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Triton-B/CS_2 mediated novel synthesis of substituted thioureas: A novel class of anti-cancer agents

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A novel method for the synthesis of substituted thioureas is being reported in this communication making use of easily available, less toxic and cost effective chemicals at normal conditions of temperature and pressure. The reaction of various aliphatic and aromatic amines with CS_2, methyl iodide using Triton-B as phase transfer catalyst led to the formation of substituted thioureas. This new method involved one flask reaction at room temperature and pressure conditions with high yield and easier separation of compound from the reaction mixture.

Keywords: Amine, Carbon disulfide, Triton-B, Substituted thiourea derivatives, Anticancer activities

Substituted thioureas are important class of compounds having numerous applications as herbicides[1], rhodenticides[2-3], analgesic[4], anti-inflammatory[5], antimicrobial[6], anticancer[7], fungicides[8], antibacterial[9], plant growth regulator[10], starting material for heterocyclic synthesis[11], cure for tuberculosois[12], as ligands in transition metal complexes[13-16], anti HIV[17] drugs (Fig. S1, Supplementary Data). The substituted thioureas have also used as a potential drug molecule for the treatment of several diseases occurring in tropical countries like India. Substituted thiourea derivatives have also been found to have anti-protozoal[18] and antileishmanial[19] activity, which are much needed in the continent of Asia and Africa.

Traditionally thioureas have been synthesized using toxic and hazardous reagents such as thiophene and isothiocyante[20-21]. Later on, the reaction of primary amine with CS_2 in pyridine/ethyl alcohol along with the heating or employing catalyst was also used to synthesize thioureas[22]. The preparation and working with isothiocyanates is complicated, hazardous, less stable, occurred at high temperature and the formation of urethane as side reaction is inevitable in ethyl alcohol medium. Attempts were made to overcome these drawbacks and several methodologies were proposed for the synthesis of symmetrical and unsymmetrical thioure as avoiding the is othiocyanates in the synthesis process[23-29].

Although several safe methods were proposed recently without using isothiocyanates and thiophosgene, still they are lacking simplicity, involve tedious work-up, occur at higher temperatures, need basic conditions and ultimately give lesser yield.Our research group has been working from past two decades for developing new methodologies for the synthesis of carbamates, dithiocarbamates and similar compounds making use of cheap, abundantly available and safe reagents like carbon dioxide and carbon disulphide, respectively[30]. Recently, we have found that Triton-B is the best catalyst for the synthesis of carbamates, dithiocarbamates, carbazates, dithiocarbazates, dithiocarbonates employing a variety of reagents and catalytic systems[31]. In the present communication, we are reporting an efficient and novel, one-pot, cleaner and greener synthesis of substituted thioureas using Triton-B/CS_2 system.

Experimental Details

Merck company reagents were used in the reaction. Identification of compounds formed was done by comparing their physical and spectral data with the

compounds already known. Infra-Red spectra (4000-450 cm^{-1}) were registered on a Perkin Elmer spectrophotometer (KBr, cm^{-1}) while NMR spectra were recorded on Brooker Advance DPX instrument spectrophotometer (400.13 MHz) with $CDCl_3$ as solvent & Tetra methyl silane as standard. Elemental analysis was done by Carlo-Erba EA 1110 CHNOS analyser which agrees approximately with calculated ones.

General procedure

3 mmol of aliphatic or aromatic amine (**1**) was taken in DMSO in a clean round bottom flask fitted with magnetic stirrer. To this 5 mmol of carbon disulphide (**2**) was added followed by 1.5 mmol of Triton-B. The mixture was stirred for 20 min and then added with 1.5 mmol of methyl iodide (**3**). The reaction mixture stirred for next 20 min and added with 1.2 mmols of amine (**4**) and continued stirring for 2-4 h. The progress of the reaction was ascertained with the help of TLC from time to time. After the formation of the product (**5a-5o**), the reaction mixture was poured into 50 mL of distilled water and extracted three times with the help of ethyl ethanoate in a batch of 20 mL each time. The product formed was concentrated and dried on rotavapour. Purification of the product was done with the help of column chromatography using silica gel in hexane for column packing.

Data analysis of few compounds

1,1 Diethyl-3-(2-trifluoromethyl-phenyl)-thiourea (5a)

$C_{12}H_{15}N_2SF_3$,yellow solid, m.p.=67-70°C; IR: 3210 (N-H), 3019, 2979 (C-H), 1216 (C=S), 759(C-F)cm^{-1}; ^{1}H NMR: δ=7.26-7.64 (4H multiplet), δ=7.097 (N-H), 3.79 (4H quartet), 1.33 (6H triplet); ^{13}C NMR: δ=111.1, 112, 120.2, 130.6, 141.5, 162.2, 179.6, 51.6, 71.1, 62.7, 14.7; Elemental analysis: %Element, found(calcd.); %C, 52.03(52.16); %H, 5.32(5.47); %N, 10.03(10.138); %S 11.39 (11.60); %F, 20.49 (20.62). Molecular weight calculated=276.318. MS: *m/z*= 276.3.

1-(3-fluoro phenyl)-3-hexyl thiourea (5b)

$C_{13}H_{19}N_2SF$,Pale yellow solid,m.p.= 73-75°C; IR: 3210 (N-H), 3019, 2979 (C-H), 1216 (C=S), 759(C-F)cm^{-1}; ^{1}H NMR: δ=7.90 (1H, NH-CH_2), 6.93-7.42(4H multiplet aromatic),δ=6.09 (N-H), 1.59 (2H quartet), 1.28 (8H quartet), 0.86 (3 H triplet); ^{13}C NMR δ=111.1, 112, 120.2, 130.6, 141.5, 162.2, 179.6, 50.6, 31.1, 28.7, 29.7, 2.2, 23.5, 14.0; Elemental analysis: %Element, found(calcd.); %C, 61.03(61.38); %H, 7.32(7.52); %N, 11.03(11.01); %S 12.39(12.60); %F, 7.32(7.46).Molecular weight calculated 254.364, MS: *m/z*= 254.3.

1-(3-chlorobenzyl)-3(3-fluoro phenyl)thioureas (5c)

$C_{14}H_{12}N_2SFCl$, white solid, m.p.=72-74°C; IR: 3210 (N-H), 3019, 2979 (C-H), 1216 (C=S), 759(C-F)cm^{-1};^{1}HNMR:δ= 4.86(2H CH_2N), 6.95-7.05 (4H aromatic, chlorobenzene), 7.20-7.40 (4H aromatic fluorobenzene), 6.42 (NH), 8.39 (NH);^{13}C NMR:δ= 179.6, 162.4, 141.0, 143.8, 133.6, 130.4, 129.7, 127.5, 126.9, 125.2, 120.9, 112.3, 111.5; Elemental analysis: %Element, found (calcd.); %C, 57.13(57.04); %H, 4.02(4.10); %N, 9.43(9.50); %S 10.63(10.87); %F, 6.28(6.44), %Cl, 11.89.(12.02). Molecular weight calculated=294.755,MS: *m/z*= 294.

1,1 Diethyl-3(3- trifluoromethyl phenyl) thioureas(5d)

$C_{12}H_{15}N_2SF_3$,cream solid, m.p.=69-71°C; IR: 3210 (N-H), 3019, 2979 (C-H), 1216 (C=S), 759(C-F) cm^{-1}; ^{1}HNMR: δ=1.31 (6H tripletCH$_3$), 3.76 (4H quartetCH$_2$), 7.05(NH, s), 7.41-7.60 (4H aromatic);^{13}C NMR:δ= 127.0, 125.6, 119.3, 179.6, 47.5, 13.4;Elemental analysis: %Element, found (calcd.); %C, 52.01(52.16); %H, 5.29(5.47); %N, 10.02(10.138); %S 11.33(11.60); %F, 20.39(20.62); = 276. Molecular weight calculated= 276.318, MS: *m/z*=276.4.

1,1 Diethyl 3-(3-fluoro phenyl) thioureas (5e)

$C_{11}H_{15}N_2SF$, grey solid, m.p.=72-74°C; IR: 3210 (N-H), 3019, 2979 (C-H), 1216 (C=S), 759 (C-F)cm^{-1}; ^{1}HNMR: δ= 1.31(6H CH_3, triplet), 3.76(4H CH_2 quartet), 6.96 (N-H, broad) 7.05-7.30 (4H aromatic);^{13}C NMR: δ=179.6, 162.4, 141.0, 130.4, 120.9, 112.3, 47.5, 13.4; Elemental analysis: %Element, found(calcd.); %C, 58.16(58.37); %H, 6.32(6.67); %N, 12.03(12.37); %S 14.39(14.16); %F, 8.03(8.39).Molecular weight calculated= 226.3117, MS: *m/z*=226.3.

1,1 Diethyl 3-(2-fluoro phenyl) thioureas (5f)

$C_{11}H_{15}N_2SF$, grey solid, m.p.=70-72 °C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ= 1.33(6H CH_3, triplet), 3.79(4H CH_2 quartet), 7.06-7.92 (aromatic 4H), 6.96 (NH, broad); ^{13}C NMR: δ= 179.6, 156.9, 126.9, 126.4, 126.1, 124.4,115.8, 47.4, 13.4; Elemental analysis: %Element, found(calcd.); %C, 57.98(58.37); %H, 6.29(6.67); %N, 12.18(12.37); %S 14.09(14.16); %F,

8.06(8.39). Molecular weight calculated== 226.3117, MS: *m/z*=225.9.

1-(2-ethoxy ethyl)-3-(3-fluorophenyl) thioureas (5g)

$C_{11}H_{15}N_2OSF$, white solid, m.p.=86-88°C; IR: 3266 (N-H), 2975 (C-H), 970 (C-O), 1285 (C=S), 759(C-F)cm^{-1};^{1}HNMR: δ= 1.19 (3H, CH_3, triplet), δ= 3.34-3.81(multiplet 6H, OCH_2, NCH_2, proton), 6.7(NH, s), 6.93-7.34 (4H aromatic H), 8.47 (NH, s); ^{13}C NMR: 179.6, 162.4, 141.0, 120.9, 130.4, 111.5, 112.3, 71.5, 62.7, 51.0, 14.7; Elemental analysis: %Element, found(calcd.); %C, 54.16(54.52); %H, 6.34(6.23); %N, 11.39(11.56); %O, 6.47(6.60); %S, 13.37(13.23); %F, 7.63(7.84);Molecular weight calculated== 242.3116, MS: *m/z*= 242.1.

1,1 Diethyl-3-(4-trifluoromethyl-phenyl)-thiourea (5h)

$C_{12}H_{15}N_2SF_3$,cream solid, m.p.=70-73°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ= 1.08 (triplet, 6H, CH_3), 3.52(quartet, 4H, CH_2), 6.43-7.24(multiplet, 4H aromatic), 8.47 (NH, broad, s); ^{13}C NMRδ= 127.0, 125.6, 119.3, 179.6, 47.5, 13.4; Elemental analysis: %Element, found (calcd.); %C, 52.01(52.16); %H, 5.29(5.47); %N, 10.02(10.138); %S 11.33(11.60); %F, 20.39 (20.62); Molecular weight calculated=276.318, MS: *m/z*=276.

1,1 Dimethyl-3-(3-trifluoromethyl-phenyl)-thiourea (5i)

$C_{10}H_{11}N_2SF_3$,white solid, m.p.=70-72°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ= 2.52 (singlet 6H CH_3), 6.52-6.98 (multiplet, 4H aromatic), 8.47 (NH, broad, s); ^{13}C NMR δ= 131.5, 121.3, 129.1, 119.3, 122.1, 128.6, 179.6, 42.3; Elemental analysis: %Element, found(calcd.); %C, 48.03(48.22); %H, 4.37(4.76); %N, 11.02(11.24); %S 12.56(12.87); %F, 22.04 (22.88); Molecular weight calculated= 249.04, MS: *m/z*=249.

1,1 Dimethyl-3-(3-fluorophenyl)-thiourea(5j)

$C_9H_{11}N_2SF$,brown solid, m.p.=74-76°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ=2.62 (singlet, 6H, CH_3), 6.15-6.99(multiplet, 4H aromatic), 8.47 (NH, broad, s); ^{13}C NMR:δ= 179.6, 162.4, 141.1, 130.4, 120.9, 112.3, 111.5, 42.3; Elemental analysis: %Element, found(calcd.); %C, 54.08(54.52); %H, 5.12(5.59); %N, 14.48(14.12); %S 15.89(16.17); %F, 9.72(9.58); Molecular weight calculated= 198.25, MS: *m/z*=198.

1,1 Dimethyl-3-(2-fluorophenyl)-thiourea(5k): $C_9H_{11}N_2SF$, brown solid, m.p.=69-71°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ=2.47 (singlet, 6H CH_3), 6.44-6.78(multiplet, 4H aromatic)8.47 (NH, broad, s); ^{13}C NMR: δ= 179.6, 158.9, 126.4, 126.1, 124.1, 115.8, 42.3; Elemental analysis: %Element, found(calcd.); %C, 54.21(54.52); %H, 5.35(5.59); %N, 14.03(14.12); %S 15.99(16.17); %F, 9.42(9.58);Molecular weight calculated= 198.25, MS: *m/z*=198.1.

1,1 Dimethyl-3-(2-trifluoromethyl-phenyl)-thiourea (5l)

$C_{10}H_{11}N_2SF_3$, yellow solid, m.p.=71-73°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ=2.47 (singlet 6H, CH_3), 6.39-7.20 (multiplet, 4H aromatic),8.47 (NH, broad, s); ^{13}C NMR: δ= 179.6, 136.8, 132.1, 127.3, 125.6, 124.8, 110.8, 42.3; Elemental analysis: %Element, found(calcd.); %C, 48.09(48.22); %H, 4.53(4.76); %N, 11.09(11.24); %S 12.67(12.87); %F, 22.19 (22.88); Molecular weight calculated= 249.04, MS: *m/z*=249.0.

1,1 Dimethyl-3-(4-fluorophenyl)-thiourea (5m)

$C_9H_{11}N_2SF$,grey solid, m.p.= 76-78°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ= 2.47(singlet, 6H CH_3), 6.44-6.72 (double doublet, 4H aromatic), 8.47 (NH, broad, s); ^{13}C NMR:179.6, 158.1, 135.0, 126.9, 115.8, 42.3;Elemental analysis: %Element, found(calcd.); %C, 54.39(54.52); %H, 5.39(5.59); %N, 14.01 (14.12); %S 15.98(16.17); %F, 9.39(9.58);Molecular weight calculated= 198.25, MS: *m/z*=198.

1,1 Dimethyl-3-(4-trifluoromethyl-phenyl)-thiourea (5n)

$C_{10}H_{11}N_2SF_3$,cream solid,m.p.=76-78°C; IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F)cm^{-1}; ^{1}HNMR: δ=2.47(singlet 6H CH_3), 6.39-7.20 (double doublet, 4H aromatic), 8.47 (NH, broad, s); ^{13}C NMR: δ= 179.6, 142.7, 127.0, 125.6, 119.3, 42.3; Elemental analysis: %Element, found(calcd.); %C, 48.15(48.22); %H, 4.62(4.76); %N, 11.13(11.24); %S 12.61(12.87); %F, 22.13(22.88); Molecular weight calculated= 249.04, MS: *m/z*=249.

1-Phenylbutyl-3-(3-fluorophenyl)-thiourea (5o)

$C_{17}H_{18}N_2SF$, yellow solid, m.p.=80-82°C;IR: 3259 (N-H), 2976 (C-H), 1261 (C=S), 756 (C-F) cm^{-1};

^{1}HNMR: δ=1.55(multiplet 2H CH_2-**CH_2**-CH_2), 1.62(multiplet 2H CH_2-**CH_2**-CH_2Ph), 2.55 (triplet 2H, Ph-CH_2), 3.45(triplet, 2H N-CH_2), 7.08-7.21(multiplet 4H aromatic), 6.17-6.99(multiplet 4H aromatic),8.47 (NH, broad, s); ^{13}C NMR:111.5, 130.4, 120.9, 162.4, 141.8, 112.3, 179.6, 50.3, 31.5, 30.1, 35.5, 128.3, 125.7, 128.3, 139.4;Elemental analysis: %Element, found(calcd.); %C, 67.58(67.74); %H, 5.97(6.01); %N, 9.13(9.29); %S 10.19(10.63); %F, 6.01(6.30); Molecular weight calculated= 301.39, MS: *m/z*=301.

Measurement of anticancer activity

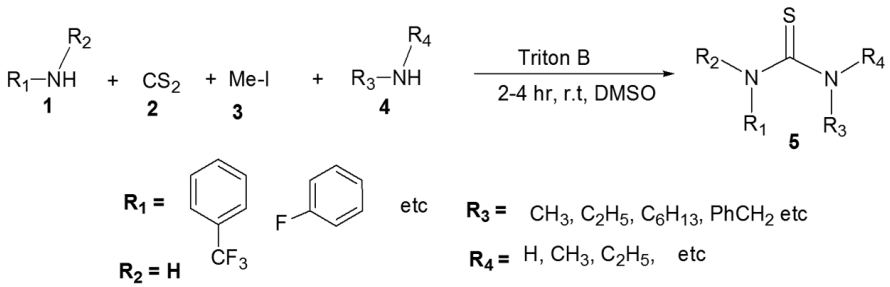
Different human cancer cell lines such as HeLa (cervical cell line), Colon cancer cell line (HCT-15); and glioblastoma (Brain cancer cells) U87-MG collected from National Cell Center, Pune, India, were used for our studies. The culturing of cell lines was done in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, US) while culturing of HCT-15 cells was done in (RPMI, Sigma-Aldrich, US). Most of the synthesized compounds were found to possess potential anticancer activity against HeLa (cervical cancer cell line), HCT-15(colon cancer cell line) and U-87 MG (brain cancer cell line). The IC_{50} values and the percentage viability of all compounds were obtained taking 100 µM, 50 µM, 25 µM and 12.5 µM and the results were very promising.

The cells of different cancers were seeded at 3 × 10^4 cells per well [in 100 µL of DMEM for HeLa and U87MG and RPMI for HCT-15 cells] containing 10% fetal bovine serum (FBS) in a 96-well tissue culture plate which were then incubated for 72 h at 37 °C, in a CO_2 incubator maintaining 90% relative humidity and 5% CO_2. After this, cancer cells were incubated with 100, 50, 25 and 12.5 µM concentrations of various synthesized trithiocarbobates prepared in DMEM/RPMI *i.e.* for 24 hr. Further, the cancer cells also incubated with 100 µL of MTT dye (0.5 mg/mL) in CO_2 incubator for next 4 h. The purple-colored formazan produced by the cells in the bottom of the wells. The culture medium was removed from each well. Following this, 100 µL of DMSO was added to each well. The formazan crystals in each well were dissolved which gave purple solution. Following this Labsystems Multiskan EX ELISA reader was used to measure the absorbance of the 96 well-plates at 570 nm against a reagent blank.IC_{50} for 50% inhibition of each drug was calculated in micromolar concentration. Simultaneously MTT assay was done in triplicate in three independent experiments.

Results and Discussion

We have synthesized the substituted thioureas by reacting substituted amines and CS_2 in presence of DMSO as solvent using Triton-B as phase transfer catalyst. The reaction was designed as given in the Scheme 1 and we tried this reaction by reacting 2-trifluoromethyl-phenyl amine with CS_2 in the presence of DMSO as solvent and Triton-B as phase transfer catalyst in the first step of the reaction in the second step methyl iodide and diethyl amine were added with constant stirring for 2 h. Simultaneously, checking was done for the formation of product with the help of TLC. The product (**5a**) thus formed was characterized and identified by spectroscopic and analytical techniques.

IR spectra of **5a** gave peaks at 1216 cm^{-1} which represent vibrations due to C=S asymmetric stretching. Vibrationof C-Sbond gives a broad band at 1080cm^{-1}. Vibrations found at 3210 cm^{-1} were due to N-H. This clearly indicates the presence of  moiety in the synthesized compounds. Proton NMR spectra of compound **5a** gave multiplet at *δ*=7.26-7.64 which is due to 4H protons of benzene ring, and broad peaks at 7.097 were due to N-H protons, while the peaks at 3.79 (2H quartet, CH_2), 1.33 (3H triplet, CH_3) which confirms the presence of two ethyl group in the compound**.** Again ^{13}C NMR gave peaks at



Scheme 1 — Synthesis of substituted thiourea by reacting substituted amines and CS_2 in presence of DMSO as solvent using Triton-B as phase transfer catalyst

δ=179.6 which confirms the formation of C=S in the compound. The elemental analysis also confirms the reported compound. Once the identity of the compound **5a** was established as 1,1 Diethyl-3-(2-trifluoromethylphenyl)-thiourea, we proceeded for the optimization of the process.

Firstly, the effect of solvents of the yield of a reaction with different solvents were studied wherein a reaction of substituted amine was tried with solvents such as dimethylformamide, dimethyl sulphoxide (DMSO), chloroform, dichloromethane, methanol, benzene, toluene, *n*-hexane, *n*-heptanesand out of them DMSO found to be most effective at room temperature (Table 1). The phase transfer catalyst makes better interaction among various reagents, thereby decreasing the time of the reaction and improves yield. During the optimization of the reaction process, study on different phase transfer catalyst was also undertaken. For this, we tried many phase transfer catalyst including tetra *n*-butyl ammonium chloride (TBAC), crown ether (18 crown 6), tetra-*n*-butyl ammonium bromide (TBAB), Basic resin (Amberlite IRA 400 in iodide form), tetra-*n*-butyl ammonium iodide (TBAI), tetra-*n*-butyl ammoniumhydrogen carbonate (TBAHC), tetra-*n*-butyl ammonium hydrogen sulphate (TBAHS) etc. The effect of phase transfer catalyst on the time and % yield of the product is given in the Table 2. It was found that Triton-B served 'best' in getting high yield of the desired substituted thioureas. The reaction was also tried in the absence of catalyst Triton B but no product formed. So it may be said that the reaction could occur only in the presence of catalyst.

Primary aliphatic amines reacted with carbon disulfide fast with better yield. Decreased nucleophilicity of primary aryl amines makes the reaction to proceed slowly and take longer time to yield product. Aromatic amines having electron donating groups increase nucleophilicity and give products in good yield. While electron withdrawing group decrease nucleophilicity and give poor yield. This process was optimized on small scale in laboratory conditions with excellent yield. The reaction is exothermic in nature and it was observed that the cooling phenomenon is required to increase the yield and decrease the reaction time. This process of synthesis of substituted thioureas can be transferred to large scale for commercial purposes.

After optimization for solvent and catalyst the reaction was performed with different types of alkyl and aryl amines and led to the formation of several substituted thiourea (compounds **5a**-**5o**) which are given in the Table 3. All the compounds were identified on the similar analogy on the basis of IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectra. The matching of IR, NMR data of the compounds obtained from the reactions agreed well with the compounds available in the literature.

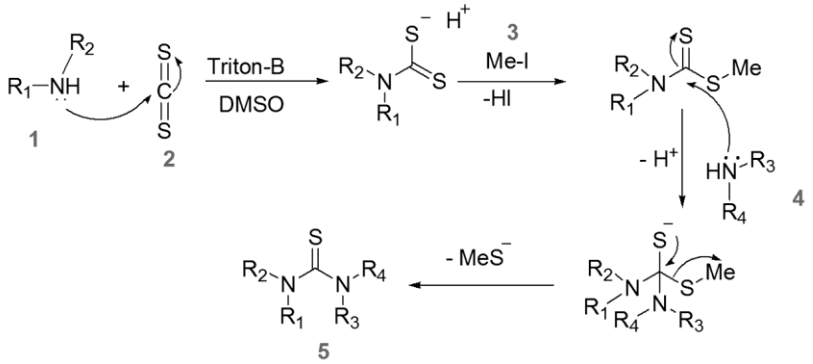
Table 1 — Effect of solvents on the yield of a reaction

S. No.	Solvent	Time (h)	Yield (%)
1	Dimethylformamide	3.0	50
2	Dimethyl sulphaoxide	2.0	66
3	Chloroform	3.5	40
4	Dichloromethane	3.2	50
5	Methanol	3.6	35
6	Benzene	4.0	38
7	Toluene	4.2	40
8	n-hexane	4.8	45
9	n-heptane	4.6	42

Table 2 — Effect of phase transfer catalysts on the yield of the reaction

S. No.	Phase transfer catalyst	Time (h)	Yield (%)
1	TBAC	3.5	45
2	TBAB	3.0	50
3	TBAI	3.1	52
4	TBAHC	3.8	40
5	TBAHS	3.9	42
6	Amberlite IRA 400 (Basic resin)	3.8	36
7	18 Crown 6	4.0	29
8	**Triton-B**	**2.0**	**66**
9	Absence of catalyst	No Reaction	0

Considering the mechanism of the reaction, it may be proposed that substituted amines (**1**) on reaction with CS_2(**2**) in the presence of phase transfer catalyst Triton-B and DMSO as medium led to the formation of dithiocarbamic acid. This dithiocarbamic acid when added with CH_3Iin the presence of DMSO led to the formation of methyl ester of dithiocarbamic acid. This ester reacted with nucleophilic centre of other added amine (**4**) and the intermediate thus formed yielded substituted thioureas on rearrangement with emission of methyl thiol. The mechanism of the reaction may be explained as given in the Scheme 2. Primary aliphatic amines reacted with carbon disulphide and gave excellent yield of thiourea in lesser time. The nucleophilic addition of primary aryl amines proceed slowly and take longer time due to decreased nucleophilicity. Aromatic amines having electron donating groups increase nucleophilicity and give products in good yield. While electron withdrawing group decrease nucleophilicity and give poor yield.



Scheme 2 — Proposed mechanism of mechanism of formation

Table 3 — Formation of various substituted thioureas (5a-5o) of general formula 5

Compound	R_1	R_2	R_3	R_4	Time (h)	Yield (%)
5a	2-trifluoromethyl-phenyl	H	C_2H_5	C_2H_5	2.0	66
5b	3-fluoro phenyl	H	n-hexyl	H	2.5	70
5c	3-chlorobenzyl	H	3-fluoro phenyl	H	3.0	73
5d	3- trifluoromethyl phenyl	H	C_2H_5	C_2H_5	3.0	74
5e	3-fluoro phenyl	H	C_2H_5	C_2H_5	4.5	72
5f	2-fluoro phenyl	H	C_2H_5	C_2H_5	4.7	70
5g	3-fluorophenyl	H	2-ethoxy ethyl	H	4.7	75
5h	4-trifluoromethyl-phenyl	H	C_2H_5	C_2H_5	4.0	78
5i	3-trifluoromethyl-phenyl	H	CH_3	CH_3	4.5	80
5j	3-fluorophenyl	H	CH_3	CH_3	2.0	66
5k	2-fluorophenyl	H	CH_3	CH_3	2.5	70
5l	2-trifluoromethyl-phenyl	H	CH_3	CH_3	3.0	73
5m	4-fluorophenyl	H	CH_3	CH_3	3.0	74
5n	4-trifluoromethyl-phenyl	H	CH_3	CH_3	4.5	72
5o	3-fluorophenyl	H	1-phenylbutyl	H	4.0	78

Anticancer activities

Various Substituted thiourea were tested for inhibition of proliferation against three human cancer cell lines *i.e.* HeLa (human cervicalcancer), HCT-15 (colon cancer) andU87-MG (brain cancer) using MTT assay. The survival of the cancer cells was reduced by specific increase in concentration ofcompounds against a fixed number of cancercells. The percentage viability of the cancer cells on giving 100 μMdose of the compounds are presented in Table 4. When the substituted thioure as derivatives were examined for *in-vitro* activity against HeLa cancer cell line it was found that almost all compounds of substituted thioureas derivatives are active against HeLa cell proliferation.Among the various compounds examined it was found that compounds**5c**, **5o** and **5b** have shown the bestinhibitory action againstthe proliferation of cervical cancer cells (HeLa). The IC_{50} values in μM of these compounds are given in Table 5. The percentage viability of HeLa cancer cells against the concentration of these substituted thioureas are given in the graphs (Fig. S2, Supplementary Data).

Table 4 — *In vitro* biological evaluation of substituted thioureas against cancer cell lines

Substituted thioureas		% Viability at 100 μM dose		
S. No.	Compound	HeLa	HCT-15	U87- MG
1	**5a**	73.63	68.14	61.83
2	**5b**	22.25	42.49	33.71
3	**5c**	21.56	42.87	38.19
4	**5d**	71.09	24.36	40.25
5	**5e**	53.90	52.49	51.42
6	**5f**	74.40	67.47	76.95
7	**5g**	65.22	71.07	75.90
8	**5h**	77.23	66.51	64.88
9	**5i**	65.68	76.33	70.65
10	**5j**	69.37	82.23	70.14
11	**5k**	8.47	81.78	70.75
12	**5l**	84.54	77.44	71.40
13	**5m**	77.90	64.84	71.58
14	**5n**	79.86	80.92	71.86
15	**5o**	21.93	53.40	40.28

it was found that almost all compounds are active against colon cancer cells (HCT-15) cell proliferation during the *in-vitro* examination, but the compounds **5d**, **5c** and **5b** have shown the highest activity to

Table 5 — Anticancer activity evaluation of compounds 5c, 5o, 5b against HeLa cancer cells

S. No.	Compound	IC_{50} HeLa
1	5c	30.26
2	5b	35.16
3	5o	30.87

Table 6 — Anticancer activity evaluation of compounds of 5d, 5c, 5b against colon cancer cells HCT-15

S. No.	Compound	IC_{50} values HCT-15
1	5c	78.25
2	5b	60.34
3	5d	49.40

Table 7 — Anticancer activity evaluation of compounds of 5d, 5c, 5b, 5o against brain cancer cells U87-MG

S. No.	Compound	IC_{50} values U-87MG
1	5d	73.62
2	5c	63.43
3	5b	62.80
4	5o	66.68

inhibit proliferation of HCT-15. The IC_{50} value of these compounds in μM for HCT-15 cells is presented in the Table 6.

The *in-vitro* examination of various compounds against Brain Cancer cell line (U87- MG), it is found that almost all compounds of substituted thioureas are active against U87-MG cell proliferation. But the compounds **5b**, **5c**, **5d** and **5o** have been found to be most active for inhibiting cell proliferation of brain cancer cell.The IC_{50} value of these compounds in μM for U87-MG cells are presented below in the Table 7. The percentage viability of U87-MG cancer cells against the concentration of the most active substituted thioureas are given in Fig. S3 (Supplementary Data).

Conclusion

We have developed an easy and efficient protocol for synthesis of substituted thioureas derivatives using cheaper and easily available CS_2 and amines using Triton-B as phase transfer reagent. This method generates substituted thioureas in good to excellent amount. This method exhibits substrate versatility, mild reaction conditions and experimental convenience. This protocol is believed to offer a more general method for formation of substituted thioureas derivatives. Many of these compounds have displayed good anticancer activity against cervical, colon and brain cancer cells. The compounds like **5b**, **5c**, **5d**, **5o** have shown exceptional anticancer activity against all three types of cancer cells and can be further assessed for *in vivo* anticancer activities.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/58776.

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