European Journal of Medicinal Chemistry 44 (2009) 1223–1229

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and *in vitro* antiproliferative activity of novel 1-benzhydrylpiperazine derivatives against human cancer cell lines

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ARTICLE INFO

Article history:
Received 3 April 2008
Received in revised form 29 July 2008
Accepted 4 September 2008
Available online 30 September 2008

Keywords:
1-Benzhydrylpiperazine derivatives
Sulfonyl chlorides
MTT assay
Antiproliferative activity
Cell proliferation
Carcinoma cell lines

ABSTRACT

In order to explore the antiproliferative effect associated with the piperazine framework, several 1-benzhydrylpiperazine derivatives **8**(**a**–**d**), **9**(**a**–**d**) and **10**(**a**–**h**) were synthesized. Variation in the functional group at N-terminal of the piperazine led to three sets of compounds, bearing the sulfonyl, amide and thiourea, respectively. Their chemical structures were confirmed by ^{1}H NMR, LCMS, IR and elemental analysis. The antiproliferative effect of the compounds were evaluated *in vitro* using the MTT colorimetric method against one normal cell line (NF-103 skin fibroblast cells) and four human cancer cell lines (MCF-7 breast carcinoma cell line, HepG-2 hepatocellular carcinoma cell line, HeLa cervix carcinoma cell line and HT-29 colon carcinoma cell line) for the time period of 24 h. Among the series, four compounds exhibited interesting growth inhibitory effects against all four cell lines.

1. Introduction

Cancer remains the leading cause of death in the World and as a result there is a pressing need for novel and effective treatments. One of the characteristic of cancer cells, that differs from their normal counterparts in a number of biochemical processes, particularly during the control of cell growth and division.

Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21st century. Because it is difficult to discover novel agents that selectively kill tumor cells or inhibit their proliferation without the general toxicity, the use of traditional cancer chemotherapy is still very limited. In the field of chemotherapeutic drugs, the search for new, more active, more selective and less toxic compounds is still very intense, and new promising anticancer approaches are being tested [1,2]. Currently, combined anticancer therapies or multi-acting drugs are clinically preferred to traditional cytotoxic treatment, with the aim of overcoming resistance and toxicity drawbacks. These events often prevent successful treatment and are responsible for reduced survival times [3,4]. In the past 50 years, the mass screening of either synthetic derivatives or natural products has led to the discovery of the currently utilized anticancer drugs.

Piperazines have been widely used in biological screening resulting in numerous applications and constitute an attractive pharmacological scaffold present in several drugs [5]. This small and rigid heterocyclic backbone could act on various pharmacological targets. Especially, piperazine nucleus could be found in a broad range of biological active compounds displaying anticancer [6–13], calcium channel blockers [14–17] and histamine antagonists [18,19]. In the continuation of previous research on synthesis and anticancer studies of bioactive heterocycles [20–23], herein, we report the synthesis and antiproliferative activity of novel 1-benzhydrylpiperazine derivatives against human cancer cell lines.

2. Chemistry

1-Benzhydrylpiperazine derivatives **8**(**a**–**d**), **9**(**a**–**d**) and **10**(**a**–**h**) were prepared by the method summarized in Scheme 1. Initially compound **2**, benzhydrol was synthesized by Grignard reaction under nitrogen atmosphere with benzaldehyde and phenyl magnesium bromide, the obtained yield was found to be 60%. Finally we synthesized the benzhydrol by reduction of benzophenone using sodium borohydride and achieved a 90% yield. Compound **2** was subsequently treated with thionyl chloride to give the corresponding benzhydryl chloride (**3**), which was directly

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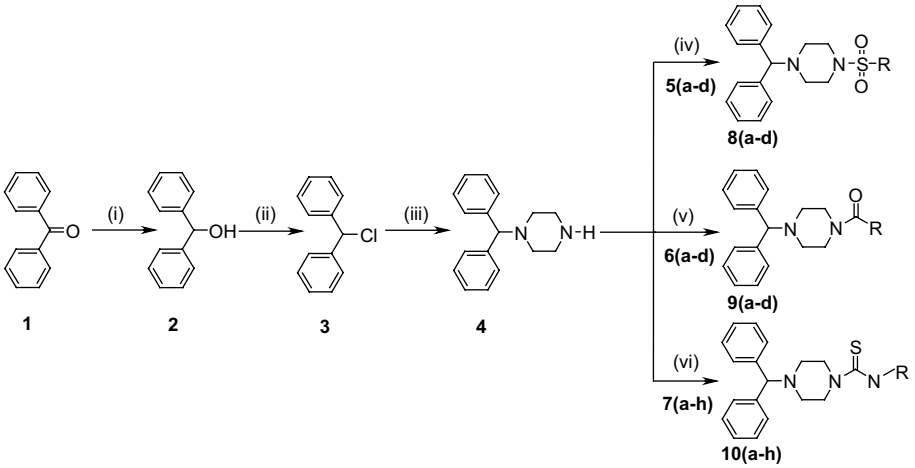
doi:10.1016/j.ejmech.2008.09.025



Scheme 1. Reagents and conditions: (i) $NaBH_4$, methanol, r.t., 5 h. (ii) Thionyl chloride, MDC, 0–5 °C, 4 h. (iii) Piperazine, K_2CO_3, DMF, 80 °C, 8 h. (iv) Sulfonyl chlorides, MDC, triethylamine, r.t., 5–6 h. (v) Acid chlorides, MDC, triethylamine, r.t., 5–6 h. (v) Isothiocyanates, MDC, triethylamine, r.t., 4–5 h. Compounds – **5a**: 4-Chloro-2-fluorobenzene-1-sulfonyl chloride; **6a**: isoxazole-5-carbonyl chloride; **5b**: camphor sulfonyl chloride; **6b**: morpholine-4-carbonyl chloride; **5c**: benzenesulfonyl chloride; **6c**: pyrrolidine-1-carbonyl chloride; **5d**: 2,2,2-trifluoro ethanesulfonyl chloride; **6d**: cyclopropanecarbonyl chloride; **7a**: 2-methoxyphenylisothiocyanate; **7b**: 3-methoxyphenylisothiocyanate; **7c**: 4-methoxyphenylisothiocyanate; **7d**: 2-chlorophenylisothiocyanate; **7e**: 3-chlorophenylisothiocyanate; **7f**: 4-chlorophenylisothiocyanate; **7g**: 4-fluorophenylisothiocyanate; **7h**: 2,4-dichlorophenylisothiocyanate.

treated with piperazine and anhydrous potassium carbonate using dimethyl formamide as a solvent at 80 °C to give the target key intermediate 1-benzhydrylpiperazine (**4**). The nucleophilic substitution reactions of 1-benzhydrylpiperazine with different sulfonyl chlorides/acid chlorides/or isothiocyanates were carried out in the presence of triethylamine and dichloromethane as solvent with a good yield ranging from 65 to 88% with good purity. Compound **8c** was structurally characterized by X-ray crystallographic studies and the data of the molecule have been published [24]. The absence of N–H proton peak in proton NMR and IR spectra confirms our products. It is also confirmed by IR data, for sulfonamide series **8**(**a**–**d**) which showed asymmetric stretching frequency of O═S═O in the range 1350–1370 cm^{-1} and symmetric stretching frequency at 1270–1290 cm^{-1} and similarly for carboxamide series **9**(**a**–**d**), IR data showed stretching frequency of –C═O at 1630–1670 cm^{-1} and 3250–3290 cm^{-1} for –NH in thioureas **10**(**a**–**h**). The products obtained were purified by column chromatography using hexane:ethyl acetate (8:2) as an eluent. The chemical structures and physical data of all the synthesized compounds are given in Table 1.

3. Experimental

Melting points were determined using SELACO-650 hot stage melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-d_6 as a solvent and TMS as internal standard (chemical shift in δ ppm). Spin multiplets are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHNS) analyses were obtained on Vario EL III Elementar. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates.

3.1. General procedure for synthesis of 1-benzhydrylpiperazine (*4*)

A solution of piperazine dihydrochloride (10.0 g, 62.86 mmol) in dimethyl formamide was taken, anhydrous potassium carbonate (43.44 g, 314.3 mmol) was added and stirred for 10 min, and then benzhydryl chloride (11.46 g, 56.58 mmol) was added. The reaction mixture was heated at 80 °C for 8 h, and monitored by TLC. After completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. Finally, water wash was given to the organic layer and dried with anhydrous sodium sulphate. The solvent was evaporated and the crude product obtained was purified by column chromatography over silica gel (60–120 mesh) using chloroform:methanol (9:1) as an eluent. Some of the compounds were recrystallised by using ethyl acetate.

3.2. General procedure for synthesis of 1-benzhydrylpiperazine derivatives *8*(*a*–*d*), *9*(*a*–*d*) and *10*(*a*–*h*)

A solution of 1-benzhydrylpiperazine (**4**) (1.0 eq) in dry dichloromethane 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 **5**(**a**–**d**)/acid chlorides **6**(**a**–**d**)/isothiocyanates **7**(**a**–**h**) (1.0 eq) were added, the reaction mixture was allowed to stir at room temperature for 4–6 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.

3.2.1. Synthesis of 1-benzhydryl-4-(4-chloro-2-fluorobenzenesulfonyl)-piperazine (*8a*)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 4-chloro-2-fluorobenzene-1-sulfonyl chloride (0.453 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was white crystalline solid (0.660 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.82 (1H, m, Ar–H), 7.74 (d, 1H, Ar–H), 7.55 (d, 1H, Ar–H), 7.17 (t, 2H, Ar–H), 7.25 (t, 4H, Ar–H), 7.37 (d, 4H, Ar–H), 4.25 (s, 1H, –CH–), 3.1 (br s, 4H, $-CH_2-$), 2.31 (br s, 4H, $-CH_2-$). MS (ESI, + ion): $m/z = 445.2$. IR (KBr, cm^{-1}): 3049, 2950, 2828, 1340, 1286. Anal. calcd. for $C_{23}H_{22}ClFN_2O_2S$ (in %): C 62.09, H 4.98, N 6.30, S 7.21. Found C 62.05, H 4.95, N 6.35, S 7.25.

Table 1
Chemical structure, physical data and purity of synthesized compounds

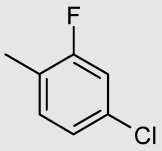
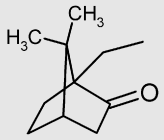
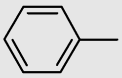
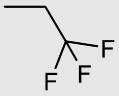
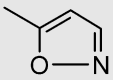
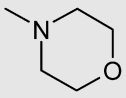
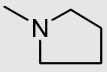
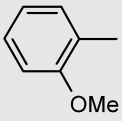
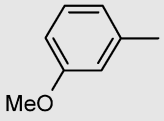
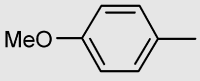
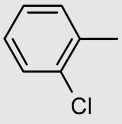
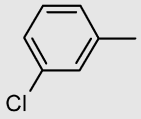
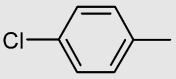
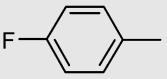
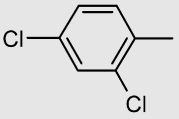
Compound	R	Yield (%)	M.P. (°C)	Purity
8a		75	192–194	98.98
8b		68	173–175	97.14
8c		80	157–159	99.1
8d		65	151–153	97.20
9a		73	143–145	98.01
9b		70	181–183	98.65
9c		77	162–164	97.36
9d		66	173–175	98.21
10a		82	158–160	97.81
10b		85	160–162	98.65
10c		88	199–201	96.85
10d		78	194–196	98.13

Table 1 (*continued*)

Compound	R	Yield (%)	M.P. (°C)	Purity
10e		75	213–215	97.90
10f		80	208–210	97.62
10g		74	189–191	98.60
10h		78	206–208	97.80

*3.2.2. Synthesis of 1-(4-benzhydrylpiperazine-1-sulfonylmethyl)-7,7-dimethyl-bicyclo [2,2,1]heptan-2-one (**8b**)*

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), (7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonyl chloride (0.496 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was white crystalline solid (0.627 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.18 (t, 2H, Ar–H), 7.27 (t, 4H, Ar–H), 7.44 (d, 4H, Ar–H), 4.37 (s, 1H, –CH–), 3.24 (br s, 4H, –CH_2–), 2.38 (br s, 4H, –CH_2–), 1.38 (m, 1H, –CH–), 1.9–2.9 (m, 12H, –CH_2–), 0.82 (s, 6H, –CH_3). MS (ESI, + ion): $m/z = 467.2$. IR (KBr, cm^{-1}): 3053, 2959, 2838, 1345, 1280. Anal. calcd. for $C_{27}H_{36}N_2O_2S$ (in %): C 71.64, H 8.02, N 6.19, S 7.08. Found C 71.60, H 8.05, N 6.15, S 7.05.

*3.2.3. Synthesis of 1-benzenesulfonyl-4-benzhydrylpiperazine (**8c**)*

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), benzenesulfonyl chloride (0.349 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product was a white crystalline solid (0.622 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.48 (d, 2H, Ar–H), 7.40 (m, 6H, Ar–H), 7.27 (t, 5H, Ar–H), 7.15 (t, 2H, Ar–H), 4.26 (s, 1H, –CH–), 3.2 (br s, 4H, –CH_2–), 2.50 (br s, 4H, –CH_2–). MS (ESI, + ion): $m/z = 393.61$. IR (KBr, cm^{-1}): 3026, 2925, 2960, 1319, 1150. Anal. calcd. for $C_{23}H_{24}N_2O_2S$ (in %): C 70.38, H 6.16, N 7.14, S 8.17. Found C 70.34, H 6.11, N 7.10, S 8.15.

*3.2.4. Synthesis of 1-benzhydryl-4-(2,2,2-trifloro-ethyanesulfonyl)-piperazine (**8d**)*

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 2,2,2-trifluoro ethanesulfonyl chloride (0.361 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was brown crystalline solid (0.512 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.18 (t, 2H, Ar–H), 7.26 (t, 4H, Ar–H), 7.45 (d, 4H, Ar–H), 4.37 (s, 1H, –CH–), 3.24 (br s, 4H, –CH_2–), 2.37 (br s, 4H, –CH_2–), 4.82 (s, 2H, –CH_2–). MS (ESI, + ion): $m/z = 399.30$. IR (KBr, cm^{-1}): 3043, 2946, 2830, 1347, 1281. Anal. calcd. for $C_{19}H_{21}F_3N_2O_2S$ (in %): C 57.27, H 5.31, N 7.03, S 8.05. Found C 57.23, H 5.27, N 7.07, S 8.10.

*3.2.5. Synthesis of 4-benzhydrylpiperazine-1-yl(isoxazol-5-yl)methanone (**9a**)*

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), isoxazole-5-carbonyl chloride (0.260 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was off-white crystalline solid (0.501 g). ^{1}H NMR (DMSO-d_6, 400 MHz)

δ: 7.38 (d, 4H, Ar–H), 7.24 (t, 4H, Ar–H), 7.17 (t, 2H, Ar–H), 5.5–5.6 (d, 2H, N–CH–CH–O–), 4.4 (s, 1H, –CH–), 3.2 (br s, 4H, $–CH_2–$), 2.35 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 348.2$. IR (KBr, cm^{-1}): 2924, 2854, 1651. Anal. calcd. for $C_{21}H_{21}N_3O_2$ (in %): C 72.60, H 6.09, N 12.09. Found C 72.56, H 6.13, N 12.05.

3.2.6. Synthesis of (4-benzhydrylpiperazin-1-yl)(morpholino)methanone (**9b**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), morpholine-4-carbonyl chloride (0.296 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was white crystalline solid (0.506 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.40 (d, 4H, Ar–H), 7.3 (t, 4H, Ar–H), 7.19 (t, 2H, Ar–H), 4.29 (s, 1H, –CH–), 3.5 (br s, 4H, $–CH_2–$), 3.0-3.1 (8H, $–CH_2–$), 2.49 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 366.0$. IR (KBr, cm^{-1}): 2935, 2863, 1660. Anal. calcd. for $C_{22}H_{27}N_3O_2$ (in %): C 72.30, H 7.45, N 11.50. Found C 72.26, H 7.40, N 11.46.

3.2.7. Synthesis of (4-benzhydrylpiperazin-1-yl)(pyrrolidin-1-yl)methanone (**9c**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), pyrrolidine-1-carbonyl chloride (0.264 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was brown crystalline solid (0.532 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.42 (d, 4H, Ar–H), 7.30 (t, 4H, Ar–H), 7.19 (t, 2H, Ar–H), 4.35 (s, 1H, –CH–), 3.3 (br s, 4H, $–CH_2–$), 3.1(br s, 4H, $–CH_2–$), 2.48 (br s, 4H, $–CH_2–$), 1.69 (b, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 350.11$. IR (KBr, cm^{-1}): 2968, 2860, 1658. Anal. calcd. for $C_{22}H_{27}N_3O$ (in %): C 75.61, H 7.79, N 12.02. Found C 75.57, H 7.75, N 12.05.

3.2.8. Synthesis of (4-benzhydrylpiperazin-1-yl)(cyclopropyl)methanone (**9d**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), cyclopropanecarbonyl chloride (0.207 g, 1.98 mmol), triethylamine (0.601 g, 5.94 mmol). The product obtained was off-white crystalline solid (0.418 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.45 (d, 4H, Ar–H), 7.28 (t, 4H, Ar–H), 7.21 (t, 2H, Ar–H), 4.32 (s, 1H, –CH–), 3.4 (br s, 2H, $–CH_2–$), 3.28 (br s, 2H, $–CH_2–$), 2.5 (br s, 2H, $–CH_2–$), 2.32 (br s, 2H, $–CH_2–$), 0.6–0.7 (m, 4H, –CH–). MS (ESI, + ion): $m/z = 321.2$. IR (KBr, cm^{-1}): 2960, 2856, 1650. Anal. calcd. for $C_{21}H_{24}N_2O$ (in %): C 78.72, H 7.55, N 8.74. Found C 78.70, H 7.52, N 8.70.

3.2.9. Synthesis of 4-benzhydryl-N-(2-methoxyphenyl)piperazine-1-carbothioamide (**10a**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 2-methoxyphenylisothiocyanate (0.354 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white crystalline solid (0.677 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.18 (s, 1H, –NH), 7.44 (d, 5H, Ar–H), 7.34 (t, 5H, Ar–H), 7.25 (t, 2H, Ar–H), 7.20 (m, 2H, Ar–H), 4.34 (s, 1H, –CH–), 3.35 (br s, 4H, $–CH_2–$), 2.39 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 418.1$. IR (KBr, cm^{-1}): 3289, 1334. Anal. calcd. for $C_{25}H_{27}N_3OS$ (in %): C 71.91, H 6.52, N 10.06, S 7.68. Found. C 71.94, H 6.55, N 10.10, S 7.70.

3.2.10. Synthesis of 4-benzhydryl-N-(3-methoxyphenyl)piperazine-1-carbothioamide (**10b**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 3-methoxyphenylisothiocyanate (0.354 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white crystalline solid (0.702 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.23 (s, 1H, –NH), 7.46 (d, 4H, Ar–H), 7.33 (t, 4H, Ar–H), 7.22–7.15 (m, 3H, Ar–H), 6.89 (t, 2H, Ar–H), 6.67 (m, 1H, Ar–H), 4.39 (s, 1H, –CH–), 3.73 (s, 3H, $–OCH_3$), 3.37 (br s, 4H, $–CH_2–$), 2.36 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 418.2$. IR (KBr, cm^{-1}): 3250, 1338. Anal. calcd. for $C_{25}H_{27}N_3OS$ (in %): C 71.91, H 6.52, N 10.06, S 7.68. Found. C 71.95, H 6.50, N 10.08, S 7.72.

3.2.11. Synthesis of 4-benzhydryl-N-(4-methoxyphenyl)piperazine-1-carbothioamide (**10c**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 4-methoxyphenylisothiocyanate (0.354 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white fluffy solid (0.726 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.13 (s, 1H, –NH), 7.46 (d, 4H, Ar–H), 7.33 (t, 4H, Ar–H), 7.22 (t, 2H, Ar–H), 7.14 (d, 2H, Ar–H), 6.86 (d, 2H, Ar–H), 4.38 (s, 1H, –CH–), 3.73 (s, 3H, $–OCH_3$), 3.89 (br s, 4H, $–CH_2–$), 2.36 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 418.1$. IR (KBr, cm^{-1}): 3158, 1385. Anal. calcd. for $C_{25}H_{27}N_3OS$ (in %): C 71.91, H 6.52, N 10.06, S 7.68. Found. C 71.94, H 6.54, N 10.06, S 7.70.

3.2.12. Synthesis of 4-benzhydryl-N-(2-chlorophenyl)piperazine-1-carbothioamide (**10d**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 2-chlorophenylisothiocyanate (0.335 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was off-white crystalline solid (0.651 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.20 (s, 1H, –NH), 7.47 (d, 4H, Ar–H), 7.36 (t, 4H, Ar–H), 7.23–7.15 (m, 3H, Ar–H), 7.05 (t, 2H, Ar–H), 6.70 (m, 1H, Ar–H), 4.38 (s, 1H, –CH–), 3.35 (br s, 4H, $–CH_2–$), 2.36 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 422$. IR (KBr, cm^{-1}): 3248, 1400. Anal. calcd. for $C_{24}H_{24}ClN_3OS$ (in %): C 68.31, H 5.73, N 9.96, S 7.60. Found. C 68.33, H 5.75, N 9.99, S 7.63.

3.2.13. Synthesis of 4-benzhydryl-N-(3-chlorophenyl)piperazine-1-carbothioamide (**10e**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 3-chlorophenylisothiocyanate (0.335 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white fluffy solid (0.626 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.23 (s, 1H, –NH), 7.45 (d, 4H, Ar–H), 7.39 (t, 4H, Ar–H), 7.27 (t, 4H, Ar–H), 6.89 (t, 2H, Ar–H), 6.67 (m, 1H, Ar–H), 4.39 (s, 1H, –CH–), 3.37 (br s, 4H, $–CH_2–$), 2.36 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 422.4$. IR (KBr, cm^{-1}): 3275, 1321. Anal. calcd. for $C_{24}H_{24}ClN_3OS$ (in %): C 68.31, H 5.73, N 9.96, S 7.60. Found. C 68.32, H 5.76, N 9.97, S 7.64.

3.2.14. Synthesis of 4-benzhydryl-N-(4-chlorophenyl)piperazine-1-carbothioamide (**10f**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 4-chlorophenylisothiocyanate (0.335 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was off-white crystalline solid (0.668 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.14 (s, 1H, –NH), 7.48 (d, 4H, Ar–H), 7.33 (t, 4H, Ar–H), 7.25 (t, 2H, Ar–H), 7.14 (d, 2H, Ar–H), 6.90 (d, 2H, Ar–H), 4.39 (s, 1H, –CH–), 3.87 (br s, 4H, $–CH_2–$), 2.38 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 422.1$. IR (KBr, cm^{-1}): 3286, 1346. Anal. calcd. for $C_{24}H_{24}ClN_3OS$ (in %): C 68.31, H 5.73, N 9.96, S 7.60. Found. C 68.35, H 5.75, N 9.97, S 7.62.

3.2.15. Synthesis of 4-benzhydryl-N-(4-fluorophenyl)piperazine-1-carbothioamide (**10g**)

This was obtained from 1-benzhydrylpiperazine (**4**) (0.5 g, 1.98 mmol), 4-fluorophenylisothiocyanate (0.303 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white fluffy solid (0.593 g). ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.17 (s, 1H, –NH), 7.45–7.40 (m, 6H, Ar–H), 7.33–7.29 (t, 4H, Ar–H), 7.22 (t, 2H, Ar–H), 7.11 (m, 2H, Ar–H), 4.35 (s, 1H, –CH–), 3.89 (br s, 4H, $–CH_2–$), 2.36 (br s, 4H, $–CH_2–$). MS (ESI, + ion): $m/z = 406.3$. IR (KBr, cm^{-1}): 3287, 1319. Anal. calcd. for $C_{24}H_{24}ClFN_3S$ (in %): C 71.08, H 5.97, N 10.36, S 7.91. Found. C 71.05, H 5.99, N 10.33, S 7.94.

3.2.16. Synthesis of 4-benzhydryl-N-(2,4-dichlorophenyl)piperazine-1-carbothioamide (**10h**)

This was obtained from 1-benzhydrylpiperazine (**6**) (0.5 g, 1.98 mmol), 2,4-dichlorophenylisothiocyanate (0.404 g, 1.98 mmol)

and triethylamine (0.601 g, 5.94 mmol). The product obtained was white fluffy solid (0.704 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.20 (s, 1H, –NH), 7.63 (s, 1H, Ar–H), 7.50–7.45 (m, 5H, Ar–H), 7.36–7.29 (m, 5H, Ar–H), 7.22 (t, 2H, Ar–H), 4.35 (s, 1H, –CH–), 3.46 (br s, 4H, $-CH_2-$), 2.40 (br s, 4H, $-CH_2-$). MS (ESI, + ion): $m/z = 456.4$. IR (KBr, cm^{-1}): 3264, 1348. Anal. calcd. for $C_{24}H_{23}Cl_2N_3S$ (in %): C 63.16, H 5.08, N 9.21, S 7.02. Found. C 63.13, H 5.11, N 9.25, S 7.05.

3.3. Biology

We tested the effect of novel synthesized compounds **8**(**a**–**d**), **9**(**a**–**d**) and **10**(**a**–**h**) on cell proliferation using five different cell lines in a cell toxicity assay and the percentage cell survived at the dose level of 100 μM for 24 h was determined. Percentage cell survival for tested compounds against NF-103, MCF-7, HepG-2, HT-29 and HeLa cells are tabulated in Table 2. The extent of inhibition of carcinoma cell lines by **8**(**a**–**d**), **9**(**a**–**d**) and **10**(**a**–**h**) were schematically presented in Figs. 1–5.

Stock solutions (100 μM) of test compounds were prepared in dimethylsulfoxide (DMSO) and stored at −20 °C. These concentrated solutions were added immediately to cell culture wells on the day of experimentation. The final DMSO concentration was 0.1% in each well and it showed no interference with the biological activities tested.

3.3.1. Cell culture

NF-103, HeLa, HepG-2, MCF-7 Cell lines were cultured in DMEM with 10% FCS, HT-29 cultured in Mc Coy's Medium with 10% FCS, CRL-170 cultured in RPMI with 10% FCS and 1% v/v antibiotic. The proliferation of skin fibroblast cells (NF-103), breast carcinoma cells (MCF-7), hepatocellular carcinoma cells (HepG-2), cervix carcinoma cells (HeLa) and colon carcinoma cells (HT-29) can be assayed at different time intervals besides 24 h. The positive control used in the experiment was wells with DMEM (10% FBS and 1% v/v antibiotic added) added while the negative controls used were wells with DMEM (1% v/v antibiotic and no FBS added). Cell cultures used were procured from the Department of Biological Sciences, National University of Singapore, Singapore. The absorbance was measured at 570 nm with a microtiter plate reader.

3.3.2. In vitro cell viability assay – MTT assay

The potential effects on cell viability were investigated by using the MTT assay [25] [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] as an indicator of metabolically active cells. A known number of MCF-7 or HT-29 or HeLa or HepG-2 cells were transferred into 96 well plates in a volume of 200 μl of culture medium and incubated for 48 h before addition of test compound. Cells were then exposed to known concentrations of the compound to be tested (100 μM expressed as final concentration) for 24 h at 37 °C. After drug exposure, the culture medium was removed and 200 μl of MTT reagent (diluted in culture medium, 1 mg/ml) was added. After incubating for 4 h, the MTT/medium was removed and DMSO (200 μl) was added to dissolve the formazan crystals. Absorbance of the colored solution was measured on a microtiter plate reader using a test wavelength of 570 nm. Results were evaluated by comparing the absorbance of the wells containing compound treated cells with the absorbance of wells containing 0.1% DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100% in the solvent control. All assays were performed in triplicate and mean ± SD values were used to estimate cell viability.

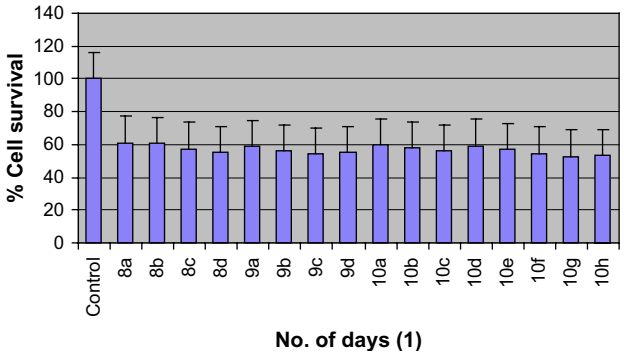


Fig. 1. MTT assay for human normal skin fibroblast cell line.

4. Results and discussion

Piperizine derivatives were shown to inhibit growth inhibition of human erythroleukemia K5-62 cells and myeloid leukemia HL-60 cells [26] and also shown to inhibit topoisomarase II activity [27]. Wilson et al reported the interaction of DNA with unfused aromatic system containing terminal piperazino substituents [9]. Bisdioxopiperazines have been reported for their anti-tumor effects against two experimental lung cancer models *in vivo* [28]. Recently, a new class of sulfonamides has been used in the treatment of diseases arising from abnormal cell growth and proliferation [29,30].

In view of the above findings, the anticancer activity was checked by carrying out the reactions of 1-benzhydrylpiperazine

Table 2
Evaluation of cytotoxicity towards carcinoma cells (% cell survival) for the synthesized compounds

Compound	NF-103	MCF-7	HepG-2	HeLa	HT-29
8a	60.95	64.03	70.14	61.89	76.27
8b	60.44	61.73	72.63	65.15	79.20
8c	57.42	68.09	66.16	61.89	69.27
8d	**54.90**	**41.11**	**47.76**	**47.69**	**43.64**
9a	**58.61**	**44.18**	**46.10**	**46.80**	**47.50**
9b	56.10	68.69	61.85	53.92	63.40
9c	53.99	71.43	66.16	64.25	70.24
9d	55.07	61.01	62.85	70.31	71.98
10a	59.70	54.49	61.02	56.79	62.10
10b	57.76	58.71	64.51	72.83	64.82
10c	55.93	63.70	**48.75**	66.04	55.59
10d	59.13	69.79	71.80	63.07	70.79
10e	56.84	73.57	69.48	69.75	69.27
10f	54.79	66.94	66.16	73.90	73.77
10g	**52.73**	**43.91**	**45.43**	**48.37**	**47.39**
10h	53.53	**45.61**	59.20	**45.45**	68.18

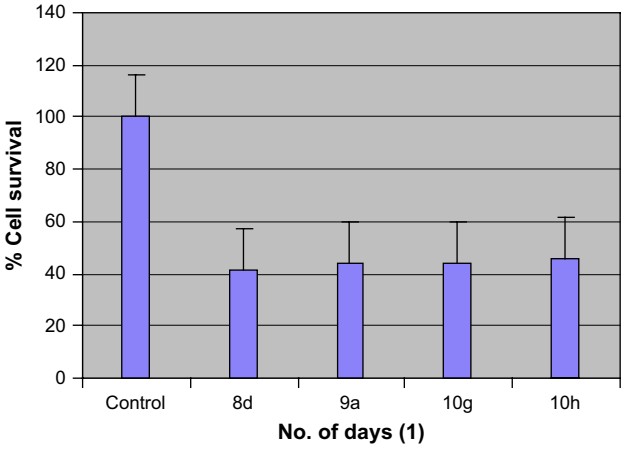


Fig. 2. MTT assay for MCF-7 cell line.

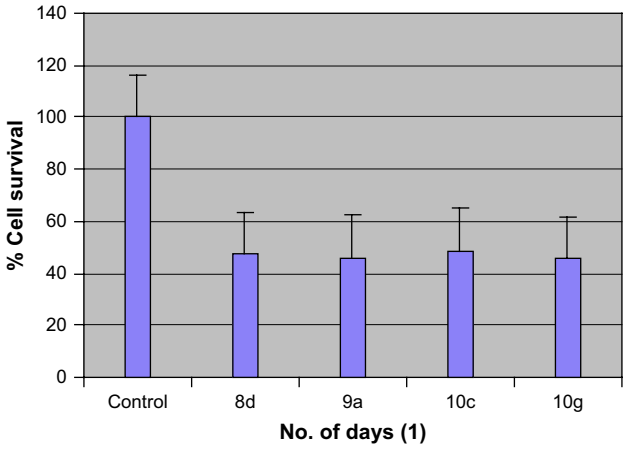


Fig. 3. MTT assay for HepG-2 cell line.

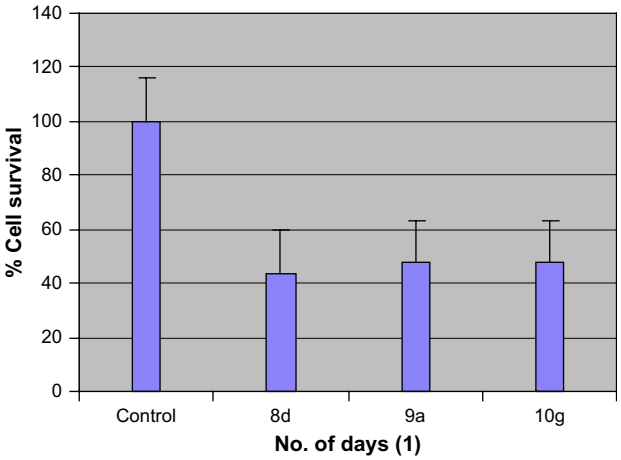


Fig. 5. MTT assay for HT-29 cell line.

with different sulfonyl chlorides/acid chlorides/isothiocyanates containing substituted aromatic rings.

The novel synthesized compounds were tested for their effect on cellular viability against human carcinoma cell lines MCF-7, HepG-2, HeLa and HT-29. All the synthesized compounds were tested for their cytotoxicity against normal skin fibroblast cells. Assays were performed *in vitro* on exponentially growing cells. The activity was evaluated by measuring the levels of surviving cells after incubation for 24 h with the test samples, using the MTT colorimetric assay, based on the ability of metabolically active cells to convert the pale yellow MTT to a blue formazan product, which is quantifiable spectrophotometrically.

Among the sulfonamide analogues **8**(**a**–**d**), compound **8d** exhibits **41.11%**, **47.76%**, **47.69%**, **43.64%** inhibitory activity against MCF-7, HepG-2, HeLa, HT-29 carcinoma cell lines, respectively, whereas compounds **8**(**a**–**c**) showed less inhibitory activity. From the data obtained, it is obvious that the nature of the *N*-terminal on the 1-benzhydrylpiperazine exerts a striking effect on the antiproliferative activity. Within the few variations considered in this study, the presence of a trifluoroethyl alkyl chain afforded a clear beneficial effect with regard to antiproliferative properties. With the exception of **8b** as alkyl camphor sulfonyl counterpart on 1-benzhydrylpiperazine was less active compare to **8d**. The good inhibition by compound **8d** could be attributed to the presence of electron withdrawing trifluoroethyl group. Introduction of an aromatic ring resulted in compounds (**8a** and **8c**) completely devoid of activity.

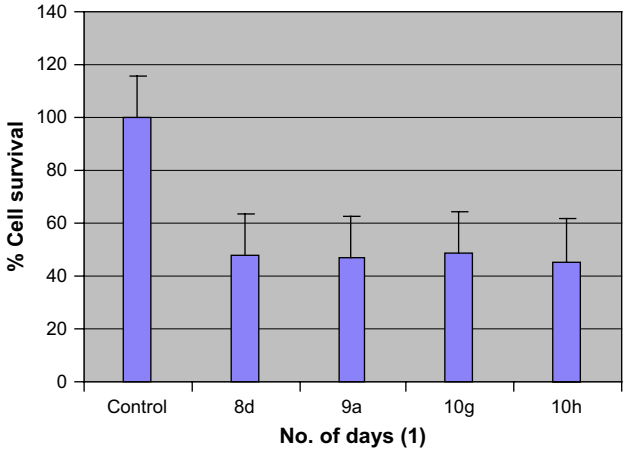


Fig. 4. MTT assay for HeLa cell line.

From benzamide derivatives **9**(**a**–**d**), compound **9a** exhibits **44.18%**, **46.10%**, **46.80%**, **47.50%** inhibitory activity against MCF-7, HepG-2, HeLa, HT-29 carcinoma cell lines, respectively. The remaining compounds **9**(**b**–**d**) exhibit less inhibitory activity. The potent inhibition of compound **9a** might be due to the presence of isoxazole moiety.

High activity of compound **9a** is connected with quite different distribution of charge in the isoxazole ring compared to other analogues. Rigidity of the isoxazole ring is essential for a more efficient binding to the active site of the enzyme. Replacing the isoxazole ring by morpholine **9b** and pyrrolidine **9c** resulted in the loss of the activity. Similarly, replacing cyclopropyl ring **9d** still decreases the antiproliferative activity.

In thioureas **10**(**a**–**h**), compound **10g** exhibits **43.91%**, **45.43%**, **48.37%**, **47.39%** inhibitory activity against MCF-7, HepG-2, HeLa, HT-29 carcinoma cell lines, respectively. Compound **10h** showed **45.61%**, **45.45%** inhibitory activity against MCF-7, HeLa carcinoma cell lines, respectively. The –NH– group in thioureas is essential for binding with the enzyme and the nature of the amide side chain is important for the exhibition of anticancer activity. Relatively high activity of compounds **10g** and **10h** can be related to the presence of the electron withdrawing fluoro and chloro substituent on the aromatic ring. Structure activity relationship can be drawn for the derivatives **10**(**d**–**h**) containing electronegative atoms, which reveals that, compound **10g** has more electronegative fluorine atom compared to **10**(**d**–**f**) having chlorine atom. In the same aspect, compound **10h** has disubstituted chlorine atom exhibits relatively good inhibition compare to **10**(**d**–**f**) having mono substituted chlorine atom. Introducing electron donating methoxy groups **10**(**a**–**c**) to the phenyl ring of the substituent at *ortho*, *meta* and *para* position resulted in the loss of the activity. However, the presence of electron withdrawing fluorine atom **10g** in the phenyl ring increased the antiproliferative efficacy.

5. Conclusion

From our experimental results, it could be concluded that the introduction of alkyl trifluoroethyl containing sulfonyl group, isoxazole ring in the amide moiety and substitution of fluoro, chloro in the aromatic ring bearing thiourea group on the 1-benzhydrylpiperazine system has great potential to get novel antiproliferative compounds. From this work we were able to identify a few active molecules which are capable of inhibiting the growth of human cancer cell lines *in vitro*. These compounds exhibited a relatively weak cytotoxicity. Our results suggest that, the antiproliferative properties induced by the 1-benzhydrylpiperazine

framework might involve distinct mechanisms. Hence, there is a need for further investigations to clarify the features underlying the antiproliferative activities of these new derivatives.

Acknowledgements

The authors are grateful to UGC, Govt. of India for financial support to K.S.R. under the project UGC-SAP (Phase I) vide No. F. 540/10/2004-05 (SAP I) UGC vide No. DV6/69/UGC/2006-07. The CHNS data from the instrument granted by DST-FIST programme Council of Scientific and Industrial Research (CSIR), for financial support under CSIR-SRF to K. Vinaya is greatly acknowledged.

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