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Synthesis and *In Vitro* Antiproliferative Activity of 2-Methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one Derivatives against Human Cancer Cell Lines

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A series of new 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one derivatives **6a-j** were synthesized by a nucleophilic substitution reaction of 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one with various sulfonyl chlorides. The compounds were characterized by different spectral studies. All the compounds were evaluated for their antiproliferative effect using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method against four human cancer cell lines (K562, Colo-205, MDA-MB 231, IMR-32) for the time period of 24 h. Among the series, compounds **6d**, **6e** and **6i** showed good activity on all cell lines except K562, whereas the other compounds in the series exhibited moderate activity. Compound **6d** could be a potential anticancer agent and therefore deserves further research.

Key words: Piperazine, Sulfonyl chlorides, MTT assay, Antiproliferative activity, Human cancer cell lines

INTRODUCTION

Cancer is becoming the most significant health hazard around the world. Development of resistance against the existing anticancer drugs keeps the research window open for the search of newer anticancer molecules. Cancer is a serious pathology and a substantial number of new antineoplastic agents have been discovered. Considerable insight has been gained into the mechanisms by which many of these compounds affect cellular growth and this knowledge has been used to design new chemotherapic drugs (Verweij and De Jonge, 2000; Verdecchia et al., 2001). 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 drawbacks often prevent successful treatment and are responsible for reduced survival times (Mencher and Wang, 2005; Jimeno and Hidalgo, 2006).

Piperazines are currently the most important building blocks in drug discovery with a high number of positive hits encountered in biological screens of this heterocycle and its congeners. Piperazine and its analogues are important pharmacophore that can be found in biologically active compounds across a number of different therapeutic areas such as antifungal (Upadhayaya et al., 2004), antibacterial, antimalarial, antipsychotic agents (Chaudhary et al., 2006), and antitumour activity against colon, prostate, breast, lung, and leukemia tumors (Hulme and Cherrier, 1999). The new class of sulfonamides (Reddy et al., 2004) has been used in the treatment of diseases arising from abnormal cell growth and proliferation. Most cancers are characterized by uncontrolled cell proliferation, lack of cell differentiation and loss of contact inhibition, which all confer upon the tumor cell a capability of invading local tissues and metastasizing. Recently,

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a series of arylsulfonanilides has been reported as potent inhibitors of various cancer cells at very low concentrations (Rubenstein et al., 2001). 3-(2-Chloroethyl)-2-methyl-pyrido[1,2-a]pyrimidin-4-one (**4**) is an intermediate in the preparation of risperidone. Risperidone contains the functional groups of benzisoxazole and piperidine as part of its molecular structure. It is a typical antipsychotic agent chemically classified as a benzisoxazole derivative with serotonin-5-HT2 and dopamine-D2 antagonist activity. In connection with such studies, the synthesis and antiproliferative activity of novel 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido [1,2-a]pyrimidin-4-one derivatives **6a-j** against human cancer cell lines have been reported in this paper.

MATERIALS AND METHODS

All solvents and reagents were purchased from Sigma Aldrich Chemicals. Melting points were determined on an electrically heated VMP-III melting point apparatus. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 elemental analyser. The FT-IR spectra were recorded using KBr discs on a Jasco FT-IR 4100 infrared spectrophotometer. The ^{1}H-NMR spectra were recorded using a Bruker DRX 400 spectrometer at 400 MHz with tetramethylsilane as the internal standard. Mass spectral data were obtained by LC/MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates.

Synthesis of 3-(1-(pyridin-2-ylamino)ethylidene) dihydrofuran-2(3H)-one (3)

The mixture of 2-aminopyridines **1** (10 mmol) and 3-acetyldihydrofuran-2(3H)-one **2** (10 mmol) was refluxed in toluene (30 mL) for 12 h in the presence of a catalytic amount of PTSA (0.02 g). The water separator was attached between the reaction flask and the water condenser. The separation of an equivalent amount of water indicates the completion of reaction. The solid obtained after cooling the reaction mixture was filtered and washed with toluene and then recrystallized in ethanol. Yield 1.69 g (83%); m.p. 87-88ºC; FT-IR (KBr, cm^{-1}) ν: 3300 (N-H), 1650 (C=O), 1150 (C-N); ^{1}H-NMR (DMSO-d_6, 400 Hz): δ 10.5 (bs, 1H, NH), 8.75 (d, 1H, CH), 8.25 (dd, 1H, CH), 6.83 (d, 1H, CH), 6.75 (dd, 1H, CH), 4.31 (t, 2H, OCH_2), 2.92 (t, 2H, CH_2), 2.50 (s, 3H, CH_3).

Synthesis of 3-(2-chloroethyl)-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (4)

Compound **3** (10 mmol) was refluxed in phosphorus oxychloride (20 mL) for 1 h (TLC check). After completion of the reaction, the excess phosphorus oxychloride was removed under reduced pressure. The residue obtained was stirred in ice-cold water (100 mL) for 30 min, then neutralized with solid sodium carbonate and was further stirred overnight. The solid precipitated was filtered and washed with water and recrystallized from ethanol. Yield 1.60 g (72%); m.p. 140-142ºC; FT-IR (KBr) ν: 1654 (C=O), 1150 (C-N), 722 (C-Cl); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 9.07 (d, 1H, CH), 7.78 (t, 1H, CH), 7.69 (d, 1H, CH), 7.15 (t, 1H, CH), 3.88 (t, 2H, CH_2Cl), 3.23 (t, 2H, CH_2), 2.55 (s, 3H, CH_3).

Synthesis of 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one (5)

A solution of 3-(2-chloroethyl)-2-methyl-4H-pyrido[1, 2-a]pyrimidin-4-one (**4**) (5.0 g, 22.45 mmol) and piperazine (2.13 g, 24.72 mmol) in DMF was prepared and K_2CO_3 (9.31 g, 67.36 mmol) was added to the reaction mixture. The reaction mixture was stirred for 6 h at room temperature. The progress of the reaction was monitored by TLC. Upon completion of the reaction, water was added and the reaction mixture was filtered and was finally washed with ether and dried under vacuum. The obtained compound was white amorphous solid with 80% yield. FT-IR (KBr, cm^{-1}) ν: 3365 (N-H), 1651 (C=O), 1160 (C-N); ^{1}H-NMR (DMSO-d_6): δ 8.81 (d, 2H, CH, J = 14.4 Hz), 7.67 (t, 1H, CH, J = 9.2 Hz), 7.51 (d, 1H, CH, J = 11.2 Hz), 7.13 (t, 1H, CH, J = 9.0 Hz), 3.38-3.04 (m, 8H), 2.54 (s, 3H, CH_3), 2.53 (t, 2H, CH_2), 2.44 (t, 2H, CH_2); Anal. Calcd. for $C_{15}H_{20}N_4O$ (%): C, 66.15; H, 7.40; N, 20.57. Found: C, 66.11; H, 7.32; N, 20.47.

General procedure for the synthesis of 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a] pyrimidin-4-one derivatives 6a-j

A solution of *2*-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one (**5**) (1.0 eq) in dry dichloromethane was prepared and cooled to 0-5ºC in an ice bath. Triethylamine (3.0 eq) was added to the cold reaction mixture and stirred for 10 min, then various sulfonyl chlorides (1.0 eq) were added and the reaction mixture was stirred at room temperature for 8 h. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and the residue was mixed in water and extracted with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution and the organic layer was finally washed with water and dried with anhydrous sodium sulphate. The solvent was evaporated to obtain the 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-(4-Benzenesulfonyl-piperazin-1-yl)-ethyl]-2-methyl-pyrido[1,2-a]pyrimidin-4-one (6a)
FT-IR (KBr, cm^{-1}) ν: 3022 (Ar-H), 1634 (C=O), 1157 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.76 (d, 1H, Ar-H, *J* = 6.81 Hz), 7.86 (t, 1H, Ar-H, *J* = 25.12 Hz), 7.75-7.63 (m, 5H, Ar-H), 7.55 (d, 1H, Ar-H, *J* = 8.88 Hz), 7.27 (t, 1H, Ar-H, *J* = 15.08 Hz), 4.13 (t, 2H, *J* = 13.32 Hz), 3.40-3.37 (m, 4H), 2.88 (t, 2H, *J* = 6.64 Hz), 2.85-2.82 (m, 4H), 2.36 (s, 3H, CH_3); MS: 412.2; Anal. Calcd. for $C_{21}H_{24}N_4O_3S$ (%): C, 61.14; H, 5.86; N, 13.58. Found: C, 61.21; H, 5.70; N, 13.75.

3-{2-[4-(4-Chloro-benzenesulfonyl)-piperazin-1-yl]-ethyl}-2-methyl-pyrido[1,2-a] pyrimidin-4-one (6b)
FT-IR (KBr, cm^{-1}) ν: 3034 (Ar-H), 1635 (C=O), 1157 (C-N), 720 (C-Cl); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.82 (d, 1H, Ar-H, *J* = 6.84 Hz), 7.89 (t, 1H, Ar-H, *J* = 16.92 Hz), 7.78 (m, 4H, Ar-H), 7.58 (d, 1H, Ar-H, *J* = 8.88 Hz), 7.30 (t, 1H, Ar-H, *J* = 14.80 Hz), 4.15 (t, 2H, *J* = 13.28 Hz), 3.42 (t, 2H, *J* = 9.48 Hz), 2.91-2.86 (m, 8H), 2.39 (s, 3H, CH_3); MS: 446.1; Anal. Calcd. for $C_{21}H_{23}ClN_4O_3S$ (%): C, 56.43; H, 5.19; N, 12.54. Found: C, 56.49; H, 5.31; N, 12.31.

2-Methyl-3-{2-[4-(2,4,6-trimethyl-benzenesulfonyl)-piperazin-1-yl]-ethyl}-pyrido[1,2-a]pyrimidin-4-one (6c)
FT-IR (KBr, cm^{-1}) ν: 3046 (Ar-H), 1638 (C=O), 1161 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.86 (d, 1H, Ar-H, *J* = 6.51 Hz), 7.91 (t, 1H, Ar-H, *J* = 18.85 Hz), 7.57 (d, 1H, Ar-H, *J* = 8.85 Hz), 7.26 (t, 1H, Ar-H, *J* = 12.0 Hz), 7.07 (s, 2H, Ar-H), 4.19 (t, 2H, *J* = 13.36 Hz), 3.35 (t, 2H, *J* = 10.41 Hz), 2.97-2.87 (m, 8H), 2.51 (s, 9H, $3CH_3$), 2.26 (s, 3H, CH_3); MS: 454.2; Anal. Calcd. for $C_{24}H_{30}N_4O_3S$ (%): C, 63.41; H, 6.65; N, 12.32. Found: C, 63.12; H, 6.71; N, 12.41.

2-Methyl-3-{2-[4-(4-trifluoromethyl-benzenesulfonyl)-piperazin-1-yl]-ethyl}-pyrido [1,2-a]pyrimidin-4-one (6d)
FT-IR (KBr, cm^{-1}) ν: 3039 (Ar-H), 1637 (C=O), 1238 (C-F), 1160 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.80 (d, 1H, Ar-H, *J* = 7.16 Hz), 8.04 (d, 2H, Ar-H, *J* = 7.96 Hz), 7.94 (d, 2H, Ar-H, *J* = 7.84 Hz), 7.87 (t, 1H, Ar-H, *J* = 15.48 Hz), 7.55 (d, 1H, Ar-H, *J* = 9.0 Hz), 7.28 (t, 1H, Ar-H, *J* = 13.56 Hz), 4.13 (t, 2H, Ar-H, *J* = 12.76 Hz), 3.40 (m, 4H), 3.32 (t, 2H, *J* = 21.20 Hz), 2.90-2.86 (m, 4H), 2.36 (s, 3H, CH_3); MS: 480.1; Anal. Calcd. for $C_{22}H_{23}F_3N_4O_3S$ (%): C, 54.99; H, 4.82; N, 11.86. Found: C, 54.79; H, 4.67; N, 11.68.

2-Methyl-3-{2-[4-(4-nitro-benzenesulfonyl)-piperazin-1-yl]-ethyl}-pyrido[1,2-a] pyrimidin-4-one (6e)
FT-IR (KBr, cm^{-1}) ν: 3081 (Ar-H), 1636 (C=O), 1547 (NO_2), 1168 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.81 (d, 1H, Ar-H, *J* = 6.54 Hz), 8.44 (d, 2H, Ar-H, *J* = 8.88 Hz), 7.99 (d, 2H, Ar-H, *J* = 8.88 Hz), 7.85 (t, 1H, Ar-H, *J* = 10.47 Hz), 7.55 (d, 1H, Ar-H, *J* = 8.85 Hz), 7.28 (t, 1H, Ar-H, *J* = 8.10 Hz), 4.13 (t, 2H, *J* = 13.41 Hz), 3.41 (t, 2H, *J* = 9.72 Hz), 2.92-2.84 (m, 8H), 2.36 (s, 3H, CH_3); MS: 457.1; Anal. Calcd. for $C_{21}H_{23}N_5O_5S$ (%): C, 51.13; H, 5.07; N, 15.31. Found: C, 51.24; H, 5.20; N, 15.45.

3-[2-(4-Methanesulfonyl-piperazin-1-yl)-ethyl]-2-methyl-pyrido[1,2-a]pyrimidin-4-one (6f)
FT-IR (KBr, cm^{-1}) ν: 1635 (C=O), 1141 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.90 (d, 1H, Ar-H, *J* = 7.08 Hz), 7.90 (t, 1H, Ar-H, *J* = 17.12 Hz), 7.60 (d, 1H, Ar-H, *J* = 8.88 Hz), 7.31 (t, 1H, Ar-H, *J* = 15.08 Hz), 4.22 (t, 2H, *J* = 13.52 Hz), 3.44-3.42 (m, 4H), 3.07-3.04 (m, 4H), 2.97 (t, 2H, *J* = 13.52 Hz), 2.87 (s, 3H, CH_3), 2.46 (s, 3H, CH_3); MS: 350.1; Anal. Calcd. for $C_{16}H_{22}N_4O_3S$ (%): C, 54.84; H, 6.33; N, 15.99. Found: C, 54.71; H, 6.53; N, 15.81.

2-Methyl-3-{2-[4-(toluene-4-sulfonyl)-piperazin-1-yl]-ethyl}-pyrido[1,2-a]pyrimidin-4-one (6g)
FT-IR (KBr, cm^{-1}) ν: 3090 (Ar-H), 1638 (C=O), 1161 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.80 (d, 1H, Ar-H, *J* = 7.0 Hz), 7.89 (t, 1H, Ar-H, *J* = 15.08 Hz), 7.59 (d, 1H, Ar-H, *J* = 8.08 Hz), 7.56 (d, 2H, Ar-H, *J* = 8.12 Hz), 7.47 (d, 2H, Ar-H, *J* = 5.68 Hz), 7.30 (t, 1H, Ar-H, *J* = 13.64 Hz), 4.13 (t, 2H, *J* = 13.28 Hz), 2.89 (t, 2H, *J* = 13.24 Hz), 2.81-2.78 (m, 8H), 2.49 (s, 3H, CH_3), 2.27 (s, 3H, CH_3); MS: 426.2; Anal. Calcd. for $C_{22}H_{26}N_4O_3S$ (%): C, 61.95; H, 6.14; N, 13.14. Found: C, 61.83; H, 6.32; N, 13.25.

3-{2-[4-(4-Methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl}-2-methyl-pyrido[1,2-a]pyrimidin-4-one (6h)
FT-IR (KBr, cm^{-1}) ν: 3064 (Ar-H), 1635 (C=O), 1151 (C-N), 1084 (C-O); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.82 (d, 1H, Ar-H, *J* = 6.96 Hz), 7.89 (t, 1H, Ar-H, *J* = 6.68 Hz), 7.67 (d, 2H, Ar-H, *J* = 14.64 Hz), 7.58 (d, 1H, Ar-H, *J* = 8.88 Hz), 7.30 (t, 1H, Ar-H, *J* = 7.0 Hz), 7.17 (d, 2H, Ar-H, *J* = 11.80 Hz), 4.15 (t, 2H, *J* = 13.32 Hz), 3.86-3.33 (m, 8H), 2.90 (t, 2H, *J* = 13.32 Hz), 2.51 (s, 3H, CH_3), 2.38 (s, 3H, OCH_3); MS: 442.2; Anal. Calcd. for $C_{22}H_{26}N_4O_4S$ (%): C, 59.71; H, 5.92; N, 12.66. Found: C, 59.67; H, 5.85; N, 12.43.

2-Methyl-3-{2-[4-(2-nitro-benzenesulfonyl)-piperazin-1-yl]-ethyl}-pyrido[1,2-a] pyrimidin-4-one (6i)
FT-IR (KBr, cm^{-1}) ν: 3059 (Ar-H), 1637 (C=O), 1528 (NO_2), 1156 (C-N); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.81 (d, 1H, Ar-H, J = 7.04 Hz), 8.01 (d, 2H, Ar-H, J = 20.32 Hz), 7.87 (d, 1H, Ar-H, J = 1.08 Hz), 7.86 (t, 2H, Ar-H, J = 14.20 Hz), 7.55 (d, 1H, Ar-H, J = 8.92 Hz), 7.25 (t, 1H, Ar-H, J = 4.04 Hz), 4.18 (t, 2H, J = 13.28 Hz), 3.42-3.14 (m, 8H), 2.92 (t, 2H, J = 13.28 Hz), 2.40 (s, 3H, CH_3); MS: 457.1; Anal. Calcd. for $C_{21}H_{23}N_5O_5S$ (%): C, 55.13; H, 5.07; N, 15.31. Found: C, 55.31; H, 5.13; N, 15.17.

3-{2-[4-(3-Methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl}-2-methyl-pyrido[1,2-a] pyrimidin-4-one (6j)
FT-IR (KBr, cm^{-1}) ν: 3054 (Ar-H), 1635 (C=O), 1151 (C-N), 1084 (C-O); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.81 (d, 1H, Ar-H, J = 7.04 Hz), 7.92 (t, 1H, Ar-H, J = 7.10 Hz), 7.87 (s, 1H, Ar-H), 7.58 (d, 1H, Ar-H, J = 9.11 Hz), 7.56 (d, 1H, Ar-H, J = 8.91 Hz), 7.31 (t, 1H, Ar-H, J = 7.10 Hz), 7.24 (t, 1H, Ar-H, J = 7.0 Hz), 7.11 (d, 1H, Ar-H, J = 7.10 Hz), 4.16 (t, 2H, J = 13.34 Hz), 3.78-3.31 (m, 8H), 2.90 (t, 2H, J = 13.32 Hz), 2.50 (s, 3H, CH_3), 2.38 (s, 3H, OCH_3); MS: 442.2; Anal. Calcd. for $C_{22}H_{26}N_4O_4S$ (%): C, 59.71; H, 5.92; N, 12.66. Found: C, 59.62; H, 5.84; N, 12.61.

Biology

Drugs and solutions

The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved (5 mg/mL) in a phosphate buffer saline (pH 7.2) and filtered before use. The RPMI 1640 cell culture medium, MTT and fetal bovine serum (FBS) were purchased from Merck chemicals.

Cell lines and culture conditions

Human metastatic breast cancer (MDA-MB 231), human chronic myeloid leukemia (K562), human colon carcinoma (Colo-205) and human neuroblastoma (IMR-32) cell lines were procured from the National Center for Cell Sciences in Pune, India. All cells were grown in RPMI-1640 supplemented with 10% heat inactivated FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. The cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C.

In vitro cell viability assay (MTT assay)

The potential effects on cell viability were investigated by using the MTT assay (Mosmann, 1983). The MTT assay is an indicator of metabolically active cells. A known number of MDA-MB 231, K562, Colo-205, and IMR-32 cells were transferred into 96 well plates in a volume of 200 µL of culture medium and incubated for 48 h before the addition of the test compound. Cells were then exposed to known concentrations of the compound to be tested (10 µM expressed as final concentration) for 24 h at 37°C. After drug exposure, the culture medium was removed and 20 ìL of MTT reagent (diluted in culture medium, 5 mg/mL) was added. After incubating for 4 h, the MTT/medium was removed and DMSO (100 µL) was added to each well and the plates were agitated for 1 min. Absorbance of the colored solution was measured on a multi-well plate reader (Victor3, Perkin Emler) 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 and the assay was performed in triplicate.

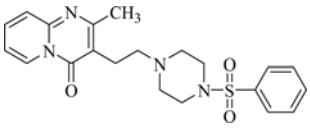
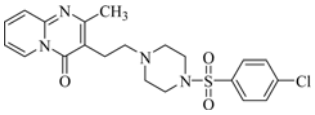
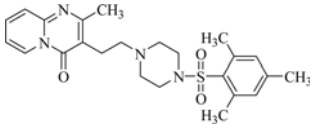
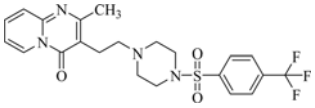
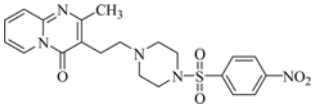
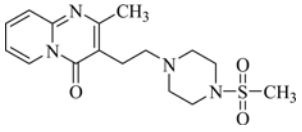
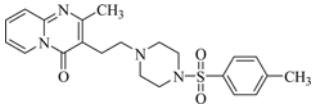
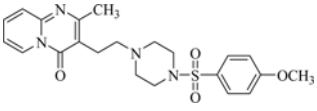
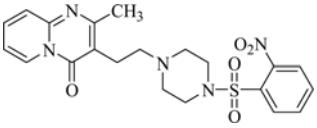
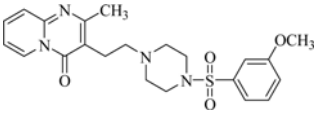
RESULTS AND DISCUSSION

In the present work, a series of ten new compounds were synthesized. The structure of the synthesized compounds was established on the basis of FT-IR, ^{1}H-NMR, and mass spectral data. The chemical structure and physical data of the novel compounds are given in Table I. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4%. 2-Methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a] pyrimidin-4-one derivatives **6a-j** were prepared by the method summarized in Scheme 1.

The FT-IR spectra of **6a-j** were recorded using KBr pellets in the range of 4000-400 cm^{-1}. The absorption bands at 3022-3090 cm^{-1} are assigned to the aromatic C-H stretch. The absorption bands at 1634-1638 cm^{-1} are due to the presence of C=O stretch. The strong bands at 1084 cm^{-1} are assigned to the C-O stretch in **6h** and **6j**. The absorption band at 3365 cm^{-1} is due to the N-H stretch in compound **5**. The absence of N-H absorption bands in **6a-j** confirmed the synthesized compounds. The strong bands at 1323-1327 cm^{-1} and 1156-1170 cm^{-1}, were attributed to SO_2 (asym. stretch) and SO_2 (sym. stretch), respectively (Hadi et al., 2009). A new band appeared at 1238 cm^{-1} (**6d**) corresponding to the C-F stretching frequency. The strong band at 720 cm^{-1} is assigned to the C-Cl stretch in **6b**. The absorptions at 1547 cm^{-1} and 1528 cm^{-1} corresponded to NO_2 (asym. stretch) in **6e** and **6i**, respectively.

The characteristic resonance peaks in ^{1}H-NMR for

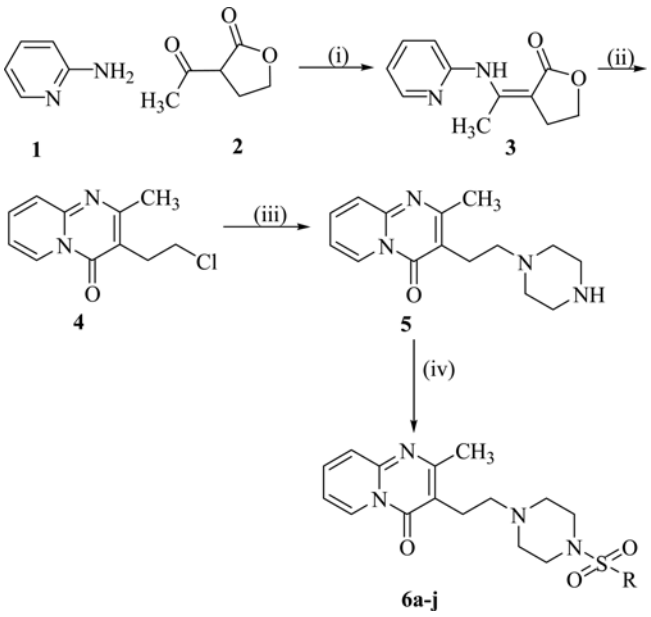
Table I. Chemical structure and physical data of **6a**-**j**

Compound	R	Structure	Yield (%)	m.p. (°C)
6a	phenyl		75	120-122
6b	4-chlorophenyl		74	179-180
6c	2,4,6-trimethyl phenyl		78	156-158
6d	trifluoromethyl phenyl		71	158-160
6e	4-nitrophenyl		79	162-163
6f	methyl		77	180-182
6g	4-methylphenyl		73	153-154
6h	4-methoxyphenyl		76	174-176
6i	2-nitrophenyl		80	158-160
6j	3-methoxyphenyl		76	170-172

the novel compounds were reported using DMSO. The expected resonances were assigned by their peak multiplicity and integration. The integration of spectra shows good agreement with the synthesized compounds. The proton NMR spectral data of NH in **5** show single resonance at δ 5.34 ppm, which is absent in the spectra of **6a**-**j**, indicating the replacement of the sulfonamide series. In addition, the resonance appearing in the range of δ 7.07-8.90 ppm as singlets, doublets, triplets and multiplates is attributed to the aromatic protons. The piperazine protons were resonated as multipletes at δ 2.79-3.86 ppm (Shafiee et al., 2009). The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The synthesized compounds were further confirmed by the appearance of a molecular ion peak in the mass spectra.

Antiproliferative activity

Piperizine derivatives were shown to inhibit growth inhibition of human erythroleukemia K-562 cells and the presence of myeloid leukemia HL-60 cells has been



Scheme 1. Reagent and reaction conditions: (i) Toluene, PTSA, 12 h; (ii) $POCl_3$, overnight; (iii) K_2CO_3, Piperazine, DMF, 80°C, 6 h; (iv) sulphonyl chlorides, DCM, 8 h.

reported (Gillet et al., 1998). A new class of sulfonamides has been used in the treatment of diseases arising from abnormal cell growth and proliferation (Medina et al., 1998). The antiproliferative action of compounds **6a-j** was tested against four different cell lines. 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. Percentage cell survival for tested compounds against MDA-MB 231, K562, Colo-205, and IMR-32 cells is tabulated in Table II. The extent of inhibition of cell lines by **6d**, **6e**, and **6i** is schematically presented in Fig. 1.

The results of the biological screening experiment revealed that within the library of compounds **6a-j,** three compounds **6d**, **6e**, and **6i** showed good activity and the remaining compounds showed moderate activity. Among the compounds **6a-j**, compound **6d** exhibited 60.61%, 65.58%, and 85.45% (at 10 μM) inhibitory activity against Colo-205, MDA-MB 231, and IMR-32 cell lines, respectively, whereas compounds **6d**, **6e**, and **6i** showed less inhibitory activity against K562. Compound **6e** exhibited 63.20%, 62.27%, and 64.38% antiproliferation against Colo-205, MDA-MB 231, and IMR-32 cell lines, respectively. Similarly, compound **6i** showed 62.75%, 61.25%, and 61.83% antiproliferation against Colo-205, MDA-MB 231, and IMR-32 cell lines, respectively. The good inhibition by

Table II. Antiproliferative activity of **6a-j** against human cancer cells determined by MTT test

Compound	% Cell survival at10 μM			
	K562	Colo-205	MDA-MB 231	IMR-32
6a	–*	51.27	53.24	35.71
6b	–*	46.50	51.53	47.43
6c	–*	59.56	55.14	50.25
6d	35.58	60.61	65.58	85.45
6e	46.51	63.20	62.27	64.38
6f	–*	30.19	41.49	33.45
6g	–*	41.32	49.52	46.48
6h	–*	53.99	54.59	49.44
6i	45.89	62.75	61.25	61.83
6j	–*	36.23	37.61	49.56
Control (0.1% DMSO)	100	100	100	100

*Represents < 30% cell survival

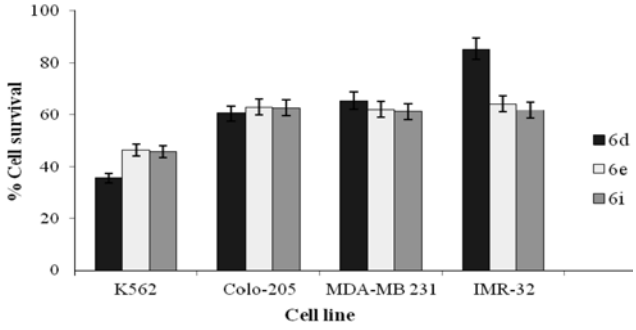


Fig. 1. MTT assay for **6d**, **6e**, and **6i** at 10 μM

compound **6d** could be attributed to the presence of an electron withdrawing trifluoromethyl group. However, the presence of electron withdrawing groups in the phenyl ring increased the antiproliferative efficacy. 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*. The results were expressed as percentage of cell proliferation compared with cells in control (cells treated with vehicle, 0.1% DMSO). The additional modification and diversification of functional groups in order to improve the anticancer activity is currently in progress.

In conclusion, a series of novel 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido[1,2-a]pyrimidin-4-one derivatives **6a-j** were synthesized and their antiproliferative activity has been evaluated. Antiproliferative assay results indicated that these derivatives have high antiproliferative activity against Colo-205, IMR-32, and MDA-MB 231. Compound **6d** containing trifluoromethyl group appeared to be the most active against the IMR-32 and MDA-MB 231 cell lines. From the

experimental results, it could be concluded that the introduction of the aryl sulfonyl moiety on the piperazine system has significant potential to obtain novel antiproliferative compounds. From this work, a few active molecules (**6d**, **6e**, and **6i**) were identified which are capable of inhibiting the growth of human cancer cell lines *in vitro*. Hence, further investigations are required to clarify the features underlying the antiproliferative activities of these new piperazine derivatives.

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