Cytotoxic Activities of some Novel Benzhydrylpiperazine Derivatives.

ARTICLE · FEBRUARY 2013

DOI: 10.1055/s-0032-1333275 · Source: PubMed

4 AUTHORS:

Ece Gürdal
Yeditepe University
4 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Mine Yarım
Yeditepe University
31 PUBLICATIONS **107** CITATIONS
SEE PROFILE

Irem Durmaz
Bilkent University
17 PUBLICATIONS **17** CITATIONS
SEE PROFILE

Rengul Cetin-Atalay
Middle East Technical University
65 PUBLICATIONS **401** CITATIONS
SEE PROFILE

Cytotoxic Activities of some Novel Benzhydrylpiperazine Derivatives

Authors
E. E. Gurdal[1], M. Yarim[1], I. Durmaz[2], R. Cetin-Atalay[2]

Affiliations
[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey
[2] Department of Molecular Biology and Genetics, BilGen, Genetics and Biotechnology Research Center, Faculty of Science, Bilkent University, Ankara, Turkey

Key words
- benzhydrylpiperazine
- benzoyl chloride
- sulfonyl chloride
- cytotoxicity
- sulphorhodamine B

Abstract

This study presents the synthesis of nineteen 1-(substitutedbenzoyl)-4-benzhydrylpiperazine and 1-[(substitutedphenyl)sulfonyl]-4-benzhydrylpiperazine derivatives. In vitro cytotoxic activities of the compounds were screened against hepatocellular (HUH-7), breast (MCF-7) and colorectal (HCT-116) cancer cell lines by sulphorhodamine B assay. Among the test compounds, benzamide derivatives had high cytotoxic activity whereas sulfonamide derivatives showed variable 50% growth inhibition (GI50).

Introduction

Cancer is the disease resulting from abnormal cells with abilities of uncontrolled dividing and invasion to other tissues through blood and lymph systems. Recently, advanced treatment opportunities are unable to overcome the major problems of chemotherapy such as drug resistance and severe side effects due to lack of specificity. Regarding issues lead the researchers to develop varying drug-like compounds targeting cancer.

Benzhydrylpiperazines are popular with their antihistaminic activities [1–6]. Literature search reveals many other activities of benzhydrylpiperazine derivatives including calcium channel blocking [7–14], dopaminergic [15–18], antimicrobial [19–36], and antiviral [37, 38] activities.

Anticancer activity of benzhydrylpiperazines has recently become important [39–46]. Kumar et al. has performed cytotoxicity assays to several 1-benzhydrylpiperazine derivatives substituted with variable sulfonyl chlorides, acid chlorides and isothiocyanates. These derivatives have potent cytotoxicity over breast cancer (MCF-7), hepatocellular carcinoma (HepG-2), cervix carcinoma (HeLa) and colon carcinoma (HT-29) cell lines [39]. Yarim et al., also synthesized some 4-chlorobenzhydrylpiperazines substituted with variable benzoyl chloride derivatives and reported their high cytotoxic activities against liver (HUH-7, FOCUS, MAHLAVU, HEPG2, HEP3B), breast (MCF-7, BT20, T47D, CAMA-1), colon (HCT-116), gastric (KATO-3) and endometrial (MFE-296) cancer cell lines [40].

In this study, we reported the synthesis, purification and characterization of some novel compounds bearing benzhydrylpiperazine backbone. Those compounds were tested for their cytotoxic activities against hepatocellular (HUH-7), breast (MCF-7) and colorectal (HCT-116) cancer cell lines with sulphorhodamine B assay. We aimed to develop a structure activity relationship for benzhydrylpiperazine derivatives in accordance with their cytotoxic activity results.

Materials and Methods

Chemistry

All chemicals and reagents used in current study were analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (°C) of the compounds were determined by using a Mettler Toledo FP62 capillary melting point apparatus (Mettler-Toledo, Greifensee, Switzerland) and are uncorrected. Ultraviolet spectra were recorded with Agilent 8453 UV-Visible Spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1, Perkin Elmer, Norwalk, CT, USA), using potassium bromide pellets, the frequencies were expressed in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), using tetramethylsilane (TMS) as the internal reference, with dimethylsulfoxide (DMSO-d_6) as solvent, the chemical shifts were reported in parts per million

received 27.11.2012
accepted 20.12.2012

Bibliography
DOI http://dx.doi.org/10.1055/s-0032-1333275
Published online: February 11, 2013
Drug Res 2013; 63: 121–128 © Georg Thieme Verlag KG Stuttgart · New York
ISSN 2194-9379

Correspondence
E. E. Gurdal, PhD
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
Yeditepe University
34755 Kayisdagi
Istanbul
Turkey
Tel.: +90/216/578 00 00-3293
Fax: +90/216/578 00 68
egurdal@yeditepe.edu.tr

(ppm). Coupling constants were recorded in Hertz (Hz). The mass spectra were recorded with a Waters 2695 Alliance Micromass ZQ LC/MS instrument (Waters Corp., Milford, MA, USA). Elemental analyses were performed on LECO 932 CHNS (LECO-932, St. Joseph, MI, USA) instrument and were within ±0.4% of the theoretical values.

General procedure for preparation of benzhydrole derivatives [44]

10 mmol (2.2 g) benzophenone was dissolved in 10 ml ethanol. In a separate flask, 11 mmol (0.4 g) sodium borohydride ($NaBH_4$) was dissolved in 2 ml ethanol. Sodium borohydride solution was slowly added to benzophenone solution with a Pasteur pipette. Reaction mixture was allowed to continue stirring for a further 30 min. For the work up of reaction, 2 ml of concentrated HCl was added to a 20 ml ice-water solution. Reaction mixture was poured into this ice cold solution slowly with stirring. White solid product was collected with vacuum filtration and washed twice with distilled water. 4-Chlorobenzophenone and 4,4′-difluorobenzophenone were also reacted with sodium borohydride to give 4-chlorobenzhydrole and 4,4′-difluorobenzhydrole respectively according to above procedure.

General procedure for preparation of Benzhydryl chloride derivatives [47]

10 mmol (1.84 g) benzhydrole was added to 15 ml of concentrated HCl. 10 mmol (1.1 g) anhydrous calcium chloride was added to the mixture to be refluxed at 85 °C for 4 h with stirring. After reaction completed, the flask was cooled to room temperature and extracted twice with 20 ml ethyl acetate. Organic layers were combined together, washed with brine and water, then dried over anhydrous sodium sulfate. Followed by the concentration under vacuo, the product was collected as brown liquid. 4-Chlorobenzhydryl chloride and 4,4′-difluorobenzhydryl chloride were also synthesized from 4-chlorobenzhydrole and 4,4′-difluorobenzhydrole according to above procedure.

General procedure for preparation of Benzhydrylpiperazine derivatives [44]

9 mmol (0.8 g) piperazine was dissolved in dimethylformamide. Anhydrous potassium carbonate was added to the solution and stirred for 10 min. Followed by the addition of 9 mmol (1.8 g) benzhydryl chloride, reaction mixture was heated at 80 °C for 8 h. After completion, dimethylformamide was removed under vacuo, then residue was taken in water and extracted with ethyl acetate. Organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated and white solid product was obtained. 1-[(4-Chlorophenyl)(phenyl)methyl]piperazine and 4,4′-benzhydrylpiperazine were also synthesized from 4-chlorobenzhydryl chloride and 4,4′-difluorobenzhydryl chloride consecutively according to above procedure.

General procedure for preparation of 1-(Substitutedbenzoyl)-4-[benzhydryl/4-chlorobenzhydryl/4,4′-difluorobenzhydryl] piperazine derivatives (5a–5i)

2 mmol (0.5 g) 1-benzhydrylpiperazine or 1.7 mmol (0.5 g) 1-[bis(4-fluorophenyl)methyl]piperazine or 0.9 mmol (0.3 g) 1-[(4-chlorophenyl)(phenyl)methyl]-piperazine was dissolved in 50 mL dry dichloromethane. Reaction flask was taken into ice bath and triethylamine (triple moles of benzhydrylpiperazine derivative) was added to the solution. 10 min later ice bath was removed and appropriate benzoyl chloride derivatives (equimolar with benzhydrylpiperazine derivative) were added. Reaction was stirred overnight at room temperature. After the reaction was completed, solution was extracted in order with water and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. The mixture was concentrated in vacuo and product was dissolved in ethyl acetate. Column chromatography was applied with *n*-hexane-ethyl acetate (80:20) mixture in silica gel column. Only oily products were dissolved in diethylether and HCl gas was passed through the solutions to obtain solid hydrochloride salt of compounds.

1-(5-Fluoro-2-methylbenzoyl)-4-(diphenylmethyl) piperazine hydrochloride (5a)

White, opaque, powdered crystals. Yield: 95% (0.402 g); m.p.: Above 300 °C. UV (MeOH, λ_{max}, nm); 205 (log ε: 5.22), 224 (log ε: 4.74). FT-IR (KBr, cm^{-1}); 3423 (N-H), 3043 (C-H, aromatic), 2955 (C-H, aliphatic), 1654 (C=O, amide), 1612 (C=C, aromatic), 1293 (C-N), 1261 (C-F). ^{1}H-NMR (DMSO, ppm); 2.13 (s, 3H, $-CH_3$); 3.2 (bs, 4H, piperazine H_3, H_5); 3.76 (bs, 4H, piperazine H_2, H_6); 5.64 (d, 1H, $(Ar)_2CH$-, *J=8.4 Hz*); 7.12 (t, 2H, diphenyl $H_{4,4'}$, *J=8.8 Hz*); 7.26–7.29 (dd, 4H, diphenyl $H_{3,5,3',5'}$, J_1*=5.2 Hz,* J_2*=2.8 Hz*); 7.31 (d, 1H, phenyl H_4, *J=7.6 Hz)*; 7.37 (d, 1H, phenyl H_3, *J=6.8 Hz*); 7.44 (s, 1H, phenyl H_6); 7.86–7.92 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=10.8 Hz,* J_2*=7.2 Hz*); (12.61 (bs, 1H, N-H salt). ^{13}C-NMR (DMSO, ppm); 37.98 (C_{25}); 43.09 ($C_{14,16}$); 51.49–51.74 ($C_{15,17}$); 75.28 (C_7); 113.31–113.54 ($C_{2,6,9,13}$); 116.36–116.56 ($C_{3,5,10,12}$); 129.17(C_4); 129.55 (C_{11}); 129.97 (C_1); 130.73 (C_8); 132.9 (C_{21}); 132.98 (C_{22}); 136.15 (C_{24}); 137.43 (C_{20}); 159.60 (C_{19}); 162.02(C_{23}); 167.86 (C_{18}). MS (m/z); 389.8 (100%, M^+ – Cl); 167.5 ($(C_6H_5)_2CH^+$). Anal. calcd. for $C_{25}H_{26}ClFN_2O$ (424.94): C, 70.66; H, 6.17; N, 6.59. Found: C, 69.77; H, 5.94; N, 6.63.

1-(2-Bromobenzoyl)-4-[bis(4-fluorophenyl)methyl] piperazine hydrochloride (5b)

White, opaque, powdered crystals. Yield: 35% (0.3 g); m.p.: 189.7 °C. UV (MeOH, λ_{max}, nm); 205 (log ε: 5.27), 223 (log ε: 4.49). FT-IR (KBr, cm^{-1}); 3423 (N-H), 3006 (C-H, aromatic), 2924 (C-H, aliphatic), 1643 (C=O, amide), 1606 (C=C, aromatic), 1292 (C-N), 1232 (C-F). ^{1}H-NMR (DMSO, ppm); 3.03 (bs, 4H, piperazine H_3, H_5); 3.67 (bs, 4H, piperazine H_2, H_6); 5.59 (s, 1H, $(Ar)_2CH$-); 7.27–7.91 (m, 17H, aromatic H's); 12.8 (bs, 1H, N-H salt). MS (m/z); 471.92 (90%, M^+ – Cl), 473.92 (89%, M+2), 203.60 (100%, $(4\text{-F-}C_6H_5)_2CH^+$). Anal. calcd. for $C_{24}H_{22}BrClF_2N_2O.H_2O$ (524.07): C, 54.82; H, 4.60; N, 5.33. Found: C, 54.45; H, 4.83; N, 5.50.

1-(3-Bromobenzoyl)-4-[bis(4-fluorophenyl)methyl] piperazine hydrochloride (5c)

White, opaque, powdered crystals. Yield: 27% (0.23 g); m.p.: 151.4 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 5.09), 223 (log ε: 4.51). FT-IR (KBr, cm^{-1}); 3425 (N-H), 3068 (C-H, aromatic), 2924 (C-H, aliphatic), 1638 (C=O, amide), 1606 (C=C, aromatic), 1290 (C-N), 1233 (C-F). ^{1}H-NMR (DMSO, ppm); 3.11 (bs, 4H, piperazine H_3, H_5); 3.67 (bs, 4H, piperazine H_2, H_6); 5.59 (bs, 1H, $(Ar)_2CH$-); 7.27–7.93 (m, 13H, aromatic H's); 12.63 (bs, 1H, N-H salt). MS (m/z); 471.91 (95%, M^+ -Cl), 473.91 (94%, M+2), 203.61 (100%, $(4\text{-F-}C_6H_5)_2CH^+$). Anal. calcd. for $C_{24}H_{22}BrClF_2N_2O.H_2O$ (524.07): C, 54.82; H, 4.60; N, 5.33. Found: C, 54.81; H, 4.59; N, 5.60.

1-(4-Bromobenzoyl)-4-[bis(4-fluorophenyl)methyl] piperazine hydrochloride (5d)

White, opaque, powdered crystals. Yield: 19% (0.1654 g); m.p.: Above 300 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 5.23), 224 (log ε: 4.46). FT-IR (KBr, cm^{-1}); 3437 (N-H), 3008 (C-H, aromatic), 2950 (C-H, aliphatic), 1640 (C=O, amide), 1606 (C=C, aromatic), 1290 (C-N), 1233 (C-F). ^{1}H-NMR (DMSO, ppm); 3.09 (bs, 4H, piperazine H_3, H_5); 3.68 (bs, 4H, piperazine H_2, H_6); 5.58 (bs, 1H, $(Ar)_2CH$-); 7.15–7.91 (m, 12H, aromatic H's); 12.69 (bs, 1H, N-H salt). MS (m/z); 471.92 (68%, M^+ – Cl), 473.92 (67%, M+2), 203.59 (100%, $(4\text{-F-}C_6H_5)_2CH^+$). Anal. calcd. for $C_{24}H_{22}BrClF_2N_2O.H_2O$ (524.07): C, 54.82; H, 4.60; N, 5.33. Found: C, 55.02; H, 4.84; N, 5.62.

1-(3-Chlorobenzoyl)-4-[bis(4-fluorophenyl)methyl] piperazine hydrochloride (5e)

Yellowish, opaque, powdered crystals. Yield: 47% (0.3663 g); m.p.: 177.5 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 5.14), 226 (log ε: 4.29). FT-IR (KBr, cm^{-1}); 3423 (N-H), 3007 (C-H, aromatic), 2925 (C-H, aliphatic), 1639 (C=O, amide), 1606 (C=C, aromatic), 1290 (C-N), 1233 (C-F). ^{1}H-NMR (DMSO, ppm); 3.13 (bs, 4H, piperazine H_3, H_5); 3.65–4.44 (m, 4H, piperazine H_2, H_6); 5.62 (bs, 1H, $(Ar)_2CH$-); 7.25–7.97 (m, 12H, aromatic H's); 12.72 (bs, 1H, N-H salt). MS (m/z); 427.97 (98%, M^+ – Cl); 429.96 (32%, M+2), 203.61 (100%, $(4\text{-F-}C_6H_5)_2CH^+$). Anal. calcd. for $C_{24}H_{22}Cl_2F_2N_2O.H_2O$ (480.12): C, 59.88; H, 5.03; N, 5.82. Found: C, 59.93; H, 5.06; N, 5.98.

1-(2-Methoxybenzoyl)-4-[(4-chlorophenyl)(phenyl) methyl]piperazine (5f)

White, shiny, needle-shaped crystals. Yield: 33% (0.140 g); m.p.: 120 °C. UV (MeOH, λ_{max}, nm); 202 (log ε: 4.78), 275 (log ε: 3.36). FT-IR (KBr, cm^{-1}); 3029 (C-H, aromatic), 2996 (C-H, aliphatic), 1626 (C=O, amide), 1602 (C=C, aromatic), 1296 (C-O), 1247 (C-N), 1000 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.18–2.33 (m, 4H, piperazine H_3, H_5); 3.63 (m, 2H, piperazine H_2); 3.14 (m, 2H, piperazine H_6); 3.75 (s, 3H, $-OCH_3$); 4.39 (s, 1H, $(Ar)_2CH$-); 6.95 (t, 1H, benzoyl H_4, *J=7.2 Hz*); 7.03 (d, 1H, benzoyl H_6, *J=8 Hz*); 7.12–7.15 (dd, 1H, benzoyl H_3, J_1*=1.6, Hz,* J_2*=6 Hz*); 7.2 (t, 1H, phenyl H_4, *J=7.2 Hz*); 7.3 (t, 2H, phenyl $H_{3,5}$, *J=7.2 Hz*); 7.35 (d, 2H, phenyl $H_{2,6}$, *J=8 Hz*); 7.38 (t, 1H, benzoyl H_5, *J=1.6 Hz*); 7.39–7.46 (dd, 4H, 4-chlorophenyl H's, J_1*=7.6 Hz,* J_2*=10.4 Hz*). Anal. calcd. for $C_{25}H_{25}ClN_2O_2$ (420.93): C, 71.33; H, 5.99; N, 6.66. Found: C, 70.78; H, 6.41; N, 6.73.

1-(3-Nitrobenzoyl)-4-[(4-chlorophenyl)(phenyl)methyl] piperazine hydrochloride (5g)

Yellowish orange, opaque, powdered crystals. Yield: 24% (0.112 g); m.p.: 196.1 °C. UV (MeOH, λ_{max}, nm); 203 (log ε: 4.33), 227 (log ε: 4.02). FT-IR (KBr, cm^{-1}); 3423 (N-H), 3063 (C-H, aromatic), 2924 (C-H, aliphatic), 1644 (C=O, amide), 1533 (N=O), 1291 (C-N), 1092 (C-Cl). ^{1}H-NMR (DMSO, ppm); 3.17 (bs, 4H, piperazine H_3, H_5); 3.83 (bs, 4H, piperazine H_2, H_6); 5.59 (s, 1H, $(Ar)_2CH$-); 7.3–8.5 (m, 13H, aromatic H's); 12.71 (s, 1H, N-H salt). Anal. calcd. for $C_{24}H_{23}Cl_2N_3O_3.H_2O$ (490.38): C, 58.78; H, 5.14; N, 8.57. Found: C, 58.57; H, 5.52; N, 8.64.

1-(3,4-Dimethoxybenzoyl)-4-[(4-chlorophenyl)(phenyl) methyl]piperazine (5h)

White, opaque, powdered crystals. Yield: 11% (0.050 g); m.p.: 148.6 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 4.59), 276 (log ε: 3.45). FT-IR (KBr, cm^{-1}); 3082 (C-H, aromatic), 2966 (C-H, aliphatic), 1621 (C=O, amide), 1583 (C=C, aromatic), 1268 (C-O), 1230 (C-N), 1027 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.31 (bs, 4H, piperazine H_3, H_5); 3.52 (bs, 4H, piperazine H_2, H_6); 3.75 (s, 3H, $-OCH_3$); 3.76 (s, 3H, $-OCH_3$); 4.39 (s, 1H, $(Ar)_2CH$-); 6.91 (d, 1H, benzoyl H_5, *J=2.8 Hz*); 6.94 (d, 1H, benzoyl H_6, *J=3.6 Hz*); 6.97 (s, 1H, benzoyl H_2); 7.21 (t, 2H, phenyl $H_{3,5}$, *J=7.6 Hz*); 7.31 (t, 1H, phenyl H_4, *J=8 Hz*); 7.35 (d, 2H, phenyl $H_{2,6}$, *J=8.4 Hz*); 7.4–7.47 (dd, 4H, 4-chlorophenyl H's, J_1*=8.8 Hz,* J_2*=10 Hz*). Anal. calcd. for $C_{26}H_{27}ClN_2O_3$ (450.96): C, 69.25; H, 6.03; N, 6.21. Found: C, 69.03; H, 6.32; N, 6.30.

1-(4-Ethylbenzoyl)-4-[(4-chlorophenyl)(phenyl)methyl] piperazine hydrochloride (5i)

Yellow, opaque, powdered crystals. Yield: 22% (0.1 g); m.p.: 206.4 °C. UV (MeOH, λ_{max}, nm): 204 (log ε: 4.56), 225 (log ε: 4.11). FT-IR (KBr, cm^{-1}); 3422 (N-H), 3010 (C-H, aromatic), 2966 (C-H, aliphatic), 1634 (C=O, amide), 1287 (C-N), 1092 (C-Cl). ^{1}H-NMR (DMSO, ppm); 1.15 (t, 3H, $-CH_3$, *J=8 Hz*); 2.63 (q, 2H, $-CH_2$-, *J=7.6 Hz*); 3.13 (s, 4H, piperazine H_3, H_5); 3.79 (s, 4H, piperazine H_2, H_6); 5.63 (d, 1H, $(Ar)_2CH$-, *J=8.4 Hz*); 7.27–7.90 (m, 13H, aromatic H's); 12.63 (s, 1H, N-H salt). Anal. calcd. for $C_{26}H_{28}Cl_2N_2O.H_2O$ (473.43): C, 65.96; H, 6.39; N, 5.92. Found: C, 66.33; H, 6.73; N, 5.98.

General procedure for preparation of 1-[(substitutedphenyl)sulfonyl]-4-[benzhydryl/4-chlorobenzhydryl/4,4′-difluorobenzhydryl]piperazines

1 mmol (0.2575 g) 1-benzhydrylpiperazine, 1.7 mmol (0.515 g) 1-[bis(4-fluorophenyl)methyl]piperazine or 0.872 mmol (0.2632 g) 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was dissolved in 20 ml dry dichloromethane. Reaction flask was taken into ice bath and triethylamine (1:3 moles) was added to the solution. 10 min later, suitable sulfonyl chloride derivative (1:1 mole) was added. Ice bath was removed 2 h later and reaction was stirred overnight at room temperature. After reaction was completed, solution was extracted in order with water and ammonium chloride solution (10%). Dichloromethane layer was washed with water again and dried with anhydrous sodium sulphate. Solvent was evaporated under vacuo and solid product was recrystallized with ethanol/water. Oily product, **6a**, was dissolved in diethylether. HCl gas was passed through the solution and solid hydrochloride salt of compound was obtained. Compound needed no further purification.

1-[2-(Trifluoromethoxy)phenylsulfonyl]-4-(diphenylmethyl)piperazine hydrochloride (6a)

White, opaque, powdered crystals. Yield: 10% (0.05 g); m.p.: 205.8 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 5.24), 226 (log ε: 4.87). FT-IR (KBr, cm^{-1}); 3445 (N-H), 3007 (C-H, aromatic), 2909 (C-H, aliphatic), 1590 (C=C, aromatic), 1353 (S=O, asym.), 1282 (C-O), 1248 (C-F), 1209 (C-N), 1167 (S=O, sym.). ^{1}H-NMR (DMSO, ppm); 3.21–3.38 (m, 4H, piperazine H_3, H_5); 3.74 (m, 4H, piperazine H_2, H_6); 5.57 (d, 1H, $(Ar)_2CH$-, *J=8.4 Hz*); 7.31–7.89 (m, 14H, aromatic H's); 12.5 (bs, 1H, N-H salt). MS (m/z); 477.8 (100%, M^+ – Cl); 167.6 ($(C_6H_5)_2CH^+$). Anal. calcd. for $C_{24}H_{24}ClF_3N_2O_3S$ (512.97): C, 56.19; H, 4.72; N, 5.46; S, 6.25. C, 55.85; H, 4.82; N, 5.80; S, 6.21.

1-[2-(Trifluoromethyl)phenylsulfonyl]-4-[(bis (4-fluorophenyl)methyl]piperazine (6b)

Colourless, shiny, prism-shaped crystals. Yield: 22% (0.112 g); m.p.: 135.6 °C. UV (MeOH, λ_{max}, nm): 203 (log ε: 5.16), 225 (log ε: 4.67). FT-IR (KBr, cm^{-1}); 3074 (C-H, aromatic), 2917 (C-H,

aliphatic), 1604 (C=C, aromatic), 1370 (S=O, asym.), 1284 (C-N), 1218 (C-F), 1144 (S=O, sym.). ^{1}H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H_3, H_5); 3.18 (bs, 4H, piperazine H_2, H_6); 4.45 (s, 1H, $(Ar)_2CH$-); 7.11 (t, 4H, diphenyl $H_{3,5,3',5'}$, *J=9.2 Hz*); 7.39–7.43 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=5.6 Hz*, J_2*=3.2 Hz*); 7.91–8.06 (m, 4H, 2-trifluoro-methylphenyl). Anal. calcd. for $C_{24}H_{21}F_5N_2O_2S$ (496.49): C, 58.06; H, 4.26; N, 5.64; S, 6.46. Found: C, 58.19; H, 4.22; N, 5.79; S, 6.58.

1-[(2,4,5-Trichlorophenyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (6c)

White, shiny, needle-shaped crystals. Yield: 11% (0.060 g), m.p.: Above 300 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 5.09), 225 (log ε: 4.79). FT-IR (KBr, cm^{-1}); 3089 (C-H, aromatic), 2969 (C-H, aliphatic), 1602 (C=C, aromatic), 1373 (S=O, asym.), 1282 (C-N), 1219 (C-F), 1153 (S=O, sym.), 1097 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.32 (bs, 4H, piperazine H_3, H_5); 3.24 (bs, 4H, piperazine H_2, H_6); 4.45 (s, 1H, $(Ar)_2CH$-); 7.11 (t, 4H, diphenyl $H_{3,5,3',5'}$, *J=8.4 Hz*); 7.39–7.43 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=5.6 Hz*, J_2*=3.2 Hz*); 8.05 (s, 1H, 2,4,5-trichlorophenyl, H_6); 8.17 (s, 1H, 2,4,5-trichlorophenyl, H_3). Anal. calcd. for $C_{23}H_{19}Cl_3F_2N_2O_2S$ (531.83): C, 51.94; H, 3.60; N, 5.27; S 6.03. Found: C, 51.73; H, 3.82; N, 5.56; S, 6.10.

1-[(3,4-Dichlorophenyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl]piperazine (6d)

White, opaque, powdered crystals. Yield: 42% (0.210 g); m.p.: 145.1 °C. UV (MeOH, λ_{max}, nm); 203 (log ε: 5.35), 227 (log ε: 4.46). FT-IR (KBr, cm^{-1}); 3088 (C-H, aromatic), 2979 (C-H, aliphatic), 1602 (C=C, aromatic), 1355 (S=O, asym.), 1286 (C-N), 1220 (C-F), 1175 (S=O, sym.), 1031 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H_3, H_5); 2.98 (bs, 4H, piperazine H_2, H_6); 4.42 (s, 1H, $(Ar)_2CH$-); 7.09 (t, 4H, diphenyl $H_{3,5,3',5'}$, *J=8.8 Hz*); 7.37–7.40 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=5.6 Hz*, J_2*=3.2 Hz*); 7.69–7.97 (m, 3H, 3,4-dichlorophenyl). Anal. calcd. for $C_{23}H_{20}Cl_2F_2N_2O_2S$ (497.38): C, 55.54; H, 4.05; N, 5.63; S, 6.45. Found: C, 55.70; H, 4.11; N, 5.92; S, 6.57.

1-[(o-Toluyl)sulfonyl]-4-[bis(4-fluorophenyl)methyl] piperazine (6e)

White, opaque, powdered crystals. Yield: 10% (0.05 g); m.p.: 117.7 °C. UV (MeOH, λ_{max}, nm); 206 (log ε: 5.32), 225 (log ε: 4.67). FT-IR (KBr, cm^{-1}); 3068 (C-H, aromatic), 2968 (C-H, aliphatic), 1601 (C=C, aromatic), 1342 (S=O, asym.), 1229 (C-N), 1221 (C-F), 1156 (S=O, sym.). ^{1}H-NMR (DMSO, ppm); 2.33 (bs, 4H, piperazine H_3, H_5); 2.55 (s, 3H, -CH_3); 3.04 (bs, 4H, piperazine H_2, H_6); 4.41 (s, 1H, $(Ar)_2CH$-); 7.10 (t, 4H, diphenyl $H_{3,5,3',5'}$, *J=8.8 Hz*); 7.38–7.41 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=5.6 Hz*, J_2*=3.2 Hz*); 7.42–7.47 (dd, 2H, 2-methylphenyl $H_{3,5}$, J_1*=5.2 Hz*, J_2*=7.6 Hz*); 7.57–7.62 (dd, 1H, 2-methylphenyl H_4, J_1*=1.2 Hz*, J_2*=7.2 Hz*); 7.75–7.77 (dd, 1H, 2-methylphenyl H_6, J_1*=1.2 Hz*, J_2*=6.8 Hz*). Anal. calcd. for $C_{23}H_{20}Cl_2F_2N_2O_2S$ (497.38); C, 65.14; H, 5.47; N, 6.33; S, 7.25. Found: C, 65.51; H, 5.30; N 6.56; S 7.37.

1-[(4-Nitrophenyl)sulfonyl]-4-[bis(4-fluorophenyl) methyl]piperazine (6f, CAS No: 1286459-36-6)

Yellowish orange, shiny, powdered crystals. Yield: 13% (0.06 g); m.p.: 224.5 °C. UV (MeOH, λ_{max}, nm): 203 (log ε: 5.23), 225 (log ε: 4.39). FT-IR (KBr, cm^{-1}); 3070 (C-H, aromatic), 2996 (C-H, aliphatic), 1604 (C=C, aromatic), 1527 (N=O), 1359 (S=O, asym.), 1224 (C-N), 1213 (C-F), 1172 (S=O, sym.). ^{1}H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H_3, H_5); 2.99 (bs, 4H, piperazine H_2, H_6); 4.42 (s, 1H, $(Ar)_2CH$-); 7.09 (t, 4H, diphenyl $H_{3,5,3',5'}$, *J=8 Hz*); 7.35–7.38 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=5.2 Hz*, J_2*=3.2 Hz*); 7.99 (d, 2H, 4-nitrophenyl $H_{2,6}$, *J=9.2 Hz*); 8.47 (d, 2H, 4-nitrophenyl $H_{3,5}$, *J=8.8 Hz*). Anal. calcd. for $C_{23}H_{21}F_2N_3O_4S$ (473.49): C, 58.34; H, 4.47; N, 8.87; S, 6.77. Found: C, 57.75; H, 4.56; N, 8.91; S, 6.83.

1-[(2,5-Dichlorophenyl)sulfonyl]-4-[bis(4-fluorophenyl) methyl]piperazine (6g)

Colourless, shiny, prism-shaped crystals. Yield: 23% (0.115 g); m.p.: 116.1 °C. UV (MeOH, λ_{max}, nm): 205 (log ε: 5.19), 224 (log ε: 4.91). FT-IR (KBr, cm^{-1}); 3003 (C-H, aromatic), 2966 (C-H, aliphatic), 1603 (C=C, aromatic), 1375 (S=O, asym.), 1284 (C-N), 1218 (C-F), 1178 (S=O, sym.), 1009 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.33 (bs, 4H, piperazine H_3, H_5); 3.23 (bs, 4H, piperazine H_2, H_6); 4.44 (s, 1H, $(Ar)_2CH$-); 7.11 (t, 4H, diphenyl $H_{3,5,3',5'}$, *J=8.4 Hz*); 7.39–7.43 (dd, 4H, diphenyl $H_{2,6,2',6'}$, J_1*=5.6 Hz*, J_2*=3.2 Hz*); 7.75–7.81 (m, 2H, 2,5-dichlorophenyl H_3, H_4); 7.9 (d, 1H, 2,5-dichlorophenyl H_6, *J=2.4 Hz*). ^{13}C-NMR (DMSO, ppm); 46.39 ($C_{14,16}$); 51.28 ($C_{15,17}$); 72.82 (C_7); 115.95–116.16 ($C_{3,10}$); 130.02–130.10 ($C_{2,9}$); 130.48 (C_{20}); 131.61 (C_{21}); 132.99 (C_{23}); 134.79 (C_{22}); 135.02 (C_{19}); 137.34 (C_{18}); 138.83–138.85 ($C_{1,8}$); 160.57–162.99 ($C_{4,11}$). MS (m/z); 497.98 (25%, M^+); 499.8 (12%, M+2); 203.5 (100%, $(4\text{-F-}C_6H_5)_2CH^+$). Anal. calcd. for $C_{23}H_{20}Cl_2F_2N_2O_2S$ (497.38): C, 55.54; H, 4.05; N, 5.63; S, 6.45. Found: C, 55.55; H, 3.89; N, 5.89; S, 6.58.

1-[(2,4,5-Trichlorophenyl)sulfonyl]-4-[(4-chlorophenyl) (phenyl)methyl]piperazine (6h)

White, opaque, powdered crystals. Yield: 19% (0.100 g); m.p.: 151.1 °C. UV (MeOH, λ_{max}, nm): 205 (log ε: 4.66), 234 (log ε: 4.09). FT-IR (KBr, cm^{-1}); 3091 (C-H, aromatic), 2967 (C-H, aliphatic), 1568 (C=C, aromatic), 1352 (S=O, asym.), 1283 (C-N), 1164 (S=O, sym.), 1066 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.29 (bs, 4H, piperazine H_3, H_5); 3.21 (bs, 4H, piperazine H_2, H_6); 4.37 (s, 1H, $(Ar)_2CH$-); 7.16 (t, 2H, phenyl $H_{3,5}$, *J=6.8 Hz*); 7.27 (t, 1H, phenyl H_4, *J=7.6 Hz*); 7.29–7.35 (dd, 4H, 4-chlorophenyl H's, J_1*=8.4 Hz*, J_2*=4.4 Hz*); 7.37 (d, 2H, phenyl $H_{2,6}$, *J=8.4 Hz*); 8.016 (s, 1H, 2,4,5-trichlorophenyl H_6); 8.14 (s, 1H, 2,4,5- trichlorophenyl H_3). Anal. calcd. for $C_{23}H_{20}Cl_4N_2O_2S$ (530.29): C, 52.09; H, 3.80; N, 5.28; S, 6.05. Found: C, 52.22; H, 4.00; N, 5.52; S, 6.19.

1-[(3,4-Dichlorophenyl)sulfonyl]-4-[(4-chlorophenyl) (phenyl)methyl]piperazine (6i)

White, opaque, powdered crystals. Yield: 25% (0.123 g); m.p.: 107.1 °C. UV (MeOH, λ_{max}, nm); 204 (log ε: 4.35), 236 (log ε: 4.11). FT-IR (KBr, cm^{-1}); 3063 (C-H, aromatic), 2965 (C-H, aliphatic), 1560 (C=C, aromatic), 1356 (S=O, asym.), 1281 (C-N), 1172 (S=O, sym.), 1033 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.36 (bs, 4H, piperazine H_3, H_5); 2.98 (bs, 4H, piperazine H_2, H_6); 4.37 (s, 1H, $(Ar)_2CH$-); 7.18 (t, 2H, phenyl $H_{3,5}$, *J=6.8 Hz*); 7.27 (t, 1H, phenyl H_4, *J=7.2 Hz*); 7.31–7.36 (dd, 4H, 4-chlorophenyl H's, J_1*=8.4 Hz*, J_2*=3.6 Hz*); 7.39 (d, 2H, phenyl $H_{2,6}$, *J=8.4 Hz*); 7.69–7.97 (m, 3H, 3,4-dichlorophenyl H's). Anal. calcd. for $C_{23}H_{21}Cl_3N_2O_2S$ (495.85): C, 55.71; H, 4.27; N, 5.65; S, 6.47. Found: C, 55.82; H, 4.35; N, 5.91; S, 6.51.

1-[(4-Nitrophenyl)sulfonyl]-4-[(4-chlorophenyl)(phenyl) methyl]piperazine (6j)

Yellowish orange, opaque, cottonlike crystals. Yield: 37% (0.175 g); m.p.: 209.3 °C. UV (MeOH, λ_{max}, nm); 202 (log ε: 4.53), 231 (log ε: 4.18), 264 (log ε: 3.82). FT-IR (KBr, cm^{-1}); 3055 (C-H,

aromatic), 2947 (C-H, aliphatic), 1606 (C=C, aromatic), 1534 (N=O), 1356 (S=O, asym.), 1281 (C-N), 1170 (S=O, sym.), 1088 (C-Cl). ^{1}H-NMR (DMSO, ppm); 2.36 (bs, 4H, piperazine H_3, H_5); 2.99 (bs, 4H, piperazine H_2, H_6); 4.38 (s, 1H, $(Ar)_2CH$-); 7.18 (t, 2H, phenyl $H_{3,5}$, *J=7.2Hz*); 7.26 (t, 1H, phenyl H_4, *J=8Hz*); 7.30–7.34 (dd, 4H, 4-chlorophenyl H's, J_1*=6.4Hz*, J_2*=2Hz*); 7.37 (d, 2H, phenyl $H_{2,6}$, *J=8.8Hz*); 8.01 (d, 2H, 4-nitrophenyl H_2, H_6, *J=8.8Hz*); 8.46 (d, 2H, 4-nitrophenyl H_3, H_5, *J=8.8Hz*). ^{13}C-NMR (DMSO, ppm); 46.67 ($C_{14,16}$); 50.81 ($C_{15,17}$); 73.69 (C_7); 125.38 ($C_{20,22}$); 127.79 (C_{11}); 128.08 ($C_{10,12}$); 129.18 ($C_{19,23}$); 129.29 ($C_{9,13}$); 129.81 ($C_{2,6}$); 129.89 ($C_{3,5}$); 132.11 (C_4); 140.99 (C_1); 141.92 (C_8); 142.29 (C_{18}); 150.76 (C_{21}). MS (m/z); 472.8 (25%, M^+); 474.8 (12%, M+2); 201.5 (100% (4-Cl-C_6H_5)-(C_6H_5)CH^+); 203.6 (38%). Anal. calcd. for $C_{23}H_{22}ClN_3O_4S$ (471.96); C, 58.53; H, 4.70; N, 8.90; S, 6.79. Found: C, 58.56; H, 4.83; N, 8.99; S, 6.84.

Cytotoxicity studies

The cytotoxic activities of the synthesized compounds were investigated on liver (HUH-7), breast (MCF-7) and colon (HCT-116) cancer cell lines, by means of sulphorhodamine B (SRB) assays in triplicate. Serial dilutions from 100µM to 2.5µM were used, 5-fluorouracil (5-FU) was the reference compound and camptothecin (CPT) was the positive control for the cytotoxic effect.

Cell culture

The human cancer cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. Each cell line was maintained in an incubator at 37°C supplied with 5% CO_2 and 95% air.

NCI-60 Sulphorhodamine B (SRB) assay

Cancer cells (range of 2000cell/well to 5000cell/well) were inoculated into 96-well plates in 200µl of media and incubated in 37°C incubators containing 5% CO_2 and 95% air. After a 24h incubation period, one plate for each cell line was fixed with 100µl 10% ice-cold trichloroacetic acid (TCA). This plate represents the behavior of the cells just prior to drug treatment and is accepted as the time-zero plate. The compounds to be tested were solubilized in DMSO to a final concentration of 40mM and stored at +4°C. While treating the cells with the stock compound solutions, the corresponding volume of the compound was applied to the cell to achieve the desired drug concentration and diluted through serial dilution. After drug treatment, the cells were incubated in 37°C incubators containing 5% CO_2 and 95% air for 72h. Following the termination of the incubation period after drug treatment, the cells were fixed with 100µl 10% ice-cold TCA and incubated in the dark at +4°C for 1h. Then the TCA was washed away with ddH_2O 5 times and the plates were left to air dry. For the final step, the plates were stained with 100µl of 0.4% sulphorhodamine B (SRB) solution in 1% acetic acid solution. Following staining, the plates were incubated in dark for 10min at room temperature. The unbound dye was washed away using 1% acetic acid and the plates were left to air dry. To measure the absorbance results, the bound stain was then solubilized using 200µl of 10mM Tris-Base. The OD values were obtained at 515nm.

Results and Discussion

Chemistry

The synthesis of the benzhydrylpiperazine derivatives **5a–6j** is outlined in **Fig. 1**.

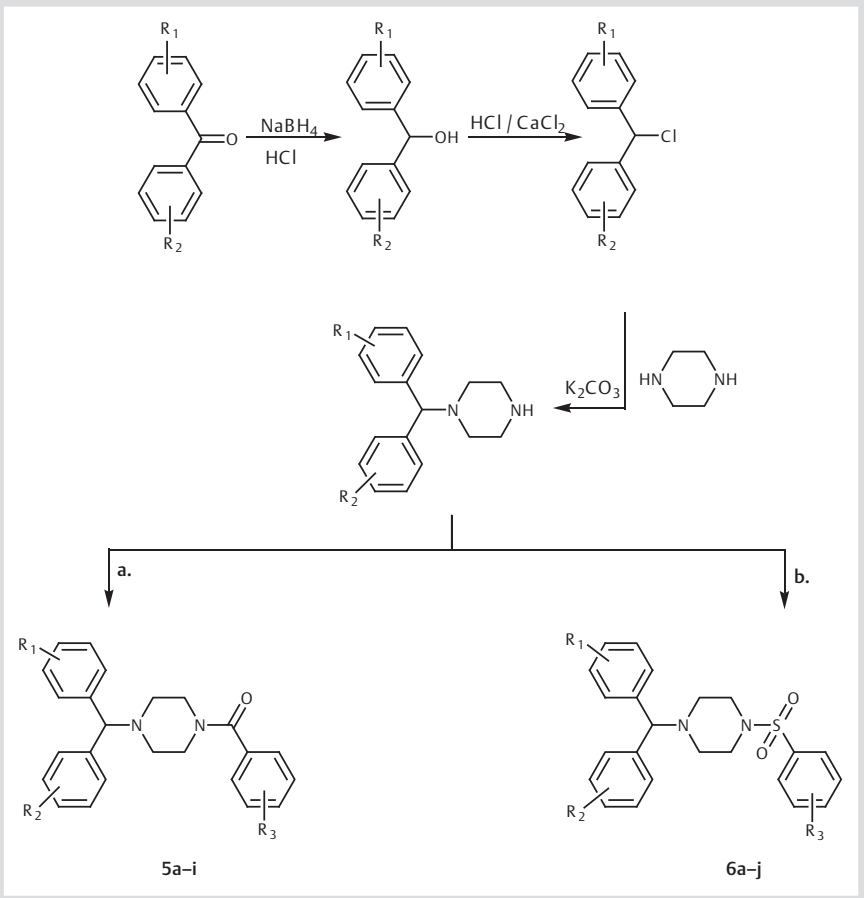


Fig. 1 Synthesis of compounds **5a–i** and **6a–j** **a** TEA, DCM, benzoyl chlorides, **b** TEA, DCM, sulfonyl chlorides.

Reduction with sodium borohydride of benzophenone, 4-chlorobenzophenone and 4,4′-difluorobenzophenone afforded benzhydrole derivatives which were chlorinated with HCl and anhydrous calcium chloride. Resulting benzhydryl chloride derivatives were used for *N*-alkylation of piperazine to give 1-benzhydrylpiperazine, 4-chlorobenzhydrylpiperazine and 4,4′-difluorobenzhydrylpiperazine. The final step was nucleophilic substitution with benzoyl chlorides or sulfonyl chlorides in order to obtain benzhydrylpiperazine derivatives **5a–6j.**

Synthesized compounds were identified with IR, UV and ^{1}H-NMR spectra. In addition, some compounds were selected for LC-MS and ^{13}C-NMR spectral evaluation. In UV spectra of test compounds there are 2 significant bands approximately at 205 and 224 nm which represent π→π* and n→π* transitions of both series. In IR spectrum of **5a–i** the stretching bands are observed nearly at 3082 cm^{-1} (C-H; aromatic), 2966 cm^{-1} (C-H; aliphatic), 1621 cm^{-1} (C=O), 1583 cm^{-1} (C=C; aromatic) and 1230 cm^{-1} (C-N). In IR spectrum of **6a–j** the stretching bands are observed nearly at 3055 cm^{-1} (C-H; aromatic), 2947 cm^{-1} (C-H; aliphatic), 1606 cm^{-1} (C=C; aromatic), 1356 (S=O; asym.), 1281 cm^{-1} (C-N) and 1170 cm^{-1} (S=O; sym.). In H^{1}-NMR spectra of **5a–i** protons of piperazine ring are seen in the range of 2.3 ppm and 3.5 ppm as broad singlets. Diphenylmethyl C-H is observed as a singlet nearly at 4 ppm. Protons of aromatic rings give multiplet peaks at 6.91–7.46 ppm. In H^{1}-NMR spectra of **6a–j** protons of piperazine moiety are observed in the range of 2.3 ppm and 3 ppm as broad singlets. Diphenylmethyl C-H is recognized as a singlet nearly at 4 ppm. Protons of chlorobenzhydryl group are seen as multiplets at 7.15–7.38 ppm. The ^{13}C-NMR spectrum of the compound **6j** shows characteristic peaks of the sulfonamide derivatives at 46.67 and 50.81 ppm for piperazine ring and 73.69 ppm for diphenylmethyl carbon. The ^{13}C-NMR spectrum of the compound **5a** shows characteristic peaks of the benzamide derivatives at 43.09 and 51.49–51.74 ppm for piperazine ring, 75.2 ppm for diphenylmethyl carbon together with 167.86 ppm for carbonyl carbon.

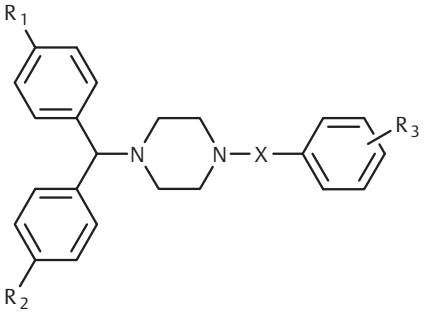
The structures of the prepared benzhydrylpiperazine derivatives are illustrated in **Table 1**.

Biological activity

The cytotoxic activity of the synthesized compounds **5a–i** and **6a–j** was investigated on liver (HUH-7), breast (MCF-7) and colon (HCT-116) cancer cell lines, by means of sulphorhodamine B (SRB) assays in triplicate. As shown in **Table 2**, all tested compounds were screened with mean 50% growth inhibition concentration (GI_{50}) in micromolar concentration range.

Generally, cytotoxic activities of sulfonamides increase when the core structure bears 4-chloro substitution on benzhydryl moiety. To exemplify this situation **6c** and **6h** or **6f** and **6j** can be compared with each other. **6c** (R_1, R_2=F, R_3=2,4,5-trichloro, X=SO_2) has no inhibition against any of the cancer cell lines. Whereas, **6h** (R_1=Cl, R_2=H, R_3=2,4,5-trichloro, X=SO_2) is slightly cytotoxic against all the cancer cell lines. Similarly, **6f** (R_1, R_2=F, R_3=4-nitro, X=SO_2) has no cytotoxicity against any of the cancer cell lines. Whereas, **6j** (R_1=Cl, R_2=H, R_3=4-nitro, X=SO_2) has variable cytotoxicity against all the cancer cell lines.

Table 1 Structural and physical information of compounds **5a–i** and **6a–j**.



Sample	X	R1	R2	R3	M. P. (°C)	Yield (%)
5a	C=O	- H	- H	5-Fluoro-2-methyl	>300 (dec.)	95
5b	C=O	- F	- F	2-Bromo	189.7	35
5c	C=O	- F	- F	3-Bromo	151.4	27
5d	C=O	- F	- F	4-Bromo	>300 (dec.)	19
5e	C=O	- F	- F	3-Chloro	177.5	47
5f	C=O	- Cl	- H	2-Methoxy	120	33
5g	C=O	- Cl	- H	3-Nitro	196.1	24
5h	C=O	- Cl	- H	3,4-Dimethoxy	148.6	11
5i	C=O	- Cl	- H	4-Ethyl	206.4	22
6a	SO_2	- H	- H	2-Trifluoromethoxy	205.8	10
6b	SO_2	- F	- F	2-Trifluoromethyl	135.6	22
6c	SO_2	- F	- F	2,4,5-Trichloro	>300 (dec.)	11
6d	SO_2	- F	- F	3,4-Dichloro	145.1	42
6e	SO_2	- F	- F	2-Methyl	117.7	10
6f*	SO_2	- F	- F	4-Nitro	224.5	13
6g	SO_2	- F	- F	2,5-Dichloro	116.1	23
6h	SO_2	- Cl	- H	2,4,5-Trichloro	151.1	19
6i	SO_2	- Cl	- H	3,4-Dichloro	107.1	25
6j	SO_2	- Cl	- H	4-Nitro	209.3	37

L (*) 6f, CAS No: 1286459-36-6

Table 2 Cytotoxic activity data for compounds **5a–i** and **6a–j**.

Sample	Cancer Cell Line GI50 (μM)		
	HUH-7	MCF-7	HCT-116
5a	10.80	10.44	11.34
5b	20.89	6.05	12.78
5c	11.72	5.95	9.10
5d	12.12	2.21	12.16
5e	11.16	5.87	8.95
5f	8.49	17.88	11.00
5g	13.23	22.72	13.85
5h	10.81	16.09	10.54
5i	10.91	–	9.45
6a	–	4.50	21.09
6b	–	–	–
6c	–	–	–
6d	15.23	56.02	–
6e	17.65	17.10	27.41
6f	–	–	–
6g	–	–	–
6h	54.41	11.16	31.41
6i	10.88	–	53.06
6j	39.95	17.22	97.74
5-FU	30.66	3.51	18.67
CPT	n.d.	n.d.	n.d.

n.d.: Not determined (Camptothecin was cytotoxic at concentrations below 2.5 μM.)

Table 3 MCF-7 (breast cancer cell line) and MCF-12A (normal-like breast epithelial cell line) cytotoxicity comparison of compounds **5d** and **6a** (μM).

	MCF-7	MCF-12A
5d	2.21	5.5
6a	4.50	9.9

Benzoylpiperazines are moderately active on HUH-7 cell line and **5f** (GI_{50} = 8.49 μM) is the most active compound of the series. Sulfonylpiperazines show low or no inhibition on HUH-7 cell line in general, however **6i** (GI_{50} = 10.88 μM) has the highest activity among the sulfonylpiperazines.

The most active compounds against MCF-7 cell line are **5d** (GI_{50} = 2.21 μM) and **6a** (GI_{50} = 4.50 μM). These highly cytotoxic compounds (**5d** and **6a**) were further evaluated for cytotoxicity against a normal-like breast epithelial cell line, MCF-12A, and found to be selective (see **Table 3**).

Against MCF-7 cell line some of the sulfonamides (**6b**, **6c**, **6f**, **6g**, **6i**) show no inhibition. Interestingly, electron withdrawing halogen substitution on phenyl ring of benzamide derivatives has elevated activity values as can be seen for compounds **5b** (GI_{50} = 6.05 μM), **5c** (GI_{50} = 5.95 μM), **5d** (GI_{50} = 2.21 μM) and **5e** (GI_{50} = 5.87 μM).

Benzamide derivatives generally show good activity values considering HCT-116 cell line. **5c** (GI_{50} = 9.10 μM), **5e** (GI_{50} = 8.95 μM) and **5h** (GI_{50} = 9.45 μM) are the most active molecules among benzoylpiperazines. However, sulfonamides present low or no inhibition on HCT-116 cell line.

Conclusion

In this study, 19 benzhydrylpiperazine derivatives with benzamide and sulfonamide moieties were prepared. In vitro cytotoxic activities were screened against hepatocellular (HUH-7), breast (MCF-7) and colorectal (HCT-116) cancer cell lines by sulphorhodamine B assay. Many compounds were found to have good inhibition values. In general, benzamide derivatives present better activities than sulfonamide derivatives. Additionally, electron withdrawing substituents on phenyl ring of benzamide derivatives increased activity against MCF-7 cancer cell line. Future synthesis of similar derivatives will take place to create a larger set of compounds in order to produce a rational quantitative structure-activity relationship (QSAR) mapping. Since 4-chloro-benzhydrylpiperazine derivatives are chiral compounds, further exploration of chiral separation methods will be performed. The primary ambition regarding future research is to evaluate the mechanism of cytotoxicity.

Conflict of Interest

The authors have declared no conflict of interest.

References

1 *Albro LP, Baltzly R, Phillips AP.* Unsymmetrically disubstituted piperazines II. Histamine antagonists. J Org Chem 1949; 14: 771–774
2 *Baltzly R, DuBreuil S, Ide WS et al.* Unsymmetrically disubstituted piperazines III. N-methyl-N'-benzhydrylpiperazines as histamine antagonists. J Org Chem 1949; 14: 775–782
3 *Iemura R, Kawashima T, Fukuda T et al.* Synthesis of 2-(4-substituted-1-piperazinyl)benzimidazoles as H_1-antihistaminic agents. J Med Chem 1986; 29: 1178–1183
4 *Beck K, Hamlin K, Weston A.* Histamine antagonists. IV. C-methyl derivatives of 1,4-disubstituted piperazines. J Am Chem Soc 1952; 74: 605–608
5 *Vandenberk J, Kennis L, Van der Aa M et al.* Piperazine derivatives, US-4250176, 1981
6 *Wang L, Wang T, Yang B et al.* Design, synthesis, and anti-allergic activities of novel (R)(-)-1-[(4-chlorophenyl)phenylmethyl]piperazine derivatives. Med Chem Res 2010; 21: 124–132
7 *Zamponi G, Feng Z, Zhang L et al.* Scaffold-based design and synthesis of potent N-type calcium channel blockers. Bioorg Med Chem Lett 2009; 19: 6467–6472
8 *Alps B.* Drugs acting on calcium channels – potential treatment for ischemic stroke. Brit J Clin Pharmaco 1992; 34: 199–206
9 *Miyake N, Fujita R, Ishikawa M et al.* Reversal of multidrug resistance in human leukemia K562 by tamolarizine, a novel calcium antagonist. Jpn J Pharmacol 2000; 82: 265–268
10 *Kimura M, Masuda T, Yamada K et al.* Novel diphenylalkyl piperazine derivatives with dual calcium antagonistic and antioxidative activities. Bioorg Med Chem Lett 2002; 12: 1947–1950
11 *Pajouhesh H, Feng Z, Ding Y et al.* Structure-activity relationships of diphenylpiperazine *N*-type calcium channel inhibitors. Bioorg Med Chem Lett 2010; 20: 1378–1383
12 *Kam Y, Rhee H, Rhim H et al.* Synthesis and T-type calcium channel blocking activity of novel diphenylpiperazine compounds, and evaluation of in vivo analgesic activity. Bioorg Med Chem 2010; 18: 5938–5944
13 *Kurokawa M, Sato F, Hatano N et al.* A new class of calcium antagonists. Synthesis and biological activity of 11-[(ω-aminoalkanoyl)amino]-6,6a,7,8,9,10,10a,11-octahydrodibenzo[b,e]thiepin derivatives. J Med Chem 1991; 34: 593–599
14 *Doddareddy M, Choo H, Cho Y et al.* 3D pharmacophore based virtual screening of T-type calcium channel blockers. Bioorg Med Chem 2007; 15: 1091–1105
15 *Sasse B, Mach U, Leppaenen J et al.* Hybrid approach for the design of highly affine and selective dopamine D-3 receptor ligands using privileged scaffolds of biogenic amine GPCR ligands. Bioorg Med Chem 2007; 15: 7258–7273
16 *Jung J, Jung S, Koh H.* Asymmetric synthesis of chiral piperazinylpropylisoxazoline ligands for dopamine receptors. Eur J Med Chem 2007; 42: 1044–1048
17 *Kimura M, Masuda T, Yamada K et al.* Synthesis of novel diphenyl piperazine derivatives and their activities as inhibitors of dopamine uptake in the central nervous system. Bioorg Med Chem 2003; 11: 1621–1630

18 *Abou-Gharbia M*, *Patel UR*, *Moyer JA et al.* Psychotropic agents: Synthesis and antipsychotic activity of substituted β-carbinoles. J Med Chem 1987; 30: 1100–1105
19 *Okachi R*, *Niino H*, *Kitaura K et al.* Synthesis and antibacterial activity of 2,2′-dithiobis(benzamide) derivatives against Mycobacterium species. J Med Chem 1985; 28: 1772–1779
20 *Punkvang A*, *Saparpakorn P*, *Hannongbua S et al.* Insight into crucial inhibitor-enzyme interaction of arylamides as novel direct inhibitors of the enoyl ACP reductase (InhA) from *Mycobacterium tuberculosis*: computer-aided molecular design. Monatsh Chem 2010; 141: 1029–1041
21 *Dinakaran M*, *Senthilkumar P*, *Yogeeswari P et al.* Antimycobacterial activities of novel 2-(sub)-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-a]quinoline-6-carboxylic acid. Bioorg Med Chem 2008; 16: 3408–3418
22 *Senthilkumar P*, *Dinakaran M*, *Banerjee D et al.* Synthesis and antimycobacterial evaluation of newer 1-cyclopropyl-1,4-dihydro-6-fluoro-7-(substituted secondary amino)-8-methoxy-5-(sub)-4-oxoquinoline-3-carboxylic acids. Bioorg Med Chem 2008; 16: 2558–2569
23 *Upadhayaya R*, *Vandavasi J*, *Kardile R et al.* Novel quinoline and naphthalene derivatives as potent antimycobacterial agents. Eur J Med Chem 2010; 45: 1854–1867
24 *Kumar A*, *Siddiqi MI*. Receptor based 3D-QSAR to identify putative binders of *Mycobacterium tuberculosis* Enoyl acyl carrier protein reductase. J Mol Model 2010; 16: 877–893
25 *Lu XY*, *Chen YD*, *You QD*. 3D-QSAR studies of arylcarboxamides with inhibitory activity on InhA using pharmacophore-based alignment. Chem Biol Drug Des 2010; 75: 195–203
26 *Sriram D*, *Senthilkumar P*, *Dinakaran M et al.* Antimycobacterial activities of novel 1-(cyclopropyl/*tert*-butyl/4-fluorophenyl)-1,4-dihydro-6-nitro-4-oxo-7-(substituted secondary amino)-1,8-naphthyridine-3-carboxylic acid. J Med Chem 2007; 50: 6232–6239
27 *Senthilkumar P*, *Dinakaran M*, *Yogeeswari P et al.* Synthesis and antimycobacterial activities of novel 6-nitroquinolone-3-carboxylic acids. Eur J Med Chem 2009; 44: 345–358
28 *Kumar A*, *Siddiqi M*. CoMFA based de novo design of pyrrolidine carboxamides as inhibitors of enoyl acyl carrier protein reductase from *Mycobacterium tuberculosis*. J Mol Model 2008; 14: 923–935
29 *Lu X*, *Chen Y*, *Jiang Y et al.* Discovery of potential new InhA direct inhibitors based on pharmacophore and 3D-QSAR analysis followed by in silico screening. Eur J Med Chem 2009; 44: 3718–3730
30 *Muddassar M*, *Jang J*, *Gon H et al.* Identification of novel antitubercular compounds through hybrid virtual screening approach. Bioorg Med Chem 2010; 18: 6914–6921
31 *Chandra J*, *Sadashiva C*, *Kavitha C et al.* Synthesis and *in vitro* antimicrobial studies of medicinally important novel *N*-alkyl and *N*-sulfonyl derivatives of 1-[bis(4-fluorophenyl)methyl]piperazine. Bioorg Med Chem 2006; 14: 6621–6627
32 *Verderame M*. 1,4-Disubstituted piperazines. 3. Piperazinylbenzothiazoles. J Med Chem 1972; 15: 693–694
33 *Aytemir M*, *Ozcelik B*. A study of cytotoxicity of novel chlorokojic acid derivatives with their antimicrobial and antiviral activities. Eur J Med Chem 2010; 45: 4089–4095
34 *Patel I*, *Parmar S*. Synthesis and studies of novel optically active Schiff's base derivatives and their antimicrobial activities. E-Journal of Chemistry 2010; 7: 617–623
35 *Srinivasan S*, *Gupta S*, *Marwah R et al.* Synthesis, characterization & in vitro biological studies of novel N-aryl piperazinyl fluoroquinolones. Res J Pharm Biol Chem Sci 2010; 1: 208–218
36 *Shivakumara K*, *Prakasha K*, *Gowda D*. Synthesis and antimicrobial activity of amino acids conjugated diphenylmethylpiperazine derivatives. E-Journal of Chemistry 2009; 6: 473–479
37 *Chern J*, *Shia K*, *Hsu T et al.* Design, synthesis, and structure-activity relationships of pyrazolo[3,4-d]pyrimidinies: a novel class of potent enterovirus inhibitors. Bioorg Med Chem Lett 2004; 14: 2519–2525
38 *Curreli F*, *Zhang H*, *Zhang X et al.* Virtual screening based identification of novel small-molecule inhibitors targeted to the HIV-1 capsid. Bioorg Med Chem 2011; 19: 77–90
39 *Kumar C*, *Prasad S*, *Vinaya K et al.* Synthesis and in vitro antiproliferative activity of novel 1-benzhydryl-piperazine derivatives against human cancer cell lines. Eur J Med Chem 2009; 44: 1223–1229
40 *Yarim M*, *Koksal M*, *Durmaz I et al.* Cancer cell cytotoxicities of 1-(4-substitutedbenzoyl)-4-(4-chlorobenzhydryl)piperazine derivatives. Int J Mol Sci 2012; 13: 8071–8085
41 *Huang W*, *Liu M*, *Li Y et al.* Design, synthesis and antitumor activity of novel chromone and aurone derivatives. Bioorg Med Chem 2007; 15: 5191–5197
42 *Huang W*, *Ding Y*, *Miao Y et al.* Synthesis and antitumor activity of novel dithiocarbamate substituted chromones. Eur J Med Chem 2009; 44: 3687–3696
43 *Gan L*, *Fang B*, *Zhou C*. Synthesis of azole-containing piperazine derivatives and evaluation of their antibacterial, antifungal and cytotoxic activities. B Kor Chem Soc 2010; 31: 3684–3692
44 *Kumar C*, *Swamy S*, *Thimmegowda N et al.* Synthesis and evaluation of 1-benzhydryl-sulfonyl-piperazine derivatives as inhibitors of MDA-MB-231 human breast cancer cell proliferation. Med Chem Res 2007; 16: 179–187
45 *Wang W*, *Xu X*, *Chen Y et al.* Apoptosis of human Burkitt's lymphoma cells induced by 2-*N*,*N*-diethylaminocarbonyloxymethyl-1-diphenylmethyl-4-(3,4,5-trimethoxybenzoyl)piperazine hydrochloride (PMS-1077). Arch Pharm Res 2009; 32: 1727–1736
46 *Demma M*, *Maxwell E*, *Ramos R et al.* SCH529074, a small molecule activator of mutant p53, which binds p53 DNA binding domain (DBD), restores growth-suppressive function to mutant p53 and interrupts HDM2-mediated ubiquitination of wild type p53. J Biol Chem 2010; 285: 198–212
47 *Venkat-Narsaiah A*, *Narsimha P*. Efficient synthesis of antihistamines clocinizine and chlorcyclizine. Med Chem Res 2011; 1–4