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Synthesis and anticancer evaluation of 2-oxo-2-(arylamino) ethyl 4-phenylpiperazine-1-carbodithioates

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Abstract: Piperazine moiety is found as an efficient pharmacological scaffold in various drugs. To explore the anticancer potential of piperazine framework, a series of novel *N*-acetamides derivatives of phenyl piperazine containing di-thio-carbamate moiety was designed and synthesized. ^{1}HNMR, ^{13}CNMR, FT-IR and mass spectrometry were used for the structures elucidation of these derivatives. *In-vitro* cytotoxic evaluation of the prepared novel compounds against lung carcinoma A-549 was carried out using standard MTT assay. All the di-thio-carbamate-piperazine derivatives exhibited moderate to excellent cytotoxic potential against A-549 cell line based on cell viability. Particularly, 6e was found to be the most potent derivative with cell viability 34.12±0.73 % at 100 µg/mL concentration and represents promising lead compound for future progress.

Keywords: *N*-Phenyl piperazine, cytotoxicity, di-thio-carbamates, human lung cancer, anti-cancer.

INTRODUCTION

Lung carcinoma is among the most common and fatal cancers with over 3.3 million cases reported annually and is the major cause of mortality worldwide (Siegel *et al.*, 2019; Howlader *et al.,* 2020). Despite the availability of several drugs for cancer control, development of new/novel potent chemotherapeutics offers a wide scope of interest in this field (Sartaj *et al.,* 2020).

Nitrogen-containing heterocycles like piperazine are interesting scaffolds of medicinal interest and exhibit wide range of pharmacological potential (Kerru *et al.,* 2020). Piperazine nucleus is well established heterocyclic moiety displaying broad spectrum biological activities *viz*. anti-viral, insecticidal, anti-cancer and anti-microbial potential (Akhtar *et al.,* 2016; Alhusadi *et al.,* 2020; Zahoor *et al.,* 2017). This firm heterocyclic backbone constitutes an important role in several pharmacologically relevant compounds such as Abemaciclib and Bosutinib which are FDA approved anticancer drugs (fig. 1) (Hino *et al*., 2020; Cortes *et al*., 2011). Literature survey reveals that *N*-substituted piperazine derivatives are an important class of well-reported therapeutic agents in medicinal chemistry (Akhtar *et al.,* 2019; Ostrowska 2020).

Di-thio-carbamates are efficient intermediates present in several pharmacologically active derivatives (Boas *et al.,* 2004; Oliveira *et al.,* 2019). Various heterocycles incorporating di-thio-carbamate moiety have been reported to be potent drugs against cancer, tuberclosis, diabetes, and Alzheimer's disease (Buac *et al.,* 2012; Winum and Supuran, 2015).

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Keeping in view the plethora of activities exhibited by *N*-phenyl piperazine and di-thio-carbamates, we were interested to synthesize piperazine-based di-thio-carbamates with cytotoxic potential against human lung carcinoma (A-549).

MATERIALS AND METHODS

General experimental part

Chemicals, *N*-phenyl piperazine, bromoacetyl bromide, carbon disulfide, pyridine, sodium hydroxide and anilines were obtained from Merck, Alfa-Aesar (Germany), Daejung and Scharlau through local suppliers. For purification of compounds silica gel was used in column chromatography system. Gallenkamp equipment was used for the determination of melting point of the target analogs. Synthesized derivatives were characterized by ^{13}CNMR and ^{1}HNMR spectra on Bruker spectrometer at 100 MHz and 500 MHz, respectively. While for FT-IR, Bruker fourier-transform spectrometer was used.

Preparation of sodium 4-phenylpiperazine-1-carbodithioate 2

Stirred the reaction mixture of phenyl piperazine (0.61 mmol) and NaOH (0.61mmol) in ethanol (4.88mL) for 15 min in ice-bath at 0°C. In the next step, added carbon disulfide (6.1mmol) to the reaction medium and allowed it to agitate for next one hour at room temperature. Formation of solid precipitates displayed the completion of reaction, which were filtered followed by washing with diethyl ether.

Common preparation method for 4-phenylpiperazine-1-carbodithioate-acetamide derivatives 6a-j

Refluxed the mixture of sodium 4-phenylpiperazine-1-carbodithioate 2 (0.03mmol) and 2-bromo-*N*-phenyl

acetamide 5a-j (0.03mmol) in acetone (5mL) for three hours. Afterwards, temperature of reaction medium was decreased to -4°C to afford the crude product as solid precipitates. The product was filtered and purified on recrystallization with ethanol.

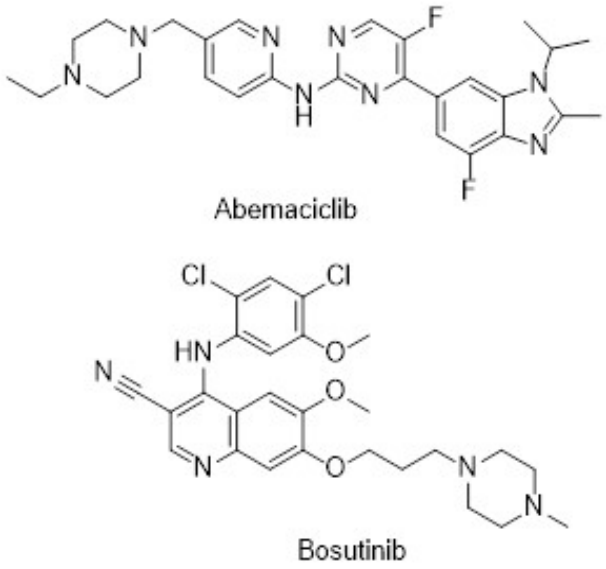


Fig. 1: Piperazine based anticancer drugs

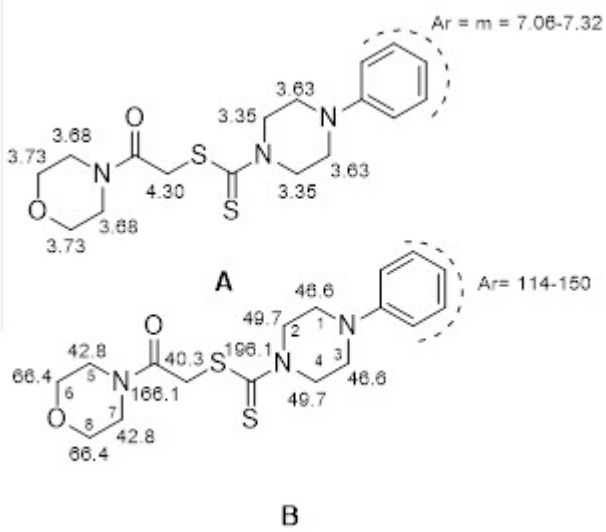


Fig 2: ^{1}HNMR A and ^{13}CNMR B Chemical shift values (ppm) of compound 6j

Cytotoxic assay

The piperazine derivatives 6a-j were investigated against lung carcinoma A-549. The corresponding cancer cells were grown at 37 °C in DMEM (Dulbecco's Modified Eagles medium) enriched by penicillin (100 units/mL) and streptomycin (100µg/mL), along with 10% FBS (Fetal Bovine Serum) with 5% Carbon dioxide (CO_2) in moistened air. Cell viability of these derivatives was calculated by standard MTT assay (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) (Rasul *et al.,* 2013; Shahzadi *et al.,* 2020). Synthesized derivatives were diluted in DMSO (di-methyl-sulfoxide). For cancer cells, 0.05% final concentration of DMSO was used for 48 hours. These DMSO treated cells were employed as control in these experimental procedures. MTT reagent (500µg/mL) was added in the cells and incubated the cells at 37°C for four hours. To examine the absorbance, solution of formazan crystals in DMSO was employed. Absorbance was recorded at 570 nm on thermo scientific microplate reader. The cell viability was calculated in % age

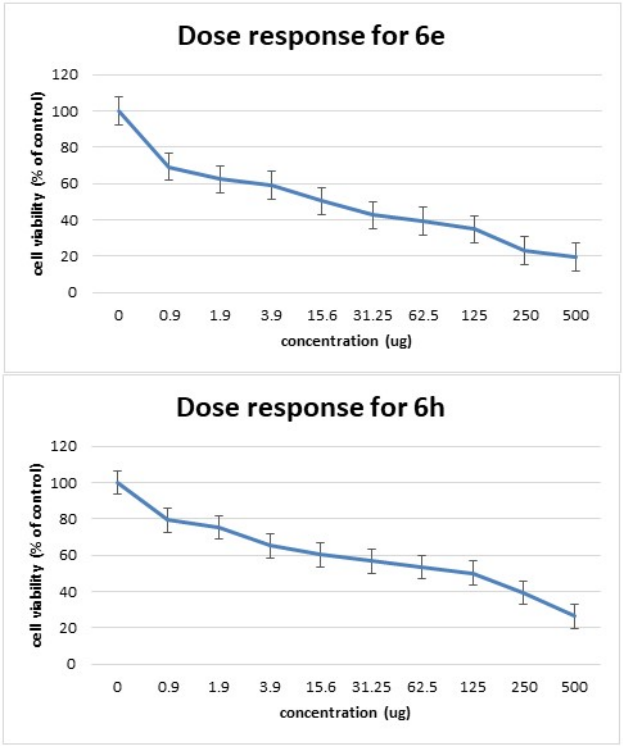


Fig. 3: Dose response graphs for 6e and 6h

STATISTICAL ANALYSIS

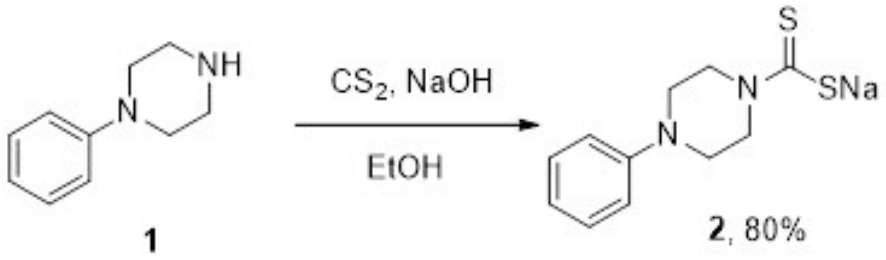
All the experiments were done in triplicate and statistical analysis was carried out by Microsoft Excel 2010. Results for cell viability are expressed as mean ± SD.

RESULTS

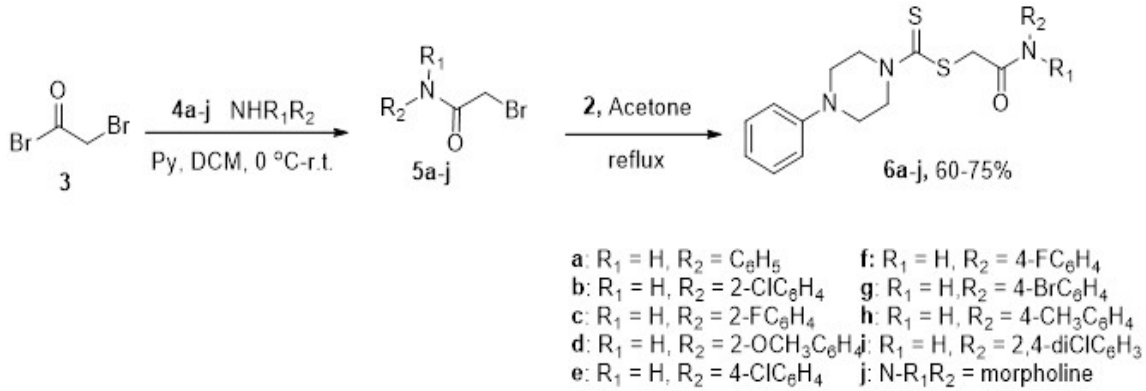
Condensation of *N*-phenyl piperazine 1 takes place in catalytic amount of (NaOH) sodium hydroxide with carbon disulfide in ethanol produced, corresponding sodium 4-phenylpiperazine-1-carbodithioate 2 in 80% yield (Scheme 1) (Mohsen, 2014). 2-Bromo-*N*-phenyl acetamide 5a-j were obtained by the reaction of anilines 4a-j with bromo acetyl bromide 3 according to known procedure (Cormier *et al.,* 2012; Faiz *et al.,* 2019). *S*-Alkylation of sodium 4-phenylpiperazine-1-carbodithioate 2 with 2-bromo-*N* phenyl acetamide 5a-j in acetone, resulted into phenyl piperazine-di-thio-carbamate acetamide derivatives 6a-j as displayed in scheme 2.

Table 1: Spectral characterization of compounds 6a-j

Compound	M.P. (°C)	Yield (%)	FT-IR (cm^{-1})ν_{max} / ^{1}HNMR (500 MHz, $CDCl_3$)/ MS (EI) (m/z)
6a	148	65	1222 (C=S), 1524 (C=C), 1664 (C=O), 3230 (N-H)/3.37 (t, 4H, J= 5 Hz, piperazine), 4.28 (s, 2H, CH_2), 4.21 and 4.60 (two bs, 4H, piperazine), 7.02-7.10 (m, 5H, Ar-H), 7.19-7.32 (m, 4H, 1H, Ar-H, N-H), 7.51 (m, 1H, Ar-H)/371.07 [M^+]
6b	115	65	752 (C-Cl), 1220 (C=S), 1524 (C=C), 1660 (C=O), 3330 (N-H)/3.34 (t, 4H, J= 5 Hz, piperazine), 4.33 (s, 2H, CH_2), 4.21 and 4.57 (two bs, 4H, piperazine), 7.02-7.10 (m, 6H, Ar-H), 7.19-7.32 (m, 2H, 1H, Ar-H, N-H), 8.31 (m, 1H, Ar-H)/405.04 [M^+]
6c	125	70	1050 (C-F), 1125 (C=S), 1624 (C=C), 1690 (C=O), 3310 (N-H)/3.32 (t, 4H, J= 5 Hz, piperazine), 4.28 (s, 2H, CH_2), 4.21 and 4.55 (two bs, 4H, piperazine), 7.02-7.10 (m, 6H, Ar-H), 7.19-7.32 (m, 2H, 1H, Ar-H, N-H), 8.31 (m, 1H, Ar-H)/389.03 [M^+]
6d	126	75	1200 (C-O), 1220 (C=S), 1590 (C=C), 1660 (C=O), 3330 (N-H)/3.35 (t, 4H, J= 5 Hz, piperazine), 4.29 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 4.21 and 4.56 (two bs, 4H, piperazine), 6.85 (m, 2H, Ar-H), 6.92-7.10 (m, 5H, Ar-H) 7.29 (m, 1H, 1H, Ar-H, N-H), 8.31 (m, 1H, Ar-H)/402.08 [M^+]
6e	179	75	750 (C-Cl), 1225 (C=S), 1600 (C=C), 1660 (C=O), 3330 (N-H)/3.34 (t, 4H, J= 5 Hz, piperazine), 4.28 (s, 2H, CH_2), 4.21 and 4.60 (two bs, 4H, piperazine), 7.02-7.10 (m, 3H, Ar-H), 7.19-7.52 (m, 6H, 1H, Ar-H, N-H)/405.02 [M^+]
6f	170	60	1050 (C-F), 1220 (C=S), 1624 (C=C), 1664 (C=O), 3310 (N-H)/3.37 (t, 4H, J= 5 Hz, piperazine), 4.23 (s, 2H, CH_2), 4.20 and 4.64 (two bs, 4H, piperazine), 6.98-7.10 (m, 5H, Ar-H), 7.32 (m, 2H, 1H, Ar-H, N-H), 7.46 (m, 1H, Ar-H)/389.07 [M^+]
6g	192	60	670 (C-Br), 1222 (C=S), 1524 (C=C), 1664 (C=O), 3350 (N-H)/3.33 (t, 4H, J= 5 Hz, piperazine), 4.24 (s, 2H, CH_2), 4.22 and 4.53 (two bs, 4H, piperazine), 6.92-6.96 (m, 3H, Ar-H), 7.22-7.34 (m, 6H, 1H, Ar-H, N-H)/449.01 [M^+]
6h	158	60	1225 (C=S), 1624 (C=C), 1664 (C=O), 3350 (N-H)/2.31 (s, 3H, CH_3), 3.27 (t, 4H, J= 5 Hz, piperazine), 4.27 (s, 2H, CH_2), 4.21 and 4.60 (two bs, 4H, piperazine), 6.98 (m, 3H, Ar-H), 7.14 (m, 2H, Ar-H), 7.33-7.43 (m, 4H, 1 H, Ar-H, N-H)/386.06 [M^+]
6i	140	65	1222 (C=S), 1624 (C=C), 1660 (C=O), 3330 (N-H)/2.21 and 2.26 (s, 6H, CH_3) 3.32 (t, 4H, J= 5 Hz, piperazine), 4.30 (s, 2H, CH_2), 4.18 and 4.55 (two bs, 4H, piperazine), 6.96 (m, 3H, Ar-H), 7.19-7.32 (m, 4H, 1 H, Ar-H, N-H), 7.61 (m, 1H, Ar-H)/400.06 [M^+]
6j	193	60	1225 (C=S), 1620 (C=C), 1660 (C=O), 3330 (N-H)/3.35 (t, 4H, J= 5 Hz, piperazine), 3.63 (t, 4H, J= 5 Hz, piperazine), 4.30 (s, 2H, CH_2), 3.68 (t, 4H, J= 6 Hz, morpholine) and 3.73 (t, 4H, J= 6 Hz, morpholine), 7.06-7.32 (m, 5H, Ar-H)/366.07 [M^+]



Scheme 1: Synthesis of sodium 4-phenylpiperazine-1-carbodithioate 2



Scheme 2: Synthesis of designed compounds 6a-6j

DISCUSSION

Spectral studies of compound 6j

Structure elucidation of the derivative 6j was carried out by Fourier transform infra-red (FT-IR), ^{1}HNMR (proton). ^{13}CNMR (Carbon) NMR spectroscopy (fig. 2). The Infra-red spectrum exhibited the absorption bands at 1225 cm^{-1} for carbon double-bond sulfur, 1620 cm^{-1} for C=C, 1660 cm^{-1} for C=O of amide group and 3330cm^{-1} for NH of amide. ^{1}HNMR of 6j gave triplet at 3.35 and 3.63 ppm for protons of piperazine. Hydrogens of CH_2 (linker) to di-thio-carbamate of piperazine appeared as singlet at 4.30 ppm. Two triplets gave chemical shift at 3.68 ppm and 3.73ppm for hydrogens of morpholine. Multiplet signal at 7.06-7.32ppm appears for 5-aryl hydrogens. ^{13}CNMR spectrum of 6j showed characteristic peak at 40.3ppm for CH_2 (methylene linker). C_1-C_3 carbons of piperazine gave peaks at chemical shift of 46.6 ppm while C_2-C_4 appears at 49.7ppm. C_5-C_7 and C_6-C_8 carbons of morpholine appeared at 42.8 and 66.4ppm respectively. Carbon of C=O of amide group is exhibited at 166.1 ppm. While di-thio-carbamate carbon associated with C=S appears at 196.1ppm. Other prepared piperazine-based derivatives were analyzed by following abovementioned approach.

Table 2: Cytotoxic potential of synthetic derivatives 6a-j against lung cancer cells (A-549).

Compounds	A-549 (% Cell viability ± SD) *
6a	68.91±3.25
6b	49.81±0.65
6c	74.86±5.68
6d	55.75±12.76
6e	34.12±0.73
6f	69.19±2.03
6g	69.96±4.30
6h	49.66±4.60
6i	82.24±1.17
6j	99.82±1.50

*Experiments were conducted in triplicate and cell viability values were compared using one-way ANOVA.

Cytotoxic activity

All the synthesized derivatives 6a-6j were evaluated for their anti-cancer potential for A-549 cell line (human lungs cancer cells). The impact of various sub stituents on phenyl ring was inspected to get comprehensive knowledge about cytotoxic activity of synthesized analogues. The results for anti-proliferative activity have been revealed in table 2 which reflect that, in general, inserting electron-donating group as sub stituent in phenyl ring helped to enhance the anti-cancer potential of synthesized derivative. Among the synthesized derivatives, compounds 6e and 6h have shown better cytotoxic activity. The viability of cells treated with 100 µg/mL of compound 6e was 34.12% and that of compound 6h was 49.66% which was lower than the other synthesized analogues. One way-ANOVA analysis of variance was used for comparison of cell viability values of different compounds (table 2). These compounds 6e and 6h were further screened for dose response by employing various doses *viz.* 0.9-500µg. 500 µg concentration of 6e and 6h exhibits best results (fig. 3).

CONCLUSION

Piperazine, a well-known nucleus used to synthesize a variety of molecules with anticancer potential. Herein, a series of *N*-phenyl acetamide derivatives of piperazine-based di-thio-carbamates 6a-6j was successfully synthesized and investigated their anti-cancer potential. Among all the synthesized derivatives, compound 6e showed significant anti-cancer potency for A-549 (Human lung carcinoma) even at low concentration of 0.9 µg, which can be possibly further improved keeping the results (obtained in present study) in view. Because of our structure activity relationship findings, further modifications on *N*-substituted piperazine derivatives can be done in order to formulate highly potent biologically active piperazine based agents.

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