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Design and Synthesis of Novel Piperazine (2-Chloroethyl)-1-nitrosourea Analogues as Anticancer Agents

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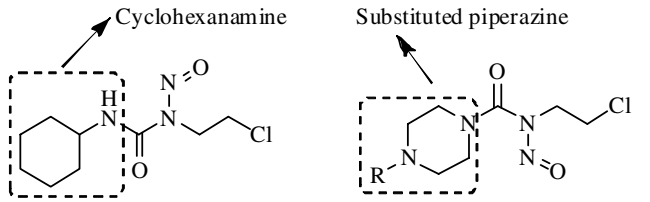
A series of piperazine (2-chloroethyl)-1-nitrosourea analogues (**6a-h**) have been designed, synthesized, characterized and screened for anticancer activity against five human cancer cell lines *viz.* human colorectal cancer (HCT-116 and HCT-15), human colon cancer (Colo-205), human breast cancer (MCF-7) and leukaemia (Molt-4). Among the screened compounds, compound **6f** exhibits potent activity against HCT-116 cell line with an IC_{50} of 1.0 µM, which regarded as promising drug candidate for the development of anticancer agents.

Keywords: Anticancer agents, Human breast cancer, Human colon cancer, Leukaemia, Piperazine, (2-Chloroethyl)-1-nitrosourea.

INTRODUCTION

During the last decade, many piperazine derivatives have been synthesized as useful chemotherapeutic agents as antibacterial [1-5], antineoplastic [3,6,7], analgesic [8], plasminogen activator inhibitor-1 (PAI-1) [9], malic enzyme inhibitors [10], cyclin-dependent kinase inhibitors [11], c-jun *N*-terminal kinase (JNK) inhibitors [12], secretory phospholipase A2 inhibitors [13], CB1 cannabinoid receptor ligands [14], melanocortin-4 receptor (MC4R) antagonists [15]. Much attention was paid in the synthesis of anticancer agents, piperazine analogues exhibit a wide range of anticancer activities [16-31].

Furthermore, nitrosoureas [32] are pharmacologically active class of alkylating compounds. *N*-(2-haloethyl)-*N*-nitrosoureas, such as, *N*-cyclohexyl-*N*-(2-chloroethyl)-*N*-nitrosourea (lomustine, CCNU), MeCCNU and *N*,*N*-bis(2-chloroethyl)-*N*-nitrosourea (carmustine, BCNU) are representatives of one of the principle classes of anticancer agents, displaying a wide range of activity in human cancers and being widely used in the treatment of brain tumours, melanomas and various leukaemias [33]. The therapeutic efficacies of nitrosourea is known to be related to their spontaneous decompositions to generate both electrophilic species, which alkylate DNA and isocyanates where carbamoylate proteins especially DNA repair proteins, contribute substantially to toxic side effects [34].



Based on the literature survey, we aimed to synthesize a single molecular frame which carry both piperazine and nitrosourea scaffolds.

EXPERIMENTAL

Chemical and reagents analytical grade were purchased from Fluka and Merck. Thin-layer chromatography (TLC) was performed on the silica gel 60 F_{254} plates (E. Merck). IR spectra were recorded on Perkin-Elmer spectrum ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with a Varian Mercury plus 400 and 100 MHz, respectively. All the chemical shifts

were reported in δ (ppm) using TMS as an internal standard. Mass spectra were recorded with a PE Sciex model API 3000 instrument.

Synthesis of piperazine compounds (3a-h): A mixture of akyl/aryl chlorides (1 mmol, **1a-h**), piperazine (5 mmol) (**2**) was stirred at 100 ºC for 8-10 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was partitioned between DCM and water at room temperature. Product was extracted with organic layer and washed with distilled water three times. DCM was evaporated under vacuum. The residue was purified by flash column chromatography using hexane:ethyl acetate.

Synthesis of *N*-(2-chloroethyl)-4-piperazine-1-carboxamide (5a-h): To a solution of *N*-substituted piperazine (1 mmol, **3a-h**) in DCM (100 mL), 2-chloroethylisocyanate (3 mmol, **4**) was added slowly at > 5 ºC. After 1 h of stirring, reaction mass was allowed to settled and again stirred for 12 h. Completion of the reaction as indicated by TLC, water was added to the reaction mass and extracted with DCM. Saturated sodium carbonate solution was added and reaction mass was again stirred for 20 min to quench the excess 2-chloroethylisocyanate. The DCM layer was separated and washed with 0.1% tartaric acid solution to remove unreacted piperazine compound followed by distilled water. Crude residue was purified by flash column chromatography using hexane:ethyl acetate.

***N*-(2-Chloroethyl)-4-methylpiperazine-1-carboxamide (5a):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.091 (s, 1H), 3.616-3.612 (t, *J* = 2.0 Hz, 2H), 3.436-3.432 (t, *J* = 2.4 Hz, 2H), 3.271-3.266 (t, *J* = 1.2 Hz, 4H), 2.630-2.621 (t, *J* = 2.4 Hz, 4H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.1, 55.2, 49.9, 43.4, 41.7, 38.9. HRMS *m/z*: calcd.: 205.0981; found: 206.1141 [M+H].

***N*-(2-Chloroethyl)-4-ethylpiperazine-1-carboxamide (5b):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.943 (s, 1H), 3.712-3.703 (t, *J* = 2.0 Hz, 2H), 3.614-3.602 (t, *J* = 4.0 Hz, 4H), 3.443-3.429 (t, *J* = 2.8 Hz, 2H), 3.279-3.268 (t, *J* = 1.2 Hz, 4H), 2.649-2.637 (t, *J* = 3.2 Hz, 3H), 2.52 (q, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.4, 53.9, 52.0, 50.7, 41.7, 40.0, 13.8. HRMS *m/z*: calcd.: 219.1138; found: 220.1247 [M+H].

***N*-(2-Chloroethyl)-4-phenylpiperazine-1-carboxamide (5c):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.572 (s, 1H), 7.012-6.783 (m, 5H), 3.731-3.620 (t, *J* = 2 Hz, 2H), 3.627-3.615 (t, *J* = 3.6 Hz, 4H), 3.529-3.515 (t, *J* = 2.4 Hz, 2H), 3.291-3.281 (t, *J* = 1.6 Hz, 4H) ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.3, 150.4, 130.0, 119.8, 116.0, 51.0, 50.7, 42.3, 41.2. HRMS *m/z*: calcd.: 267.1138; found: 268.1203 [M+H].

4-Benzyl-*N*-(2-chloroethyl)piperazine-1-carboxamide (5d): ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.473 (s, 1H), 7.541-7.212 (m, 5H), 3.547 -3.483 (m, 4 H), 3.581-3.570 (t, *J* = 2.0, 2H), 3.321-3.277 (m, 4H), 2.641-2.593 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.9, 136.9, 131.4, 129.8, 128.2, 127.5, 126.0, 55.7, 53.9, 41.2, 40.9. HRMS *m/z*: calcd.: 281.1294; found: 282.0931 [M+H].

***N*-(2-Chloroethyl)-4-((4-chlorophenyl)(phenyl)methyl)-piperazine-1-carboxamide (5e):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.825 (s, 1H), 7.421-7.241 (m, 9H), 5.021 (s, 1H), 4.402-4.340 (t, *J* = 6.0 Hz, 2H), 3.732-3.705 (t, *J* = 5.4 Hz, 4H), 3.486-3.516 (t, *J* = 6 Hz, 2H), 2.466-2.439 (t, *J* = 5.6 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.4, 141.8, 140.8, 131.9, 128.7, 128.9, 128.8, 128.7, 127.4, 82.0, 52.1, 51.7, 42.6, 40.2. HRMS *m/z*: calcd.: 392.3221; found: 393.0988 [M+H].

4-(Benzo[*d*]isothiazol-3-yl)-*N*-(2-chloroethyl)piperazine-1-carboxamide (5f): ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.421 (s, 1H), 7.911-7.830 (m, 4H), 4.274-4.239 (m, 8H), 3827-3.792 (m, 4H) ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.5, 154.1, 15.1, 127.4, 126.1, 124.0, 123.9, 49.2, 46.1, 42.9, 40.3, HRMS *m/z*: calcd.: 324.0811; found: 325.5830 [M+H].

***N*-(2-Chloroethyl)-4-(2,3-dihydrobenzo[*b*][1,4]dioxine-2-carbonyl)piperazine-1-carboxamide (5g):** ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.976 (s, 1H), 7.175-6.954 (m, 4H), 4.742-4.722 (t, *J* = 4, 1H), 4.432-4.4.412 (d, *J* = 8 Hz, 2H), 4.328-3.926 (m, 4H), 3.717-3.591 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.1, 155.2, 142.1, 140.9, 122.9, 121.6, 116.9, 81.2, 65.1, 52.4, 49.6, 42.9, 39.8, HRMS *m/z*: calcd.: 353.1142; found: 354.0774 [M+H].

Methyl 2-(4-(2-chloroethylcarbamoyl)piperazin-1-yl)-2-(2-chlorophenyl)acetate (5h): ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.429 (s, 1H), 7.217- 6.961 (m, 4H), 4.972 (s, 1H), 3.69 (s, 3H), 3.742-3.734 (t, *J* = 3.2 Hz, 2H), 3.641-3.633 (t, *J* = 2.4 Hz, 2H), 3.349-3.341 (t, *J* = 3.2 Hz, 4H), 2.251-2.243 (t, *J* = 1.6 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.2, 156.4, 136.5, 135.7, 134.9, 130.0, 129.1, 127.1, 65.7, 51.6, 50.2, 49.6, 42.1, 40.8. HRMS *m/z*: calcd.: 374.2622; found: 375.1206 [M+H].

Synthesis of *N*-(2-chloroethyl)-4-*N*-nitrosopiperazine-1-carboxamide (6a-h): A solution of *N*-(2-chloroethyl)-4-piperazine carbonyl urea compounds (2 mmol, **5a-h**) in 99% HCOOH (10 mL) was cooled at 0-5 ºC. To this, dry $NaNO_2$ powder (6 mmol) was added lotwise over a period of 60 min. The dark brown solution was stirred for 1 h at 0-5 ºC and then allowed to stir the same solution at room temperature for 3 h. Water was added to the reaction mass and contents were extracted with DCM (3 × 50 mL). The combined DCM extracts were washed with diluted 1% sodium bicarbonate solution and dried over anhydrous sodium sulphate, filtered and evaporated to give crude compounds (**6a-h**). Crude residue was purified by flash column chromatography using hexane:ethyl acetate.

***N*-(2-Chloroethyl)-4-methyl-*N*-nitrosopiperazine-1-carboxamide (6a):** Colourless oil; yield: 87%; HPLC purity = 99.21%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.629-3.625 (t, *J* = 2.0 Hz, 2H), 3.463-3.451 (t, *J* = 2.4 Hz, 2H), 3.274-3.268 (t, *J* = 1.2 Hz, 4H), 2.638-2.629 (t, *J* = 2.4 Hz, 4H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.1, 56.0, 50.1, 43.0, 42.1, 39.6. FTIR (KBr, cm^{-1}): 2997, 2927, 1663, 1342, 1073, 820, 754, 630. HRMS *m/z*: calcd.: 234.0883; found: 235.1114 [M+H].

***N*-(2-Chloroethyl)-4-ethyl-*N*-nitrosopiperazine-1-carboxamide (6b):** Colourless oil; yield: 84%; HPLC purity = 98.99%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.701-3.692 (t, *J* = 1.6 Hz, 2H), 3.608-3.596 (t, *J* = 4.0 Hz, 4H), 3.446-3.432 (t, *J* = 2.8 Hz, 2H), 3.281-3.271 (t, *J* = 1.2 Hz, 4H), 2.612-2.600 (t, *J* = 2.8 Hz, 3H) 2.43 (q, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0, 53.1, 51.8, 50.3, 41.5, 39.6, 14.0. FTIR (KBr, cm^{-1}): 2995, 2938, 1650, 1420, 1336, 1082, 763. HRMS *m/z*: calcd.: 248.1040; found: 249.1274 [M+H].

***N*-(2-Chloroethyl)-*N*-nitroso-4-phenylpiperazine-1-carboxamide (6c):** Colourless oil; yield: 75%; HPLC purity = 99.56%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.101-7.021 (m, 2H), 6.613-6.598 (m, 3H), 3.711-3.698 (m, 6H), 3.422-3.381(m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.1, 150.2, 129.8, 119.1, 115.3, 51.5, 51.0, 42.0, 41.2. FTIR (KBr, cm^{-1}): 2936, 2823, 2712, 1678, 1452, 1368, 1452, 1370, 1245, 1076, 891, 736, 487. HRMS *m/z*: calcd.: 296.1040; found: 297.1265 [M+H].

4-Benzyl-*N*-(2-chloroethyl)-*N*-nitrosopiperazine-1-carboxamide (6d): Colourless oil; yield: 89%; HPLC purity = 99.82%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.226-7.012 (m, 5H), 3.672-3.611 (m, 4H), 3.456-3.447 (t, *J* = 2.0, 2H), 3.286-3.272 (m, 4H), 2.712-2.698 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.2, 136.2, 129.1, 129, 128.6, 127.0, 54.2, 52.5, 42.2, 41.6. FTIR (KBr, cm^{-1}): 2928, 2856, 2690, 1620, 1475, 1374, 1462, 1380, 1255, 820, 465. HRMS *m/z*: calcd.: 310.1196; found 311.0957 [M+H].

***N*-(2-Chloroethyl)-4-((4-chlorophenyl)(phenyl)methyl)-*N*-nitrosopiperazine-1-carboxamide (6e):** White solid, yield: 83%, m.p.: 78-80 ºC. HPLC purity = 99.48%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.376-7.189 (m, 9H), 4.956 (s, 1H), 4.295-4.239 (t, *J* = 6.4 Hz, 2H), 3.835-3.808 (t, *J* = 5.6 Hz, 4H), 3.582-3.552 (t, *J* = 6 Hz, 2H), 2.387-2.360 (t, *J* = 5.6 Hz, 4H) ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.0, 141.3, 140.5, 132.8, 129.0, 128.9, 128.8, 128.7, 127.6, 81.8, 51.7, 51.5, 42.5, 40.0. FTIR (KBr, cm^{-1}): 2955, 2923, 2854, 1463, 1377, 722. HRMS *m/z*: calcd.: 420.1119; found: 421.1198 $[M+H]^+$.

4-(Benzo[*d*]isothiazol-3-yl)-*N*-(2-chloroethyl)-*N*-nitrosopiperazine-1-carboxamide (6f): White solid, yield: 81%, m.p.: 102-104 ºC. HPLC purity = 99.81%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.911-7.830 (dd, *J* = 8.4, 8.4 Hz, 2H), 7.518-7.481(t, *J* = 7.6 Hz, 1H), 7.410-7.373 (t, *J* = 7.2, Hz, 1H), 4.174-4.145 (t, *J* = 5.6 Hz, 2H), 3.935(m, 4H), 3.648- 3.617 (t, *J* = 6.4 Hz 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 154.3, 152.8, 127.7, 127.6, 124.1, 123.4, 49.7, 46.6, 42.5, 40.1. FTIR (KBr, cm^{-1}): 2920, 2851, 2727, 2680, 1588, 1463, 1377, 1168,1076, 1012, 772, 669, 451. HRMS *m/z*: calcd. 353.0713; found: 354.0758 $[M+H]^+$.

***N*-(2-Chloroethyl)-4-(2,3-dihydrobenzo[*b*][1,4]dioxine-2-carbonyl)-*N*-nitrosopiperazine-1-carboxamide (6g):** Yellowish white solid, yield: 90%, m.p.: 110-114 ºC. HPLC purity = 99.91%. ^{1}HNMR (400 MHz, $CDCl_3$): δ 6.919-6.845 (m, 4H), 4.863-4.844 (t, *J* = 2.4 Hz, 1H), 4.554-4.534 (d, *J* = 8 Hz, 2H), 4.167-3.885 (m, 6H), 3.706-3.573 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 154.4, 143.2, 142.1, 122.4, 121.6, 117.1, 80.6, 64.9, 51.7, 49.7, 42.5, 40.1. FTIR (KBr, cm^{-1}): 2922, 2853, 2727, 1693, 1659, 1462, 1377, 1235, 1162, 1082, 893, 722, 447. HRMS *m/z*: calcd.: 382.1044; found: 383.1122 $[M+H]^+$.

Methyl 2-(4-((2-chloro ethyl)(nitroso)carbamoyl)-piperazin-1-yl)-2-(2-chloro phenyl)acetate (6h): Colourless oil; yield: 72%; HPLC purity = 98.62%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.102-7.096 (m, 1H), 7.016-6.998 (m, 3H), 4.82 (s, 1H), 3.71 (s, 3H), 3.694-3.686 (t, *J* = 3.0 Hz, 2H), 3.564-3.556 (t, *J* = 2.4 Hz, 2H), 3.296-3.291 (t, *J* = 1.6 Hz, 4H), 2.722-2.719 (t, *J* = 1.6 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 156.0, 136.0, 135.4, 134.3, 130.1, 129.4, 128.1, 65.2, 52.6, 50.6, 50.2, 42.0, 40.2. FTIR (KBr, cm^{-1}): 3096, 2965, 2896, 2712, 1482, 1388, 1468, 1396, 1288, 972,836, 720. HRMS *m/z*: calcd. 403.2604; found: 404.1222 [M+H].

Anticancer activity

Cell lines and cell culture: Human cancer cell lines colorectal cancer (HCT-15, HCT116), colon cancer (Colo-205), breast cancer (MCF-7) and Molt-4 (Leukaemia) were obtained from ECACC, England. The cells were grown on a monolayer in 25 cm^2 or 72 cm^2 depending on the requirement of tissue culture flask and maintained in RPMI-1640/MEM medium, supplemented with 10% FCS and 100 units penicillin/100 µg streptomycin per mL medium as antibiotic solution at 37 ºC with 95% humidity and 5% CO_2 gas environment in incubator. The media of cell culture flask were observed daily for pH changes based on the colour phenol red. This may be due to normal media utilization by cells or abnormal level of O_2/CO_2 tension in incubator or contamination. Contamination or overgrowth of cells also results in cloudiness of media. Cells were observed daily under inverted microscope for attained health and growth confluence, phenotype and possible contamination or other abnormalities are recorded. Media was changed after 2-3 days as per requirement. Cell grown to 70-80% or more confluence (number of cells over flask area) were sub-cultured into new flask. Over confluence or other stress such as inadequate media change, abnormal O_2/CO_2 tension, *etc*. lead to change in cell characteristics making it unsafe for experiments in *in vitro* models.

Test concentrations and cytotoxicity assessment: The *in vitro* cytotoxicity studies of piperazine clubbed nitrosourea derivatives (**6a-h**) were performed on five different cell line, HCT-15 (colorectal cancer), HCT116 (colorectal cancer), Colo-205 (colon cancer), MCF-7 (breast cancer) and Molt-4 (leukaemia). The nitrosourea derivatives (**6a-h**) dissolved in DMSO as a 1 mg/mL stock solution and diluted to required concentration with FCS (phosphate buffer saline). Dihydrorhodamine 123 (DHR123), propidium iodide (PI), DNAase-free RNAase, 3-(4,5,-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT assay was performed as per standard protocols by varying concentrations of compounds were used to treat specific number of cancer cells. Cells were seeded in 96-well tissue culture plates. plates and when 70-75% confluent (for adherent cells) and 15000 cells/well (for suspension cells), cells were treated with 1, 10, 30, 50 and 100 µM of compounds **6a-h** for 48 h time period. MTT dye was added 3 h prior to experiment termination. After incubation of 4 h at 37 ºC in a 5% CO_2 incubator, microscopic visualization for the formation of formazan was confirmed. To this, 150 µL DMSO was added to dissolve the salt. The OD was measured at 570 nm (reference wave length 620 nm) % growth inhibition was calculated by comparing the absorbance of treated verses untreated cells. The activities of the test compounds were compared to that of reference standard drug lomustine. The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data were subjected to linear regression analysis and the regression

lines were plotted for the best straight-line fit. The IC_{50} concentrations were calculated using the respective regression equation.

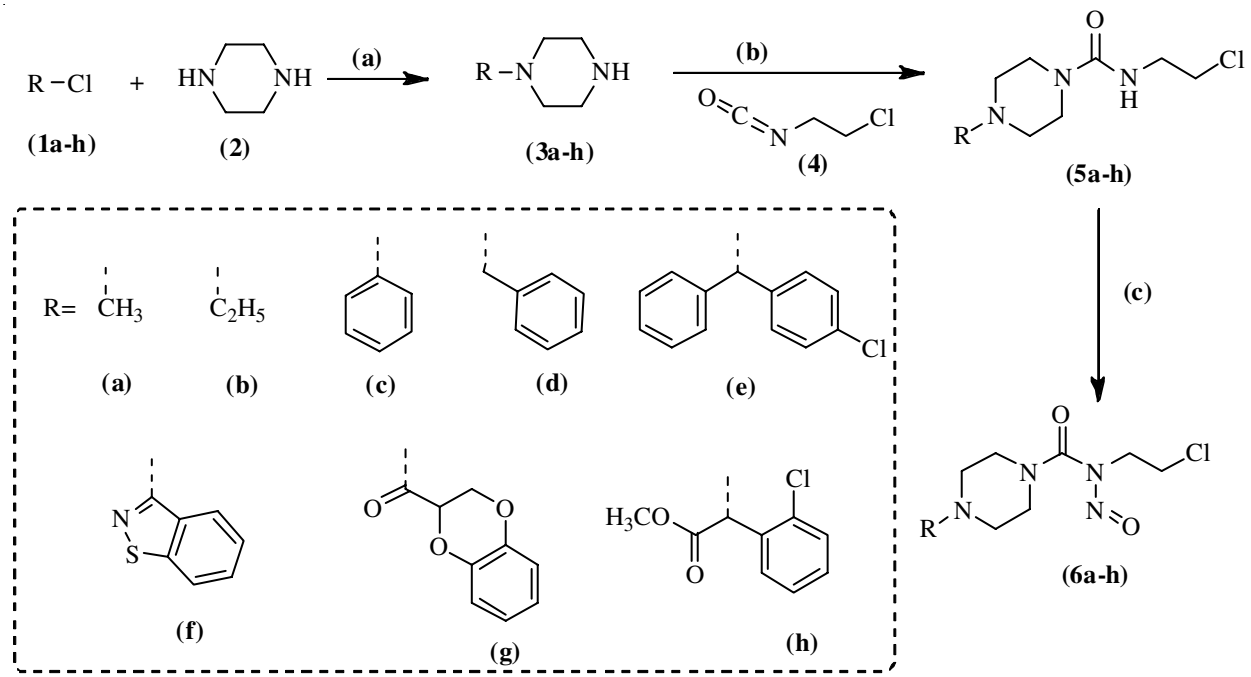
RESULTS AND DISCUSSION

In order to synthesize more potent anticancer agents *viz.* substituted piperazine containing 2-chloroethyl-1-nitrosourea derivatives (**6a-h**) have been accomplished by adopting the synthetic route which is presented in **Scheme-I**. In this synthesis, various chloro compounds (**1a-h**) were reacted with piperazine (**2**) in DCM and refluxed at 80 ºC to obtain *N*-substituted piperazines (**3a-h**). *N*-substituted piperazines (**3a-h**) on reaction with 2-chloroethyl isocyanate (**4**) in dichloromethane to obtain *N*-(2-chloroethyl)-4-piperazine carbonyl urea derivatives (**5a-h**). These substituted urea derivatives (**5a-h**) reacted with sodium nitrite in presence of formic acid at 0-5 ºC to yield *N*-(2-chloroethyl)-4-*N*-nitrosopiperazine-1-carboxamides (**6a-h**).

All the synthesized compounds were well characterized by advanced spectroscopic techniques and the spectral data of the synthesized compounds are in accordance with the structures.

***in vitro* Anticancer activity:** The newly synthesized piperazine (2-chloroethyl)-1-nitrosourea analogues (**6a-h**) were evaluated to investigate their anti-proliferative/cytotoxic activities on five different types of human cancer cell lines, *viz.* human colorectal cancer (HCT-116, HCT-15), human colo cancer (Colo-205), human breast cancer (MCF-7) and leukaemia (Molt-4) using the MTT assay. The cytotoxicity of piperazine analogues (**6a-h**) against the screened cell lines were compared with the reference drug (lomustine) and the activity was expressed in terms of IC_{50} values as summarized in Table-1.

Most of the compounds have shown significant to moderate cancer cell growth inhibition. The effect of various substituents



Reagents and reaction conditions: (a) dichloromethane, 80 °C, 10 h, (b) dichloromethane, 0-5 °C, 12 h
(c) dichloromethane, HCOOH, $NaNO_2$, 0-5 °C, 5 h

Scheme-I: Synthesis of piperazine analogues carrying (2-chloroethyl)-1-nitrosourea **(6a-h)**

TABLE-1
In vitro CYTOTOXICITY PROFILES OF PIPERAZINE ANALOGUES CARRYING (2-CHLOROETHYL)-1-NITROSOUREA (**6a-h**)

Entry	Compound	Cytotoxicity IC_{50} (μM)[a]				
		HCT-116[b]	HCT-15[b]	Colo-205[c]	MCF-7[d]	Molt-4[e]
1	**6a**	8.0	30.3	25.0	12.5	25.0
2	**6b**	5.0	33.4	10.4	7.0	18.0
3	**6c**	21	40.0	36.0	27.6	44.0
4	**6d**	18.7	25.0	21.5	13.0	34.3
5	**6e**	10	23.5	25.6	2.0	> 100
6	**6f**	1.0	33.2	90.3	9.5	52.1
7	**6g**	20.2	10.5	36.6	18.2	25.0
8	**6h**	22.0	55.0	86.7	11.0	77.1
9	Lomustine	3.0	13.0	3.5	4.0	10

[a]IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor; [b]Colorectal cancer; [c]Colon cancer; [d]Breast cancer; [e]Leukemia

or groups on piperazine was examined. The structure-activity relationship (SAR) study revealed that among the aliphatic analogues [methyl (**6a**) and ethyl (**6b**)], ethyl analogue was found to be potent against all the cell lines (except HCT-15). Among the tested compounds against colo-205 and Molt-4 cell lines, ethyl analogue (**6b**) was the lead compound with an IC_{50} value of 10.4 and 18.0 µM, respectively. Considering the aromatic [phenyl (**6c**) and benzyl (**6d**)] analogues, phenyl analogue (**6c**) showed potent activity than the corresponding benzyl analogue (**6d**), but none of them were potent against all the screened cell lines. By replacing the benzyl group with (4-chlorophenyl)(phenyl)methane (**6e**), an increases in the activity against colorectal (HCT-116 and HCT-15) and breast cancer cell lines (MCF7) was observed. Among the tested compounds against breast cancer cell line (MCF7), compound **6e** was found to be promising with an IC_{50} of 2.0 µM and methyl-2-(2-chlorophenyl)acetate analogue (**6h**) showed a moderate activity. By observing the IC_{50} values of heterocyclic analogues *viz*. benzo[*d*]isothiazol (**6f**) and 2,3-dihydrobenzo[*b*][1,4]-dioxine-2-carboxyl (**6g**) revealed that these compounds were found to be potent against colorectal cell line, specifically compound **6f** was active against HCT-116, while compound **6g** was active against HCT-15 with an IC_{50} value of 1.0 and 10.5 µM, respectively. The cytotoxicity of newly synthesised compounds (**6a-h**) was also compared with the previously reported piperazine analogues as presented in Table-2.

TABLE-2
COMPARISON OF CYTOTOXICITY OF NOVEL PIPERAZINE AND PREVIOUSLY REPORTED PIPERAZINE ANALOGUES

Cell lines	IC_{50} (µM)[a]	IC_{50} (µM)[b]	Ref.
HCT-116	1.0-22.0	3.4 – 42.1	[9]
		30 – >100	[10]
		2.2 – >20	[14]
		16.5 – >50	[23]
		60.4 – 79.4	[26]
		16.5 – >50	[27]
		12.8 – >100	[28]
		4.9 – >40.4	[29]
		9.5	[30]
		36.4 – >100	[31]
HCT-15	10.5–55	>100	[30]
		9.3 – >100	[31]
Colo-205	10.4–90.3	>100	[30]
		5.5 – >100	[31]
MCF-7	2.0–27.6	7.4 – 52.0	[9]
		6.0 – 21.4	[29]
		>100	[30]
		5.6 – >100	[31]
Molt-4	18.0 – >100	>100	[30]
		>100	[31]

[a]Range of IC_{50} values for piperazine carrying (2 – chloroethyl)-1-nitrosourea analogues; [b]Range of IC_{50} values for previously synthesized piperazine analogues.

Conclusion

In conclusion, eight novel piperazine (2-chloroethyl)-1-nitrosourea analogues (**6a-f**) were synthesized and characterized. The anticancer study was undertaken to evaluate the effects of substituent. All the synthesized compounds exhibited good cytotoxicity against the five human cancer cell lines. Among the screened compounds, compound **6f** exhibit potent activity against HCT-116 cell line with an IC_{50} of 1.0 µM.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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