C), 41.40, 58.10, 113.40, 116.80, 124.40, 126.70 (2C), 127.20, 128.10, 128.50 (2C), 128.80 (2C), 130.10 (2C), 130.70 (2C), 135.40, 137.70, 148.12, 152.20, 154.20, 155.50, 197.20. Anal. Calc. for $C_{28}H_{25}NO_3$: C, 79.41; H, 5.95; N, 3.31. Found: C, 79.43; H, 5.96; N, 3.38.

*4.1.4.4. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-allylketoxime **(6d)***. IR (KBr) (υ, cm^{-1}), 1644 (C=C stretching), 1493 (C=N stretching), 1030 (ether C–O stretching), 1010–919 (=CH plane out bending), 990 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.5–2.8 (m, 4H, cyclobutane CH_2), 3.9 (p, 1H, cyclobutane CH), 4.7 (d, 2H,=CH_2), 5.3 (dd, 2H, allylic CH_2), 6.1 (m, 1H,=CH), 7.1–7.9 (m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 32.40, 33.40, 40.50 (2C), 41.20, 77.80, 113.40, 115.50, 118.70, 124.10, 125.40, 126.80, 127.20, 127.80 (2C), 130.10, 131.10 (2C), 136.00, 148.60, 150.80, 154.30, 155.40. Anal. Calc. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 79.99; H, 6.68; N, 4.13.

*4.1.4.5. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-carbetoxymethylketoxime **(6e)***. IR (KBr) (υ, cm^{-1}); 1753 (C=O stretching), 1601 (C=C stretching), 1493 (C=N stretching), 1126 (ether C–O stretching), 1021 (N–O stretching), ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.2 (t, 3H, C–CH_3), 1.6 (s, 3H, CH_3), 2.4–2.7 (m, 4H, cyclobutane CH_2), 3.9 (p, 1H, cyclobutane CH), 4.2 (q, 2H, O–CH_2–C), 4.8 (s, 2H, O–CH_2–C=O), 7.1–7.8 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) δ: 18.16, 32.30, 33.20, 40.50 (2C), 41.13, 62.85, 73.60, 113.40, 118.40, 124.30, 125.10, 126.70, 127.20, 128.10, 130.10 (2C), 130.30 (2C), 148.10, 152.20, 154.20, 155.50, 171.50. Anal. Calc. for $C_{24}H_{25}NO_4$: C, 73.64; H, 6.44; N, 3.58. Found: C, 73.66; H, 6.48; N, 3.61.

*4.1.5. A general method for the syntheses of compounds **7a–f***

The compound **4** (1 mmol) and 2 mmol secondary amines (or NaN_3 for **7b**) were placed one-necked flask with a reflux condenser. The reaction mixture was refluxed with 50 ml appropriate solvent for 4 h (with water for **7a**, acetone for **7b–d** and THF for **7e, f**). The excess of secondary amine was removed by under reduced pressure. After filtration and removal of the solvent by using rotary evaporator, the compounds **(7a–f)** were crystallized from appropriate the solvent. The following products were obtained.

*4.1.5.1. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-(2-hydroxy-3-izopropylamino) propyl ketoxime **(7a)***. IR (KBr) (υ, cm^{-1}); 3475–3200 (OH stretching), 1610 (C=C stretching), 1449 (C=N stretching), 1580 (N–H bending), 1114–1099 (C–N stretching), 997 (N–O stretching); ^{1}H-NMR

($CHCl_3$-d, 200 MHz) δ (ppm), 1.1 (d, 6H, CH_3), 1.6 (s, 3H, CH_3), 2.3 (broad, 2H, OH and NH), 2.4–2.8 (m, 7H, cyclobutane 2 × CH_2, CH_2 and CH), 3.8–4.1 (m, 2H, cyclobutane CH and CH–O), 4.3 (d, 2H, O–CH_2–CH), 7.1–7.81(m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 25.00 (2C), 33.20, 40.60 (2C), 40.70, 41.20, 50.80, 51.30, 71.50, 78.90, 113.40, 115.70, 124.20, 125.10, 126.70, 127.30, 128.00, 130.10, 130.20 (2C), 130.50, 148.40, 151.30, 154.20, 155.50. Anal. Calc. for $C_{26}H_{32}N_2O_3$: C, 74.26; H, 7.67; N, 6.66. Found: C, 74.31; H, 7.69; N, 6.69.

*4.1.5.2. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-(3-azido-2-hidroxy) propylketoxime **(7b)***. IR (KBr) (υ, cm^{-1}); 3423 (broad OH stretching), 2099 (N_3 asymmetric stretching), 1620 (C=C stretching), 1493 (C=N stretching), 1302 (N_3 symmetric stretching), 1112 (C–N stretching), 980 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.4 (d, 2H, CH_2N_3) 2.4–2.6 (m, 4H, cyclobutane CH_2), 3.2 broad, 1H, OH), 3.9 (p, 1H, cyclobutane CH), 4.1(m, 1H, CH–OH), 4.3 (d, 2H, OCH_2), 7.1–7.7 (m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) δ: 32.40, 33.40, 40.50 (2C), 41.30, 55.30, 72.00, 77.30, 113.50, 116.50, 124.30, 125.30, 126.70, 127.40 (2C), 128.40, 130.10, 130.20 (2C), 148.00, 152.20, 154.00, 155.00 . Anal. Calc. for $C_{23}H_{24}N_4O_3$: C, 68.30; H, 5.98; N, 13.85. Found: C, 68.33; H, 5.99; N, 13.87.

*4.1.5.3. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-(2-hydroxy-3-piperidino) propyl ketoxime **(7c)***. IR (KBr) (υ, cm^{-1}), 3411(broad OH stretching), 1640 (C=C stretching), 1460 (C=N stretching), 1112–1110 (C–N piperidine ring), 987 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm) 1.4–1.6 (m, 6H), 1.6 (s, 3H,CH_3), 2.3–2.7 (m, 10H), 3.4 (broad, 1H, OH), 3.9 (p, 1H, cyclobutane CH), 4.1–4.3 (m, 3H, OCH_2OH), 7.1–7.7 (m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 25.80, 27.50 (2C), 32.30, 33.30, 40.60 (2C), 41.10, 56.80 (2C), 63.60, 67.90, 78.20, 113.40, 115.70, 124.20, 125.00, 126.70, 127.20, 127.30, 127.90, 130.10, 130.30 (2C), 148.50, 151.10, 154.30, 155.50. Anal. Calc. for $C_{28}H_{34}N_2O_3$: C, 75.31; H, 7.67; N, 6.27. Found: C, 75.32; H, 7.68; N, 6.25.

*4.1.5.4. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-[2-hydroxy-3-(N-methylpiperazino)] propylketoxime **(7d)***. IR (KBr) (υ, cm^{-1}), 3135 (broad OH stretching), 1603 (C=C stretching), 1493(C=N stretching), 1153–1085 (C–N piperazine ring stretching), 981 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.3 (s, 3H, piperazine CH_3), 2.4–2.8 (m, 14H, cyclobutane 2 × CH_2, piperazine CH_2 and CH_2), 3.5 (broad, 1H, OH), 3.9 (p, 1H, cyclobutane CH), 4.1 (m, 1H, CH–OH), 4.3 (d, 2H, OCH_2), 7.1–7.6 (m, 10H, aromatic protons) ^{13}C-NMR ($CDCl_3$) δ: 32.30, 33.30, 40.50 (2C), 41.10, 47.90, 55.30 (2C), 57.10 (2C), 62.60, 68.40, 79.30, 113.30, 115.70, 124.10, 125.00, 126.70 (2C), 127.20, 128.00 (2C), 130.10, 130.30, 148.50, 151.10, 154.30, 155.50. Anal. Calc. for $C_{28}H_{35}N_3O_3$: C, 72.86; H, 7.64; N, 9.10. Found: C, 72.89; H, 7.66; N, 9.11.

*4.1.5.5. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-[2-hydroxy-3-(N-phenylpiperazino)] propylketoxime **(7e)***. IR (KBr) (υ, cm^{-1}), 3300 (broad OH stretching), 1620 (C=C stretching), 1496 (C=N stretching), 1285 (Ar–N piperazine ring stretching), 1234 (broad, OH bending), 1151–1082 (C–N piperazine ring stretching), 996 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm), 1.6 (s, 3H, CH_3), 2.4–2.8 (m, 7H, cyclobutane 2 × CH_2, CH_2–N and OH), 3.0–3.2 m, 8H, piperazine CH_2), 3.9 (p, 1H, cyclobutane CH), 6.8–7.7 (m, 15H, aromatic protons); ^{13}C-NMR ($CDCl_3$) *δ*: 32.40, 33.30, 41.20, 40.80 (2C), 51.20, 52.40 (2C), 55.40 (2C), 62.70, 68.40, 113.40, 115.80, 118.10 (4C), 120.70, 124.20, 125.20, 126.70, 127.30, 128.10, 130.20 (2C), 131.30 (2C), 148.40, 151.20, 153.20, 154.30, 155.50. Anal. Calc. for $C_{33}H_{37}N_3O_3$: C, 75.69; H, 7.12; N, 8.02. Found: C, 75.71; H, 7.15; N, 7.98.

*4.1.5.6. (Benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl)-O-(2-hydroxy-3-morfolino)propylketoxi me **(7f)***. IR (KBr) (υ, cm^{-1}), 3448 (broad OH stretching), 1600 (C=C stretching), 1493 (C=N stretching), 1297 (broad, OH bending), 1038 (C–O morpholine), 997 (N–O stretching); ^{1}H-NMR ($CHCl_3$-d, 200 MHz) δ (ppm) 1.6 (s, 3H, CH_3), 2.3–2.9 (m, 14H cyclobutane 2 × CH_2, morpholine 4 × CH_2 and CH_2), 3.9 (p, 1H, cyclobutane CH), 4.2 (m, 1H, CH–OH), 4.3 (d, 2H, OCH_2), 7.1–7.8 (m, 10H, aromatic protons); ^{13}C-NMR ($CDCl_3$) δ: 32.40, 33.30, 40.50 (2C), 41.10, 55.80 (2C), 63.20, 63.30, 68.80 (2C), 78.40, 113.40, 115.50, 115.80, 124.20, 125.10, 126.70, 127.30, 128.10, 130.20 (4C), 148.30, 151.20, 154.20. Anal. Calc. for $C_{27}H_{32}N_2O_4$: C, 72.30; H, 7.19; N, 6.25. Found: C, 72.26; H, 7.24; N, 6.28.

4.2. Biological activity studies

4.2.1. Antimicrobial activity

Disk diffusion method was used for antimicrobial activity. Antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi, Shigella flexneri, Proteus mirabilis* ATCC 14153 were investigated. Mueller–Hinton agar (Difco, Detroit, USA) for all bacterial strains were used. The media were melted at 100 °C and after cooled to 50 °C were poured into plates of 9 cm diameter in quantities of 20 ml, and left on a flat surface to solidify and the surface of media was dried at 37 °C. Then, the inoculum was prepared using a 4–6 h Mueller–Hinton broth adjusted to a turbidity equivalent to a 0.5 McFarland standard (10^8 cfu ml^{-1}). A sterile cotton swab was dipped in the inoculum and the surfaces of the Mueller–Hinton agar were inoculated by streaking the swab over the surface. The surface of the media was allowed to dry 3–5 min at room temperature. The 10 mg ml^{-1} (in DMSO, E. Merck), compound impregnated disks were applied to the surface of inoculated plates .The Mueller–Hinton agar plates were incubated at 35 °C for 18–24 h. The plates were examined and the diameter of the inhibition zone was measured [18].

The plates were examined and compounds, which were found effective against the strains, were selected in order to determine the minimum inhibitory concentrations (MIC).

4.2.1.1. Determination of the MICs. MIC was determined by micro broth dilutions technique using Mueller–Hinton broth for bacteria, RPMI-1640 medium for yeast strain. Serial two-fold dilutions ranging from 5000 to 0.031 mg ml^{-1} were prepared in media. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 Mc Farland standard, diluted in broth media to give a final concentration of 5×10^5 cfu ml^{-1} for bacteria and 0.5×10^3–2.5×10^3 cfu ml^{-1} for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller–Hinton broth were incubated at 35 °C for 18–20 h and the trays containing RPMI-1640 medium were incubated at 35 °C for 46–50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth [17,19].

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