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# Cytotoxic Activities of some Novel Benzhydrylpiperazine Derivatives

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## 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<sup>-1</sup>. The <sup>1</sup>H- and <sup>13</sup>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<sub>6</sub>) 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

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(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  $\pm 0.4\%$  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 ( $\text{NaBH}_4$ ) 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 Benzhydrolyl 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  $85^\circ\text{C}$  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-Chlorobenzhydrolyl chloride and 4,4'-difluorobenzhydrolyl chloride were also synthesized from 4-chlorobenzhydrole and 4,4'-difluorobenzhydrole according to above procedure.

#### General procedure for preparation of Benzhydrolylpiperazine 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) benzhydrolyl chloride, reaction mixture was heated at  $80^\circ\text{C}$  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'-benzhydrolylpiperazine were also synthesized from 4-chlorobenzhydrolyl chloride and 4,4'-difluorobenzhydrolyl chloride consecutively according to above procedure.

#### General procedure for preparation of 1-(Substitutedbenzoyl)-4-[benzhydrolyl/4-chlorobenzhydrolyl/4,4'-difluorobenzhydrolyl] piperazine derivatives (5a–5i)

2 mmol (0.5 g) 1-benzhydrolylpiperazine 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 benzhydrolylpiperazine derivative) was added to the solution. 10 min later ice bath was

removed and appropriate benzoyl chloride derivatives (equimolar with benzhydrolylpiperazine 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  $300^\circ\text{C}$ . UV (MeOH,  $\lambda_{\text{max}}$ , nm); 205 (log  $\epsilon$ : 5.22), 224 (log  $\epsilon$ : 4.74). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 2.13 (s, 3H,  $-\text{CH}_3$ ); 3.2 (bs, 4H, piperazine  $\text{H}_3, \text{H}_5$ ); 3.76 (bs, 4H, piperazine  $\text{H}_2, \text{H}_6$ ); 5.64 (d, 1H,  $(\text{Ar})_2\text{CH}-$ ,  $J=8.4\text{Hz}$ ); 7.12 (t, 2H, diphenyl  $\text{H}_{4,4'}$ ,  $J=8.8\text{Hz}$ ); 7.26–7.29 (dd, 4H, diphenyl  $\text{H}_{3,5,3',5'}$ ,  $J_1=5.2\text{Hz}$ ,  $J_2=2.8\text{Hz}$ ); 7.31 (d, 1H, phenyl  $\text{H}_4$ ,  $J=7.6\text{Hz}$ ); 7.37 (d, 1H, phenyl  $\text{H}_3$ ,  $J=6.8\text{Hz}$ ); 7.44 (s, 1H, phenyl  $\text{H}_6$ ); 7.86–7.92 (dd, 4H, diphenyl  $\text{H}_{2,6,2',6'}$ ,  $J_1=10.8\text{Hz}$ ,  $J_2=7.2\text{Hz}$ ); (12.61 (bs, 1H, N-H salt).  $^{13}\text{C-NMR}$  (DMSO, ppm); 37.98 ( $\text{C}_{25}$ ); 43.09 ( $\text{C}_{14,16}$ ); 51.49–51.74 ( $\text{C}_{15,17}$ ); 75.28 ( $\text{C}_7$ ); 113.31–113.54 ( $\text{C}_{2,6,9,13}$ ); 116.36–116.56 ( $\text{C}_{3,5,10,12}$ ); 129.17 ( $\text{C}_4$ ); 129.55 ( $\text{C}_{11}$ ); 129.97 ( $\text{C}_1$ ); 130.73 ( $\text{C}_8$ ); 132.9 ( $\text{C}_{21}$ ); 132.98 ( $\text{C}_{22}$ ); 136.15 ( $\text{C}_{24}$ ); 137.43 ( $\text{C}_{20}$ ); 159.60 ( $\text{C}_{19}$ ); 162.02 ( $\text{C}_{23}$ ); 167.86 ( $\text{C}_{18}$ ). MS (m/z); 389.8 (100%,  $\text{M}^+ - \text{Cl}$ ); 167.5 ( $(\text{C}_6\text{H}_5)_2\text{CH}^+$ ). Anal. calcd. for  $\text{C}_{25}\text{H}_{26}\text{ClF}_2\text{N}_2\text{O}$  (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.:  $189.7^\circ\text{C}$ . UV (MeOH,  $\lambda_{\text{max}}$ , nm); 205 (log  $\epsilon$ : 5.27), 223 (log  $\epsilon$ : 4.49). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 3.03 (bs, 4H, piperazine  $\text{H}_3, \text{H}_5$ ); 3.67 (bs, 4H, piperazine  $\text{H}_2, \text{H}_6$ ); 5.59 (s, 1H,  $(\text{Ar})_2\text{CH}-$ ); 7.27–7.91 (m, 17H, aromatic H's); 12.8 (bs, 1H, N-H salt). MS (m/z); 471.92 (90%,  $\text{M}^+ - \text{Cl}$ ), 473.92 (89%,  $\text{M}+2$ ), 203.60 (100%,  $(4\text{-F-C}_6\text{H}_5)_2\text{CH}^+$ ). Anal. calcd. for  $\text{C}_{24}\text{H}_{22}\text{BrClF}_2\text{N}_2\text{O}\cdot\text{H}_2\text{O}$  (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.:  $151.4^\circ\text{C}$ . UV (MeOH,  $\lambda_{\text{max}}$ , nm); 204 (log  $\epsilon$ : 5.09), 223 (log  $\epsilon$ : 4.51). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 3.11 (bs, 4H, piperazine  $\text{H}_3, \text{H}_5$ ); 3.67 (bs, 4H, piperazine  $\text{H}_2, \text{H}_6$ ); 5.59 (bs, 1H,  $(\text{Ar})_2\text{CH}-$ ); 7.27–7.93 (m, 13H, aromatic H's); 12.63 (bs, 1H, N-H salt). MS (m/z); 471.91 (95%,  $\text{M}^+ - \text{Cl}$ ), 473.91 (94%,  $\text{M}+2$ ), 203.61 (100%,  $(4\text{-F-C}_6\text{H}_5)_2\text{CH}^+$ ). Anal. calcd. for  $\text{C}_{24}\text{H}_{22}\text{BrClF}_2\text{N}_2\text{O}\cdot\text{H}_2\text{O}$  (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% (0.1654g); m.p.: Above 300°C. UV (MeOH,  $\lambda_{\max}$ , nm); 204 (log  $\epsilon$ : 5.23), 224 (log  $\epsilon$ : 4.46). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 3.09 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.68 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 5.58 (bs, 1H, (Ar)<sub>2</sub>CH-); 7.15–7.91 (m, 12H, aromatic H's); 12.69 (bs, 1H, N-H salt). MS (m/z); 471.92 (68%, M<sup>+</sup> – Cl), 473.92 (67%, M+2), 203.59 (100%, (4-F-C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>CH<sup>+</sup>). Anal. calcd. for C<sub>24</sub>H<sub>22</sub>BrClF<sub>2</sub>N<sub>2</sub>O.H<sub>2</sub>O (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% (0.3663g); m.p.: 177.5°C. UV (MeOH,  $\lambda_{\max}$ , nm); 204 (log  $\epsilon$ : 5.14), 226 (log  $\epsilon$ : 4.29). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 3.13 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.65–4.44 (m, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 5.62 (bs, 1H, (Ar)<sub>2</sub>CH-); 7.25–7.97 (m, 12H, aromatic H's); 12.72 (bs, 1H, N-H salt). MS (m/z); 427.97 (98%, M<sup>+</sup> – Cl); 429.96 (32%, M+2), 203.61 (100%, (4-F-C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>CH<sup>+</sup>). Anal. calcd. for C<sub>24</sub>H<sub>22</sub>Cl<sub>2</sub>F<sub>2</sub>N<sub>2</sub>O.H<sub>2</sub>O (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% (0.140g); m.p.: 120°C. UV (MeOH,  $\lambda_{\max}$ , nm); 202 (log  $\epsilon$ : 4.78), 275 (log  $\epsilon$ : 3.36). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 2.18–2.33 (m, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.63 (m, 2H, piperazine H<sub>2</sub>); 3.14 (m, 2H, piperazine H<sub>6</sub>); 3.75 (s, 3H, -OCH<sub>3</sub>); 4.39 (s, 1H, (Ar)<sub>2</sub>CH-); 6.95 (t, 1H, benzoyl H<sub>4</sub>,  $J=7.2\text{Hz}$ ); 7.03 (d, 1H, benzoyl H<sub>6</sub>,  $J=8\text{Hz}$ ); 7.12–7.15 (dd, 1H, benzoyl H<sub>3</sub>,  $J_1=1.6\text{Hz}$ ,  $J_2=6\text{Hz}$ ); 7.2 (t, 1H, phenyl H<sub>4</sub>,  $J=7.2\text{Hz}$ ); 7.3 (t, 2H, phenyl H<sub>3,5</sub>,  $J=7.2\text{Hz}$ ); 7.35 (d, 2H, phenyl H<sub>2,6</sub>,  $J=8\text{Hz}$ ); 7.38 (t, 1H, benzoyl H<sub>5</sub>,  $J=1.6\text{Hz}$ ); 7.39–7.46 (dd, 4H, 4-chlorophenyl H's,  $J_1=7.6\text{Hz}$ ,  $J_2=10.4\text{Hz}$ ). Anal. calcd. for C<sub>25</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>2</sub> (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% (0.112g); m.p.: 196.1°C. UV (MeOH,  $\lambda_{\max}$ , nm); 203 (log  $\epsilon$ : 4.33), 227 (log  $\epsilon$ : 4.02). FT-IR (KBr,  $\text{cm}^{-1}$ ); 3423 (N-H), 3063 (C-H, aromatic), 2924 (C-H, aliphatic), 1644 (C=O, amide), 1533 (N=O), 1291 (C-N), 1092 (C-Cl).  $^1\text{H-NMR}$  (DMSO, ppm); 3.17 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.83 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 5.59 (s, 1H, (Ar)<sub>2</sub>CH-); 7.3–8.5 (m, 13H, aromatic H's); 12.71 (s, 1H, N-H salt). Anal. calcd. for C<sub>24</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>3</sub>.H<sub>2</sub>O (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% (0.050g); m.p.: 148.6°C. UV (MeOH,  $\lambda_{\max}$ , nm); 204 (log  $\epsilon$ : 4.59), 276 (log  $\epsilon$ : 3.45). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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).  $^1\text{H-NMR}$  (DMSO, ppm); 2.31 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.52 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 3.75 (s, 3H, -OCH<sub>3</sub>); 3.76 (s, 3H, -OCH<sub>3</sub>); 4.39 (s, 1H, (Ar)<sub>2</sub>CH-); 6.91 (d, 1H, benzoyl H<sub>5</sub>,  $J=2.8\text{Hz}$ ); 6.94 (d, 1H, benzoyl H<sub>6</sub>,  $J=3.6\text{Hz}$ ); 6.97 (s, 1H, benzoyl H<sub>2</sub>); 7.21 (t, 2H, phenyl H<sub>3,5</sub>,  $J=7.6\text{Hz}$ ); 7.31 (t, 1H, phenyl H<sub>4</sub>,  $J=8\text{Hz}$ ); 7.35 (d, 2H, phenyl H<sub>2,6</sub>,  $J=8.4\text{Hz}$ ); 7.4–7.47 (dd, 4H, 4-chlorophenyl H's,  $J_1=8.8\text{Hz}$ ,  $J_2=10\text{Hz}$ ). Anal. calcd. for C<sub>26</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>3</sub> (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% (0.1g); m.p.: 206.4°C. UV (MeOH,  $\lambda_{\max}$ , nm); 204 (log  $\epsilon$ : 4.56), 225 (log  $\epsilon$ : 4.11). FT-IR (KBr,  $\text{cm}^{-1}$ ); 3422 (N-H), 3010 (C-H, aromatic), 2966 (C-H, aliphatic), 1634 (C=O, amide), 1287 (C-N), 1092 (C-Cl).  $^1\text{H-NMR}$  (DMSO, ppm); 1.15 (t, 3H, -CH<sub>3</sub>,  $J=8\text{Hz}$ ); 2.63 (q, 2H, -CH<sub>2</sub>-,  $J=7.6\text{Hz}$ ); 3.13 (s, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.79 (s, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 5.63 (d, 1H, (Ar)<sub>2</sub>CH-,  $J=8.4\text{Hz}$ ); 7.27–7.90 (m, 13H, aromatic H's); 12.63 (s, 1H, N-H salt). Anal. calcd. for C<sub>26</sub>H<sub>28</sub>Cl<sub>2</sub>N<sub>2</sub>O.H<sub>2</sub>O (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 (0.2575g) 1-benzhydrylpiperazine, 1.7 mmol (0.515g) 1-[bis(4-fluorophenyl)methyl]piperazine or 0.872 mmol (0.2632g) 1-[(4-chlorophenyl)(phenyl)methyl]piperazine was dissolved in 20ml 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 2h 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% (0.05g); m.p.: 205.8°C. UV (MeOH,  $\lambda_{\max}$ , nm); 204 (log  $\epsilon$ : 5.24), 226 (log  $\epsilon$ : 4.87). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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.).  $^1\text{H-NMR}$  (DMSO, ppm); 3.21–3.38 (m, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.74 (m, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 5.57 (d, 1H, (Ar)<sub>2</sub>CH-,  $J=8.4\text{Hz}$ ); 7.31–7.89 (m, 14H, aromatic H's); 12.5 (bs, 1H, N-H salt). MS (m/z); 477.8 (100%, M<sup>+</sup> – Cl); 167.6 ((C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>CH<sup>+</sup>). Anal. calcd. for C<sub>24</sub>H<sub>24</sub>ClF<sub>3</sub>N<sub>2</sub>O<sub>3</sub>S (512.97): C, 56.19; H, 4.72; N, 5.46; S, 6.25. Found: 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% (0.112g); m.p.: 135.6°C. UV (MeOH,  $\lambda_{\max}$ , nm); 203 (log  $\epsilon$ : 5.16), 225 (log  $\epsilon$ : 4.67). FT-IR (KBr,  $\text{cm}^{-1}$ ); 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.). <sup>1</sup>H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.18 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.45 (s, 1H, (Ar)<sub>2</sub>CH-); 7.11 (t, 4H, diphenyl H<sub>3,5,3',5'</sub>, *J*=9.2 Hz); 7.39–7.43 (dd, 4H, diphenyl H<sub>2,6,2',6'</sub>, *J*<sub>1</sub>=5.6 Hz, *J*<sub>2</sub>=3.2 Hz); 7.91–8.06 (m, 4H, 2-trifluoro-methylphenyl). Anal. calcd. for C<sub>24</sub>H<sub>21</sub>F<sub>5</sub>N<sub>2</sub>O<sub>2</sub>S (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<sub>max</sub>, nm); 204 (log ε: 5.09), 225 (log ε: 4.79). FT-IR (KBr, cm<sup>-1</sup>); 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). <sup>1</sup>H-NMR (DMSO, ppm); 2.32 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.24 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.45 (s, 1H, (Ar)<sub>2</sub>CH-); 7.11 (t, 4H, diphenyl H<sub>3,5,3',5'</sub>, *J*=8.4 Hz); 7.39–7.43 (dd, 4H, diphenyl H<sub>2,6,2',6'</sub>, *J*<sub>1</sub>=5.6 Hz, *J*<sub>2</sub>=3.2 Hz); 8.05 (s, 1H, 2,4,5-trichlorophenyl, H<sub>6</sub>); 8.17 (s, 1H, 2,4,5-trichlorophenyl, H<sub>3</sub>). Anal. calcd. for C<sub>23</sub>H<sub>19</sub>Cl<sub>3</sub>F<sub>2</sub>N<sub>2</sub>O<sub>2</sub>S (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<sub>max</sub>, nm); 203 (log ε: 5.35), 227 (log ε: 4.46). FT-IR (KBr, cm<sup>-1</sup>); 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). <sup>1</sup>H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 2.98 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.42 (s, 1H, (Ar)<sub>2</sub>CH-); 7.09 (t, 4H, diphenyl H<sub>3,5,3',5'</sub>, *J*=8.8 Hz); 7.37–7.40 (dd, 4H, diphenyl H<sub>2,6,2',6'</sub>, *J*<sub>1</sub>=5.6 Hz, *J*<sub>2</sub>=3.2 Hz); 7.69–7.97 (m, 3H, 3,4-dichlorophenyl). Anal. calcd. for C<sub>23</sub>H<sub>20</sub>Cl<sub>2</sub>F<sub>2</sub>N<sub>2</sub>O<sub>2</sub>S (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-Toluy)l)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<sub>max</sub>, nm); 206 (log ε: 5.32), 225 (log ε: 4.67). FT-IR (KBr, cm<sup>-1</sup>); 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.). <sup>1</sup>H-NMR (DMSO, ppm); 2.33 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 2.55 (s, 3H, -CH<sub>3</sub>); 3.04 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.41 (s, 1H, (Ar)<sub>2</sub>CH-); 7.10 (t, 4H, diphenyl H<sub>3,5,3',5'</sub>, *J*=8.8 Hz); 7.38–7.41 (dd, 4H, diphenyl H<sub>2,6,2',6'</sub>, *J*<sub>1</sub>=5.6 Hz, *J*<sub>2</sub>=3.2 Hz); 7.42–7.47 (dd, 2H, 2-methylphenyl H<sub>3,5</sub>, *J*<sub>1</sub>=5.2 Hz, *J*<sub>2</sub>=7.6 Hz); 7.57–7.62 (dd, 1H, 2-methylphenyl H<sub>4</sub>, *J*<sub>1</sub>=1.2 Hz, *J*<sub>2</sub>=7.2 Hz); 7.75–7.77 (dd, 1H, 2-methylphenyl H<sub>6</sub>, *J*<sub>1</sub>=1.2 Hz, *J*<sub>2</sub>=6.8 Hz). Anal. calcd. for C<sub>23</sub>H<sub>20</sub>Cl<sub>2</sub>F<sub>2</sub>N<sub>2</sub>O<sub>2</sub>S (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<sub>max</sub>, nm): 203 (log ε: 5.23), 225 (log ε: 4.39). FT-IR (KBr, cm<sup>-1</sup>); 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.). <sup>1</sup>H-NMR (DMSO, ppm); 2.35 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 2.99 (bs, 4H, pip-

erazine H<sub>2</sub>, H<sub>6</sub>); 4.42 (s, 1H, (Ar)<sub>2</sub>CH-); 7.09 (t, 4H, diphenyl H<sub>3,5,3',5'</sub>, *J*=8 Hz); 7.35–7.38 (dd, 4H, diphenyl H<sub>2,6,2',6'</sub>, *J*<sub>1</sub>=5.2 Hz, *J*<sub>2</sub>=3.2 Hz); 7.99 (d, 2H, 4-nitrophenyl H<sub>2,6</sub>, *J*=9.2 Hz); 8.47 (d, 2H, 4-nitrophenyl H<sub>3,5</sub>, *J*=8.8 Hz). Anal. calcd. for C<sub>23</sub>H<sub>21</sub>F<sub>2</sub>N<sub>3</sub>O<sub>4</sub>S (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<sub>max</sub>, nm): 205 (log ε: 5.19), 224 (log ε: 4.91). FT-IR (KBr, cm<sup>-1</sup>); 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). <sup>1</sup>H-NMR (DMSO, ppm); 2.33 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.23 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.44 (s, 1H, (Ar)<sub>2</sub>CH-); 7.11 (t, 4H, diphenyl H<sub>3,5,3',5'</sub>, *J*=8.4 Hz); 7.39–7.43 (dd, 4H, diphenyl H<sub>2,6,2',6'</sub>, *J*<sub>1</sub>=5.6 Hz, *J*<sub>2</sub>=3.2 Hz); 7.75–7.81 (m, 2H, 2,5-dichlorophenyl H<sub>3</sub>, H<sub>4</sub>); 7.9 (d, 1H, 2,5-dichlorophenyl H<sub>6</sub>, *J*=2.4 Hz). <sup>13</sup>C-NMR (DMSO, ppm); 46.39 (C<sub>14,16</sub>); 51.28 (C<sub>15,17</sub>); 72.82 (C<sub>7</sub>); 115.95–116.16 (C<sub>3,10</sub>); 130.02–130.10 (C<sub>2,9</sub>); 130.48 (C<sub>20</sub>); 131.61 (C<sub>21</sub>); 132.99 (C<sub>23</sub>); 134.79 (C<sub>22</sub>); 135.02 (C<sub>19</sub>); 137.34 (C<sub>18</sub>); 138.83–138.85 (C<sub>1,8</sub>); 160.57–162.99 (C<sub>4,11</sub>). MS (m/z); 497.98 (25%, M<sup>+</sup>); 499.8 (12%, M+2); 203.5 (100%, (4-F-C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>CH<sup>+</sup>). Anal. calcd. for C<sub>23</sub>H<sub>20</sub>Cl<sub>2</sub>F<sub>2</sub>N<sub>2</sub>O<sub>2</sub>S (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<sub>max</sub>, nm): 205 (log ε: 4.66), 234 (log ε: 4.09). FT-IR (KBr, cm<sup>-1</sup>); 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). <sup>1</sup>H-NMR (DMSO, ppm); 2.29 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 3.21 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.37 (s, 1H, (Ar)<sub>2</sub>CH-); 7.16 (t, 2H, phenyl H<sub>3,5</sub>, *J*=6.8 Hz); 7.27 (t, 1H, phenyl H<sub>4</sub>, *J*=7.6 Hz); 7.29–7.35 (dd, 4H, 4-chlorophenyl H's, *J*<sub>1</sub>=8.4 Hz, *J*<sub>2</sub>=4.4 Hz); 7.37 (d, 2H, phenyl H<sub>2,6</sub>, *J*=8.4 Hz); 8.016 (s, 1H, 2,4,5-trichlorophenyl H<sub>6</sub>); 8.14 (s, 1H, 2,4,5-trichlorophenyl H<sub>3</sub>). Anal. calcd. for C<sub>23</sub>H<sub>20</sub>Cl<sub>4</sub>N<sub>2</sub>O<sub>2</sub>S (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<sub>max</sub>, nm); 204 (log ε: 4.35), 236 (log ε: 4.11). FT-IR (KBr, cm<sup>-1</sup>); 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). <sup>1</sup>H-NMR (DMSO, ppm); 2.36 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 2.98 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.37 (s, 1H, (Ar)<sub>2</sub>CH-); 7.18 (t, 2H, phenyl H<sub>3,5</sub>, *J*=6.8 Hz); 7.27 (t, 1H, phenyl H<sub>4</sub>, *J*=7.2 Hz); 7.31–7.36 (dd, 4H, 4-chlorophenyl H's, *J*<sub>1</sub>=8.4 Hz, *J*<sub>2</sub>=3.6 Hz); 7.39 (d, 2H, phenyl H<sub>2,6</sub>, *J*=8.4 Hz); 7.69–7.97 (m, 3H, 3,4-dichlorophenyl H's). Anal. calcd. for C<sub>23</sub>H<sub>21</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>2</sub>S (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<sub>max</sub>, nm); 202 (log ε: 4.53), 231 (log ε: 4.18), 264 (log ε: 3.82). FT-IR (KBr, cm<sup>-1</sup>); 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). <sup>1</sup>H-NMR (DMSO, ppm); 2.36 (bs, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>); 2.99 (bs, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>); 4.38 (s, 1H, (Ar)<sub>2</sub>CH-); 7.18 (t, 2H, phenyl H<sub>3,5</sub>, *J*=7.2 Hz); 7.26 (t, 1H, phenyl H<sub>4</sub>, *J*=8 Hz); 7.30–7.34 (dd, 4H, 4-chlorophenyl H's, *J*<sub>1</sub>=6.4 Hz, *J*<sub>2</sub>=2 Hz); 7.37 (d, 2H, phenyl H<sub>2,6</sub>, *J*=8.8 Hz); 8.01 (d, 2H, 4-nitrophenyl H<sub>2</sub>, H<sub>6</sub>, *J*=8.8 Hz); 8.46 (d, 2H, 4-nitrophenyl H<sub>3</sub>, H<sub>5</sub>, *J*=8.8 Hz). <sup>13</sup>C-NMR (DMSO, ppm); 46.67 (C<sub>14,16</sub>); 50.81 (C<sub>15,17</sub>); 73.69 (C<sub>7</sub>); 125.38 (C<sub>20,22</sub>); 127.79 (C<sub>11</sub>); 128.08 (C<sub>10,12</sub>); 129.18 (C<sub>19,23</sub>); 129.29 (C<sub>9,13</sub>); 129.81 (C<sub>2,6</sub>); 129.89 (C<sub>3,5</sub>); 132.11 (C<sub>4</sub>); 140.99 (C<sub>1</sub>); 141.92 (C<sub>8</sub>); 142.29 (C<sub>18</sub>); 150.76 (C<sub>21</sub>). MS (m/z); 472.8 (25%, M<sup>+</sup>); 474.8 (12%, M+2); 201.5 (100% (4-Cl-C<sub>6</sub>H<sub>5</sub>)-(C<sub>6</sub>H<sub>5</sub>)CH<sup>+</sup>); 203.6 (38%). Anal. calcd. for C<sub>23</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>4</sub>S (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 100 μM to 2.5 μM 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<sub>2</sub> and 95% air.

### NCI-60 Sulphorhodamine B (SRB) assay

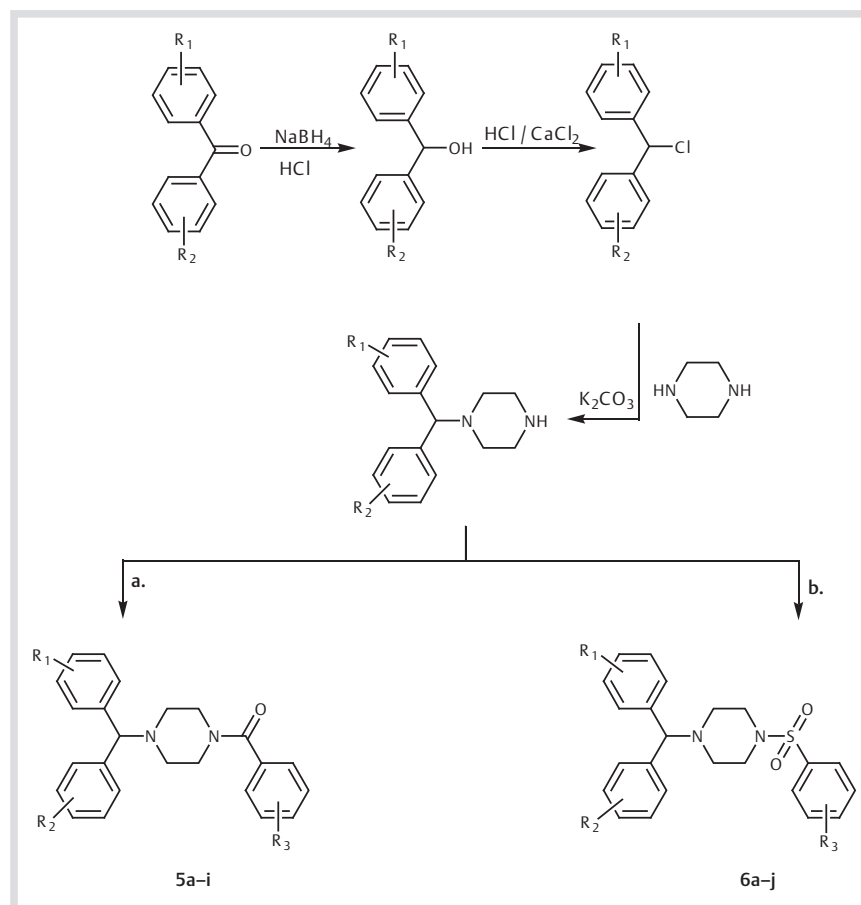
Cancer cells (range of 2000 cell/well to 5000 cell/well) were inoculated into 96-well plates in 200 μl of media and incubated in 37 °C incubators containing 5% CO<sub>2</sub> and 95% air. After a 24 h incubation period, one plate for each cell line was fixed with 100 μl 10% ice-cold trichloroacetic acid (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 40 mM 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<sub>2</sub> and 95% air for 72 h. Following the termination of the incubation period after drug treatment, the cells were fixed with 100 μl 10% ice-cold TCA and incubated in the dark at +4 °C for 1 h. Then the TCA was washed away with ddH<sub>2</sub>O 5 times and the plates were left to air dry. For the final step, the plates were stained with 100 μl of 0.4% sulphorhodamine B (SRB) solution in 1% acetic acid solution. Following staining, the plates were incubated in dark for 10 min 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 200 μl of 10 mM Tris-Base. The OD values were obtained at 515 nm.

## 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 benzhydre 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 <sup>1</sup>H-NMR spectra. In addition, some compounds were selected for LC-MS and <sup>13</sup>C-NMR spectral evaluation. In UV spectra of test compounds there are 2 significant bands approximately at 205 and 224 nm which represent  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions of both series. In IR spectrum of **5a–i** the stretching bands are observed nearly at 3082 cm<sup>-1</sup> (C-H; aromatic), 2966 cm<sup>-1</sup> (C-H; aliphatic), 1621 cm<sup>-1</sup> (C=O), 1583 cm<sup>-1</sup> (C=C; aromatic) and 1230 cm<sup>-1</sup> (C-N). In IR spectrum of **6a–j** the stretching bands are observed nearly at 3055 cm<sup>-1</sup> (C-H; aromatic), 2947 cm<sup>-1</sup> (C-H; aliphatic), 1606 cm<sup>-1</sup> (C=C; aromatic), 1356 (S=O; asym.), 1281 cm<sup>-1</sup> (C-N) and 1170 cm<sup>-1</sup> (S=O; sym.). In <sup>1</sup>H-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 <sup>1</sup>H-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 <sup>13</sup>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 <sup>13</sup>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.

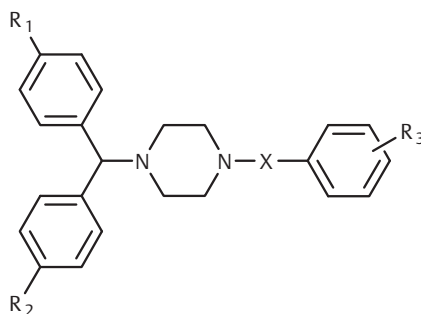
The structures of the prepared benzhydrylpiperazine derivatives are illustrated in **Table 1**.

### 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<sub>50</sub>) 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<sub>1</sub>, R<sub>2</sub>=F, R<sub>3</sub>=2,4,5-trichloro, X=SO<sub>2</sub>) has no inhibition against any of the cancer cell lines. Whereas, **6h** (R<sub>1</sub>=Cl, R<sub>2</sub>=H, R<sub>3</sub>=2,4,5-trichloro, X=SO<sub>2</sub>) is slightly cytotoxic against all the cancer cell lines. Similarly, **6f** (R<sub>1</sub>, R<sub>2</sub>=F, R<sub>3</sub>=4-nitro, X=SO<sub>2</sub>) has no cytotoxicity against any of the cancer cell lines. Whereas, **6j** (R<sub>1</sub>=Cl, R<sub>2</sub>=H, R<sub>3</sub>=4-nitro, X=SO<sub>2</sub>) 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 (%)
<b>5a</b>	C=O	-H	-H	5-Fluoro-2-methyl	>300 (dec.)	95
<b>5b</b>	C=O	-F	-F	2-Bromo	189.7	35
<b>5c</b>	C=O	-F	-F	3-Bromo	151.4	27
<b>5d</b>	C=O	-F	-F	4-Bromo	>300 (dec.)	19
<b>5e</b>	C=O	-F	-F	3-Chloro	177.5	47
<b>5f</b>	C=O	-Cl	-H	2-Methoxy	120	33
<b>5g</b>	C=O	-Cl	-H	3-Nitro	196.1	24
<b>5h</b>	C=O	-Cl	-H	3,4-Dimethoxy	148.6	11
<b>5i</b>	C=O	-Cl	-H	4-Ethyl	206.4	22
<b>6a</b>	SO <sub>2</sub>	-H	-H	2-Trifluoromethoxy	205.8	10
<b>6b</b>	SO <sub>2</sub>	-F	-F	2-Trifluoromethyl	135.6	22
<b>6c</b>	SO <sub>2</sub>	-F	-F	2,4,5-Trichloro	>300 (dec.)	11
<b>6d</b>	SO <sub>2</sub>	-F	-F	3,4-Dichloro	145.1	42
<b>6e</b>	SO <sub>2</sub>	-F	-F	2-Methyl	117.7	10
<b>6f*</b>	SO <sub>2</sub>	-F	-F	4-Nitro	224.5	13
<b>6g</b>	SO <sub>2</sub>	-F	-F	2,5-Dichloro	116.1	23
<b>6h</b>	SO <sub>2</sub>	-Cl	-H	2,4,5-Trichloro	151.1	19
<b>6i</b>	SO <sub>2</sub>	-Cl	-H	3,4-Dichloro	107.1	25
<b>6j</b>	SO <sub>2</sub>	-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 GI <sub>50</sub> (μM)		
	HUH-7	MCF-7	HCT-116
<b>5a</b>	10.80	10.44	11.34
<b>5b</b>	20.89	6.05	12.78
<b>5c</b>	11.72	5.95	9.10
<b>5d</b>	12.12	2.21	12.16
<b>5e</b>	11.16	5.87	8.95
<b>5f</b>	8.49	17.88	11.00
<b>5g</b>	13.23	22.72	13.85
<b>5h</b>	10.81	16.09	10.54
<b>5i</b>	10.91	–	9.45
<b>6a</b>	–	4.50	21.09
<b>6b</b>	–	–	–
<b>6c</b>	–	–	–
<b>6d</b>	15.23	56.02	–
<b>6e</b>	17.65	17.10	27.41
<b>6f</b>	–	–	–
<b>6g</b>	–	–	–
<b>6h</b>	54.41	11.16	31.41
<b>6i</b>	10.88	–	53.06
<b>6j</b>	39.95	17.22	97.74
<b>5-FU</b>	30.66	3.51	18.67
<b>CPT</b>	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
<b>5d</b>	2.21	5.5
<b>6a</b>	4.50	9.9

Benzoylpiperazines are moderately active on HUH-7 cell line and **5f** (GI<sub>50</sub>=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<sub>50</sub>=10.88 μM) has the highest activity among the sulfonylpiperazines.

The most active compounds against MCF-7 cell line are **5d** (GI<sub>50</sub>=2.21 μM) and **6a** (GI<sub>50</sub>=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<sub>50</sub>=6.05 μM), **5c** (GI<sub>50</sub>=5.95 μM), **5d** (GI<sub>50</sub>=2.21 μM) and **5e** (GI<sub>50</sub>=5.87 μM).

Benzamide derivatives generally show good activity values considering HCT-116 cell line. **5c** (GI<sub>50</sub>=9.10 μM), **5e** (GI<sub>50</sub>=8.95 μM) and **5h** (GI<sub>50</sub>=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 sulforhodamine 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.

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