ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Synthesis, characterization, molecular docking and *in vitro* anticancer activity of 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide

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Article History:

Received on: 15 Mar 2020
Revised on: 01 Apr 2021
Accepted on: 12 Apr 2021

Keywords:

Anticancer,
Molecular docking,
Pyrazoline,
SRB Assay and Molegro Virtual Docker

ABSTRACT

In the present study, eight numbers of new 3- (4-methoxy phenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h) have been synthesized from 1- (4-methoxy phenyl)-3- (substituted phenyl)-prop-2-en-1-one (3a-h) and structurally characterized by using FT-IR, ^{1}H NMR, ^{13}C NMR, Mass and Elemental analysis. The synthesized molecules were biologically evaluated for their *in vitro* anticancer activity against human breast adenocarcinoma (MCF-7), liver cancer (Hep-G2) and leukaemia cancer (K-562) cell line using Sulforhodamine B (SRB) bioassay technique. From the all synthesized compounds 5a, 5c, 5d, and 5e exhibited potent anticancer activity (GI_{50}= <10μg/ml) as compared to the controlled drug 5-Fluorouracil (5-FU) (GI_{50}=44.5μg/ml) and Adriamycin (ADR) (GI_{50}= <10μg/ml) on MCF-7 cell lines. Besides this, all the synthesized compounds have exhibited moderate activity against human liver cancer (Hep-G2) and leukaemia cancer (K-562) cell lines. In addition, molecular docking studies were also explored in order to study the probable binding specificity into the active site of *Epidermal Growth Factor Receptor tyrosine kinase* (EGFR) (PDB ID: 1M17) using Molegro Virtual Docker Evaluation 2013 6.0.1 (MVD). Based on the molecular docking result, it was found that compound 5a exhibited the best interaction with the above target (i.e., EGFR) by interacting with specific amino acid residues such as: *Thr* 766, *Gin* 767, *Thr* 830, *Cys* 575, *Ala* 719 and *Met* 769.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i2.4759

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INTRODUCTION

Globally, cancer is one of the serious and dreadful diseases characterized by the uncontrolled, rapid, and pathological proliferation of cells (Chavan *et al.*, 2018). In underdeveloped countries incidence and mortality rate due to cancer increases day to day because of the growth and aging of the population (Suma *et al.*, 2019). As per a survey by GLOBOCAN in 2018, over 18.1 million new cancer cases and 9.6 million deaths till now and the cases may gradually raise up to 23.6 million by 2030 (Suma *et al.*, 2019; Santosh *et al.*, 2019; Jemal *et al.*, 2011).

Despite surgery, radiation and chemotherapy, cancer is still the second leading cause of death after

cardiovascular disorders. Treatment of cancer through chemotherapy is effective but, in most cases, the toxicity of chemotherapeutic agents and the occurrence of drug resistance hinder the successfulness of medication (Patel *et al.*, 2011).

Therefore, the search for novel anticancer agents is urgently needed to prevent drug toxicity. Now a day the role of synthetic organic chemistry is immense vital for the deployment of the drug for anticancer by adopting multidisciplinary intervention (Denny *et al.*, 2002).

Pyrazoline is 5-membered nitrogen containing a heterocyclic ring. It was reported that maximally treatments are with nitrogen containing chemical entity only. Studies revealed that pyrazoline derivatives possess therapeutic potential like antimicrobial (Siddiqui *et al.*, 2011; Turan-Zitouni *et al.*, 2007), analgesic (Samshuddin *et al.*, 2012; James and Bhat, 2012), anti-inflammatory (Sharma *et al.*, 2012), antiameobic (Wani *et al.*, 2012), antitubercular (Taj *et al.*, 2011), antimalarial (Acharya *et al.*, 2010), anticonvulsant, antidepressant (Özdemir *et al.*, 2007; Palaska, 2001), antioxidant (Isloor *et al.*, 2013), antiparkinsonism (Amr *et al.*, 2008), antileishmanial (Rizvi *et al.*, 2012), antihyperglyceamic (Ovais *et al.*, 2013), hepatoprotective (Khalilullah *et al.*, 2011), angiotensin converting enzyme inhibitor (Bonesi *et al.*, 2010), MOA inhibitors (Mathew *et al.*, 2014), B-Raf kinase inhibitor (Jeong *et al.*, 2004), β-Ketoacyl-acyl carrier protein synthase III inhibitor (Blackburn *et al.*, 2010), EGFR kinase inhibitor (Duffey *et al.*, 2010), GluN2C/GluN2D selective antagonist (Qin *et al.*, 2015), 5-Hydroxytryptamine 6 receptor antagonist (Acker *et al.*, 2013), NMDA receptor antagonist (Loevezijn *et al.*, 2011), Cannabinoid receptor antagonist (Tamborini *et al.*, 2013) and anticancer activity (Bashir *et al.*, 2011; Hayat *et al.*, 2010; Wang *et al.*, 2013).

In our earlier publication, we have noticed that the pyrazoline derivatives exhibited noticeable anticancer activity, where the synthesized compounds have been evaluated *in vitro* in the MCF-7 cancer cell line (Zimenkovsky *et al.*, 2009; Johnson *et al.*, 2007; Khalil *et al.*, 2013). Based on the previous research, anticancer evaluation and docking study encourages us to plan and synthesize eight numbers of a new compound, 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h), structurally characterized, molecular docking and conformation of anticancer activity by *in vitro* model against MCF-7, Hep-G2 and K-562 cell line by SRB assay. (Havrylyuk *et al.*, 2011; Shaharyar *et al.*, 2010; Sharma *et al.*, 2014).

MATERIALS AND METHODS

Material

The chemicals utilized during the experiments were of GR standard reagent and were procured from Sigma Aldrich. Pvt. Ltd. Bangalore, SpectrochemPvt. Ltd., Mumbai and Merck specialities Pvt. Ltd., Mumbai, and utilized during the experiments without any purification.

Instruments

Veego melting point apparatus (VMP-DS) had been used to determine the melting points of synthesized compounds. Moreover, Thin-layer chromatography (TLC) was used to test the purity of the compound on a silica gel-G plate of 0.5 mm, and the developed plate was identified by iodine vapour or UV light.

For confirmatory, Fourier transforms infrared (FT-IR) spectra were analyzed on a Brucker FT-IR ALPHA in the range 4000-400cm^{-1}.^{1}H NMR and ^{13}C NMR spectra were observed by using Brucker Ultra shield 300 MHz spectrometer by utilizing Deuterated chloroform ($CDCl_3$) as solvent and Tetramethylsilane (TMS) as internal standard.

The mass spectrum was observed on a G2 QTOF XEVO mass spectrometer. CHN spectral data was observed by using Perkin Elmer 2400 Series II.

Synthesis of 1-(4-methoxyphenyl)-3-(substituted phenyl)-prop-2-en-1-one (3a-h)

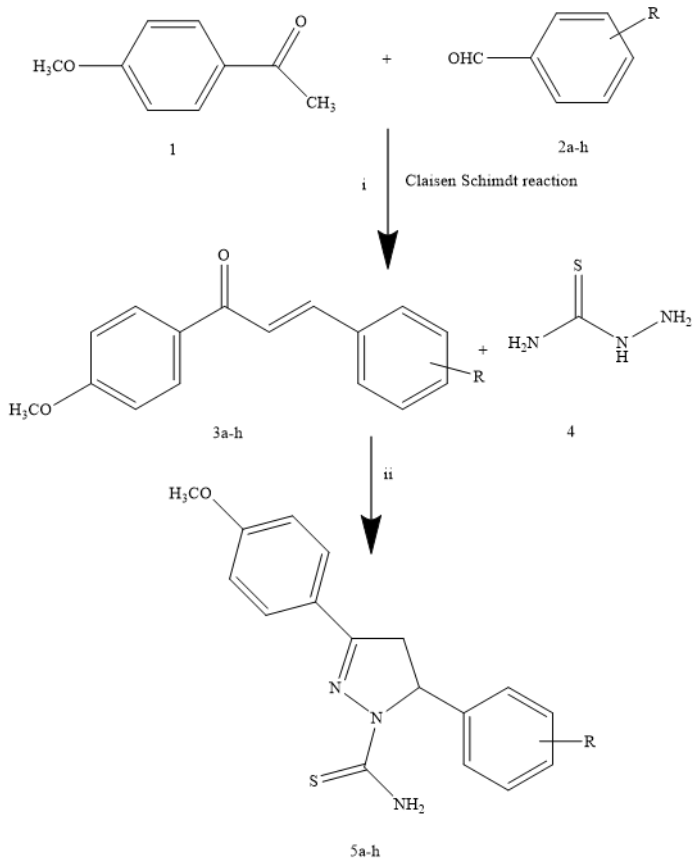
This synthesized mixture concentration was 4-methoxy acetophenone (0.01mole) 1 with 10-15 ml of ethanol in combination with substituted aromatic aldehydes (0.01mole) 2a-h were taken in 250 ml of the round-bottom flask.

After 30 minutes of stirring, then 10 ml of sodium hydroxide solution (10%) was mixed drop by drop into the reaction with vigorous stirring for 5-6 hr. Then the solution was neutralized by 0.1N HCl, and the precipitate was formed, which further undergoes filtration.

After it was air drying and then go for recrystallization by ethyl alcohol to obtain 1-(4-methoxyphenyl)-3-(substituted phenyl)-prop-2-en-1-one (3a-h). The entire reaction was conducted between 10-15^{0}C by using an ice water bath (Choudhary and Juyal, 2011).

Synthesis of 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h)

The equimolar concentration of 1-(4-methoxyphenyl)-3-(substituted phenyl)-prop-2-en-1-one (0.01 mole), 3a-h and thiosemicarbazide (0.01 mole), 4 were taken into 250 ml of the round-bottom flask and mixed in 20 ml of dry ethanol used as a catalyst. After 30 minutes of reflux then sodium



Scheme 1: Synthesis of 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h). R=-C_6H_5(a), -C_6H_4 (p-OCH_3)(b), -C_6H_4 (m-OCH_3)(c), -C_6H_4 (p-Cl)(d), -C_6H_4(p-CH_3)(e), -C_6H_4 (p-$N(CH_3)_2$)(f), -C_6H_4 (p-Br)(g), -C_6H_4(m-Br)(h).
Reagentand conditions: (i)NaOH, C_2H_5OH, stirring, 6-8 hr, (ii)C_2H_5OH,NaOH, reflux, 16-18 hr.

hydroxide (10%) was mixed into the prepared reaction. Then reflux was continuing for 16-18 hr.

Then the reaction mixture was allowed to cool. After that, the products was poured into ice water and kept overnight and then go for filtration. After that, again dried and recrystallized with ethyl alcohol to obtain 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h) (Turan-Zitouni *et al.*, 2007; Wang *et al.*, 2018).

3-(4-Methoxyphenyl)-5-phenyl-2-pyrazoline-1-carbothioamide (5a)

White crystalline solid; yield: 65%, m.p: 178-179^{0}C, R_f 0.75, Chemical formula: $C_{17}H_{17}N_3OS$, Exact mass: 311.11, Molecular weight: 311.40, FT-IR V_{max} cm^{-1} (NH_2)3432, (aromatic C-H) 2931, (aliphatic C-H) 2832, (pyrazoline C=N) 1667, (OCH_3)1073. ^{1}H NMR (300MHz, $CDCl_3$ δ, ppm): 3.82[2H, d, H-4 (pyrazoline)],3.88 [1H, t, H-5 (pyrazoline)], 3.84 [3H, t, OCH_3-4'], 6.99 [2H, d, H-3', 5'], 7.09[2H, d, H-2,6], 7.25[1H, d, H-4], 7.30 [2H, d, H-3,5], 7.78 [2H, d, H-2'6'], 7.95[2H, d, H-1].^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 40.00 (3-C), 55.84 (17-C), 63.19 (2-C), 114.03 (7-C, 9-C), 125.76 (12-C, 16-C), 127.35 (14-C), 128.94 (13-C, 15-C), 129.33 (5-C, 6-C, 10-C), 143.35 (11-C), 155.32 (4-C), 161.06 (8-C), 176.24 (1-C). Elemental analysis: C=65.57, H=5.50, N=13.49, O=5.14, S=10.30%, Found C=65.75, H=5.44, N=13.37%.

3,5-Bis(4-methoxyphenyl)- 2-pyrazoline -1-carbothioamide (5b)

White crystalline solid, yield: 70%, m.p:171-172^{0}C, R_f 0.77, Chemical formula: $C_{18}H_{19}N_3O_2S$, Exact mass: 341.12, Molecular weight: 341.43, FT-IR V_{max} cm^{-1} (NH_2)3403, (aromatic C-H) 2998, (aliphatic C-H) 2898, (pyrazoline C=N) 1667, (OCH_3) 1097. ^{1}H NMR (300MHz, $CDCl_3$ δ, ppm): 3.78[2H, d, H-4 (pyrazoline)], 3.82 [3H, t, OCH_3-4], 3.84 [3H, t, OCH_3-4'], 3.84[1H, t, H-5 (pyrazoline)], 6.94 [2H, d, H-3,5], 6.91 [2H, d, H-3',5'], 7.16 [1H, d, H-2,6], 7.65 [2H, d, H-2'6'], 7.68[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 40.26 (3-C), 55.50 (17-C), 55.85 (18-C), 62.65 (2-C), 114.26 (13-C, 15-C), 114.62 (7-C, 9-C), 127.09 (12-C, 16-C), 129.31 (5-C, 6-C, 10-C), 135.55 (11-C), 155.32 (4-C), 158.63 (14-C), 161.64 (8-C), 176.12 (1-C). Elemental analysis: C=63.32, H=5.61, N=12.31, O=9.37,

S=9.39%, Found C=63.15, H=6.07, N=12.47%

5-(3-Methoxyphenyl)-3-(4-methoxyphenyl)-2-pyrazoline-1-carbothioamide (5c)

Light yellow crystalline solid, yield: 65%, m.p: 118^{0}C; R_f 0.68, Chemical formula: $C_{18}H_{19}N_3O_2S$, Exact mass: 341.12, Molecular weight: 341.43, FT-IR V_{max} cm^{-1} (NH_2) 3413, (aromatic C-H) 2930, (aliphatic C-H) 2836, (pyrazoline C=N) 1661, (OCH_3) 1084. ^{1}H NMR (300MHz, $CDCl_3$ δ, ppm): 3.77 [3H, t, OCH_3-3], 3.80 [3H, t, OCH_3-4'],3.86 [1H, t, H-5 (pyrazoline)],3.82[2H, d, H-4 (pyrazoline)], 6.98[2H, d, H-2,6], 7.17 [2H, d, H-3',5'],7.80 [2H, d, H-2'6'], 7.96[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 40.27 (3-C), 55.41 (17-C), 55.84 (18-C), 63.10 (2-C), 112.26 (12-C, 14-C), 114.62 (7-C, 9-C), 117.68 (16-C), 127.09 (12-C, 16-C), 129.32 (5-C, 6-C, 10-C), 130.15 (15-C), 135.55 (11-C), 145.10 (11-C), 155.32 (4-C), 159.79 (13-C), 161.66 (8-C), 176.26 (1-C). Elemental analysis: C=63.32, H=5.61, N=12.31, O=9.37, S=9.39%, Found C=63.29, H=5.55, N=12.28%

5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-pyrazoline-1-carbothioamide (5d)

Yellow crystalline solid, yield 68%, m.p: 205^{0}C, R_f 0.73, Chemical formula: $C_{17}H_{16}ClN_3OS$, Exact mass: 345.07, Molecular weight: 345.85, FT-IR V_{max} cm^{-1} (NH_2) 3424, (aromatic C-H) 2927, (aliphatic C-H) 2832, (pyrazoline C=N) 1589, (OCH_3) 1081, (C-Cl) 806. ^{1}H NMR (300MHz, $CDCl_3$ δ, ppm): 3.78[2H, d, H-4 (pyrazoline)],3.82 [3H, t, OCH_3-4'], 3.84 [1H, t, H-5 (pyrazoline)], 7.11[2H, d, H-3', 5'], 7.33[2H, d, H-3, 5], 7.78[2H, d, H-2, 6], 7.80[2H, d, H-2', 6'], 7.98[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 40.27 (3-C), 55.85 (17-C), 62.63 (2-C), 114.62 (7-C, 9-C), 117.68 (16-C), 127.81 (12-C, 16-C), 128.92 (13-C, 15-C), 129.37 (5-C, 6-C, 10-C), 131.83 (14-C), 142.51 (11-C), 155.32 (4-C), 161.70 (8-C), 176.18 (1-C). Elemental analysis: C=59.04, H=4.66, Cl=10.25, N=12.15, O=4.63, S=9.27%, Found C=58.75, H=3.65,N=11.84%

3-(4-Methoxyphenyl)-5-(p-tolyl)-2-pyrazoline-1-carbothioamide (5e)

Light yellow crystalline solid, yield: 75%, m.p: 178^{0}C, R_f 0.75, Chemical formula: $C_{18}H_{19}N_3OS$, Exact mass: 325.12, Molecular weight: 325.43, FT-IR V_{max} cm^{-1}(NH_2) 3408, (aromatic C-H) 2920, (CH_3) 2850, (aliphatic C-H) 2820, (pyrazoline C=N) 1669, (OCH_3) 1080. ^{1}H NMR (300MHz, $CDCl_3$ δ, ppm):2.22[3H, t, CH_3-4]; 3.07 [3H, t, OCH_3-4']; 3.36 [2H, d, H-4 (pyrazoline)],3.77 [1H, t, H-5 (pyrazoline)], 7.00[2H, d, H-3', 5'], 7.06[2H, d, H-2, 6], 7.08[2H, d, H-3, 5], 7.80[2H, d, H-2', 6'], 7.98[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 21.30 (17-C), 40.25 (3-C), 55.89 (18-C), 63.01 (2-C), 114.67 (7-C, 9-C), 125.76 (12-C, 16-C), 129.35 (13-C, 15-C), 129.50 (5-C, 6-C, 10-C), 136.47 (14-C), 140.61 (11-C), 155.38 (4-C), 161.69 (8-C), 176.23 (1-C). Elemental analysis: C=66.43, H=5.88, N=12.91, O=4.92, S=9.85%, Found C=65.75, H=5.81, N=12.84%

5-(4-(Dimethylamino)phenyl)-3-(4-methoxyphenyl)-2-pyrazoline-1-carbothioamide (5f)

Yellow crystalline solid, yield: 65%, m.p: 187-180^{0}C,R_f 0.72, Chemical formula: $C_{19}H_{22}N_4OS$, Exact mass: 354.15, Molecular weight: 354.47, FT-IR V_{max} cm^{-1} (NH_2) 3406, (aromatic C-H) 2999, (CH_3) 2801, (aliphatic C-H) 2882, (pyrazoline C=N) 1603, (C-N) 1243, (OCH_3)1085. ^{1}H NMR (300MHz, $CDCl_3$ δ, ppm):2.90[6H, t, CH_3-4], 3.75 [3H, t, OCH_3-4'], 3.79 [2H, d, H-4 (pyrazoline)],3.84 [1H, t, H-5 (pyrazoline)], 6.67[2H, d, H-3, 5], 7.08[2H, d, H-3', 5'], 7.11[2H, d, H-2, 6], 7.65[2H, d, H-2', 6'], 7.68[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm):40.20 (3-C),40.73 (17-C, 18-C), 55.89 (19-C), 62.80 (2-C), 112.88 (13-C, 15-C), 114.68 (7-C, 9-C), 126.71 (12-C, 16-C), 129.32 (5-C, 6-C, 10-C), 131.16 (11-C), 150.04 (14-C), 155.55 (4-C), 161.66 (8-C), 176.09 (1-C). Elemental analysis: C=64.38, H=6.26, N=15.81, O=4.51, S=9.05%, Found C=63.97 H=5.25, N=15.45%

5-(4-Bromophenyl)-3-(4-methoxyphenyl)-2-pyrazoline-1-carbothioamide (5g)

Milk white crystalline solid, yield: 68%, m.p 175^{0}C, R_f 0.65, Chemical formula: $C_{17}H_{16}BrN_3OS$, Exact mass: 389.02, Molecular weight: 390.30, FT-IR V_{max} cm^{-1}(NH_2) 3402, (aromatic C-H) 2940, (aliphatic C-H) 2840, (pyrazoline C=N) 1662, (OCH_3)1072, (C-Br) 575.^{1}H NMR (300MHz, $CDCl_3$ δ, ppm):3.83[3H, t, OCH_3-4'], 3.85 [2H, d, H-4 (pyrazoline)],3.89 [1H, t, H-5 (pyrazoline)], 7.06 [2H, d, H-3', 5'], 7.47[2H, d, H-2, 6], 7.78[2H, d, H-3, 5], 7.81[2H, d, H-2', 6'], 7.99[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 40.25 (3-C), 55.91 (17-C), 62.74 (2-C), 114.68 (7-C, 9-C), 120.38 (14-C), 128.22 (12-C, 16-C), 129.41 (5-C, 6-C, 10-C), 131.89 (13-C, 15-C), 142.98 (11-C), 155.34 (4-C), 161.75 (8-C), 176.23 (1-C). Elemental analysis: C=52.31, H=4.13, Br=20.47, N=10.77, O=4.10, S=8.22%, Found C=48.19 H=3.85, N=12.33%

5-(3-Bromophenyl)-3-(4-methoxyphenyl)-2-pyrazoline-1-carbothioamide (5h)

Milk white crystalline solid, yield: 70%, m.p: 165^{0}C, R_f 0.68, Chemical formula: $C_{17}H_{16}BrN_3OS$, Exact mass: 389.02, Molecular weight: 390.30, FT-IR V_{max} cm^{-1}(NH_2) 3408, (aromatic C-H) 2952, (aliphatic C-H) 2849, (pyrazoline C=N) 1667, (OCH_3) 1073, (C-Br) 578.^{1}H NMR (300MHz, $CDCl_3$ δ, ppm):3.74[3H, t, OCH_3-4'], 3.89 [2H, d, H-4

(pyrazoline)], 3.91 [1H, t, H-5 (pyrazoline)], 6.92 [2H, d, H-3', 5'], 6.94[1H, d, H-5], 7.25 [2H, d, H-2, 6], 7.77[2H, d, H-2', 6'], 8.33[2H, d, H-1]. ^{13}C NMR (300MHz, $CDCl_3$ δ, ppm): 40.25 (3-C), 55.91 (17-C), 62.33 (2-C), 114.64 (7-C, 9-C), 121.22 (13-C), 126.32 (16-C), 128.50 (5-C, 6-C, 10-C), 129.43 (14-C, 16-C), 133.33 (12-C), 141.89 (11-C), 155.32 (4-C), 161.75 (8-C), 176.17 (1-C). Elemental analysis: C=52.31, H=4.13, Br=20.47, N=10.77, O=4.10, S=8.22%, Found C=50.23, H=3.50, N=10.19%.

ANTIPROLIFERATIVE ACTIVITY

In vitro Sulforhodamine B (SRB) assay

The cancer cell lines (MCF-7, Hep-G2 and K562) were grown in RPMI 1640 medium using 96 microtiter well plates capacity to have 100 μL. The plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h before the addition of experimental drugs (5a-h) and 5-FU, ADR is a positive control.

The above procure followed after cell inculcation. All the prepared compounds were solubilised in dimethyl sulfoxide (DMSO) at a dilution of 100 mg/ml and further diluted to 1mg/ml by utilizing distilled water which was stored frozen before use. At the time of drug addition, the concentration (1 mg/ml) was thawed and again diluted to 100 μg/ml, 200 μg/ml, 400 μg/ml and 800 μg/ml, respectively. Then 10 μl of these of drug dilution were added to this specific micrometer well which is already content 90 μl of the medium.

This result in final concentration such as 10 μg/ml, 20 μg/ml, 40 μg/ml, 80 μg/ml then plate were incubated for 48hr. After the addition of the experimental compound (5a-h). Then it needs to fixed cell lines by addition of 50 μl of cold 30.5 % w/v of trichloroacetic acids. The superannuated was discarded after incubation for 1hr and the palate was washed and air dries completely. Then each of the wells were received a 50 μl solution of Sulforhodomine B (0.4% w/v) in acetic acid (1%), followed by incubating the all plates for about 20 minutes at room temperature. The unbound dye was recovered, and the residual dye was washed five times with 1% acetic acid solution after staining.

Then the plates were air-dried. Then the bound stain was subsequently eluted with a 10 mM trizma base, and the absorbance was recorded on a microplate reader at a wavelength of 540 nm.

Then on a plate-by-plate basis for test wells relative to control wells, the percentage of growth was calculated:

$$\% \text{ Growth} = [Average\ absorbance\ of\ the\ tested\ drug\ in\ well / Average\ absorbance\ of\ the\ control\ drug\ in\ well] \times 100$$

The percentage growth has been calculated, which measures at each of the drug concentration level. (Vanicha Vichai and Kirtikara, 2006; Skehan *et al.*, 1990).

$$\%\ growth\ inhibition = [Ti/C]\ x\ 100\ \%$$

MOLECULAR DOCKING STUDIES

Molegro Virtual Docker, Version (*MVD 2013 6.0.1*) has been used in this molecular docking interaction study. During this study, both the protein and ligand molecules were taken in their 3D format. The chemical structures of the screened compounds were drawn by using chem draw software. Before performing the docking study, the target protein was prepared using the protein preparation wizard module of MVD software through removing the crystallographic water molecules, ligand and cofactors followed it all the bonds, bond orders, hybridization and charges were assigned. The main objective of protein preparation was to remove errors like bond order, bond position, explicitly hydrogen, flexible torsions etc. During the molecular docking study, the parameters like binding radius, grid resolution and maximum iterations were set to 15 Å, 0.3 Å and 2,000, respectively. The docking algorithm was set to the simplex evolution population size of 50 and energy minimization was performed to optimize the molecules up to their lowest stable state of energy and 5 independent runs were conducted. After performing docking, the cluster having negative lowest-energy and similar poses was removed, keeping the best scoring one. The clusters were screened on the basis of their docking interaction energy, the best lowest binding free energy pose was selected for the analysis of the docking results and the other docking complex were also analyzed for various intermolecular interactions.

In the beginning, a total of five different cavities were predicted using cavity prediction wizard of MVD software with different surface area, and volume was mapped in *epidermal growth factor receptor tyrosine kinase* (EGFR) (1M17). Cavity 1, with the highest volume (178.20 Å3) and largest surface area (741.31Å2), was selected as the origin for the binding site (Venkateshan *et al.*, 2018). Finally, the docking data were compiled with the data obtained by Qiu *et al.* (2012).

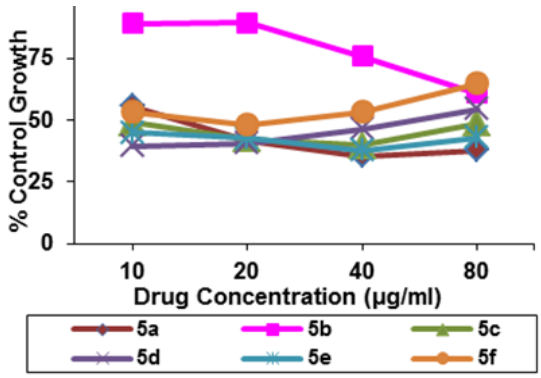


Figure 1: Growth Curve: Human Breast Cancer Cell Line MCF-7

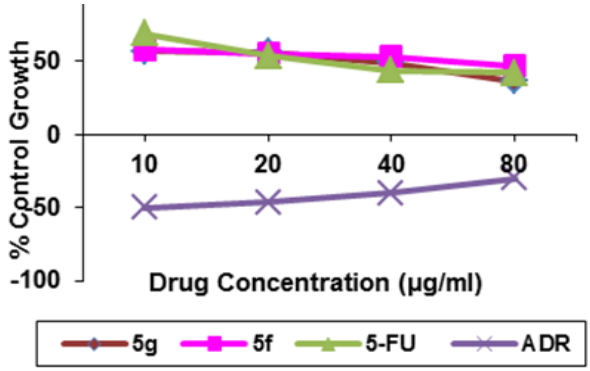


Figure 2: Growth Curve: Human Breast Cancer Cell Line MCF-7

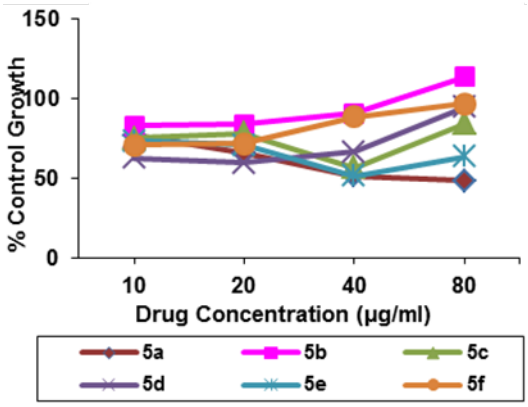


Figure 3: Growth Curve: Human Hepatoma Cell Line Hep-G2

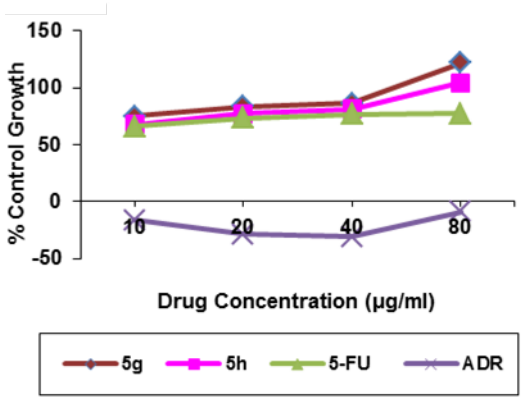


Figure 4: Growth Curve: Human Hepatoma Cell Line Hep-G2

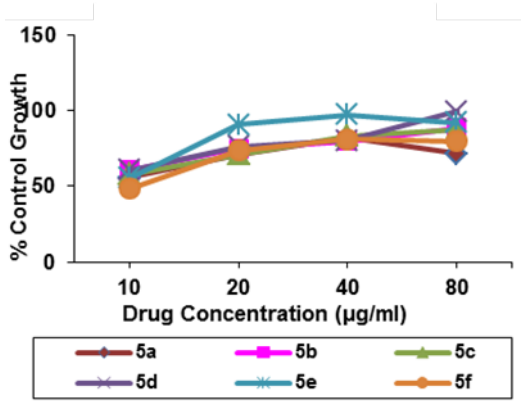


Figure 5: Growth Curve: Human Leukemia Cell Line K-562

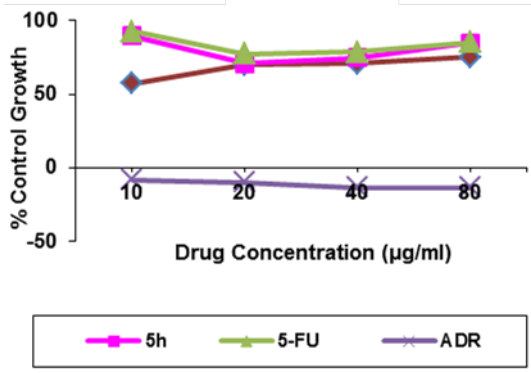


Figure 6: Growth Curve: Human Leukemia Cell Line K-562

RESULTS AND DISCUSSION

At present studies, eight numbers of new 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h) have been synthesized by the synthetic route outlined in Scheme 1. The resulting synthesized compounds were elucidated structurally by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and Elemental studies. The FT-IR spectrum of all the compounds (5a-h) showed bands ranging from 1097-1072 cm^{-1} for OCH_3. However, all the compound displayed bands at 1667-1603 cm^{-1} for the pyrazoline C=N group. Besides, compounds also showed bands range at 2898-2820 cm^{-1} for aliphatic C-H and bands range at 2998-2920 cm^{-1} for aromatic ring and bands range at 3432-3403 cm^{-1} for NH_2 group.

The ^{1}H NMR spectra of all compounds (5a-h) showed 1H, d, H-4 (pyrazoline) as doublet signal at 1.65-1.95 ppm and 1H, d, H-5 (pyrazoline) as triplet at 3.90-3.95 ppm, which proves 2-pyrazoline ring

Table 1: LC_{50}, TGI, GI_{50} values of the tested compound against MCF-7, Hep-G2, K-562 cell line by SRB analysis

Compounds	MCF-7 Cell line			Hep-G2 Cell line			K-562 Cell line		
	LC_{50} (μg/ml)	TGI (μg/ml)	GI_{50}* (μg/ml)	LC_{50} (μg/ml)	TGI (μg/ml)	GI_{50}* (μg/ml)	LC_{50} (μg/ml	TGI (μg/ml)	GI_{50}* (μg/ml)
5a	-	-	<10	-	-	>66.2	-	-	-
5b	-	-	80	-	-	-	-	-	-
5c	-	-	<10	-	-	>80	-	-	-
5d	-	-	<10	-	-	-	-	-	-
5e	-	-	<10	-	-	>80	-	-	-
5f	-	-	13	-	-	>80	-	-	-
5g	-	-	36.9	-	-	>88	-	-	-
5h	-	-	61.2	-	-	>80	-	-	-
5-FU	-	-	44.5	-	-	>80	-	-	-
ADR	-	<10	<10	-	<10	<10	-	<10	<10

Where, LC_{50} = it is the concentration of drug to kill cell at 50%; GI_{50}= it is the concentration of drug to inhabits cell growth at 50%; TGI= it is the concentration of drug for total inhibition of cell growth; 5-FU=5- Fluorouracil; ADR= Adriamycin is a positive control; - =Non-evaluable data

formation. The OCH_3 appeared as a triplet at 3.87-3.91 ppm and aromatic hydrogen showed triplet at 6.99-9.00 ppm. ^{13}C NMR spectra of all compounds have shown that aliphatic-Cs resonate at 76-77 ppm while aromatic-Cs appear at 120-140 ppm. All the compounds were shown one doublet at 1.65-1.95 ppm, assigned for H-4 (pyrazoline) and one triplet at 3.90-3.95 ppm, assigned for H-5 (pyrazoline) which proves 2-pyrazoline ring formation.^{13}C NMR spectra of all compounds have shown that aliphatic-Cs resonate at 76-77 ppm while aromatic-Cs appear at 120-140 ppm. Then the confirmed compounds (5a-h) have been screened for cytotoxicity activity (*in vitro*) against MCF-7, Hep-G2, K-562 cell line by utilizing the SRB assay method as a tool.

The *in vitro* results summary are tabulated under in Table 1.

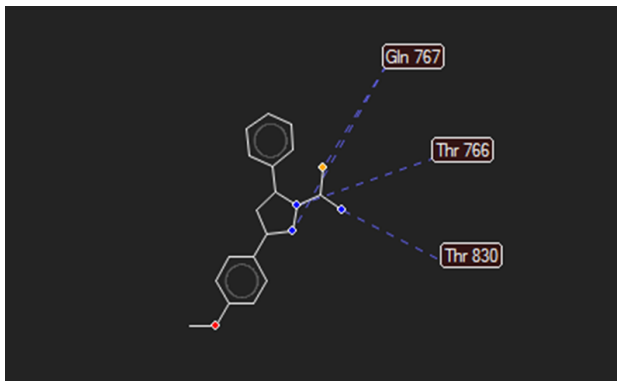


Figure 8: Hydrogen bond interactions of most active compound 5a with 1M17

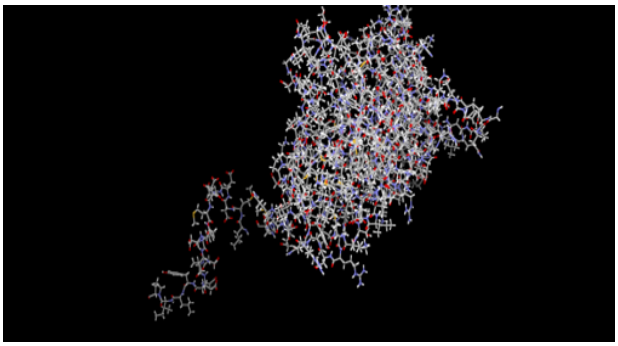


Figure 7: Structure of the Protein (1M17)

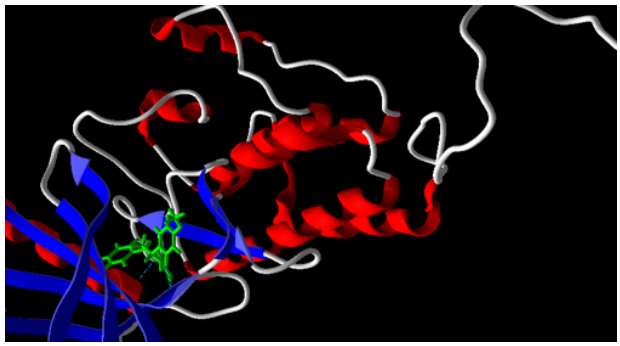


Figure 9: Secondary view of complex [compound 5a (green colour) with1M17 having PDB id: 1M17 (Red as α-helices and Blue as β-sheets)]

Screening results of *in vitro* anticancer study (Table 1) revealed that the compound 5a, 5c, 5d and 5e with (GI_{50}=<10μg/ml) exhibited the most potent anticancer properties while as compiling with drug

Table 2: Docking score of all the synthesized compounds (5a-h) with EGFR (1M17)

Compound	Mol-Dock Score	Re-rank Score	H-Bond score
5a	-117.141	-88.0349	-0.653391
5b	-113.894	-67.79	-1.55824
5c	-114.071	-81.3722	-5.55235
5d	-111.878	-90.3553	-0.859569
5e	-105.219	-18.9256	-5.88098
5f	-105.201	-84.1836	-2.74627
5g	103.021	-90.9255	-1.4398
5h	-107.533	-82.4842	-5.58533

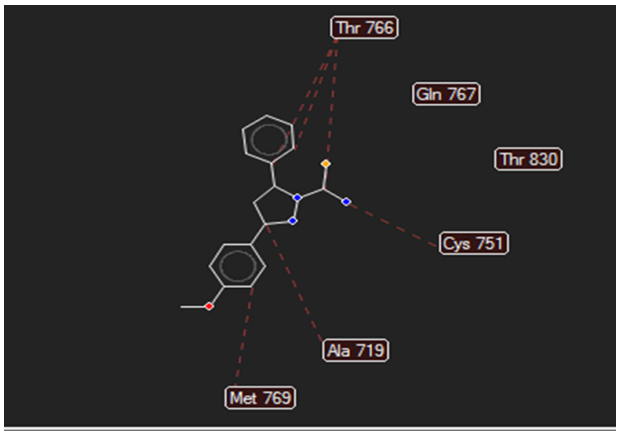


Figure 10: Steric interaction study of compound 5a with 1M17

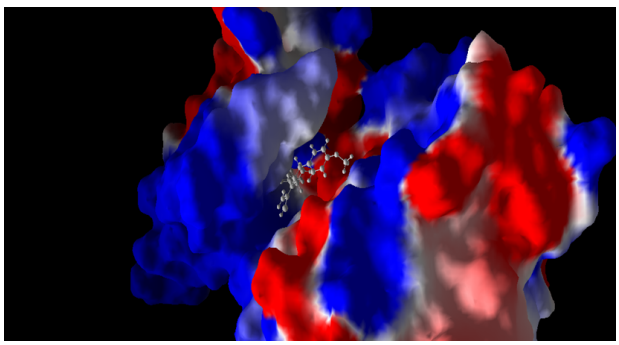


Figure 11: Hydrophobic interactions of the compound 5a with 1M17 enzyme showing hydrophobic and hydrophilic surface

5-FU, ADR with (GI_{50}=44.5, <10μg/ml) against MCF-7 cell line and other drug 5b, 5f, 5g, 5h are shown moderate activity. Moreover, compounds of 5a, 5c, 5e, 5f, 5g and 5h with (GI_{50}= >80μg/ml) displayed lower activities against Hep-G2 cell line with (GI_{50}= <10μg/ml). But in the case of the K-562 cell line screening study, compounds have no effect with respect to 5-FU and ADR.

From the aforementioned result (Table 1), it is recommended that the compounds 5a, 5c, 5d and 5e have shown cell growth inhibition of 50% at a concentration of 10 μl/ml, which is preferred for their *in vitro* anticancer activity on MCF-7, Hep-G2 and K-562 cell line via SRB assay protocols. Moreover, the growth curves of the MCF-7 cell line (Figure 1 and Figure 2), Hep-G2 cell line (Figure 3 and Figure 4) and K-562 cell line (Figure 5 and Figure 6) were given below.

In molecular docking studies, the entire confirmed compounds (5a-h) interacted with *EGFR tyrosine kinase enzyme* (1M17) and the consequence is given in Table 2. Docking study consequence reveals that the compound 5a having the highest mole dock scoring (-117.141), which is further proven for it's significant anticancer activity, it forms H-bond and steric interaction with *Thr* 766, *Gln* 767, *Thr* 830, *Cys* 575, *Ala* 719, and *Met* 769 amino acid residues of the 1M17 enzyme. The enzyme, hydrogen bond interaction, secondary view of the complex, steric interactions and hydrophobic interactions of compound 5a with 1M17 enzyme have been shown in Figure 7, Figure 8, Figure 9, Figure 10 and Figure 11 respectively. From this study, it has noticed that the binding interaction obtained from docking results may be the reason for the better activity while comparing with other compounds.

CONCLUSION

In the present research work, eight numbers of new 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide (5a-h) were synthesized by cyclization of 1-(4-methoxyphenyl)-3-(substituted phenyl)-prop-2-en-1-one (3a-h) with thiosemicarbazide (4) and the synthesised, resulting compounds were confirmed by virtue of their spectral analysis data. Further, the compounds were being assayed for evaluation of anticancer activity (*in vitro*) against MCF-7, Hep-G2 and K-562 cell line using SRB assay, and results have been evaluated. The results revealed that compounds 5a, 5c, 5d and 5e(GI_{50}=<10μg/ml) exhibit noticeable cytotoxicity activity against cancer cell MCF-7cell

lines as compared to 5-FU, ADR with (GI_{50}= 44.5 <10μg/ml). Moreover, docking analysis of all the synthesized compounds were performed with the *EGFR tyrosine kinase enzyme* (1M17). We found that the compound 3-(4-methoxyphenyl)-5-phenyl-2-pyrazoline-1-carbothioamide (5a) was having better interaction with *Thr* 766, *Gln* 767, *Thr* 830, *Cys* 575, *Ala* 719 and *Met* 769 amino acid residues and it can be concluded that compounds 5a, 5c, 5d and 5e could be considered as bioactive molecules for future development and research and hope to get more target-specific, less toxic and promising anticancer activity.

ACKNOWLEDGEMENT

The authors are highly thankful to the Department of Pharmaceutical Technology, Jadavpur University for their cooperation and providing adequate facilities to carry out the present work. The authors also obelised to University Grant Commission (New Delhi) for the grant of National fellowship.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding Support

The authors declare that they have no funding support for this study.

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