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Original Article

Design, Synthesis, Characterization, and Anti-Cancer Activity Evaluation of Novel Thiosemicarbazide Analogs

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ABSTRACT

Aim/Background: Across the world disease of cancer is one of the factors of death and to identify the emerging role of thiosemicarbazide analogs for the cancer disease it was decided to design, and synthesize novel thiosemicarbazide derivatives (5a-i) with *in-vitro* anticancer activity evaluation. **Materials and Methods:** Substituted benzoic acid, thionyl chloride, hydrazine hydrate, and ammonium thiocyanate were used to create a new series of thiosemicarbazide derivatives (5a-i). The final derivatives were characterized using ^{13}C and ^{1}H NMR, FTIR, and mass spectroscopy. The compounds were then analyzed for anticancer action with the *in-vitro* MTT assay method as well as by measuring the percentage of tumor growth on the B16F10 melanoma cell line, a clone of B16 cells. **Results:** Compounds 5a, 5b, and 5e outperformed and were comparable to the reference drug, doxorubicin. All three compounds have an electron-withdrawing group substitution and have shown remarkable anticancer effects. When compared to other substituted derivatives, the drug-likeness score evaluated by Swiss ADME online software confirmed the same fact that thiosemicarbazides with electron-withdrawing groups substitution like halogens, and nitro groups at different positions could work as excellent and promising anticancer agents. **Conclusion:** The study and the result of the study revealed that thiosemicarbazide moiety represents an important core and could be used as a template for further development of analogs having promising anti-cancer effects.

Keywords: Anticancer, B16F10 cell line, Melanoma, Thiosemicarbazide, MTT assay.

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INTRODUCTION

Cancer is a chief cause of death in every country and a significant impediment to extending life expectancy. In 2018, the World Health Organization (WHO) said that 18.1 million people across the globe had tumors along with 9.6 million died from the disease. By the end of 2040, these accounts will nearly double, with the fastest growth in the middle- and low-income nations, where more than two-thirds of the globe's cancers will occur. It is the cause of about 30 percent of immediate deaths in adults aged 30 to 69 years due to non-communicable diseases.[1-2] Skin tumor is the most frequent type of malignancy with a total of 106,110 new melanomas diagnosed this year (about 62,260 in men and 43,850 in women), and melanoma is estimated to kill about 7,180 people. It occurs globally in all races, though the risk is significantly higher in Caucasians due to the photoprotective effect of epidermal melanin.[3-5] About 75 to 80% of fair-skinned individuals with non-melanoma skin tumors have basal cell carcinomas and up to 25% have squamous cell carcinomas.[6]

Melanoma is a type of skin tumor that develops in cells that produce melanin (a type of indolic polymer found in the skin of both humans and animals).[7-9] Melanin is created by melanocytes, which are pigment cells in the skin that store them in membrane-bound organelles called melanosomes.[10] Vitiligo and albinism are two diseases caused by abnormal melanin synthesis (the lack of melanin synthesis).[11-12]

Thiosemicarbazide, a thiourea-based active nucleus, is an important metal ion chelator because of the presence of "self" donor atoms such as nitrogen and sulfur which further results in promising anti-cancer action of the thiosemicarbazide moiety possessing derivatives.[13-14] Due to their ability to chelate iron and copper ions in cancer cells, thiosemicarbazide derivatives were often thought to act as tumor inhibitors.[15-18] Many thiosemicarbazone derivatives have recently been synthesized, and their antitumor properties have been published such as 3-Aminopyridine (Triapine) which is a thiosemicarbazide analog that has been progressed into clinical trials and has potent anticancer and ribonucleotide reductase inhibitory activity.[19-20] Other thiosemicarbazides, such as Bp44Mt, DpC,

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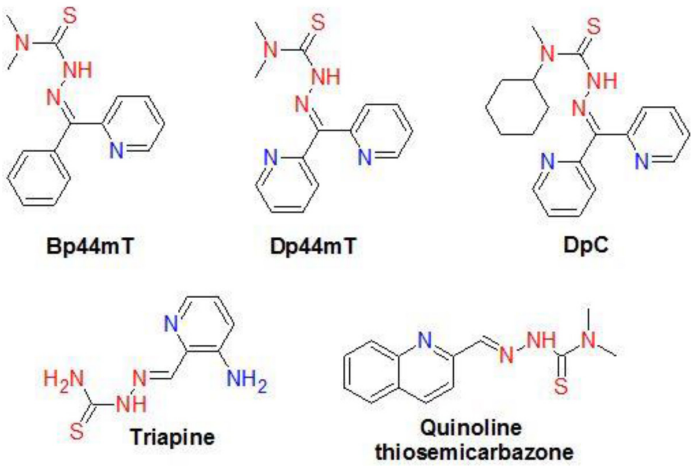

Figure 1: Examples of potent anticancer agents with thiosemicarbazide moiety.

and Dp44Mt, structures of them are shown in Figure 1, have potent anticancer activity.[21-22] As a result, thiosemicarbazide has emerged as a promising source for developing new anticancer drugs. Thiosemicarbzide inhibits cancer cell growth via different mechanisms, including deactivation of the ribonucleotide reductase enzyme,[23] cell cycle inhibition,[24] reactive oxygen species (ROS) production,[25] chelation of essential metal ions,[26] and its effect on proteins involved in cell life and death processes.[27]

Thiosemicarbazide analogs exhibited the different number of potential pharmacological potency such as antifungal,[28,29] antibacterial,[30-32] antiprotozoal,[33] antituberculosis,[34,35] anti-Trypanosoma,[36] anti-Alzheimers,[37] anti-inflammatory,[38] anti-urease,[39,40] antioxidant,[41,42] antiviral,[43,44] anticancer,[45,22] anticonvulsant,[46,47] tyrosinase inhibitory activities.[48] Melanoma remains the most aggressive skin cancer despite recent advances in therapeutic approaches, showing a five-year survival rate of only 15 to 20%.[49]

Based on the fast incidence of skin tumors and the lack of efficient drugs as well as drug delivery systems, it is necessary to explore possible ways to cure or prevent the disease. Thiosemicarbazide analogs are also reported to have good anticancer activity because of their metal-chelating action thus it was decided to carry some novel route synthesis of thiosemicarbazide derivatives further with their *in-vitro* activity of anticancer evaluation on skin cancer cell line of melanoma type.

MATERIALS AND METHODS

The required chemicals and solvents were bought from E. Merck India Ltd. and CDH (Central Drug House) and used without additional purification. Clean and sterile laboratory equipment was utilized. M.Ps of all the compounds were recorded by a digital-melting- point device (Lab India Melting Point instrument) using single-end open capillary tubes and were not corrected. The reaction progress and the homogeneity of the derivatives were observed on thin-layer-chromatography (TLC) plates through a solvent system of n-hexane and ethyl acetate. Proton nuclear magnetic-resonance (^{1}HNMR) spectra of the pure compounds were obtained on a Bruker Advance AVIII HD-300MHz spectrophotometer. The chemical shift value (δ (delta)) was observed in parts-per-million (ppm). The infra-red (IR) spectrum of the compounds were obtained on a Perkin-Elmer 65 FTIR spectrophotometer by employing potassium-bromide (KBr) pellets and the Mass-spectrometry spectral data of the compounds were obtained on a UPLC-TQD mass spectrometer instrument. A Perkin-Elmer 240 analyzer was equipped for elemental analysis (hydrogen (H), carbon (C), nitrogen (N)). The physicochemical characterization data for the final compounds (5a-5i) were provided in Table 1.

General Procedure for preparation of substituted carbamothioyl methanehydrazonate (substituted thiosemicarbazide derivatives) (5a-i)

Synthesis of substituted thiosemicarbazide analogs (5a-5i) was done in accordance with the scheme shown in Figure 2. During the process of synthesis substituted benzoic acid (1a-i) (0.1 mol) was treated with 5gm concentrated sulfuric acid in the presence of 50 ml absolute methanol at a temperature of 75-80°C for 4-5hr to produce the first intermediate substituted methyl benzoate (2a-i), which was further reacted with thionyl chloride (0.01 mol) with continuous stirring at 5-10°C for 30-35 min in the ice bath. Intermediates were obtained (3a-i) which were treated with hydrazine- hydrate (0.25 mol) in the presence of 50 ml absolute methanol at 80°C for 4-5 hr resulting in the formation of substituted benzoyl methanehydrazonate (4a-4i). Compounds (4a-i) (0.035 mol) were further reacted with ammonium thiocyanate (0.1 mol) and absolute methanol (50 ml) in the round bottom flask at 75-81°C for 3-4hr after which the final substituted benzoyl carbamothioyl methane hydrazonate (5a-i) compounds were obtained. The completion of the reaction is assessed by utilizing TLC with an ethyl-acetate solvent system and n-hexane in a ratio of 6:2. Then the solid was thoroughly washed with ethyl-acetate and filtered. All the synthesized compounds were recrystallized from absolute methanol. The yield was 50-80%.

Table 1: *In vitro* Anticancer Activity of synthesized compounds by MTT test.

Concentration	5a	5b	5e	5g
10	77.56	54.25	75.03	66.74
1	54.28	32.65	51.2	32.51
0.1	18.62	12.62	17.89	12.59
0.01	12.55	5.65	11.78	2.36
0.001	3.22	3.15	3.17	1.44
IC_{50} value (µg/ml)	0.7	6	0.9	3
Doxorubicin	0.6			

Physical and spectral data of the compounds (5a-5i)

Synthesis of 4-chlorobenzoyl carbamothioylmethanehydrazonate (5a)

Yield 82%, melting point (M.P):178-179°C, Retention factor (Rf): 0.56, IR (KBr) (CM^{-1}): 3308.02 (N-H), 3211.47 (N-H), 3016.84 (C-H Ar), 2897.63 (C-H Alkene), 1711 (C=O), 1615.48 (C=N), 1558.36 (C=S), 1108.06 (C-O), 772.06 (C-Cl); ^{1}H-NMR (dimethyl sulphoxide d6(DMSO-d6) δ/ppm: 9.86 (s, 1H, CH=N), 7.87 (d, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.46 (d, 1H, Ar-H), 4.53 (s, 1H, NH), 3.17 (s, 2H, NH);^{13}C-NMR(dimethyl sulphoxide d6(DMSO-d6) δ/ppm: 172.45 (C=O), 186.92 (C=S), 164.95 (CH=N), 128.7-131.5 (Ar, 6C), 139.0 (C-Cl); electrospray-ionization mass-spectrometry (ESI-MS): 257 ($M^{+}H^{+}$).

Synthesis of 3,5-dinitrobenzoyl carbamothioylmethanehydrazonate (5b)

Yield 79%, melting point (M.P): 174-175°C, Retention factor (Rf): 0.42, IR (KBr) (cm^{-1}): 3384.52 (N-H), 3304.53 (N-H), 3187.32 (C-H Ar), 2913.74 (C-H Alkene), 1636.95 (C=O), 1602.95 (C=N), 1570.83 (C=S), 1273.80 (C-O), 1510.8 (NO_2); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 9.92 (s, 1H, CH=N), 7.74 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 4.55 (s, 1H, NH), 3.16 (s, 2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 165.58 (C=O), 185.85 (C=S), 149.56 (CH=N), 101.81-120.09 (Ar, 4C), 147.56 (C-NO_2); electrospray-ionization mass-spectrometry (ESI-MS): 313 ($M^{+}H^{+}$).

Synthesis of 3,4-dichlorobenzoyl carbamothioylmethanehydrazonate (5c)

Yield 68%, melting point (M.P): 177-178°C, Retention factor (Rf): 0.58; IR (KBr) (CM-1): 3351.96 (N-H), 3298.72 (N-H), 3078.45 (C-H Ar), 2932.91 (C-H Alkene), 1709.44 (C=O), 1603.85 (C=N), 1563.89 (C=S), 1150.87 (C-O), 779.65 (C-Cl); 1H-NMR (dimethyl sulphoxide d6(DMSO-d6) δ/ppm: 9.89 (s, 1H, CH=N), 7.90 (s, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.62 (d, 1H, Ar-H), 4.58 (s, 1H, NH), 3.42 (s, 2H, NH); 13C-NMR(dimethyl sulphoxide d6(DMSO-d6) δ/ppm: 174.49 (C=O), 183.96 (C=S), 149.85 (CH=N), 122.28-136.94 (Ar, 6C), 47.80 (C-Cl); electrospray-ionization mass-spectrometry (ESI-MS): 290 (M+H+).

Synthesis of 2-chlorobenzoyl carbamothioylmethanehydrazonate (5d)

Yield 61%, melting point (M.P): 169-170°C, Retention factor (Rf): 0.57, IR (KBr) (cm^{-1}): 3304.69 (N-H), 3332.78 (N-H), 3064.71 (C-H Ar), 2885.32 (C-H Alkene), 1712.1 (C=O), 1617.83 (C=N), 1562.98 (C=S), 1258.79 (C-O), 754.30 (C-Cl); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 9.74 (s, 1H, CH=N), 7.88 (d, 1H, Ar-H), 7.79 (t, 1H, Ar-H), 7.52 (t, 1H, Ar-H), 7.44 (d, 1H, Ar-H), 4.59 (s, 1H, NH), 3.25 (s, 2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 169.58 (C=O), 179.94 (C=S), 147.36 (CH=N), 136.48-125.92 (Ar, 6C), 54.65 (C-Cl); electrospray-ionization mass-spectrometry (ESI-MS): 256 ($M^{+}H^{+}$).

Synthesis of 4-bromobenzoyl carbamothioylmethanehydrazonate (5e)

Yield 72%, melting point (M.P): 176-177°C, Retention factor (R_f): 0.71, IR (KBr) (cm^{-1}): 3378.64 (N-H), 3317.93 (N-H), 3023.93 (C-H Ar), 2889.41 (C-H Alkene), 1716.20 (C=O), 1611.34 (C=N), 1565.89 (C=S), 1150.33 (C-O), 611.78 (C-Br); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 99.83 (s, 1H, CH=N), 7.82 (d, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.49 (d, 1H, Ar-H), 4.60 (s, 1H, NH), 3.27 (s, 2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: = 178.45 (C=O), 181.92 (C=S), 162.71 (CH=N), 119.7-131.5 (Ar, 6C), 60.8 (C-Br);electrospray ionization mass spectrometry (ESI-MS): 300 ($M^{+}H^{+}$).

Synthesis of 2-chloro-4-fluorobenzoyl carbamothioylmethanehydrazonate (5f)

Yield 66%, melting point (M.P): 191-192°C, Retention factor (R_f): 0.23, IR (KBr) (cm^{-1}): 3348.56 (N-H), 3295.85 (N-H), 3035.33 (C-H Ar), 2907.33 (C-H Alkene), 1702.66 (C=O), 1608.74 (C=N), 1557.42 (C=S), 1274.37 (C-O), 1223.2 (C-F), 754.52 (C-Cl); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 9.98 (s, 1H, CH=N), 7.73 (d, 1H, Ar-H), 7.52 (d, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 4.58 (s, 1H, NH), 3.20 (s, 2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 177.47 (C=O), 180.94 (C=S), 164.74 (CH=N), 128.7-131.5 (Ar, 6C), 39.0 (C-Cl), 52.36 (C-F); electrospray-ionization mass-spectrometry (ESI-MS): 274 ($M^{+}H^{+}$).

Synthesis of 4-aminobenzoyl carbamothioylmethanehydrazonate (5g)

Yield 75%, melting point (M.P): 181-182°C, Retention factor (R_f): 0.36, IR (KBr) (cm): 3382.69 (N-H), 3321.65 (N-H), 3089.54 (C-H Ar), 2920.14 (C-H Alkene), 1690.52 (C=O), 1623.80 (C=N), 1548.85 (C=S), 1180.97 (C-O); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 8.60 (s, 1H, CH=N), 7.61 (d, 1H, Ar-H), 7.58 (d, 1H, Ar-H), 7.14 (d, 1H, Ar-H), 7.09 (d, 1H, Ar-H), 6.55 (s, 1H, NH), 3.47 (s, 2H, NH) 3.15 (s, 2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 166.62 (C=O), 182.32 (C=S), 150.69 (CH=N), 110.7-120.5 (Ar, 6C), 151.9 (C-NH_2); electrospray-ionization mass-spectrometry (ESI-MS): 238 ($M^{+}H^{+}$).

Synthesis of 2-methoxybenzoyl carbamothioylmethanehydrazonate (5h)

Yield 79%, melting point (M.P): 176-177°C, Retention factor (R_f): 0.63, IR (KBr) (cm^{-1}): 3382.74 (N-H), 3289.11 (N-H), 3065.10 (C-H Ar), 2896.04, 2835.5 (C-H Alkene), 1750.40 (C=O), 1603.87 (C=N), 1564.78 (C=S), 1254.12 (C-O); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 9.96 (s, 1H, CH=N), 7.89 (d, 1H, Ar-H), 7.81 (t, 1H, Ar-H), 7.54 (t, 1H, Ar-H), 7.39 (d, 1H, Ar-H), 4.61 (s, 1H, NH), 4.08 (s, 3H, OCH_3), 3.35 (s,

2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 173.45 (C=O), 184.22 (C=S), 154.23 (CH=N), 114.7-131.1 (Ar, 6C), 56.12 (C-CH_3); electrospray-ionization mass-spectrometry (ESI-MS): 252 (M^+H^+).

Synthesis of 4-methoxybenzoyl carbamothioylmethanehydrazonate (5i)

Yield 77%, melting point (M.P): 171-172°C, Retention factor (R_f): 0.66, IR (KBr) (cm^{-1}): 3390.46 (N-H), 3301.61 (N-H), 3071.49 (C-H Ar), 2890.78, 2840.2 (C-H Alkene), 1738.78 (C=O), 1610.27 (C=N), 1566.32 (C=S), 1240.39 (C-O); ^{1}H-NMR (dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 10.09 (s, 1H, CH=N), 7.84 (d, 1H, Ar-H), 7.78 (d, 1H, Ar-H), 7.60 (d, 1H, Ar-H), 7.44 (d, 1H, Ar-H), 4.63 (s, 1H, NH), 4.12 (s, 3H, OCH3), 3.39 (s, 2H, NH); ^{13}C-NMR(dimethyl sulphoxide d_6(DMSO-d6) δ/ppm: 172.38 (C=O), 179.23 (C=S), 141.50 (CH=N), 112.7-127.3 (Ar, 6C), 50.12 (C-CH_3); electrospray-ionization mass-spectrometry (ESI-MS): 253 (M^+H^+)

PHARMACOLOGICAL EVALUATION

All experimental treatments were carried out according to CPCSEA guidelines and licensed by the foundation's IAES (1410/c/11/ CPCSEA). Chemicals utilized in pharmacological studies were obtained from Merck and Himidia. To acclimatize, at laboratory levels, the animals were kept in individual cages for a week. They were given free access to water and food.

In-vitro Anticancer Activity Evaluation

The anticancer activity of the synthesized derivatives was done by the use of a melanoma cancer cell line which was B16F10 cell line and activity was evaluated by two methods:

MTT Assay Method

MTT [(3-(4,5-dimethylthizol-2-yl)-2,5- diphenyl tetrazolium bromide)] is a light-yellow substrate from which dark blue formazan is extracted by live cells. This procedure is based on the fact that even recently dead cells do not break down MTT substrate. Therefore, MTT is proportionate to the viable cell number present, as determined by colorimetric methods. This was done by utilizing the standard operating procedure, in brief, derivatives were dissolved in DMSO and the concentration was sequentially diluted with a medium to obtain a test concentration range. The concentration of DMSO remained more than 0.1 percent in all B16F10 samples, seeded in plates of 96-well and treated with various concentrations of the test samples, after that incubated at 5% CO_2, 37°C for 96 hr. Then the MTT reagent was added to the wells and further incubated for 4 hr. Under the safety cabinet, the formation of dark-blue formazan product produced by the cells takes place which was dissolved in DMSO and read at 570nm.

Inhibition of tumor growth evaluation

The percentage inhibition was determined and plotted against the concentrations that were utilized to derive the IC_{50} values.[50] Reduction in the % of tumor growth was also evaluated on B16F10 cell line. For the same method, mice were housed in a separately ventilated cage system on a 12hr light-dark cycle. The animals were handled in a laminar-airflow environment. The humidity and noise levels were controlled and the animals were given free access to fed autoclaved commercial pellets and water. Mice aged 8 to 10 weeks were utilized in the experiment. B16F10 cells 1×105 were injected into the backs of mice and allowed to initiate palpable tumors. Tumors in experimental animals were minced and regrafted.[51] The administered test samples were taken once the tumor had reached palpable size. Doxorubicin and test samples were administered for 18 days. The volume of the tumor was assessed with digital vernier callipers (Mitutoyo JAPAN).

Tumor–volume calculated by volume = (width) 2 × length /2.

RESULTS

Chemistry

The synthesis of the titled compounds substituted benzoyl carbamothioylmethanehydrazonate (5a-5i) was finished as per the scheme shown in Figure 2. The ^{1}H NMR spectra were verified for the proton-related signals of the titled compounds and the structures were established. The IR spectrum of all compounds clearly disclosed the absorption of the amino group in the range of 3390.46-3211.47 cm^{-1}, and absorption in the range of 1570.83-1548.85 cm^{-1} indicates the formation of C=S, which represents the formation of thiosemicarbazide moiety. In the mass spectra, the molecular ion peaks correspond to the molecular weights of all derived compounds (5a–5i). Elemental analysis data for all compounds agreed with theoretical values and detailed spectral data is provided in the experimental protocol.

Evaluation of Pharmacological action *In-vitro* Anticancer activity Evaluation

The MTT assay results are shown in Table 1, Figure 3, and Figure 4. In the MTT test performed it was analyzed from the result obtained that the compound 4-chlorobenzoyl carbamothioyl methane hydrazonate (5a) and 4-bromobenzoyl carbamothioyl methane hydrazonate (5e) exhibited significant activity with IC_{50} value 0.7 and 0.9 µg/mL when compared with reference drug doxorubicin IC_{50} value 0.6 µg/mL. For the anticancer activity evaluation, another in vitro method for checking the anticancer activity of two compounds 5a and 5b in the form of a percentage of tumor growth in B16F10 cancer cell line was also used. The result of the test was also related to the standard doxorubicin. 8-10 weeks old mice were utilized for the experiment. B16F10 cells 1× 105 were injected into the back of mice and allowed to form palpable tumors. Tumors in experimental animals were

minced and regrafted. Test samples were administered once palpable tumor size was reached. Doxorubicin and test samples were administered for 18 days. The volume of the tumor was assessed with digital vernier calipers (Mitutoyo, JAPAN).

Tumor-volume calculated by volume = $(\text{width})^2 \times \text{length}/2$.

The result of the study was given in Table 2, Figure 5 and Figure 6.

Drug likeness Score

The drug-likeness scores of the derived compounds were estimated using the SwissADME online server tool (data stated in Table 3).

DISCUSSION

In the scheme, various substituted benzoic acid was treated with concentrated sulfuric acid in the presence of absolute methanol to produce the first intermediate substituted methyl benzoate, which on further reaction with thionyl chloride by dropper with continuous stirring led to the formation of the intermediate substituted chloromethyl benzoate which was then reacted with hydrazine hydrate in the presence of absolute methanol resulting in the formation of various substituted benzoyl methanehydrazonate (4a-4i). Compounds (4a-i) on further reaction with ammonium thiocyanate yielded the corresponding final compounds (5a-5i). The chemical composition of the synthesized compounds was in complete agreement with the spectral data obtained.

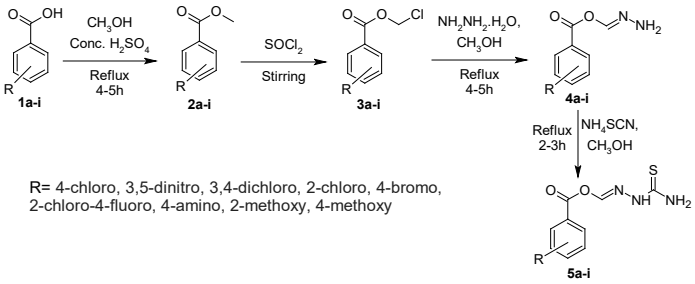

Figure 2: Scheme for the synthesis of thiosemicarbazide derivatives.

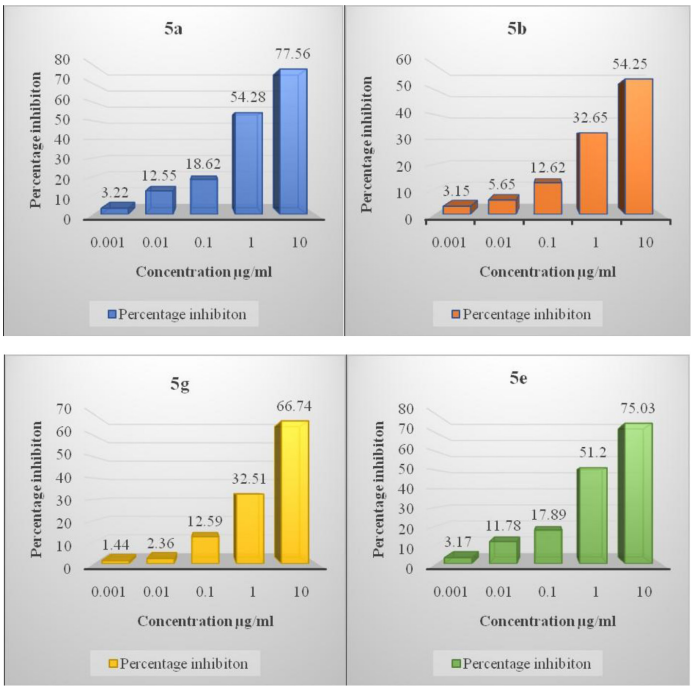

Figure 3: Percentage inhibition of cell growth by synthesized compounds.

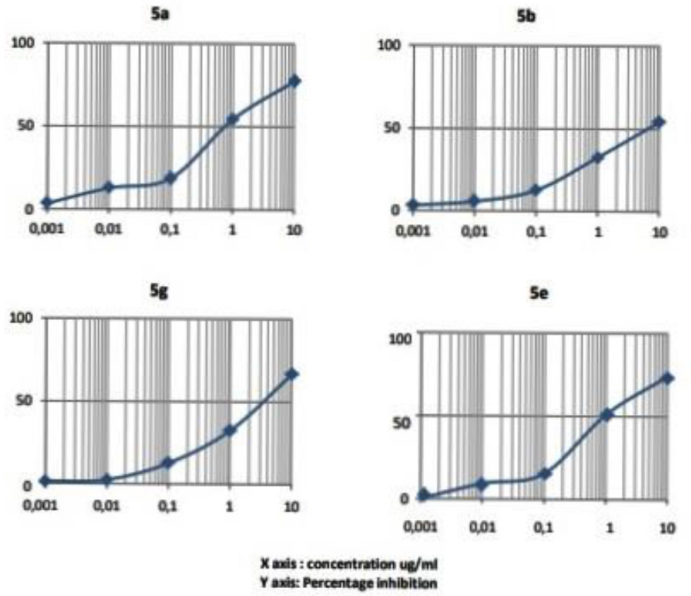

Figure 4: Graph showing percentage inhibition of cell growth by synthesized compounds.

Table 2: Anticancer Activity against B16F10 Melanoma Cell Line.

	% Tumor Growth			
Days	UT	Dox	5a	5b
0	100.0	100.0	100.0	100.0
10	266.9	126.7	208.2	222.4
18	392.6	202.8	268.3	296.7

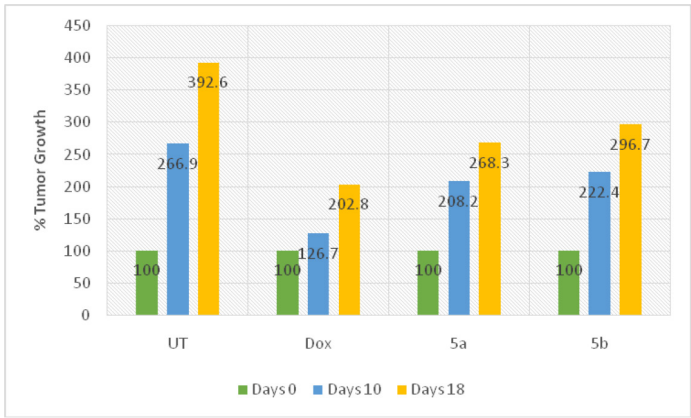

Figure 5: Reduction of tumor growth in B16F10 cell line by comparison with standard drug doxorubicin.

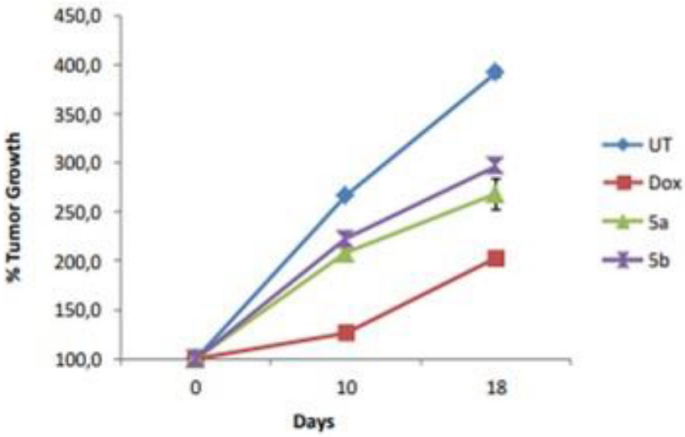

Figure 6: Graph showing inhibition of tumor growth in B16F10 cell line by comparison with standard drug doxorubicin.

Table 3: Drug Likeness Score of the Title Compounds.

Sl. No.	Drug	Drug likeness score
1	5a	0.56
2	5b	0.55
3	5c	0.07
4	5d	0.46
5	5e	0.54
6	5f	0.46
7	5g	0.04
8	5h	0.47
9	5i	0.25

The synthesized molecules (5a, 5b, 5e, 5g) were further screened for *in vitro* anticancer potency using MTT Assay and by calculating the percentage of tumor growth in the B16F10 cancer cell line by taking doxorubicin as a standard drug. The compound 4-chlorobenzoyl carbamothioylmethanehydrazonate (5a) and 4-bromobenzoyl carbamothioylmethanehydrazonate (5e) exhibited significant activity with IC_{50} value 0.7 and 0.9 µg/mL when compared with reference drug doxorubicin IC_{50} value 0.6 µg/mL. The tested compounds 5a and 5b demonstrated a significant reduction in the percentage of tumor growth as 268.3 and 296.7 after a total of 18 days when compared with tumor growth of untreated and standard drug doxorubicin-treated animals.

Results of the drug-likeness scores of the derived compounds showed that among all of the synthesized thio semi carbazide analogs good drug-likeness scores of 0.56, 0.55, and 0.54, respectively, were acquired for compounds 5a, 5b and 5e with chloro, dinitro and bromo substitutions. This result adds support to the fact that among all the synthesized analogs higher anticancer activity evaluated from both *in vitro* methods was observed to be highest for electron-withdrawing substituted compounds.

CONCLUSION

Thus, in the conclusion of the whole study, novel thiosemicarbazide analogs (5a-5i) were synthesized, characterized, and screened for anticancer activity by two *in vitro* methods. From both *in vitro* study results, it was observed that three compounds 5a, 5b, and 5e showed potent anticancer activity when compared to standard drug doxorubicin. The same result of potency was also supported through the drug likeliness score which settles the fact that synthesized thiosemicarbazide derivatives with electron-withdrawing group substitution act as potent anticancer compounds when compared with analogs having electron-donating substitutions. The research study accentuates the importance and scope of thiosemicarbazide pharmacophore as anti-cancer agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IR: Infrared; **^{1}H NMR:** Proton nuclear magnetic resonance; **^{13}C NMR:** Carbon-13 nuclear magnetic resonance; **TLC:** Thin layer chromatography; **h:** Hour; **%:** Percentage; **°C:** Degree Celsius; **min:** Minute; **mol:** Mole; **M.P:** Melting point; **R_f:** Retardation factor; **µg:** Microgram; **M:** Molar; **ml:** Milliliter; **nm:** Nanometer; **KBr:** Potassium bromide; **MHz:** Megahertz; **DMSO:** Dimethyl sulfoxide; **d:** Doublet; **MS:** Spectroscopy; **MTT:** [(3-(4,5-dimethythizol-2-yl)-2,5-diphenyl tetrazolium bromide)]; **UV:** Ultra violet; **UT:** Untested; **Dox:** Doxorubicin; **ppm:** Parts per million; **ESI:** Electrospray Ionization; **Dp44mT:** di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone; **DpC:** di-2- pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone; **Bp44mT:** 2-benzoylpyridine 4,4-dimethyl-3-thiosemicarbazone.

SUMMARY

The work discussed in the paper describes the novel scheme which was adopted for the synthesis of nine new thiosemicarbazide derivatives which were synthesized in 4 different sequential steps. The structural confirmation of all new compounds was done by spectral analysis. Then the novel compounds were screened for anti-cancer potential by two *in-vitro* tests among which first was the MTT test and the other was done to find out the percentage of tumor growth on the B16F10 melanoma cell line, a clone of B16 cells. In the result of both the anticancer tests, it was observed that thiosemicarbazide analogs with electron-withdrawing group

substitutions showed good anti-cancer activity when compared to compounds with electron releasing groups substitution. The drug-likeness score when obtained with online Swiss ADME software also confirmed the same fact that thiosemicarbazide compounds when attached to halogens, nitro groups could serve as promising anti-cancer agents thus whole study result could be used as a template in the future for more *in-vivo* studies for the development of more potential analogs with optimum activity.

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