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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro antiproliferative activity of diphenyl(sulphonylpiperidin-4-yl)methanol derivatives

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Abstract A series of novel diphenyl(piperidin-4-yl)methanol derivatives 10(a–n) were synthesized and characterized by ^{1}H NMR, LC/MS, FTIR, and elemental analyses. All the synthesized compounds were evaluated for cell proliferation by MTT assay. The antiproliferative effects of the synthesized compounds were tested against viable human skin fibroblast cell line and carcinoma cell lines, namely HeLa cells, HT-29 cells, MCF-7 cells, and HepG-2 cells in comparing the positive and negative control. Among the synthesized compounds, (10b) and (10g) have been identified as potent antiproliferative agents.

Keywords Diphenyl(pyridin-4-yl)methanol · Sulfonyl chloride · MTT assay · Antiproliferative activity · Cancer therapy · Cell proliferation

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. Cancer cells differ from their normal cells in a number of biochemical processes, particularly during the control of cell growth and division. Antiproliferative and cytotoxic drugs play a major role in cancer therapy, whether used alone or in combination with other treatment modalities, such as surgery, radiation, and biological therapy. During the past 50 years, the mass screening of synthetic derivatives or natural products has led to the discovery of the currently utilized anticancer drugs. In the field of chemotherapeutic drugs, the search

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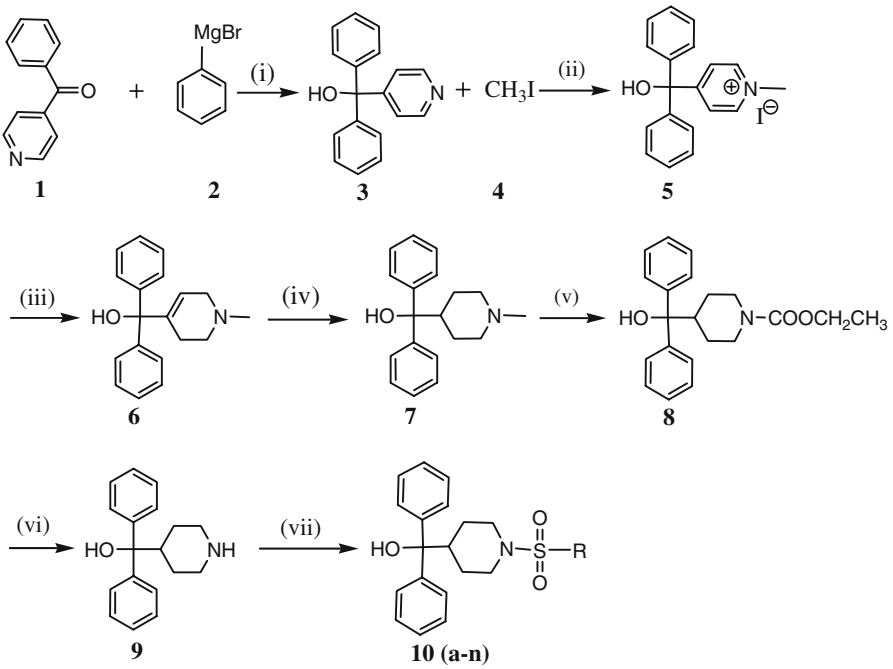
for new, more active, more selective, and less toxic compounds is still very intense, and new promising anticancer approaches are being tested (Sawyers, 2004; Li and Xu, 2005). Currently, combined anticancer therapies or multiacting drugs are clinically preferred to traditional cytotoxic treatment, with the goal of overcoming resistance and toxicity drawbacks. These events often prevent successful treatment and are responsible for reduced survival times (Jimeno and Hidalgo 2006; Mencher and Wang, 2005).

The accentuated interest in the piperidine class of opiate analgesics continues to be expressed in the field of pharmaceuticals, and biological properties of these agents have been the subject of ongoing investigations (Colapret *et al.*, 1989). The importance of nitrogen heterocycles, especially piperidine type, as subunits of bioactive molecules stimulates the chemist for the development of new synthetic methods (Cordell, 2000). During the last decade, combinatorial chemistry has represented the most promising approach to allow entry to a great number of biological targets arising from molecular biology and genomic studies (Ganesan, 2002). The piperidine scaffold is wide-ranging in its therapeutic uses and is ubiquitously found in drugs. It is a key structural component of successful Parkinson's drug (Klockgether *et al.*, 1996) and displays antipsychotic (Stephen *et al.*, 2002), metabolic (Lihu *et al.*, 1998), antimicrobial (Baikenova *et al.*, 2004; Anna *et al.*, 2002), antidepressants (Amat *et al.*, 1996), antimalarial (Maniyan *et al.*, 2006), and anticonvulsant activity (Reddy *et al.*, 1997; Matthew *et al.*, 1998). Recently, a class of sulfonamides has been used for the treatment of diseases arising from abnormal cell growth and proliferation (Medina *et al.*, 1998; Shan *et al.*, 1999). Based on the diverse biological activities of the piperidine sulfonamides and a recent report from our laboratory (Anil Kumar *et al.*, 2007a; Anil Kumar *et al.*, 2007b; Ananda Kumar *et al.*, 2007; Priya *et al.*, 2007), we present the antiproliferative activity of the synthesized compounds against four human cancer cell lines.

Chemistry

Synthesis of the target key intermediate (9) as outlined in Scheme 1 was accomplished in high yield. Compound diphenyl(pyridin-4-yl)methanol (3) was prepared from 4-benzoylpyridine by Grignard reaction (Dhananjay *et al.*, 1993). In the second step *N*-methylation of compound (3), 4-(Hydroxy-diphenyl-methyl)-1-methyl-pyridinium iodide (5) of its salt was obtained. Sodium borohydride reduction of compound (5) yielded (1,2,3,6-tetrahydro-1-methylpyridin-4-yl)diphenylmethanol (6) (Dhananjay *et al.*, 1993), which on catalytic transfer hydrogenation (Ra–Ni/Cyclohexanol) gave (1-methyl piperidin-4-yl)diphenylmethanol (7). In the fifth step *N*-demethylation of (7) with ethylchlorformate (Abdel-Menem and Portoghese, 1972) yielded the ethyl 4-(hydroxydiphenylmethyl)piperidine-1-carboxylate (8), which on hydrolysis gave diphenyl(piperidin-4-yl)methanol (9). Compound (9) thus prepared is used for the synthesis of its derivatives. Although the method involves several stages, the reactions can be conveniently performed and may be scaled up. The method avoids the use of high pressure. Diphenyl(piperidin-4-yl)methanol derivatives 10(a–n) were



Scheme 1 *Reagents and Conditions:* (i). Dry THF, r.t, 3 hours. (ii). acetone, r.t, 3–4 hours. (iii). $NaBH_4$, MeOH, r.t, 4 hours. (iv). Ra–Ni/Cyclohexanol, reflux, 2 hours (v). $CHCl_3$, K_2CO_3, C_2H_5COOCl, r.t, overnight. (vi). C_2H_5OH, KOH, reflux under N_2, 24 hours. (vii). R–SO_2–Cl, TEA, MDC, 4 hours

prepared by the nucleophilic substitution reaction of diphenyl(piperidin-4-yl)methanol (9) with different substituted aromatic, aliphatic and heterocyclic sulfonyl chlorides (R–SO_2–Cl) were performed in presence of triethylamine and dichloromethane as solvent with a good yield ranging from 82–91%. The absence of –N–H proton peak in synthesized derivatives 10(a–n) in proton NMR confirms our products. It also is confirmed by IR data, for sulfonamide series 10(a–n), 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}. All the compounds were characterized by ^{1}H-NMR spectroscopy and LCMS. The yield, physical data, and chemical structures of the synthesized compounds are shown in Table 1.

Results and discussion

New classes of sulfonamides have been used for the treatment of diseases arising from abnormal cell growth and proliferation (Medina *et al.*, 1998; Shan *et al.*, 1999). Most cancers are characterized by uncontrolled cell proliferation, lack of cell differentiation, and loss of contact inhibition, which confers upon the tumor cell a capability to invade local tissues and metastasize. The novel synthesized diphenyl(piperidin-4-yl)methanol analogues 10(a–n) were evaluated for their cell

Table 1 Chemical structure, yield, and melting point of the synthesized compounds 10(a–n)

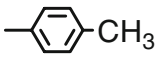
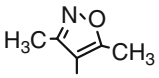
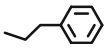
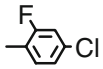
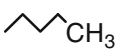
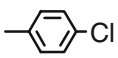
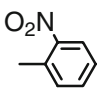
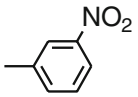
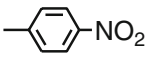
Compound	R	Yield (%)	M. P (°C)
10a		90	165–167
10b		89	185–187
10c		85	237–239
10d		90	172–174
10e		89	142–144
10f		88	135–137
10g		87	215–217
10h		91	236–238
10i		83	205–207
10j		88	275–277
10k		83	180–182
10l		87	194–196

Table 1 continued

Compound	R	Yield (%)	M. P (°C)
10m		82	–
10n		86	161–163

antiproliferative activity against human MCF-7 breast carcinoma cell line, HT-29 colon carcinoma cell line, HeLa cervix carcinoma cell line, and HepG-2 hepatocellular carcinoma cell line. All the synthesized compounds were tested for their cytotoxicity against normal skin fibroblast cells. In vitro cytotoxicity was evaluated after 24 hours of cell treatment performing MTT assay. The percentage of cells survived in all the test compounds are listed in Table 2.

Among the tested compounds, the minimum 38.68% cell survival activity was shown by (10b) against MCF-7 carcinoma cell line. Compound (10j) exhibited 39.11% antiproliferation against HepG-2 carcinoma cell line. Similarly, compound (10g) showed 39.22% antiproliferation against HeLa carcinoma cell line. Compound (10g) showed good potency for all the carcinoma cell lines and exhibits 44.16%, 46.32%, 47.17%, and 39.22% cell survival activity against MCF-7, HepG-

Table 2 Evaluation of cytotoxicity toward carcinoma cells for the synthesized compounds 10(a–n)

Compound	NF-103	MCF-7	HepG-2	HT-29	HeLa
10a	65.63	70.24	62.67	45.11	71.21
10b	53.82	38.68	52.18	73.61	39.89
10c	63.98	60.54	60.85	74.70	64.36
10d	63.0	65.80	68.30	73.88	68.29
10e	59.98	68.65	62.56	74.21	73.12
10f	59.98	70.46	64.66	77.63	78.50
10g	51.76	44.16	46.32	47.17	39.22
10h	NT	NT	NT	NT	NT
10i	53.53	66.02	59.13	45.16	56.06
10j	59.81	69.69	39.11	76.49	70.14
10k	52.96	61.36	52.18	69.59	49.71
10l	63.41	73.75	57.20	78.55	64.30
10m	57.36	63.39	64.16	74.97	70.31
10n	53.59	69.75	68.74	70.84	54.04

NT = not tested

2, HT-29, and HeLa cell lines, respectively. Similarly, compound (10b) showed potent antiproliferative activity and exhibits 38.68% and 39.89% cell survival activity against MCF-7 and HeLa cell lines, respectively. Compounds (10a), (10i), and (10k) showed moderate activity against HT-29, HT-29, and HeLa cell lines and exhibits 45.11%, 45.16%, and 49.71% cell survival, respectively. Moreover, the other tested compounds 10(c–f) and 10(l–n) showed very little inhibitory activity. The extent of inhibition of carcinoma cell lines by 10(a–n) are schematically presented in Figs. 1, 2, 3, 4, 5.

The inhibition by compound (10g) (39.22% cell survival) could be attributed to electron donating fluorine and chlorine atoms present on the substituted benzene at ortho and para position. The compound (10b) exhibited 38.68% cell survival, which may be due to the presence of electron-donating dimethyl groups present on the substituted isoxazole moiety. The hydrophobicity of chloro fluorophenyl, dimethyl, and rigidity of the isoxazole ring is essential for a more efficient binding to the active site of the enzyme.

Initial structure–activity relationship (SAR) studies were focused on the effects of the substitutions on the aromatic ring. Compounds with electron-donating substituents (10a, 10b, 10d, 10e, and 10g) showed enhanced activity compared with

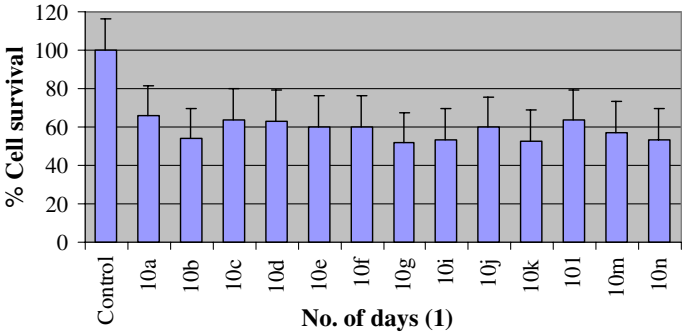
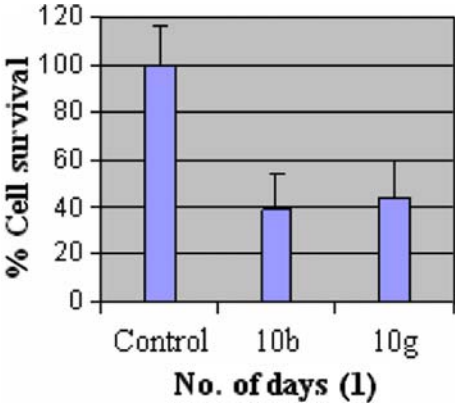


Fig. 1 MTT assay for human normal skin cell line

Fig. 2 MTT assay for MCF-7 cell line



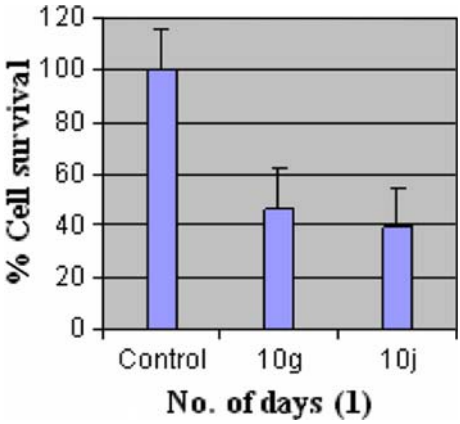


Fig. 3 MTT assay for HepG-2 cell line

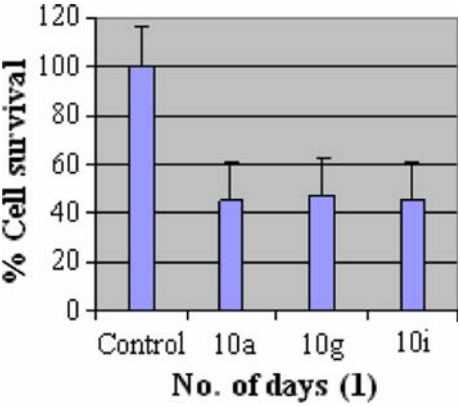


Fig. 4 MTT assay for HT-29 cell line

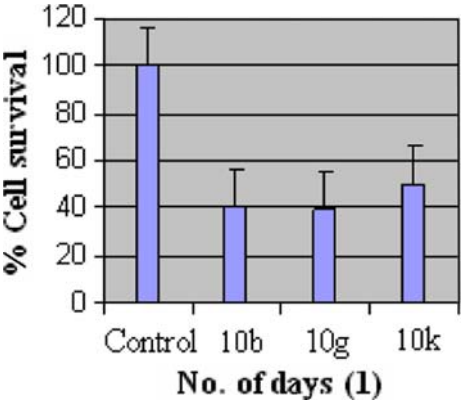


Fig. 5 MTT assay for HeLa cell line

those bearing electron-withdrawing substituents 10(l–n). As shown in Table 2, chloro fluorophenyl (10g) and dimethyisoxazole ring (10b) derivatives showed a pronounced effect on all the cell lines. These results suggest that the electronic

features of the various substituents have an effect on how easily the drug can interact with bioactive molecules. We synthesized compounds (10c), (10f), (10h), and (10i) to understand the effect of alkyl chain substitution in compound (9). It was observed that as the length of alkyl chain increases, decrease in the antiproliferative activity was noted. Therefore, length of carbon side chain has shown no effect on the increase in the antiproliferative activity. On the other hand, replacement of the alkyl side chain by aromatic and heterocyclic substitution substantially increased the antiproliferative activity.

Conclusions

Based on the experimental results, it seems that the introduction of an aryl sulfonyl and alkyl moieties on the diphenyl(piperidin-4-yl)methanol system is of potential interest to obtain novel antiproliferative compounds. This work allowed us to identify a few active molecules able to inhibit in vitro growth of human cancer cell lines. These compounds displayed a relatively weak cytotoxicity. All of these findings support the need for further investigations to clarify the features underlying the antiproliferative activities of these new diphenyl(piperidin-4-yl)methanol derivatives. This work is under progress.

Experimental

Melting points were determined using Veego model VMP-III melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM-400, and chemical shifts are expressed in parts per million (ppm, for δ) relative to tetra methyl silane as an internal standard and DMSO-d_6 as solvent. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHNS) analysis was 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. All of the reagents and chemicals were purchased from Sigma Aldrich Chemicals Pvt Ltd.

Synthesis of diphenyl(pyridin-4-yl)methanol (3)

To a solution of compound (1) (1.0 equiv) in dry THF add phenylmagnesium bromide (2) (1.0 equiv) and the mixture stirred for 2 hours at room temperature under N_2 atmosphere. The reaction was monitored by TLC, and after completion of the reaction, reaction mass was quench with water filtered through celite bed. The filtrate was extracted with ethyl acetate and organic layer was evaporated under reduced pressure and got the crude product. The product was purified by column chromatography.

Synthesis of *N*-methylation of compound (5)

The compound (3) (1.0 equiv) was taken in the tenfold volume of acetone; then methyl iodide (4.0 equiv) was added and the mixture was stirred for 3–4 hours. After completion of the reaction, the reaction was monitored by TLC, and the solvent was evaporated under reduced pressure. A yellow pyridinium salt (5) was obtained.

Synthesis of (1,2,3,6-tetrahydro-1-methylpyridin-4-yl)diphenylmethanol (6)

The quaternary salt (5) (15 g, 37 mmol) was dissolved in 60 ml of methanol and treated with sodium borohydride (4.19 g, 11 mmol) in small portions. Temperature was maintained at <20 °C during the addition. The reaction was monitored by TLC; after completion of the reaction, the solvent was evaporated under reduced pressure. The reaction mixture was diluted with water (150 ml), and the precipitated solid filtered, washed with water, and air dried. Recrystallization from chloroform:methanol (1:1) gave compound 6 (10.2 g, 95%), mp: 178–180 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.13 (m, 10H, Ar–H), 2.10 (t, 2H, –CH_2), 2.16 (s, 3H, N–CH_3), 2.4 (t, 2H, –CH_2), 2.86 (d, 2H, N–CH_2), 3.0 (bs, 1H, O–H), 5.2 (t, 1H, vinylic-H). IR (KBr, cm^{-1}): 3500 (–OH). Anal. calc. for $C_{19}H_{21}NO$ (in %): C-81.68, H-7.58, N-5.01. Found: C-81.65, H-7.54, N-4.96.

Synthesis of (1-methyl piperidin-4-yl)diphenylmethanol (7)

To a solution of (6) (10.0 g, 35 mmol) in cyclohexanol (75 ml) was added Raney Nickel (2.05 g, 3.5 mmol, previously washed with *t*-butanol) and the mixture stirred under reflux for 2 hours. The reaction mixture was cooled to room temperature and filtered. Cyclohexanol was concentrated under reduced pressure and completion of the reaction was monitored by TLC. The residue was extracted with chloroform (3 × 25 ml) and the combined chloroform extracts were dried over anhydrous sodium sulphate and recovered. Recrystallization from benzene-petether (3:15) gave compound (7) (7.1 g, 85%) as a crystalline solid. mp: 131–133 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7–7.5 (m, 10H, Ar–H), 3.14 (d, 2H, –CH_2), 2.62 (t, 2H, –CH_2), 2.38 (m, 1H, –CH), 2.25 (s, 1H, –OH), 2.13 (s, 3H, N–CH_3), 1.62 (d, 4H, –CH_2). IR (KBr, cm^{-1}): 3500 (–OH). Anal. calc. for $C_{19}H_{23}NO$ (in %): C-81.10, H-8.24, N-4.98. Found: C-81.08, H-8.23, N-4.96.

Synthesis of ethyl 4-(hydroxydiphenylmethyl)piperidine-1-carboxylate (8)

Compound (7) (6 g, 21.3 mmol) was dissolved in 50 ml of chloroform, and to this solution added K_2CO_3 (8.87 g, 63.96 mmol) and followed by ethyl chloroformate (5.7 g, 21.3 mmol). The mixture was stirred overnight at room temperature. The chloroform layer was washed successively with water and dilute HCl. Dried over anhydrous Na_2SO_4, the completion of the reaction was monitored by TLC and the mixture concentrated under reduced pressure gave compound (8) (5.1 g, 70%) as a crystalline solid. mp: 152–154 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7–7.5 (m, 10H,

Ar–H), 1.2 (t, 3H, $–OCH_2CH_3$), 1.5–2 (m, 5H, $–CH_2–CH–CH_2$), 2.4–3 (m, 4H, $–CH_2–N–CH_2$), 4.03 (q, 2H, $–OCH_2–CH_3$), 4.25 (bs, 1H, OH). IR (KBr, cm^{-1}): 3500 (–OH), 1680 (NCOOEt). Anal. calc. for $C_{21}H_{25}NO_3$ (in %): C-74.31, H-7.42, N-4.13. Found: C-74.27, H-7.40, N-4.11.

Synthesis of diphenyl(piperidin-4-yl)methanol (9)

Ethyl 4-(hydroxydiphenylmethyl)piperidine-1-carboxylate (8) (5.0 g, 14.7 mmol) was dissolved in 50 ml of ethanol, K_2CO_3 solution (30 ml, 50%) was added. The reaction mixture was refluxed under N_2 for 24 hours. The reaction mixture 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 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. Recrystallization from isopropanol-ether gave 9 (3 g, 75%). The presence of proton peak at 2.00 ppm in ^{1}H NMR spectrum and N–H stretching frequency at 3300 cm^{-1} in IR spectrum confirms the formation of the product. M.P: 160–162°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.48 (m, 4H, Ar–H), 7.30 (m, 4H, Ar–H), 7.18 (m, 2H, Ar–H), 3.1 (d, 2H, $–CH_2$), 2.68 (t, 2H, $–CH_2$), 2.4 (m, 1H, $–CH_2$), 2.2 (s, 1H, –OH), 2.0 (s, 1H, –NH), 1.5 (d, 4H, $–CH_2$). IR (KBr, cm^{-1}): 3500 (–OH), 3300 (NH). MS (ESI) *m/z*: 268.16 ($M + H^+$). Anal. calc. for $C_{18}H_{21}NO$ (in %): C-80.86, H-7.92, N-5.24. Found: C-80.82, H-7.88, N-7.87.

General procedure for the synthesis of diphenyl(sulphonylpiperidin-4-yl)methanol derivatives 10(a–n)

A solution of diphenyl(piperidin-4-yl)methanol (9) (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 minutes, then different sulfonyl chloride (1.0 eq) was added. The reaction mixture was allowed to room temperature under stirring for 5–6 hours. The reaction mixture 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.

Synthesis of diphenyl-[1-(toluene-4-sulfonyl)-piperidin-4-yl]-methanol (10a)

The general synthetic method described earlier afforded (10a); the product obtained was pale brown crystalline solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), *p*-toluenesulfonyl chloride (0.338 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.7 (m, 4H, Ar–H), 7.48 (m, 4H, Ar–H), 7.35 (m, 4H, Ar–H), 7.18 (t, 2H, Ar–H), 3.13 (d, 2H, $–CH_2$), 2.65 (t, 2H, $–CH_2$), 2.46 (m, 1H, –CH), 2.31 (s, 3H, $–CH_3$), 2.2 (s, 1H, –OH), 1.5 (d, 4H, $–CH_2$).

IR (KBr, cm^{-1}): 3500, 2856, 1350, 1276. MS (ESI) *m/z*: 422.17 ($M + H^+$). Anal. calcd. for $C_{25}H_{27}NO_3S$ (in %): C-71.23, H-6.46, N-3.32, S-7.61. Found C-71.20, H-6.40, N-3.28, S-7.58.

Synthesis of [1-(3,5-dimethyl-2,3-dihydro-isoxazole-4-sulfonyl)-piperidin-4-yl]-diphenyl-methanol (10b)

The general synthetic method described earlier afforded (10b); the product obtained was pale yellow crystalline solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), 3,5-dimethyl-2,3-dihydro-isoxazole-4-sulfonyl chloride (0.349 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^1H NMR (DMSO, 400 MHz) δ: 7.47 (m, 4H, Ar–H), 7.23 (m, 4H, Ar–H), 7.09 (t, 2H, Ar–H), 3.1 (d, 2H, $–CH_2$), 3.61 (d, 2H, $–CH_2$), 2.68 (t, 2H, $–CH_2$), 2.62 (s, 3H, $–CH_3$), 2.56 (s, 3H, $–CH_3$), 2.4 (m, 1H, –CH), 2.31 (s, 2H, $–CH_2$), 2.2 (s, 1H, –OH). IR (KBr, cm^{-1}): 3515, 1470, 1376, 1356, 1276. MS (ESI) *m/z*: 427.00 ($M + H^+$). Anal. calcd. for $C_{23}H_{26}N_2O_4S$ (in %): C-64.77, H-6.14, N-6.57, S-7.52. Found C-64.75, H-6.10, N-6.53, S-7.48.

Synthesis of (1-methanesulfonyl-piperidin-4-yl)-diphenyl-methanol (10c)

The general synthetic method described earlier afforded (10c); the product obtained was white amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), methane sulfonyl chloride (0.202 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^1H NMR (DMSO, 400 MHz) δ: 7.48 (m, 4H, Ar–H), 7.30 (m, 4H, Ar–H), 7.18 (m, 2H, Ar–H), 3.12 (d, 2H, $–CH_2$), 2.8 (s, 3H, $–CH_3$), 2.68 (t, 2H, $–CH_2$), 2.4 (m, 1H, –CH), 2.2 (s, 1H, –OH), 1.5 (d, 4H, $–CH_2$). IR (KBr, cm^{-1}): 3522, 1479, 1383, 1346, 1266. MS (ESI) *m/z*: 346.14 ($M + H^+$). Anal. calcd. for $C_{19}H_{23}NO_3S$ (in %): C-66.06, H-6.71, N-4.05, S-9.28. Found C-66.01, H-6.67, N-4.01, S-9.26.

Synthesis of (1-benzenesulfonyl-piperidin-4-yl)-diphenyl-methanol (10d)

The general synthetic method described earlier afforded (10d); the product obtained was white amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), benzenesulfonyl chloride (0.312 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^1H NMR (DMSO, 400 MHz) δ: 7.9 (d, 2H, Ar–H), 7.55 (m, 3H, Ar–H), 7.48 (m, 4H, Ar–H), 7.30 (m, 4H, Ar–H), 7.18 (m, 2H, Ar–H), 3.1 (d, 2H, $–CH_2$), 2.68 (t, 2H, $–CH_2$), 2.4 (m, 1H, –CH), 2.2 (s, 1H, –OH), 1.5 (d, 4H, $–CH_2$). IR (KBr, cm^{-1}): 3493, 1361, 1263. MS (ESI) *m/z*: 408.16 ($M + H^+$). Anal. calcd. for $C_{24}H_{25}NO_3S$ (in %): C-70.73, H-6.18, N-3.44, S-7.87. Found C-70.71, H-6.15, N-3.40, S-7.84.

Synthesis of diphenyl-[1-(4-propyl-benzenesulfonyl)-piperidin-4-yl]-methanol (10e)

The general synthetic method described earlier afforded (10e); the product obtained was white amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g,

1.77 mmol), 4-propyl-benzenesulfonyl chloride (0.387 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.6 (d, 2H, Ar–H), 7.5 (d, 2H, Ar–H), 7.48 (m, 4H, Ar–H), 7.30 (m, 4H, Ar–H), 7.18 (m, 2H, Ar–H), 3.1 (d, 2H, –CH_2), 2.68 (t, 2H, –CH_2), 2.4 (m, 1H, –CH), 2.2 (s, 1H, –OH), 2.15 (t, 2H, –CH_2), 1.64 (m, 2H, –CH_2), 1.5 (d, 4H, –CH_2), 0.92 (t, 3H, –CH_3). IR (KBr, cm^{-1}): 3532, 1479, 1375, 1356, 1281. MS (ESI) *m/z*: 450.2 (M + H^+). Anal. calcd. for $C_{27}H_{31}NO_3S$ (in %): C-72.13, H-6.95, N-3.14, S-7.13. Found C-72.09, H-6.90, N-3.10, S-7.09.

Synthesis of diphenyl-[1-(propane-2-sulfonyl)-piperidin-4-yl]-methanol (10f)

The general synthetic method described earlier afforded (10f); the product obtained was pale brown amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), propane-2-sulfonyl chloride (0.252 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.42 (m, 4H, Ar–H), 7.35 (m, 4H, Ar–H), 7.22 (m, 2H, Ar–H), 3.4 (m, 1H, –CH), 3.13 (d, 2H, –CH_2), 2.62 (t, 2H, –CH_2), 2.43 (m, 1H, –CH), 2.26 (s, 1H, –OH), 1.52 (d, 4H, –CH_2), 1.4 (s, 6H, –$(CH_3)_2$). IR (KBr, cm^{-1}): 3528, 1466, 1380, 1351, 1275. MS (ESI) *m/z*: 374.17 (M + H^+). Anal. calcd. for $C_{21}H_{27}NO_3S$ (in %): C-67.53, H-7.29, N-3.75, S-8.58. Found C-67.49, H-7.25, N-3.70, S-8.55.

Synthesis of [1-(4-chloro-2-fluoro-benzenesulfonyl)-piperidin-4-yl]-diphenyl-methanol (10g)

The general synthetic method described earlier afforded (10g); the product obtained was white crystalline solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), 4-chloro-2-fluoro-benzenesulfonyl chloride (0.405 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.57 (m, 4H, Ar–H), 7.27 (m, 6H, Ar–H), 7.12 (t, 2H, Ar–H), 7.10 (d, 1H, Ar–H), 3.32 (m, 4H, –CH_2), 2.76 (m, 4H, –CH_2), 2.43 (m, 1H, –CH), 2.2 (s, 1H, –OH). IR (KBr, cm^{-1}): 3505, 1359, 1040, 1263, 712. MS (ESI) *m/z*: 460.11 (M + H^+). Anal. calcd. for $C_{24}H_{23}ClFNO_3S$ (in %): C-62.27, H-5.04, N-3.05, S-6.97. Found C-62.22, H-5.01, N-3.01, S-6.93.

Synthesis of [1-(butane-1-sulfonyl)-piperidin-4-yl]-diphenyl-methanol (10h)

The general synthetic method described earlier afforded (10h); the product obtained was white amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), butane-1-sulfonyl chloride (0.277 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.45 (m, 4H, Ar–H), 7.39 (m, 4H, Ar–H), 7.23 (m, 2H, Ar–H), 3.56 (t, 2H, –CH_2), 3.07 (d, 2H, –CH_2), 2.61 (t, 2H, –CH_2), 2.46 (m, 1H, –CH), 2.22 (s, 1H, –OH), 1.85 (m, 2H, –CH_2), 1.58 (d, 4H, –CH_2), 1.3 (t, 2H, –CH_2), 0.94 (t, 3H, –CH_3). IR (KBr, cm^{-1}): 3528, 1466, 1380, 1351, 1275. MS (ESI) *m/z*: 388.19 (M + H^+). Anal. calcd. for $C_{22}H_{29}NO_3S$ (in %): C-68.18, H-7.54, N-3.61, S-8.27. Found C-68.15, H-7.50, N-3.58, S-8.22.

Synthesis of (1-chloromethanesulfonyl-piperidin-4-yl)-diphenyl-methanol (10i)

The general synthetic method described earlier afforded (10i); the product obtained was pale brown solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), chloro-methane sulfonyl chloride (0.263 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.40 (m, 4H, Ar–H), 7.38 (m, 4H, Ar–H), 7.23 (m, 2H, Ar–H), 5.3 (s, 2H, –CH_2), 3.09 (d, 2H, –CH_2), 2.72 (t, 2H, –CH_2), 2.39 (m, 1H, –CH), 2.2 (s, 1H, –OH), 1.58 (d, 4H, –CH_2). IR (KBr, cm^{-1}): 3500, 1450, 1386, 1360, 1286, 728. MS (ESI) *m/z*: 380.10 (M + H$^+$). Anal. calcd. for $C_{19}H_{22}ClNO_3S$ (in %): C-60.07, H-5.84, N-3.69, S-8.44. Found C-60.02, H-5.80, N-3.65, S-8.39.

Synthesis of diphenyl-(1-phenylmethanesulfonyl-piperidin-4-yl)-methanol (10j)

The general synthetic method described earlier afforded (10j); the product obtained was white amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), phenyl-methanesulfonyl chloride (0.337 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.52 (m, 4H, Ar–H), 7.33 (m, 4H, Ar–H), 7.25 (m, 2H, Ar–H), 7.14 (m, 5H, Ar–H), 4.72 (s, 2H, –CH_2), 3.16 (d, 2H, –CH_2), 2.63 (t, 2H, –CH_2), 2.44 (m, 1H, –CH), 2.2 (s, 1H, –OH), 1.56 (d, 4H, –CH_2). IR (KBr, cm^{-1}): 3509, 1445, 1377, 1369, 1286. MS (ESI) *m/z*: 422.17 (M + H$^+$). Anal. calcd. for $C_{25}H_{27}NO_3S$ (in %): C-71.23, H-6.46, N-3.23, S-7.61. Found C-71.19, H-6.41, N-3.20, S-7.58.

Synthesis of [1-(4-chloro-benzenesulfonyl)-piperidin-4-yl]-diphenyl-methanol (10k)

The general synthetic method described earlier afforded (10k); the product obtained was white crystalline solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), 4-chloro-benzenesulfonyl chloride (0.373 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.6 (m, 4H, Ar–H), 7.42 (m, 4H, Ar–H), 7.39 (m, 4H, Ar–H), 7.21 (m, 2H, Ar–H), 3.14 (d, 2H, –CH_2), 2.63 (t, 2H, –CH_2), 2.42 (m, 1H, –CH), 2.2 (s, 1H, –OH), 1.55 (d, 4H, –CH_2). IR (KBr, cm^{-1}): 3500, 1360, 1286, 722. MS (ESI) *m/z*: 442.12 (M + H$^+$). Anal. calcd. for $C_{23}H_{24}ClNO_3S$ (in %): C-65.22, H-5.47, N-3.17, S-7.26. Found C-65.18, H-5.42, N-3.13, S-7.21.

Synthesis of [1-(2-nitro-benzenesulfonyl)-piperidin-4-yl]-diphenyl-methanol (10l)

The general synthetic method described earlier afforded (10l); the product obtained was white crystalline solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), 2-nitrobenzene-1-sulfonyl chloride (0.391 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.94 (d, 1H, Ar–H), 7.6–7.7 (m, 3H, Ar–H), 7.42 (m, 4H, Ar–H), 7.29 (m, 4H, Ar–H), 7.20 (t, 2H, Ar–H), 2.77 (m, 4H, –CH_2), 2.48 (m, 1H, –CH_2), 1.65–1.5 (m, 4H, –CH_2), 2.18

(s, 1H, –OH). IR (KBr, cm^{-1}): 3518, 1526, 1353, 1330, 1286. MS (ESI) *m/z*: 453.14 ($M + H^+$). Anal. calcd. for $C_{24}H_{24}N_2O_5S$ (in %): C-63.70, H-5.35, N-6.19, S-7.09. Found C-63.64, H-5.31, N-6.15, S-7.05.

Synthesis of [1-(3-nitro-benzenesulfonyl)-piperidin-4-yl]-diphenyl-methanol (10m)

The general synthetic method described earlier afforded (10m); the product obtained was pale brown amorphous solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), 3–nitrobenzene-1-sulfonyl chloride (0.391 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.91 (d, 1H, Ar–H), 7.6–7.7 (m, 3H, Ar–H), 7.48 (m, 4H, Ar–H), 7.21 (m, 4H, Ar–H), 7.23 (t, 2H, Ar–H), 2.70 (m, 4H, $–CH_2$), 2.42 (m, 1H, $–CH_2$), 1.65–1.5 (m, 4H, $–CH_2$), 2.11 (s, 1H, –OH). IR (KBr, cm^{-1}): 3511, 1516, 1356, 1339, 1276. MS (ESI) *m/z*: 453.14 ($M + H^+$). Anal. calcd. for $C_{24}H_{24}N_2O_5S$ (in %): C-63.70, H-5.35, N-6.19, S-7.09. Found C-63.64, H-5.31, N-6.15, S-7.05.

Synthesis of [1-(4-nitro-benzenesulfonyl)-piperidin-4-yl]-diphenyl-methanol (10n)

The general synthetic method described earlier afforded (10n); the product obtained was pale yellow solid from diphenyl(piperidin-4-yl)methanol (9) (0.5 g, 1.77 mmol), 4-nitrobenzene-1-sulfonyl chloride (0.391 g, 1.77 mmol), and triethylamine (0.537 g, 5.31 mmol). ^{1}H NMR (DMSO, 400 MHz) δ: 7.86 (d, 1H, Ar–H), 7.6–7.7 (m, 3H, Ar–H), 7.39 (m, 4H, Ar–H), 7.28 (m, 4H, Ar–H), 7.31 (t, 2H, Ar–H), 2.76 (m, 4H, $–CH_2$), 2.40 (m, 1H, $–CH_2$), 1.65–1.5 (m, 4H, $–CH_2$), 2.15 (s, 1H, –OH). IR (KBr, cm^{-1}): 3500, 1521, 1359, 1342, 1296. MS (ESI) *m/z*: 453.14 ($M + H^+$). Anal. calcd. for $C_{24}H_{24}N_2O_5S$ (in %): C-63.70, H-5.35, N-6.19, S-7.09. Found C-63.64, H-5.31, N-6.15, S-7.05.

Biology

We synthesized novel diphenyl(piperidin-4-yl)methanol derivatives 10(a–n) and evaluated for their efficacy as antiproliferative agents on the growth of the five different cell lines in a cell toxicity assay for percentage cell survival at 100 μM. Percentage cell survival for compounds against NF-103, MCF-7, HepG-2, HT-29, and HeLa cell proliferation at the concentration of 100 μM for 24 hours are shown in Table 2.

Stock solutions (100 μM) of test compounds were first prepared in dimethylsulfoxide (DMSO) and stored at −20 °C. On the day of experiment, these concentrated solutions were diluted to the desired final concentrations immediately before addition to cell culture wells. The final DMSO concentration was 0.1% in each well and showed no interference with the biological activities tested.

Cell culture and in vitro cell viability assay—MTT assay

NF-103, HeLa, HepG-2, and MCF-7 cells were cultured in DMEM with 10% FCS. HT-29 cells were cultured in McCoy'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), HeLa cells, HepG-2 cells, HT-29 cells, and MCF-7 cells can be assayed at different time points besides 24 hours. The positive control used was wells with DMEM (10% FBS added; 1% v/v antibiotic added) added, whereas the negative control used was wells with DMEM (1% v/v antibiotic added; no FBS added). Cell cultures were maintained in the Department of Biological Sciences, National University of Singapore, Singapore. The absorbance was measured at 570 nm with a microtiter plate reader.

The potential effects on cell viability were investigated by using the MTT assay (Scudiero *et al.*, 1988) [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] as an indicator of metabolically active cells (Ananda Kumar *et al.*, 2009). 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. Mean ± standard deviation values were used to estimate cell viability.

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