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Article · January 2021

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ARTICLE

Design, Synthesis, Characterization, Evaluation for Anticancer and Cytotoxic Properties of New Pyrazole Carbothioamides

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Asian Journal of Organic & Medicinal Chemistry

Volume: 6 Year: 2021
Issue: 1 Month: January–March
pp: 53–58
DOI: https://doi.org/10.14233/ajomc.2021.AJOMC-P291

Received: 19 December 2020
Accepted: 3 March 2021
Published: 24 March 2021

ABSTRACT

Chemotherapy against specific molecular targets is one of the most effective approaches used to treat cancer patients. However, lack of selectivity and development of drug-resistance reduces the efficacy of cancer chemotherapy. Therefore, development of effective and safe anticancer agents with high potency and less toxicity is a major focus for researchers across the world. In the current article, the utility of a reverse ligand similarity based approach to identify potential targets for a new series of synthesized pyrazole carbothioamides that demonstrate the potent anticancer activities against MCF-7 cells compared to other structurally related molecules and controls is discussed. Further, *in silico* docking analysis provided insights into their sight of binding. Thus, these compounds show promise for development as next generation anticancer drugs.

KEYWORDS

Annulation, Anticancer, Chemotherapy, Citrus, Pyrazoline.

INTRODUCTION

Cancer is a disease characterized by the progressive, persistent, abnormal and uncontrolled proliferation of malignant cells. It is the second cause of mortality in the world and continuing to be a major health hazard all over the world [1]. The existing chemotherapeutic agents, like 5-fluorouracil, cisplatin, *etc*. used to treat cancer have side-effects and difficult to discriminate the drug target across the malignant and normal cells [2]. Therefore, design and development of small molecules with potent anticancer activity with lesser side effects with higher bioactivities is important for the treatment of cancer [3,4]. Heterocycles, like pyrazole carbothioamides, represent important scaffolds for medicinal and pharmaceutical applications due to their ability to mimic structures of peptides and their property of reversibly binding to proteins [5]. Modification of pyrazole carbothioamide nucleus by varying substitutions led to a number of lead molecules with selective CB1 antagonists [6], PKM2 activation [7], anti-tubercular [8], antitumor [9], antifungal [10], antidiabetic [11] and anti-inflammatory [12] activities.

Literature reveals that the pyrazoles were synthesized by the reaction of cyanothioacetamide with pyrazole-3(5)-diazonium chlorides to afford pyrazolo[5,1-*c*][1,2,4]triazine-3-carbothio-

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amides [13], Vilsmeier-Haack reaction of acetophenone phenylhydrazines [14], 1,3-dipolar cycloaddition (3+2) diaroyl hydrazones/hydrazineto alkenes [15]. Phenylhydrazines to *N*-aryl maleimides [16] using chloramine-T as catalytic dehydrogenating agent. Recent reports on the pyrazole synthesis involves, the copper-catalyzed condensation reaction of phenylhydrazine and 1,3-diketones [17], regioselective synthesis by the reactions of *N*-alkylated tosylhydrazones and terminal alkynes mediated by aluminium chloride [18], one-pot synthesis involving ketones, aldehydes and hydrazine hydrochloride through *in situ* oxidation with bromine [19], silver-mediated [3+2] cycloaddition of *N*-isocyanoiminotriphenyl phosphorane to terminal alkynes [20].

It was emphasized that pyrazole scaffolds are thought to be promising molecules with potential applications in medicinal chemistry. We report herein the synthesis of a series of pyrazole carbothioamides, *in vitro* evaluation for their anticancer and citotoxic properties, detailed ligand-similarity search and *in silico* docking analysis.

EXPERIMENTAL

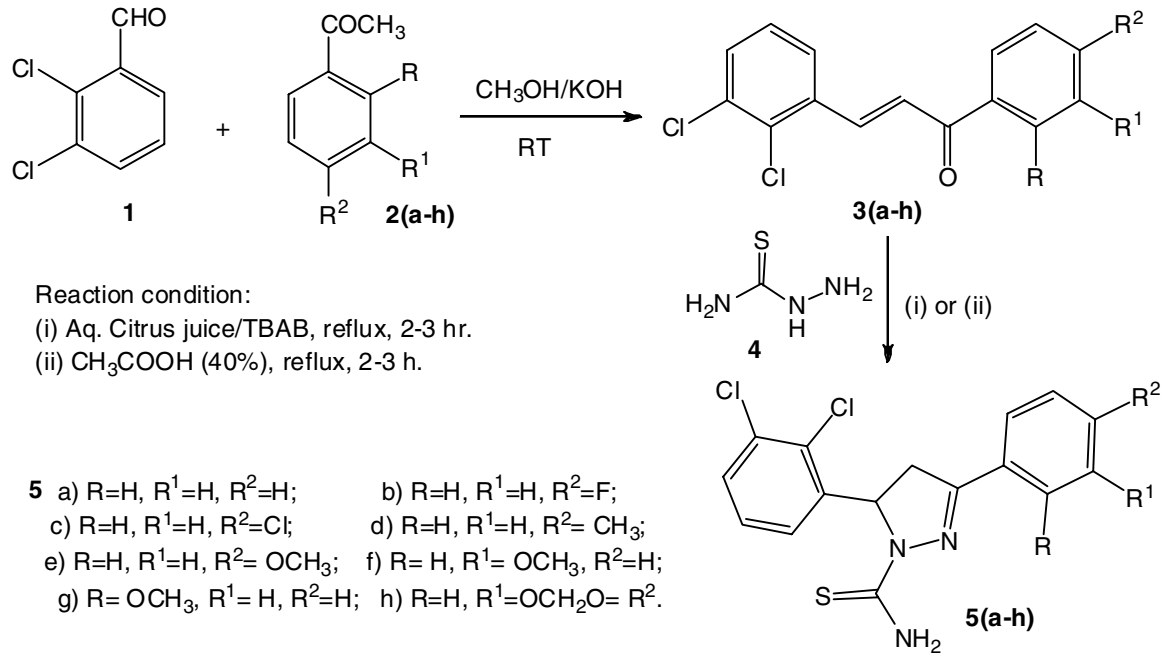
In search of new potent anticancer small molecules, herein we report the environmentally benign synthesis of pyrazole carbothioamides. Initially, the required intermediate chalcones (**3a-h**) were synthesized by the base catalyzed Claisen-Schmidt reaction of 2,3-dichlorobenzaldehyde (**1**) and substituted acetophenone (**2a-h**) in methyl alcohol according to our earlier reports [21]. The reaction of chalcones (**3a-h**) and thiosemicarbazide (**4**) in citrus extract medium in the presence of tetrabutylammonium bromide (TBAB) under reflux conditions produced pyrazole carbothioamides (**5a-h**). Alternatively, the reaction of **3a-h** with **4** in methyl alcohol and 5-6 drops of acetic acid (40%) under reflux conditions yielded the target pyrazoline carbothioamides (**5a-h**) in good yields (**Scheme-I**).

Extraction of juice (**citrus extract**): Orange lemons bought from the locally grown lemon trees. Lemons were squeezed to urge the pulp juice (100 mL) into a beaker, diluted with water (50 mL) and then well agitated to a fine solution with the help of mechanical stirrer. The solution warmed for 30 min at 45-50 ºC and filtered to urge fine juice and diluted to 30% with water [22].

Synthesis of 3-aryl-5-(2,3-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamides (5a-h): A solution mixture of chalcones (**3a-h**) (10 mmol), thiosemicarbazide (**4**, 15 mmol), and tetrabutylammonium bromide (TBAB) (0.001mmol) in freshly prepared juice (30 mL, aq. 40%), was mixture refluxed on a water bath for 2-3 h. After the completion, the reaction mixture was filtered and the filtrate quenched into the crushed ice. The separated solids were filtered and washed successively with 5% $NaHCO_3$ and water; the crude solids crystallized from ethyl alcohol to get target molecules **5a-h**. Alternatively, the reaction was conducted with a solution mixture of chalcones (**3a-h**) (10 mmol) and thiosemicarbazide (**4**, 15 mmol) in acetic acid (40%) under reflux conditions for 2-3 h.

5-(2,3-Dichlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**5a**): Yield 78%; m.p.: 109-112 ºC; IR (KBr, ν_{max}, cm^{-1}): 3226 (NH), 2949 (CH), 1570 (C=N), 1254 (C=S), 1021 (C–N), 743 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.976 (dd, 1H, J = 9.0, 18.0 Hz, C_4-H_a), 3.946 (dd, 1H, J = 8.1, 16.0 Hz, C_4-H_b), 5.572 (dd, 1H, J = 7.1, 15.6 Hz, C_5-H), 6.980-7.481 (m, 8H, Ar-H), 7.720 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 45.83 (1C, C-4), 55.59 (1C, C-5), 126.26 (1C), 127.31 (1C), 127.92 (1C), 128.03 (1C), 128.13 (1C), 128.18 (1C), 128.76 (1C), 128.81 (1C), 132.33 (1C), 134.25 (1C), 135.44 (1C), 143.61 (1C), 158.16 (1C, C-3), 174.87(1C, C=S). MS (*m/z*): 353.01 (M+4, 13), 351.02 (M+2, 64), 349.02 (M+, 100); Anal. calcd. (found) % for $C_{16}H_{13}N_3SCl_2$: C, 54.86 (54.77); H, 3.74 (3.63); N, 12.00 (11.89).

5-(2,3-Dichlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**5b**): Yield 89%; m.p.: 120-122 ºC; IR (KBr, ν_{max}, cm^{-1}): 3210 (NH), 2930 (CH), 1554 (C=N), 1233 (C=S), 1320 (C-F), 1016 (C–N), 724 (C-Cl); ^{1}H



5 a) R=H, R^1=H, R^2=H; b) R=H, R^1=H, R^2=F; c) R=H, R^1=H, R^2=Cl; d) R=H, R^1=H, R^2= CH_3; e) R=H, R^1=H, R^2= OCH_3; f) R= H, R^1= OCH_3, R^2=H; g) R= OCH_3, R^1= H, R^2=H; h) R=H, R^1=OCH_2O= R^2.

Scheme-I: Synthesis of pyrazole carbothioamides, **5**(**a-h**)

NMR ($CDCl_3$; δ ppm): 2.965 (dd, 1H, *J* = 8.5, 17.4 Hz, C_4-H_a), 3.930 (dd, 1H, *J* = 8.4, 16.9 Hz, C_4-H_b), 5.561 (dd, 1H, *J* = 7.7, 15.0 Hz, C_5-H), 6.991-7.456 (m, 7H, Ar-H), 7.790 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): (1C, C-4), 54.45 (1C, OCH_3), (1C, C-5), (1C, C-3), (1C, C=S). 45.88 (1C, C-4), 55.55 (1C, C-5), 114.66 (1C), 114.71 (1C), 126.86 (1C), 127.22 (1C), 128.25 (1C), 128.56 (1C), 129.67 (1C), 129.72 (1C), 132.45 (1C), 134.10 (1C), 143.80 (1C), 158.25 (1C, C-3), 165.41 (1C), 174.96 (1C, C=S). MS (*m/z*): 370.01 (M+4, 11), 369.01 (M+2, 64), 367.01 (M+, 100); Anal. calcd. (found) % for $C_{16}H_{12}N_3SCl_2F$: C, 52.18 (52.10); H, 3.28 (3.21); N, 11.41 (11.30).

5-(2,3-Dichlorophenyl)-3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5c):Yield 90%; m.p.: 156-159 ºC; IR (KBr, ν_{max}, cm^{-1}): 3222 (NH), 2944 (CH), 1565 (C=N), 1250 (C=S), 1023 (C–N), 734 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.990 (dd, 1H, *J* = 9.3, 18.9 Hz, C_4-H_a), 3.940 (dd, 1H, *J* = 8.0, 16.3 Hz, C_4-H_b), 5.570 (dd, 1H, *J* = 7.3, 14.9 Hz, C_5-H), 6.894-7.375 (m, 7H, Ar-H), 7.770 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 45.90 (1C, C-4), 55.50 (1C, C-5), 126.80 (1C), 127.21 (1C), 128.16 (1C), 128.21 (1C), 128.55 (1C), 128.61 (1C), 128.68 (1C), 128.70 (1C), 133.14 (1C), 134.42 (1C), 135.90 (1C), 144.10 (1C), 158.15 (1C, C-3), 174.95 (1C, C=S). MS (*m/z*): 388.97 (M+6, 4.5), 386.98 (M+4, 31), 384.98 (M+2, 100), 382.98 (M+, 99.2); Anal. calcd. (found) % for $C_{16}H_{12}N_3SCl_3$: C, 49.95 (49.79); H, 3.14 (3.04); N, 10.92 (10.90).

5-(2,3-Dichlorophenyl)-3-(4-methylphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5d): Yield 75%; m.p.: 165-166 ºC; IR (KBr, ν_{max}, cm^{-1}): 3240 (NH), 2935 (CH), 1562 (C=N), 1248 (C=S), 1027 (C–N), 739 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.234 (s, 3H, CH_3), 2.975 (dd, 1H, *J* = 9.1, 18.4 Hz, C_4-H_a), 3.926 (dd, 1H, *J* = 8.8, 16.5 Hz, C_4-H_b), 5.579 (dd, 1H, *J* = 7.5, 15.1 Hz, C_5-H), 6.902-7.390 (m, 7H, Ar-H), 7.699 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 20.85 (1C, CH_3), 45.89 (1C, C-4), 55.43 (1C, C-5), 126.10 (1C), 126.66 (1C), 126.71 (1C), 127.84 (1C), 128.11 (1C), 128.18 (1C), 129.12 (1C), 129.20 (1C), 133.22 (1C), 134.50 (1C), 141.24 (1C), 145.10 (1C), 158.19 (1C, C-3), 174.93 (1C, C=S). MS (*m/z*): 367.03 (M+4, 13), 365.03 (M+2, 65), 363.04 (M+, 100); Anal. calcd. (found) % for $C_{17}H_{15}N_3SCl_2$: C, 56.05 (56.00); H, 4.15 (4.10); N, 11.53 (11.44).

5-(2,3-Dichlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5e): Yield 75%; m.p.: 100-103 ºC; IR (KBr, ν_{max}, cm^{-1}): 3233 (NH), 2943 (CH), 1568 (C=N), 1254 (C=S), 1029 (C–N), 741 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.987 (dd, 1H, *J* = 9.5, 18.0 Hz, C_4-H_a), 3.822 (s, 3H, -OCH_3), 3.934 (dd, 1H, *J* = 8.4, 16.2 Hz, C_4-H_b), 5.584 (dd, 1H, *J* = 7.0, 14.5 Hz, C_5-H), 6.788-7.384 (m, 7H, Ar-H), 7.658 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 45.82 (1C, C-4), 54.45 (1C, OCH_3), 55.43 (1C, C-5), 111.55 (1C), 120.60 (1C), 120.80 (1C), 122.22 (1C), 129.06 (2C), 130.12 (1C), 131.33 (1C), 133.34 (1C), 139.46 (1C), 151.99 (1C), 155.40 (1C), 158.10 (1C, C-3), 174.90 (1C, C=S). MS (*m/z*): 383.17 (M+4, 11), 381.17 (M+2, 64), 379.16 (M+, 100); Anal. calcd. (found) % for $C_{17}H_{15}N_3OSCl_2$: C, 53.69 (53.57); H, 3.98 (3.88); N, 11.05 (10.95).

5-(2,3-Dichlorophenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5f): Yield 85%; m.p.: 123-125 ºC; IR (KBr, ν_{max}, cm^{-1}): 3254 (NH), 2956 (CH), 1571 (C=N), 1257 (C=S), 1038 (C–N), 745 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.982 (dd, 1H, *J* = 9.0, 18.2 Hz, C_4-H_a), 3.836 (s, 3H, OCH_3), 3.935 (dd, 1H, *J* = 8.4, 16.1 Hz, C_4-H_b), 5.581 (dd, 1H, *J* = 7.1, 14.7 Hz, C_5-H), 6.812-7.372 (m, 7H, Ar-H), 7.660 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 45.99 (1C, C-4), 54.42 (1C, OCH_3), 55.60 (1C, C-5), 113.46 (1C), 115.80 (1C), 119.33 (1C), 126.81 (1C), 127.80 (1C), 128.54 (1C), 128.72 (1C), 129.10 (1C), 129.18 (1C), 133.60 (1C), 145.22 (1C), 151.97 (1C), 158.32 (1C, C-3), 174.78 (1C, C=S). MS (*m/z*): 383.06 (M+4, 11), 381.05 (M+2, 66), 379.05 (M+, 100); Anal. calcd. (found) % for $C_{17}H_{15}N_3OSCl_2$: C, 53.69 (53.55); H, 3.98 (3.84); N, 11.05 (10.91).

5-(2,3-Dichlorophenyl)-3-(2-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5g):Yield 68%; m.p.: 119-121 ºC; IR (KBr, ν_{max}, cm^{-1}): 3251 (NH), 2950 (CH), 1566 (C=N), 1253 (C=S), 1024 (C–N), 742 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.982 (dd, 1H, *J* = 9.0, 18.2 Hz, C_4-H_a), 3.845 (s, 3H, OCH_3), 3.942 (dd, 1H, *J* = 8.0, 16.6 Hz, C_4-H_b), 5.579 (dd, 1H, *J* = 7.2, 15.3 Hz, C_5-H), 6.903-7.421 (m, 7H, Ar-H), 7.659 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 45.86 (1C, C-4), 54.47 (1C, OCH_3), 55.48 (1C, C-5), 114.50 (1C), 115.75 (1C), 120.36 (1C), 127.21 (1C), 127.93 (1C), 128.42 (1C), 128.86 (1C), 129.12 (1C), 129.66 (1C), 133.98 (1C), 145.06 (1C), 153.33 (1C), 158.22 (1C, C-3), 174.98 (1C, C=S). MS (*m/z*): 383.05 (M+4, 11), 381.06 (M+2, 66), 379.07 (M+, 100); Anal. calcd. (found) % for $C_{17}H_{15}N_3OSCl_2$: C, 53.69 (53.59); H, 3.98 (3.88); N, 11.05 (10.97).

3-(Benzo[*d*][1,3]dioxol-5-yl)-5-(2,3-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (5h): Yield 88%; m.p.: 139-142 ºC; IR (KBr, ν_{max}, cm^{-1}): 3237 (NH), 2938 (CH), 1565 (C=N), 1247 (C=S), 1031 (C–N), 729 (C-Cl); ^{1}H NMR ($CDCl_3$; δ ppm): 2.981 (dd, 1H, *J* = 9.2, 18.1 Hz, C_4-H_a), 3.938 (dd, 1H, *J* = 8.5, 16.1 Hz, C_4-H_b), 5.581 (dd, 1H, *J* = 7.5, 15.2 Hz, C_5-H), 6.050 (s, 2H, OCH_2O), 6.996-7.404 (m, 6H, Ar-H), 7.670 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ ppm): 45.88 (1C, C-4), 55.61 (1C, C-5), 100.82 (1C), 112.34 (1C), 114.80 (1C), 121.45 (1C), 125.90 (1C), 127.65 (1C), 127.81 (1C),127.94 (1C), 128.26 (1C), 132.60 (1C),144.24 (1C), 147.30 (1C), 149.87 (1C), 158.14 (1C, C-3), 174.80 (1C, C=S). MS (*m/z*): 397.00 (M+4, 14), 395.01 (M+2, 64), 393.01 (M+, 100); Anal. calcd. (found) % for $C_{17}H_{13}N_3O_2SCl_2$: C, 51.79 (51.67); H, 3.32 (3.20); N, 10.66 (10.55).

Cell culture and *in-vitro* compounds treatment: MCF-7 breast cancer cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. The MCF-7 cells were routinely maintained in DMEM (Sigma, St. Louis, MO, USA) supplemented with 10% heat inactivated FCS (Himdeia, Mumbai, India). EAT cells were collected from JSS college of Pharmacy, Mysore, India.

RESULTS AND DISCUSSION

The (3+2) annulation reactions involving the chalcones and hydrazines in the synthesis of five membered heterocycles was achieved in the presence of protic solvents and/or Lewis acids [23]. Earlier, we have demonstrated that an acidic citrus extract facilitates the cycloaddition reaction of chalcone and phenylhydrazine to yields substituted pyrazoles in good yields [22]. In this study, we were successful in extending the use of

the citrus extract as protic medium for the cycloaddition reaction involving chalcones and thiosemicarbazide in the synthesis of pyrazole carbothioamides (**5a-h**), instead of the more conventional acid catalyzed (CH_3COOH or MeOH/HCl) conditions [24].

The spectroscopic and elemental analysis provides the structural verification of new compounds. IR spectra of the compounds **5a-h** recorded on FT-IR Agilent spectrophotometer by the KBr pellet method, shows the absorption bands in the region for NH_2 at 3210-3254 cm^{-1}, CH at 2956-2930 cm^{-1}, C=N at 1571-1554 cm^{-1}, C=S at 1257-1233 cm^{-1}, C-N 1038-1016 cm^{-1} and C-Cl at 745-724 cm^{-1}. These values were in agreement with the reported similar compounds [25]. The moderate intensity bands observed in the range 1257-1233 cm^{-1} for C=S stretching is due to less electronegative sulphur than the oxygen atom and C=S group is not as polar as C=O group.

^{1}H NMR spectra recorded on Agilent-NMR 400 MHz spectrometer shows that CH_2 proton of C-4 atom of compounds **5a-h** are diastereotopic. Compounds **5a-h** shows a doublet of doublets for C_4-H_a at δ 2.965-2.990 (J = 8.5-9.5 Hz and 17.4-18.9 Hz) ppm; C_4-H_b at δ 3.926-3.946 (J = 8.0-8.8 Hz and 16.0-16.9 Hz) ppm; C_5-H at 5.561-5.584 (J = 7.0-7.7 Hz and 14.5-15.6 Hz) ppm. The singlets appear at δ 7.658-7.790 ppm for $CSNH_2$ protons and the signals for aromatic and substituent protons in the respective regions. The ^{13}C NMR spectra of compounds **5a-h** recorded on Agilent-NMR 100 MHz spectrometer shows signals for C-4, C-5 and C-3 carbons at δ 45.82-45.99, 55.43-55.61 and 158.10-158.32 ppm, respectively. The signals for C=S carbons at δ 174.78-174.98 ppm and other signals in the aromatic and substituent carbon region. The mass spectra of all designed series of compounds recorded on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMSACQUITY UPLC model spectrometer show *m/z* peaks at their respective molecular masses and demonstrated satisfactory elemental analyses performed on Thermo Finnigan Flash EA 1112 CHN analyser.

Anticancer and cytotoxicity studies: After the synthesis and structural confirmation, the synthetic pyrazole carbothioamide molecules were assessed for their anticancer and anti-angiogenic activities. In order to evaluate the anti-angiogenic activity of the pyrazoline carbothioamides (**5a-h**), the *in vitro* cytotoxic effect on MCF-7 breast cancer cell lines following 48 h exposure using trypan blue dye exclusion assay and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [26] were assessed. Tamoxifen (TMX) was used as a standard and DMSO as negative control; the results of the screening are summarized in Table-1. In the trypan blue assay, compounds **5c** and **5d** showed excellent cell growth inhibition with IC_{50} values of 8.6 and 9.3 μM, respectively in comparison with the standard tamaxifen 11.4 μM. The effect of compounds on cell proliferation tested using MTT assay revealed that compounds **5c** and **5d** have maximum cytotoxic effect with an IC_{50} values of 8.1 and 9.0 μM, respectively, these results are in agreement with those reported with trypan blue (Table-1).

From the results, it can be clearly understood that the compound **5c** with 4-chloro phenyl substitution, exhibited highest activity against MCF-7 cells in trypan blue and MTT assay with IC_{50} values of 8.6 and 8.1 μM, respectively, when compared with the standard. The result shows that compounds **5g** and **5h** have no cytotoxic effect, compounds **5b** and **5e** possesses moderate effects; while compounds **5a** and **5f** shows lesser cytotoxic effect on MCF-7 cells. Amongst the synthesized series, compounds **5c** and **5d** were regarded as lead molecules for further investigation. The cytotoxic effects of synthesized pyrazole analogues **5a-h** were compared with the effects shown by structurally related pyrazoles on MCF-7 cell lines, and found that the values are good agreement with the reported ones [27].

TABLE-1
IC_{50} VALUES OF DESIGNED SERIES OF COMPOUNDS **5**(**a-h**) ON TRYPAN BLUE AND MTT ASSAY AT 48 h IN MCF-7 CELL LINES

Compound	Trypan blue assay IC_{50} value (μM)	MTT assay IC_{50} value (μM)
Control	–	–
5a	34.1 ± 0.21	33.7 ± 0.28
5b	12.6 ± 0.16	13.1 ± 0.18
5c	8.6 ± 0.11	8.1 ± 0.12
5d	9.3 ± 0.14	9.0 ± 0.16
5e	17.4 ± 0.21	17.0 ± 0.15
5f	54.3 ± 0.20	55.0 ± 0.25
5g	74.5 ± 0.38	75.4 ± 0.33
5h	> 100	> 100
Standard	11.4 ± 0.07	11.4 ± 0.09

To study the morphological features of the cells, the EAT cells treated with compounds **5c** and **5d** and stained with Giemsa and ethidium bromide/acridine orange staining procedure [26]. The experiments were observed under fluorescent microscope with blue filter and photographed employing Nikon D3200 camera. The results confirmed the pro-apoptotic activity of these compounds as understood by high nuclear condensation with clumping of nuclear chromatin. Blebbing of the nuclear and cytoplasmic membranes and few apoptotic bodies were seen (Fig. 1a-b). The apoptotic morphology clearly indicated the potential cytotoxic effect of compounds **5c** and **5d**, respectively.

Conclusion

In summary, a successful attempt for the synthesis and assessment of small-molecules with anticancer and angiogenic effect especially on EAT cell lines is made. Amongst the synthesized compounds, compounds **5c** and **5d** show potent anticancer activity against MCF-7 cells with IC_{50} value of 8.6, and 9.3 μM respectively, compared to other structurally related molecules of the designed series. Further, we demonstrate the utility of reverse ligand-similarity based search methods to understand the molecular targets of a small-molecule inhibitor. This work presents an important advance in designing and synthesis of molecules, in particular, pyrazoles with carboxamide and carbothioamide substitutions that have potent anticancer activities and thus, paves way for the novel anticancer agents.

ACKNOWLEDGEMENTS

The authors are grateful to the IOE, University of Mysore for recording spectral analysis, Dr. N.D. Rekha, Department of Biotechnology, JSS College for Arts, Science and Commerce, Mysore, India for helping in the biological assays.

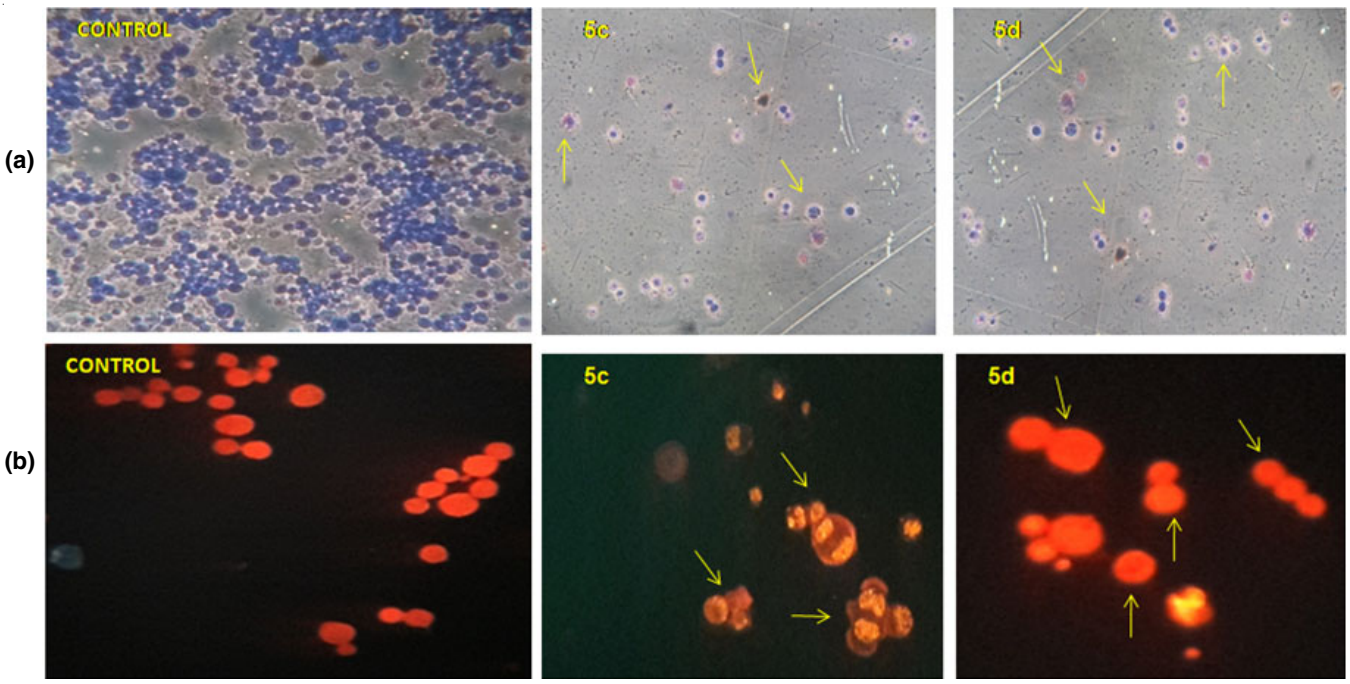


Fig. 1. Anticancer and cytotoxic effects. (a) Giemsa staining of normal cells and cells treated with compounds **5c** and **5d** highlights the apoptotic morphology of the cells; (b) Ethidium bromide/acridine orange staining of normal cells and cells treated with compounds **5c** and **5d** highlights the apoptotic morphology of the cells

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