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# Synthesis, antimicrobial and antioxidant activities of 1-(1,4-benzodioxane-2-carbonyl)piperazine derivatives

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ABSTRACT

A series of novel 1-(1,4-benzodioxane-2-carbonyl)piperazine derivatives (**6a-h**) and (**7a-e**) were synthesized by nucleophilic substitution reaction of 1-(1,4-benzodioxane-2-carbonyl)piperazine (**3**) with various sulfonyl and acid chlorides. The newly synthesized compounds were characterized by elemental analyses, UV-visible, FT-IR, $^1$H NMR and LC-MS spectral studies. All compounds were evaluated for *in vitro* antibacterial, antifungal and antioxidant activities. Compound, 4-(2-trifluoromethyl)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine (**6b**) exhibited significant antimicrobial activity against tested pathogenic bacterial and fungal strains. Compound, 4-(3-methoxy)-benzoyl-1-(1,4-benzodioxane-2-carbonyl)piperazine (**7a**) showed moderate antioxidant activity compared to standard drug by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method.

## 1. Introduction

Diseases caused by microbial infections are a serious menace to the health of human being and often have connection to some other diseases whenever the body system gets debilitated. Developing antimicrobial drugs and maintaining their potency in opposition to resistance by different classes of microorganisms as well as a broad spectrum of antibacterial activity are some of the major concerns of research in this area. In recent years, there has been an increased interest in the application of antioxidants to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. Free radicals play a role in the pathogenesis of chronic degenerative diseases including cancer, inflammatory, cardiovascular and neurodegenerative diseases [1-3]. It is also known that oxidative stress can be induced by a wide range of environmental factors including UV stress, pathogen invasion, herbicide action and oxygen shortage [4]. Owing to these facts, synthetic and natural compounds with potential antioxidant activity are receiving increased attention in biological research, medicine and pharmacy [5].

Piperazine is currently the most important building block used in drug discovery with a high number of positive hits encountered in biological screens of this heterocycle and its congeners. A literature survey revealed that piperazine derivatives are important pharmacophores across a number of different therapeutic areas [6] and act as antifungal [7], antibacterial, antimalarial, antipsychotic [8] and anti-HIV protease [9]. Piperazine sulfonamides are most widely used antibacterial agents [10] in the world, chiefly because of their low cost, low toxicity, and excellent activity against common bacterial disease. 1,4-Benzodioxane-2-carboxylic acid (**1**) is very important entity in medicinal chemistry since it has chiral building blocks in the design and synthesis of chiral therapeutic agents [11]. Highly efficient resolutions of **1** with *p*-substituted 1-phenylethylamines [12], crystallographic, theoretical and morphological approach of (*S*)-1-phenylethylamine *p*-substitution on the resolution of **1** have been reported [13]. Enzymatic resolution of ethyl 1,4-benzodioxane-2-carboxylate catalyzed by a microbial esterase has been reported to produce optically active **1** in good yield [14]. Compound **1** has been used for the preparation of doxazosin mesylate which is an alpha blocker used to treat high blood pressure and benign prostatic hyperplasia. Doxazosin mesylate has been developed as an oral solid dosage form for benign prostatic hyperplasia (BPH) and antihypertensive activity [15]. Literature data showed that doxazosin mesylate can crystallize in different polymorphic forms [16,17]. In connection with such studies, the present paper reporting for the first time on the synthesis, antibacterial, antifungal and antioxidant activities of novel 1-(1,4-benzodioxane-2-carbonyl)piperazine derivatives (**6a-h**) and (**7a-e**).

## 2. Experimental

### *2.1. Instrumentation*

All solvents and reagents were purchased from Sigma Aldrich Chemicals. Melting points were determined on an electrically heated VMP-III melting point apparatus (Veego, India). The elemental analyses of the compounds were performed on a Perkin Elmer 2400 Elemental Analyser (Waltham, MA, USA). The UV-visible spectra were recorded on Analytik Jena Specord 50 UV-vis spectrophotometer with quartz cell of 1.0 cm path length in Dimethyl sulfoxide (DMSO). The FT-IR spectra were recorded using KBr discs on FT-IR 4100 Infrared spectrophotometer (Jasco, USA).


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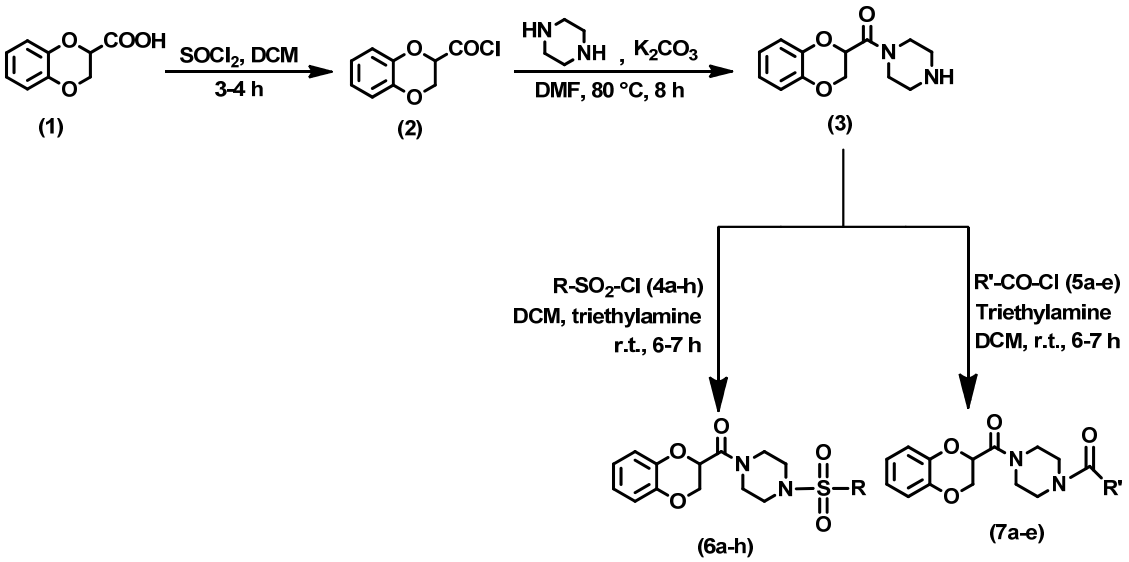



**Scheme 1**

The NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz for $^1$H NMR (Bruker AG, Germany) with tetramethylsilane as the internal standard. Mass spectral data were obtained by LC/MSD Trap XCT (Agilent Technologies, USA). Silica gel column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck-made TLC plates.

### *2.2. Synthesis of 1,4-benzodioxan-2-carboxylic acid (1)*

Initially 1,4-benzodioxan-2-carboxylic acid (**1**) was synthesized by condensation of catechol with ethyl 2,3-dibromopropionate in dry acetone in the presence of anhydrous potassium carbonate and successive saponification of the intermediate ester according to a literature method [18]. Colour: white. Yield: 76%. M.p.: 126-128 °C. FT-IR (KBr, ν, cm$^{-1}$): 3171 (br, OH carboxylic), 1718 (C=O acid), 1153 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 4.51 (dd, 1H, $J$ = 9.6, 2.8 Hz, 3-CH$_2$), 4.94 (d, 1H, $J$ = 7.4 Hz, 3-CH$_2$), 5.12 (t, 1H, $J$ = 12.6 Hz, 2-CH), 6.81-6.91 (m, 4H, Ar-H), 10.64 (s, 1H, COOH).

### *2.3. Synthesis of 1,4-benzodioxan-2-carbonyl chloride (2)*

Compound **2** was synthesized by reaction of 1,4-benzodioxan-2-carboxylic acid (**1**) with thionyl chloride in dichloromethane (DCM). The reaction mixture was stirred for 3-4 h at room temperature. Finally washed with ether and dried under vacuum (Scheme 1). Colour: white. Yield: 78%. M.p.: 133-135 °C. FT-IR (KBr, ν, cm$^{-1}$): 1653 (C=O acid), 1154 (C-O 1,4-benzodioxane), 721 (C-Cl carbonyl chloride). $^1$H NMR (DMSO-$d_6$, δ ppm): 4.52 (dd, 1H, $J$ = 9.7, 2.7 Hz, 3-CH$_2$), 4.95 (d, 1H, $J$ = 7.3 Hz, 3-CH$_2$), 5.14 (t, 1H, $J$ = 11.5 Hz, 2-CH), 6.83-6.91 (m, 4H, Ar-H).

### *2.4. Synthesis of 1-(1,4-benzodioxane-2-carbonyl)piperazine (3)*

A solution of 1,4-benzodioxan-2-carbonyl chloride (**2**) (10 g, 55.50 mmol) and piperazine (5.26 g, 61.05 mmol) in *N*,*N*-dimethylformamide was taken. $K_2CO_3$ (23.01 g, 166.5 mmol) was added to the reaction mixture. The reaction mixture was stirred for 8 h at 80 °C. The progress of the reaction was monitored by thin layer chromatography (TLC). Upon completion of the reaction, water was added and the reaction mixture was filtered. Finally washed with ether and dried under vacuum (Scheme 1). Colour: white. Yield: 86%. M.p.: 138-140 °C. FT-IR (KBr, ν, cm$^{-1}$): 3365 (N-H piperazine), 1651 (C=O acid), 1160 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 3.04-3.38 (m, 8H, piperazine-H), 4.19 (dd, 1H, $J$ = 9.0, 3.4 Hz, 3-CH$_2$), 4.38 (d, 1H, $J$ = 14.4 Hz, $J$ = 8.0 Hz, 3-CH$_2$), 5.22 (t, 1H, $J$ = 9.2 Hz, 2-CH), 5.34 (s, 1H, NH, piperazine-H), 6.81-6.91 (m, 4H, Ar-H). Anal. Calcd. for $C_{13}H_{16}N_2O_3$ (in %): C, 62.89; H, 6.50; N, 11.28. Found: C, 62.71; H, 6.42; N, 11.47.

### *2.5. General procedure for the synthesis of 1-(1,4-benzo dioxane-2-carbonyl)piperazine derivatives (6a-h) and (7a-e)*

A solution of 1-(1,4-benzodioxane-2-carbonyl)piperazine (**3**) (1.0 eq) in DCM was taken and cooled to 0-5 °C in an ice bath. Triethylamine (3.0 eq) was added to the cold reaction mixture and stirred for 10 min, then different sulfonyl chlorides (**4a-h**)/acid chlorides (**5a-e**) (1.0 eq) were added. The reaction mixture was allowed to stir at room temperature for 6-7 h. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with 10 % ammonium chloride solution and finally water wash was given to organic layer and dried with anhydrous sodium sulphate. The solvent was evaporated to get crude product which was purified by column chromatography over silica gel (60-120 mesh) using hexane: ethyl acetate (8:2) as an eluent (Scheme 1).

*4-(4-Methyl)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine* (**6a**): White. Yield: 75%. M.p.: 146-148 °C. FT-IR (KBr, ν, cm$^{-1}$): 3071 (arom CH), 1652 (C=O acid), 1162 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$) δ ppm: 2.38 (s, 3H, CH$_3$), 2.80-3.65 (m, 8H, piperazine-H), 4.11 (dd, 1H, $J$ = 9.2, 2.6 Hz, 3-CH$_2$), 4.29 (d, 1H, $J$ = 14.4 Hz, 3-CH$_2$), 5.12 (t, 1H, $J$ = 8.9 Hz, 2-CH), 6.79-6.81 (m, 4H, Ar-H), 7.43-7.45 (d, 2H, $J$ = 8.0 Hz, Ar-H), 7.59-7.61 (d, 2H, $J$ = 8.4 Hz, Ar-H). EI/MS (m/z): 403 (M$^+$+1). Anal. Calcd. for $C_{20}H_{22}N_2O_5S$ (in %): C, 59.69; H, 5.51; N, 6.96. Found: C, 59.91; H, 5.40; N, 6.75.

*4-(2-Trifluoromethyl)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine* (**6b**): Off white. Yield: 74%. M.p.: 141-142 °C. FT-IR (KBr, ν, cm$^{-1}$): 3052 (arom CH), 1655 (C=O acid), 1270 (C-F), 1167 (C-N piperazine), 1153 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 3.05 (m, 4H, piperazine-H), 3.67 (m, 4H, piperazine-H), 4.12 (dd, 1H, $J$ = 9.0, 2.9 Hz, 3-CH$_2$), 4.28 (d, 1H, $J$ = 13.6 Hz, 3-CH$_2$), 5.13 (t, 1H, $J$ =

7.6 Hz, 2-CH), 6.76-6.81 (m, 4H, Ar-H), 7.96 (t, 2H, *J* = 8.0 Hz, Ar-H), 8.04 (d, 2H, *J* = 8.4 Hz, Ar-H). EI/MS (m/z): 457 ($M^+$+1). Anal. Calcd. for $C_{20}H_{19}F_3N_2O_5S$ (in %): C, 52.63; H, 4.20; N, 6.14. Found: C, 52.49; H, 4.41; N, 6.31.

*4-(4-Chloro)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine* (**6c**): White. Yield: 78%. M.p.: 144-146 °C. FT-IR (KBr, ν, $cm^{-1}$): 3052 (arom CH), 1653 (C=O acid), 1164 (C-N piperazine), 1152 (C-O 1,4-benzodioxane), 721 (C-Cl). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.86- 3.54 (m, 8H, piperazine-H), 4.12 (dd, 1H, *J* = 9.0, 3.1 Hz, 3-$CH_2$), 4.29 (d, 1H, *J* = 15.4 Hz, 3-$CH_2$), 5.13 (t, 1H, *J* = 8.8 Hz, 2-CH), 6.77-6.81 (m, 4H, Ar-H), 7.70 (d, 2H, *J* = 2.4 Hz, Ar-H), 7.75 (d, 2H, *J* = 3.2 Hz, Ar-H). EI/MS (m/z): 423 ($M^+$+1). Anal. Calcd. for $C_{19}H_{19}ClN_2O_5S$ (in %): C, 53.96; H, 4.53; N, 6.62. Found: C, 54.02; H, 4.71; N, 6.71.

*4-Benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**6d**): White. Yield: 71%. M.p.: 129-131 °C. FT-IR (KBr, ν, $cm^{-1}$): 3075 (arom CH), 1654 (C=O acid), 1170 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.83-3.66 (m, 8H, piperazine-H), 4.11 (dd, 1H, *J* = 9.4, 3.9 Hz, 3-$CH_2$), 4.29 (d, 1H, *J* = 14.4 Hz, 3-$CH_2$), 5.13 (t, 1H, *J* = 8.8 Hz, 2-CH), 6.78-6.81 (m, 4H, Ar-H), 7.63-7.75 (m, 5H, Ar-H). EI/MS (m/z): 389 ($M^+$+1). Anal. Calcd. for $C_{19}H_{20}N_2O_5S$ (in %): C, 58.75; H, 5.19; N, 7.21. Found: C, 58.49; H, 5.07; N, 7.41.

*4-Methanesulfonyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**6e**): Brown. Yield: 70%. M.p.: 118-120 °C. FT-IR (KBr, ν, $cm^{-1}$): 1654 (C=O acid), 1169 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.85 (s, 3H, $CH_3$), 2.91-3.67 (m, 8H, piperazine-H), 4.12 (dd, 1H, *J* = 9.0, 4.5 Hz, 3-$CH_2$), 4.29 (d, 1H, *J* = 14.4 Hz, 3-$CH_2$), 5.13 (t, 1H, *J* = 8.9 Hz, 2-CH), 6.72-6.83 (m, 4H, Ar-H). EI/MS (m/z): 327 ($M^+$+1). Anal. Calcd. for $C_{14}H_{18}N_2O_5S$ (in %): C, 51.52; H, 5.56; N, 8.58. Found: C, 51.71; H, 5.40; N, 8.77.

*4-(2-Nitro)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine* (**6f**): Yellow. Yield: 77%. M.p.: 139-141 °C. FT-IR (KBr, ν, $cm^{-1}$): 3071 (arom CH), 1653 (C=O acid), 1540 ($NO_2$), 1164 (C-N piperazine), 1153 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.91-3.67 (m, 8H, piperazine-H), 4.12 (dd, 1H, *J* = 9.3, 2.8 Hz, 3-$CH_2$), 4.27 (d, 1H, *J* = 14.2 Hz, 3-$CH_2$), 5.11 (t, 1H, *J* = 8.4 Hz, 2-CH), 6.71-6.83 (m, 4H, Ar-H), 7.96-8.04 (m, 4H, Ar-H). EI/MS (m/z): 434 ($M^+$+1). Anal. Calcd. for $C_{19}H_{19}N_3O_7S$ (in %): C, 52.65; H, 4.42; N, 9.69. Found: C, 52.71; H, 4.73; N, 9.51.

*4-(2,5-Dichloro)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine* (**6g**): Off-White. Yield: 73%. M.p.: 127-130 °C. FT-IR (KBr, ν, $cm^{-1}$): 3078 (arom CH), 1651 (C=O acid), 1165 (C-N piperazine), 1154 (C-O 1,4-benzodioxane), 722 (C-Cl). $^1$H NMR (DMSO-$d_6$, δ ppm): 3.15-3.65 (m, 8H, piperazine-H), 4.15 (dd, 1H, *J* = 11.4, 5.2 Hz, 3-$CH_2$), 4.34 (d, 1H, *J* = 14.0 Hz, 3-$CH_2$), 5.18 (t, 1H, *J* = 8.4 Hz, 2-CH), 6.78-6.86 (m, 4H, Ar-H), 7.92 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.93 (s, 1H, Ar-H). EI/MS (m/z): 458 ($M^+$+1). Anal. Calcd. for $C_{19}H_{18}Cl_2N_2O_5S$ (in %): C, 49.90; H, 3.97; N, 6.13. Found: C, 50.03; H, 3.73; N, 6.37.

*4-(4-Tert-butyl)-benzenesulfonyl-1-(1,4-benzodioxane-2-carbonyl)piperazine* (**6h**): White. Yield: 74%. M.p.: 137-139 °C. FT-IR (KBr, ν, $cm^{-1}$): 3090 (arom CH), 1652 (C=O acid), 1162 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 1.35 (s, 9H, $3CH_3$), 2.92-3.67 (m, 8H, piperazine-H), 4.15 (dd, 1H, *J* = 11.2, 5.1 Hz, 3-$CH_2$), 4.25 (d, 1H, *J* = 14.3 Hz, 3-$CH_2$), 5.12 (t, 1H, *J* = 8.4 Hz, 2-CH), 6.97-7.20 (m, 4H, Ar-H), 7.57 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.86 (d, 2H, *J* = 8.1 Hz, Ar-H). EI/MS (m/z): 445 ($M^+$+1). Anal. Calcd. for $C_{23}H_{28}N_2O_5S$ (in %): C, 62.14; H, 6.35; N, 6.30. Found: C, 62.27; H, 6.25; N, 6.43.

*4-(3-Methoxy)-benzoyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**7a**): White. Yield: 76%. M.p.: 114-116 °C. FT-IR (KBr, ν, $cm^{-1}$3060 (arom CH), 1651 (C=O acid), 1167 (C-N piperazine), 1152 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 3.32-3.63 (m, 8H, piperazine-H), 3.79 (s, 3H, $OCH_3$), 4.20 (dd, 1H, *J* = 10.3, 4.7 Hz, 3-$CH_2$), 4.40 (d, 1H, *J* = 14.2 Hz, 3-$CH_2$), 5.22 (s, 1H, 2-CH), 6.81-6.90 (m, 4H, Ar-H), 6.98 (d, 2H, *J* = 8.1 Hz, Ar-H),7.03 (s, 1H, Ar-H), 7.38 (t, 1H, *J* = 16.1 Hz, Ar-H). EI/MS (m/z): 383 ($M^+$+1). Anal. Calcd. for $C_{21}H_{22}N_2O_5$ (in %): C, 65.96; H, 5.80; N, 7.33. Found: C, 65.81; H, 5.73; N, 7.17.

*4-(4-Tert-butyl)-benzoyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**7b**): Off white. Yield: 74%. M.p.: 135-137 °C. FT-IR (KBr, ν, $cm^{-1}$): 3080 (arom CH), 1650 (C=O acid), 1169 (C-N piperazine), 1153 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 1.35 (s, 9H, $3CH_3$), 2.91-3.67 (m, 8H, piperazine-H), 4.13 (dd, 1H, *J* = 11.4, 5.4 Hz, 3-$CH_2$), 4.25 (d, 1H, *J* = 14.4 Hz, 3-$CH_2$), 5.12 (t, 1H, *J* = 8.4 Hz, 2-CH), 6.95-7.05 (m, 4H, Ar-H), 7.57 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.86 (d, 2H, *J* = 8.4 Hz, Ar-H). EI/MS (m/z): 409 ($M^+$+1). Anal. Calcd. for $C_{24}H_{28}N_2O_4$ (in %): C, 70.57; H, 6.91; N, 6.86. Found: C, 70.72; H, 6.84; N, 6.91.

*4-(3,5-Dinitro)-benzoyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**7c**): Yellow. Yield: 65%. M.p.: 148-150 °C. FT-IR (KBr, ν, $cm^{-1}$): 3075 (arom CH), 1650 (C=O acid), 1540 ($NO_2$), 1169 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.92-3.67 (m, 8H, piperazine-H), 4.12 (dd, 1H, *J* = 10.5, 4.3 Hz, 3-$CH_2$), 4.28 (d, 1H, *J* = 14.3 Hz, 3-$CH_2$), 5.13 (t, 1H, *J* = 8.4 Hz, 2-CH), 6.73-6.81 (m, 4H, Ar-H), 7.61 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H). EI/MS (m/z): 443 ($M^+$+1). Anal. Calcd. for $C_{20}H_{18}N_4O_8$ (in %): C, 54.30; H, 4.10; N, 12.66. Found: C, 54.38; H, 4.20; N, 12.82.

*4-(2-Fluoro)-benzoyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**7d**): White. Yield: 65%. M.p.: 126-128 °C. FT-IR (KBr, ν, $cm^{-1}$3060 (arom CH), 1650 (C=O acid), 1299 (C-F), 1169 (C-N piperazine), 1154 (C-O 1,4-benzodioxane). $^1$H NMR (DMSO-$d_6$, δ ppm): 1.96-2.82 (m, 8H, piperazine-H), 4.12 (dd, 1H, *J* = 10.4, 3.3 Hz, 3-$CH_2$), 4.42 (d, 1H, *J* = 14.5 Hz, 3-$CH_2$), 5.13 (s, 1H, 2-CH), 7.13 (d, 1H, *J* = 6.2 Hz, Ar-H), 7.22-7.25 (m, 4H, Ar-H), 7.41 (t, 2H, *J* = 7.8 Hz, Ar-H), 8.02 (d, 1H, *J* = 8.5 Hz, Ar-H). EI/MS (m/z): 371 ($M^+$+1). Anal. Calcd. for $C_{20}H_{19}FN_2O_4$ (in %): C, 64.86; H, 5.17; N, 7.56. Found: C, 64.72; H, 5.21; N, 7.31.

*4-(3,4-Dichloro)-benzoyl-1-(1,4-benzodioxane-2-carbonyl) piperazine* (**7e**): White. Yield: 70%. M.p.: 120-122 °C. FT-IR (KBr, ν, $cm^{-1}$): 3085 (arom CH), 1651 (C=O acid), 1167 (C-N piperazine), 1153 (C-O 1,4-benzodioxane), 721 (C-Cl). $^1$H NMR (DMSO-$d_6$, δ ppm): 2.60-3.59 (m, 8H, piperazine-H), 4.15 (dd, 1H, *J* = 9.4, 2.6 Hz, 3-$CH_2$), 4.34 (d, 1H, *J* = 14.0 Hz, 3-$CH_2$), 5.18 (t, 1H, *J* = 8.4 Hz, 2-CH), 6.78-6.87 (m, 4H, Ar-H), 7.56 (d, 2H, *J* = 2.4 Hz, Ar-H), 7.58 (s, 1H, Ar-H). EI/MS (m/z): 422 ($M^+$+1). Anal. Calcd. for $C_{20}H_{18}Cl_2N_2O_4$ (in %): C, 57.02; H, 4.31; N, 6.65. Found: C, 57.13; H, 4.47; N, 6.85.

### *2.6. Antibacterial activity*

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 7443)) and Gram-negative bacteria (*Xanthomonas campestris* (MTCC 7908) and *Escherichia coli* (MTCC 7410)) in *N*,*N*-dimethylformamide (DMF) by disc diffusion method on nutrient agar medium [19]. The sterile medium (Nutrient Agar Medium, 15 mL) in each petri-plates was uniformly smeared with cultures of Gram positive and Gram-negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) were made in each of the petriplates, to which 50 µL (1 mg/mL i.e., 50 µg/disc) of the different synthesized compounds were added. The treatments also included 50 µL of DMF as negative, 10 µL of bacteromycin and gentamycin (1 mg/mL i.e., 10 µg/disc) as positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 25 ± 2 °C for 24 h and the size of the resulting zone of inhibition, if any, was determined.

### 2.7. Antifungal activity

The synthesized compounds were screened for their antifungal activity against *Fusarium oxysporum* (MTCC 2480) in DMF by poisoned food technique [20]. Potato Dextrose Agar (PDA) media was prepared and about 15 mL of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the center of the petriplates and incubated at 26 °C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin (concentration was 500 µL i.e., 0.1 mg/mL) was used as standard. All the synthesized compounds were tested (at the dosage of 500 µL of the novel compounds/petriplate, where concentration was 0.1 mg/mL) by poisoned food technique.

### 2.8. Antioxidant activity

The free radical scavenging activity of the synthesized compounds was studied *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method [21]. Stock solution of the drug was diluted to different concentrations in the range of 100-200 µg/mL in methanol. Methanol solution of the synthesized compounds (3 mL) was added to methanol solution of DPPH (1 mL). The samples were kept in the dark for 30 min after which the optical density was measured at 517 nm and the percentage of scavenging activity was calculated. Ascorbic acid was used as standard. The inhibition ratio (I %) of the tested compounds was calculated according to the following equation: I % = (Ac-As) / Ac × 100, where Ac is the absorbance of the control and as is the absorbance of the sample. The concentration of compounds providing 50 % scavenging of $DPPH^{\cdot}$ ($IC_{50}$) was calculated from the plot of percentage inhibition against concentration (µg/mL) [22,23]. All tests and analyses were done in triplicate and the results were averaged.

## 3. Results and discussion

### 3.1. Chemistry

In the present work, a series of thirteen new compounds were synthesized. Scheme 1 illustrates the way the target compounds were prepared. The synthesis of 1,4-benzodioxan-2-carboxylic acid (**1**) was carried out by the condensation of catechol with ethyl 2,3-dibromopropionate in dry acetone. 1,4-Benzodioxan-2-carbonyl chloride (**2**) was prepared by reacting **1** with thionyl chloride in dichloromethane (DCM). 1-(1,4-Benzodioxane-2-carbonyl)piperazine (**3**) was synthesized from **2** with piperazine in dry DMF at 80 °C for 8 h. The progress of the reaction was monitored by TLC. Further, the intermediate **3** was reacted with various sulfonyl chlorides (R-$SO_2$-Cl, **4a-h**) and acid chlorides (R'-CO-Cl, **5a-e**) in DCM to obtain 1,4-benzodioxan-2-carboxylic acid derivatives (**6a-h**) and (**7a-e**) in high yields (65-78 %). These synthesized compounds were characterized by elemental analyses, UV-visible, FT-IR, $^1$H NMR and LC-MS spectral studies. Antimicrobial and antioxidant activities were reported. The chemical structure and physical data of novel compounds are given in Table 1. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4 %. The electronic absorption spectral data showed that the synthesized compounds were very similar and displayed main features at 310-410 nm using DMSO.

The FT-IR spectra of (**6a-h**) and (**7a-e**) were recorded using KBr pellets in the range of 4000-400 $cm^{-1}$. The absorption bands at 3052-3093 $cm^{-1}$ are assigned to the aromatic C-H stretch. The bands at 1650-1655 $cm^{-1}$ are due to the presence of C=O stretch. The strong bands at 1153-1154 $cm^{-1}$ are assigned to the C-O stretch. The absorption band at 3365 $cm^{-1}$ is due to the N-H stretch in compound **3**. The absence of N-H absorption band in (**6a-h**) and (**7a-e**) confirmed the synthesized compounds. The strong bands at 1323-1327 $cm^{-1}$ and 1156-1170 $cm^{-1}$ are attributed to $SO_2$ (*asym*. stretch) and $SO_2$ (*sym*. stretch), respectively [24]. New bands appeared at 1270 $cm^{-1}$ (**6b**) and 1299 $cm^{-1}$ (**7d**) corresponding to C-F stretching frequency. The strong bands at 721-722 $cm^{-1}$ are assigned to the C-Cl stretch in **6c**, **6g** and **7e**. The absorption at 1540 $cm^{-1}$ is corresponding to $NO_2$ (*asym*. stretch) in **6f** and **7c**.

The characteristic resonance peaks in $^1$H NMR for the novel compounds were reported using DMSO. The expected resonances were assigned by their peak multiplicity and integration. The integration of spectra shows good agreement with the synthesized compounds. The proton NMR spectral data of NH in **3** show single resonance at δ 5.34 ppm which is absent in the spectra of (**6a-h**) and (**7a-e**), indicating the replacement of the sulfonamide series and carboxamide series. In addition, the resonance appeared in the range of δ 6.71-8.05 ppm as singlets, doublets, triplets and multiplates are attributed to the aromatic protons. The piperazine protons were resonated as multipletes at δ 2.60-3.67 ppm [25]. The dioxane ring protons were resonated in the region δ 4.11-5.22 ppm. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. A strong signal assigned to the carbon atoms in piperazine ring appeared at 51.6-55.2 ppm. All the compounds were further confirmed by the appearance of molecular ion peak in mass spectra. Mass spectra of the newly synthesized compounds showed $M^+$ fragmentation peak in agreement with their molecular formula.

### 3.2. In vitro biological activity

The investigation of antibacterial screening data revealed that all tested compounds showed antibacterial activity against four pathogenic bacterial strains. Among the series (**6a-h**), compound **6b** exhibited an elevated antibacterial activity against Gram positive (zone of inhibition 28-32 mm) and Gram negative (zone of inhibition 30-33 mm) bacteria. Compounds **6c**, **6f**, **6g**, **7c**, **7e** and **7d** showed significant antibacterial activity against all the tested organisms. Compounds **6h**, **7a** and **7b** showed moderate inhibitory activity against *S. aureus* in comparison to standard drugs. Compounds **6a**, **6d** and **6e** showed no inhibition against *X. campestris*. The results were compared with standard drugs bacteromycin and gentamycin as depicted in Table 2.

The *in vitro* antifungal activity of the synthesized compounds (**6a-h**) and (**7a-e**) were studied against *Fusarium oxysporum*. The results were compared with the standard drug nystatin as collected in Table 2. Compound **6b** showed significant antifungal activity with 88.5 % inhibition when compared with other compounds in the series against *F. oxysporum*. Compounds **6a**, **6d** and **6e** were found to be moderately active against tested fungal strain. Compounds **6c**, **6f**, **6g**, **6h** and (**7a-e**) have been demonstrated significant antifungal activity against *F. oxysporum*. From the results it is evident that most of the compounds showed significant activity and few are moderately active. The compounds (**6a-h**) and (**7a-e**) showed antimicrobial activity in the order: **6b** > **6g** > **7e** > **7c** > **6c** > **7d** > **6f** > **7a** > **6h** > **7b** > **6a** > **6d** > **6e** against tested microbial strains.

The *in vitro* assay of DPPH scavenging radicals were performed spectrophotometrically with ascorbic acid as positive control, and the results are shown in Table 3. All the compounds showed DPPH scavenger activity at the 200 µg/mL with a scavenging effect in the range, 30.5-58.0 % (**6a-h**) and 35.3-70.5 % (**7a-e**).

**Table 1.** Chemical structure and physical data of 1-(1,4-benzodioxane-2-carbonyl)piperazine derivatives (**6a-h**) and (**7a-e**).

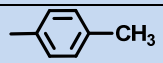
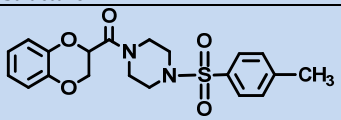
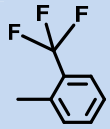
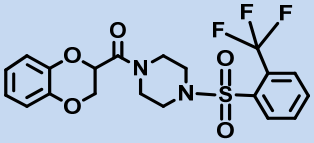
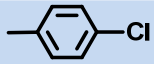
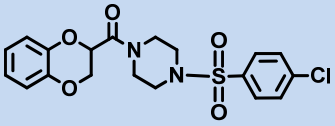
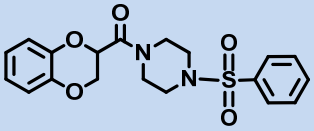
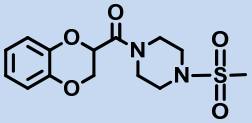
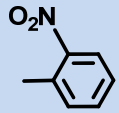
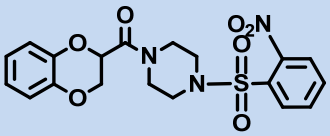
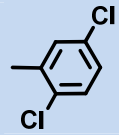
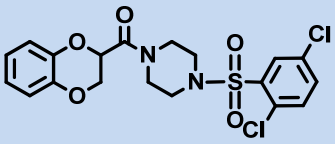
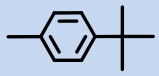
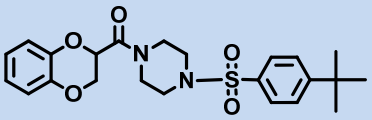
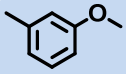
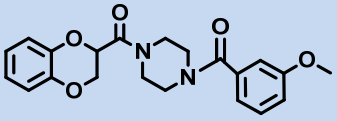
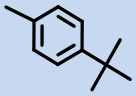
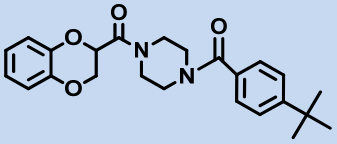
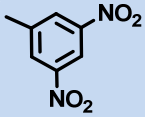
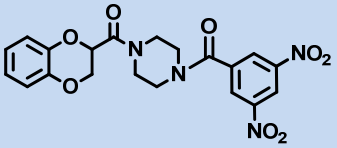
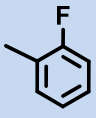
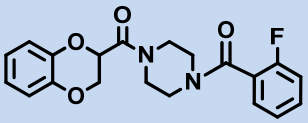
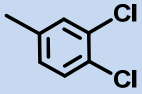
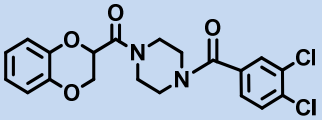
| Compound | R | R' | Structure | UV-visible ($\lambda_{max}$) |
|---|---|---|---|---|
| 6a |  | - |  | 325 |
| 6b |  | - |  | 310 |
| 6c |  | - |  | 330 |
| 6d |  | - |  | 326 |
| 6e |  | - |  | 410 |
| 6f |  | - |  | 458 |
| 6g |  | - |  | 312 |
| 6h |  | - |  | 384 |
| 7a | - |  |  | 345 |
| 7b | - |  |  | 383 |
| 7c | - |  |  | 468 |
| 7d | - |  |  | 405 |
| 7e | - |  |  | 355 |

**Table 2**. *In vitro* antibacterial and antifungal activities of (**6a-h**) and (**7a-e**)[a].

| Compound | Zone of inhibition in diameter (mm)[b] | | | | % Inhibition[b] |
|---|---|---|---|---|---|
| | Bs | Sa | Xc | Ec | Fo |
| 6a | 14 (± 0.53) | 13 (±0.49) | NA[c] | 13 (±0.49) | 59.5 (±0.81) |
| 6b | 32 (± 0.60) | 28 (±1.06) | 33 (±1.24) | 30 (±1.13) | 88.5 (±0.69) |
| 6c | 19 (±0.69) | 18 (±0.68) | 20 (±0.75) | 21 (±0.79) | 78.6 (±0.58) |
| 6d | 13 (±0.81) | 12 (±0.45) | NA | 12 (±0.45) | 55.4 (±0.56) |
| 6e | 12 (±0.45) | 11 (±0.54) | NA | 12 (±0.45) | 53.2 (±0.94) |
| 6f | 17 (±0.98) | 16 (±0.60) | 19 (±0.69) | 20 (±0.72) | 68.7 (±0.99) |
| 6g | 24 (±0.75) | 21 (±0.79) | 25 (±0.94) | 26 (±0.98) | 79.3 (±0.87) |
| 6h | 14 (±0.49) | 17 (±0.98) | 13 (±0.81) | 15 (±0.56) | 63.4 (±0.77) |
| 7a | 15 (±0.56) | 16 (±0.60) | 14 (±0.53) | 16 (±0.60) | 67.1 (±0.71) |
| 7b | 14 (±0.76) | 16 (±0.60) | 12 (±0.45) | 14 (±0.53) | 63.2 (±0.64) |
| 7c | 20 (±0.72) | 19 (±0.69) | 21 (±0.79) | 23 (±0.87) | 75.4 (±0.57) |
| 7d | 17 (±0.68) | 17 (±0.68) | 20 (±0.72) | 20 (±0.72) | 73.0 (±0.84) |
| 7e | 23 (±0.93) | 20 (±0.72) | 23 (±0.93) | 25 (±0.94) | 79.2 (±0.88) |
| Bacteromycin | - | - | 34 (±1.28) | - | - |
| Gentamycin | 35 (±1.32) | 30 (±1.13) | - | 35 (±1.32) | - |
| Nystatin | - | - | - | - | 100 (±0.69) |

[a] Mean value (±SEM).
[b] Bs: *Bacillus subtilis*; Sa: *Staphylococcus aureus*; Xc: *Xanthomonas campestris*; Ec: *Escherichia coli*; Fo: *Fusarium oxysporum.*
[c] NA: Not active.

**Table 3**. Results of DPPH radical scavenging assay of (**6a-h**) and (**7a-e**).

| Compound | % Scavenging effect | | | $IC_{50}$ µg/mL |
|---|---|---|---|---|
| | 100 µg/mL | 150 µg/mL | 200 µg/mL | |
| 6a | 35.4 | 40.5 | 46.0 | - |
| 6b | 36.9 | 41.8 | 47.2 | - |
| 6c | 22.8 | 28.0 | 33.2 | - |
| 6d | 38.6 | 43.8 | 49.5 | - |
| 6e | 21.4 | 26.7 | 30.5 | - |
| 6f | 28.7 | 33.2 | 38.9 | - |
| 6g | 21.7 | 27.0 | 32.4 | - |
| 6h | 45.8 | 52.4 | 58.8 | 120.7 |
| 7a | 58.6 | 64.7 | 70.5 | 35.2 |
| 7b | 45.9 | 52.8 | 58.3 | 121.0 |
| 7c | 34.1 | 40.4 | 45.8 | - |
| 7d | 24.9 | 30.8 | 35.3 | - |
| 7e | 27.9 | 32.8 | 38.3 | - |
| Ascorbic acid | 86.4 | 91.3 | 98.7 | 10.6 |

Compound **7a** exhibited significant antioxidant activity with $IC_{50}$ value of 35.2 µg/mL when compared to other compounds in the series. **6h**, **7a** and **7b** showed more than 50 % antioxidant activity at 200 µg/mL. The compounds (**6a**-**g**) and (**7c**-**e**) showed less antioxidant activity.

The initial structure activity relationship (SAR) can be drawn for the compounds (**6a**-**h**) and (**7a**-**e**). In the present study, different electron withdrawing and electron donating groups attached to phenyl ring as substituent which is linked to sulfonyl and carbonyl groups were studied for antimicrobial and antioxidant efficacy. The **6b** produce comparable antimicrobial activity with commercially available standard antibiotics. The presence of same substituents at the same position of the phenyl ring of the series, **6h** and **7b**, the relatively more antimicrobial and antioxidant activities of **6h** may be due the presence of sulfonamide functionalized derivative. The same substituents in the series, (**6f**,**7c**) and (**6c**,**7e**) at the different position emphasizes that the electron withdrawing group increases the potency of the compound, and hence carboxamide functionalized derivatives showed relatively more antimicrobial activity. Changing the substituent on the phenyl ring (**6a**,**7a**) reveals that the carboxamide functionalized derivatives showed relatively more antimicrobial and antioxidant activities. The compound **7a** showed higher radical inhibition activity which is due to the presence of methoxy group in the aromatic ring [26]. The above SAR correlation study reveals that, the nature of the functional linkage (-$SO_2$- or -CO-) and substituent on phenyl ring influences the antimicrobial and antioxidant activities.

## 4. Conclusion

Series of novel 1-(1,4-benzodioxane-2-carbonyl)piperazine derivatives (**6a**-**h**) and (**7a**-**e**) were synthesized and their antimicrobial and antioxidant activities have been evaluated. Trifluoromethyl group (**6b**) produced significant changes in activity against Gram-positive and Gram-negative bacteria. Electron donating methoxy group (**7a**) exhibited the highest free radical scavenging activity compared to other compounds in the series. The SAR studies revealed that, the nature of functional linkage (-$SO_2$- and -CO-) and substituents (electron withdrawing and electron donating groups) on phenyl ring are crucial for antimicrobial and antioxidant activities. On the basis of their activity, these derivatives were identified as viable leads for further studies.

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