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Design, synthesis, antioxidant and anticancer activity of novel pyrazole derivatives

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ABSTRACT

Docking studies of a series of pyrazole derivatives were performed with the help of VLife MDS 4.2 software using Epidermal Growth Factor Receptor kinase domain (PDB ID: 1M17) as a target. Docked compounds were analyzed for hydrogen bonding, hydrophobic bonding and vander Waal's interactions. Based on the docking results, novel pyrazole derivatives were synthesized and characterized by IR, ^{1}H NMR,^{13}CNMR and Mass spectroscopy. These compounds were screened for antioxidant activity by DPPH radical scavenging activity and anticancer activity against breast cancer cell line (MCF-7) and lung cancer cell line (A549) with MTT assay. Compounds P9, P21 and P22 showed significant antioxidant activity. Compounds P1, P6, P7, P11 and P12 showed significant anticancer activity against MCF-7 and A549 cell lines which was comparable to the positive control doxorubicin.

Keywords: Anticancer, Antioxidant, Pyrazole, Docking, MTT assay.

INTRODUCTION

Cancer is currently second leading cause of death after cardiovascular disease. Consequently, there is great unmet medical need for new anticancer small molecule therapeutics[1]. The past two decades have witnessed a remarkable revolution in the field of tumor chemotherapy [2, 3]. On the basis of exhaustive literature review, it has been found that pyrazole derivatives have good potential to exhibit anticancer activity.

Heterocycles and medicines are both interrelated because humans are totally dependent on the drugs derived from heterocyclic rings [4]. Heterocyclesand their derivatives have attracted the attention of chemists, mainly because of broad spectrum biological and pharmacological activities associated with this class of compounds specially having nitrogen, sulphur and oxygen atoms [5]. Pyrazole derivatives are well-known and important nitrogen-containing five-membered heterocyclic compounds and have occupied a unique position in the design and synthesis of novel biologically active agents [6]. They display various biological activities such as antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-tubercular, antioxidant, anti-inflammatory and analgesic properties [7, 8, 9]. Due to these interesting activities of pyrazole derivatives, considerable attention has been focused on this classof compounds.
The thiourea and urea derivatives were also play an important role as anticancer agents because of their good inhibitory activity against receptor tyrosine kinases (RTKs), Protein tyrosine kinases (PTKs), and NADH oxidase, which play critical roles in many aspects of tumorigenesis [10, 11, 12].Thus In the present study, an attempt has been made to synthesize pyrazole derivatives containing thiourea skeleton as anticancer agents.

MATERIALS AND METHODS

Docking Studies:

Docking was done by GRIP batch docking method with the help of Vlife MDS 4.2 software. The crystal structure of Epidermal Growth Factor Receptor kinase domain (PDB ID: 1M17) for anticancer docking studies and was obtained from the protein data bank [13, 14, 15]. The parameter fixed for docking simulation was number of placements: 50, rotation angle: 10º, ligand flexible, exhaustive method, scoring function: dock score. The ligand forming most stable drug-receptor complex was the one which was having minimum dock score. After docking simulation, the best docked conformer of each ligand was checked for various interactions with receptor like hydrogen bonding, hydrophobic bonding and van der Waal's interaction.

Table 1: Docking scores of the synthesized pyrazole derivatives

Compound	Dock Score
P1	-67.47
P2	-65.77
P3	-72.19
P4	-65.26
P5	-67.55
P6	-71.03
P7	-69.83
P8	-71.22
P9	-65.72
P10	-65.42
P11	-71.16
P12	-73.75
P13	-63.07
P14	-72.10
P15	-69.87
P16	-70.68
P17	-68.03
P18	-65.35
P19	-62.17
P20	-61.92
P21	-68.01
P22	-69.47
P23	-63.40
P24	-67.37
P25	-62.12

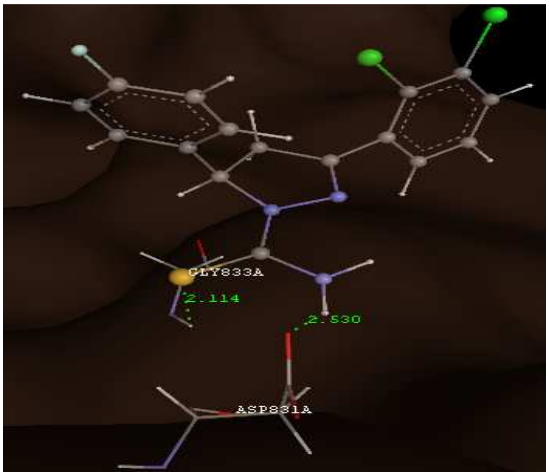


Fig. 1: Hydrogen interaction of P12 with receptor(green dotted line)

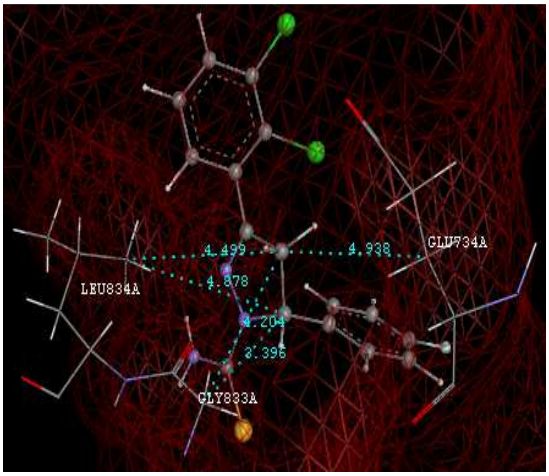


Fig. 2: Hydrophobic interaction of P12 with receptor(blue dotted line)

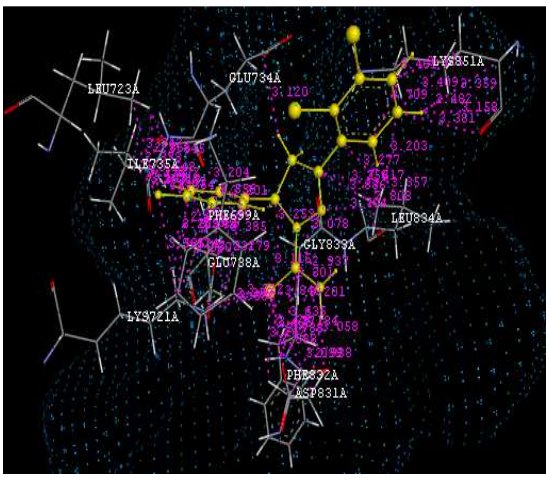


Fig. 3: vander Waal's interaction of P12 with receptor (pink dotted line)

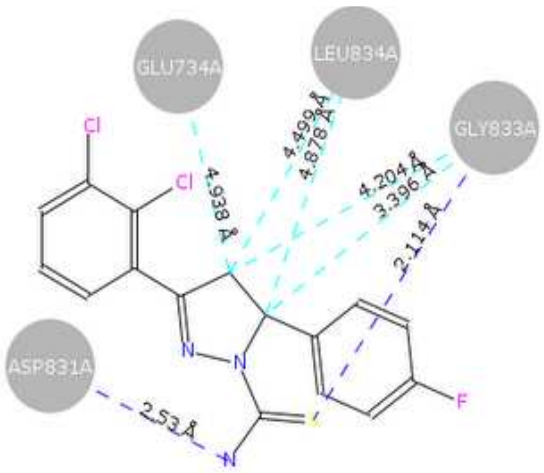
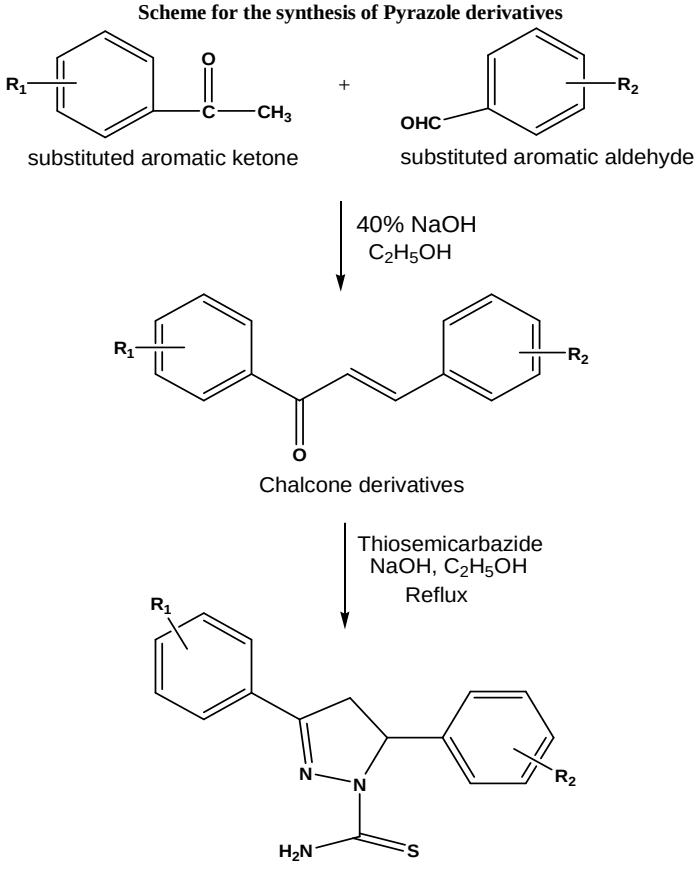


Fig. 4: 2D image of P12showing hydrogen and hydrophobic interaction with receptor

Scheme for the synthesis of Pyrazole derivatives



Synthesis

The Solvents, reagents and chemicals used in the present work were purchased from Aldrich, E. Merck, Spectrochem, and S. D. Fine Chem., HI-MEDIA and used without further purification. The purity of the synthesized compounds was checked by TLC on silica gel 60 F254 (E. Merck) aluminum plates. Melting points were determined

using laboratory melting point apparatus (Toshniwal P. Ltd.) and were uncorrected. IR spectra of the synthesized compounds were recorded on FT-IRAffinity-1 (Shimadzu) IR Spectrometer. Mass spectra were recorded on GC-MS-QP5050*A* (Shimadzu). NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-*d*6 as the solvent.

General procedure for the synthesis of chalcones

Equimolar portions of the substituted aromatic aldehydes and ketones were dissolved in 15 ml of ethanol. The mixture was allowed to stir for several minutes at 5–10°C. 10 ml of40% aqueous sodium hydroxide solution was then slowly added drop wise to the reaction flask. The reaction solution was allowed to stir at room temperature for about 4 h. Most commonly, a precipitate formed which was then collected by suction filtration.

General procedure for the synthesis of pyrazole derivatives (P1–P25)

A mixture of chalcone (0.01 mol), thiosemicarbazide (0.01 mol), and NaOH (0.025 mol) was refluxed in ethanol (25 ml) for 8 hr. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol.Yields and physical characteristics are listed in Table 2.

Table 2: Physical data of synthesized Pyrazolederivaties

Compound	R1	R2	% Yield	M.P(˚C)	Rf Value	Log P
P1	4-F	2,3-2Cl	78	205-207	0.45	4.35
P2	4-F	2,4-2Cl	72	220-223	0.63	4.38
P3	4-F	2,3-2OCH3	65	196-198	0.49	2.93
P4	4-F	2,4-2OCH3	63	170-172	0.56	2.89
P5	4-F	4-F	55	249-252	0.61	3.39
P6	4-OCH3	2,3-2Cl	74	217-219	0.36	3.83
P7	4-OCH3	2,4-2Cl	70	168-171	0.48	3.80
P8	4-OCH3	2,3-2OCH3	52	208-211	0.42	2.27
P9	4-OCH3	2,4-2OCH3	49	190-192	0.75	2.34
P10	4-OCH3	4-F	88	241-243	0.70	3.11
P11	2,3-2Cl	4-F	70	209-212	0.59	4.27
P12	2,4-2Cl	4-F	67	224-226	0.53	4.30
P13	2,3-2OCH3	4-F	62	193-195	0.44	2.83
P14	2,4-2OCH3	4-F	65	178-181	0.42	2.95
P15	2-F	4-F	46	240-243	0.54	3.29
P16	2,3-2Cl	4-OCH3	77	227-229	0.69	3.88
P17	2,4-2Cl	4-OCH3	81	175-178	0.73	3.78
P18	2,3-OCH3	4-OCH3	64	216-218	0.38	2.30
P19	2,4-2OCH3	4-OCH3	56	201-203	0.46	2.37
P20	4-F	4-OCH3	84	246-249	0.55	3.21
P21	2-Cl,4-F	2,3-2Cl	58	235-237	0.78	4.75
P22	2-Cl,4-F	2,4-2Cl	53	229-231	0.31	4.62
P23	2-Cl,4-F	2,3-OCH3	60	236-239	0.40	3.16
P24	2-Cl,4-F	2,4-OCH3	66	249-251	0.37	3.08
P25	2-Cl,4-F	4-F	75	228-230	0.72	3.95

Spectral data of pyrazole derivatives

3-(4-fluorophenyl)-5-(2,3-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P1) FTIR (KBr, cm^{-1},): 3400.50(N-H), 2924.09(C-H), 1593.20(C=N), 1575.84(C=C), 1352.10(C-N), 1199.72(C-F), 1138.00(C=S), 756.10(C-Cl); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.90(d, 2H), 2.08(s, 2H), 3.93(m, 1H), 6.94-7.03(m, 4H), 7.10-7.62(m, 4H); GCMS (EI,*m/z*):369(M+1).

3-(4-fluorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P2) FTIR (KBr, cm^{-1},): 3444.87(N-H), 2802.57(C-H), 1593.20(C=N), 1537.27(C=C), 1363.67(C-N), 1195.87(C-F), 1132.21(C=S), 788.89(C-Cl); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.85(d, 2H), 2.21(s, 2H), 3.62(m, 1H), 6.94-7.03(m, 4H), 7.0-7.60(m, 4H); GCMS(EI,*m/z*): 369(M+1).

3-(4-fluorophenyl)-5-(2,3-dimethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P3) FTIR (KBr, cm^{-1},): 3441.01(N-H), 2827.64(C-H), 1593.20(C=N), 1539.20(C=C), 1398.39(C-N), 1188.15(C-F), 1138.00(C=S), 1031.92(C-O); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.89(d, 2H), 2.20(s, 2H), 3.73(s, 6H), 3.90(m, 1H), 6.98-7.07(m, 4H), 7.25-7.69(m, 4H); GCMS (EI,*m/z*): 359(M+).

3-(4-fluorophenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P4) FTIR (KBr, cm^{-1},): 3442.94(N-H), 2835.36(C-H), 1600.92(C=N), 1467.83(C=C), 1394.53(C-N), 1188.15(C-F), 1078.71(C=S), 1066.64(C-O); GCMS (EI,*m/z*): 359(M+).

3-(4-fluorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P5) FTIR (KBr, cm^{-1},): 3473.80(N-H), 2937.59(C-H), 1575.84(C=N), 1506.41(C=C), 1228.66(C-N), 1136.07(C-F), 1066.64(C=S); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.81(d, 2H), 1.96(s, 2H), 3.88(m, 1H), 6.82-7.23(m, 4H), 6.92-7.44(m, 4H); GCMS (EI,*m/z*): 317(M+).

3-(4-methoxyphenyl)-5-(2,3-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P6) IR FTIR (KBr, cm^{-1},): 3454.51(N-H), 2902.77(C-H), 1543.05(C=N), 1402.25(C=C), 1278.81(C-N), 1138.00(C=S), 1068.56(C-O), 812.03(C-Cl); GCMS (EI,*m/z*): 380(M+).

3-(4-methoxyphenyl)-5-(2,4-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P7) FTIR (KBr, cm^{-1},): 3442.94(N-H), 2833.43(C-H), 1597.06(C=N), 1535.34(C=C), 1361.74(C-N), 1255.56(C=S), 1174.65(C-O), 833.25(C-Cl); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.99(d, 2H), 2.18(s, 2H), 3.74(s, 3H), 4.02(m, 1H), 7.04-7.23(m, 4H), 6.90-7.44(m, 4H); GCMS (EI,*m/z*): 380(M+).

3-(4-methoxyphenyl)-5-(2,3-dimethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P8) FTIR (KBr, cm^{-1},): 3439.08(N-H), 2833.43(C-H), 1593.20(C=N), 1473.62(C=C), 1361.74(C-N), 1259.52(C=S), 1174.65(C-O); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.72(d, 2H), 1.86(s, 2H), 3.83(s, 9H), 4.11(m, 1H), 6.48-6.66(m, 4H), 7.87-7.48(m, 4H); GCMS (EI,*m/z*): 371(M+).

3-(4-methoxyphenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P9) FTIR (KBr, cm^{-1},): 3439.08(N-H), 2833.43(C-H), 1598.99(C=N), 1533.41(C=C), 1367.53(C-N), 1209.37(C=S), 1122.57(C-O); GCMS (EI,*m/z*): 371(M+).

3-(4-methoxyphenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P10) FTIR (KBr, cm^{-1},): 3354.21(N-H), 2835.36(C-H), 1571.99(C=N), 1510.26(C=C), 1361.74(C-N), 1257.59(C-F), 1215.15(C=S), 1091.71(C-O); ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.20(d, 2H), 2.33(s, 2H), 3.68(s, 3H), 3.96(m, 1H), 6.92-7.10(m, 4H), 7.14-7.68(m, 4H); GCMS (EI,*m/z*): 329(M+).

3-(2,3-dichlorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P11) FTIR (KBr, cm^{-1},): 3427.51(N-H), 3005.13(C-H), 1595.13(C=N), 1635.64(C=C), 1280.73(C-N), 1205.51(C-F), 1138.00(C-F), 756.10(C-Cl); GCMS (EI,*m/z*): 368(M+).

3-(2,4-dichlorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P12) FTIR (KBr, cm^{-1},): 3441.01(N-H), 2943.97(C-H), 1595.13(C=N), 1406.11(C=C), 1319.31(C-N), 1176.58(C-F), 1176.58(C=S), 783.25(C-Cl); GCMS (EI,*m/z*): 368(M+).

3-(2,3-dimethoxyphenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P13) FTIR (KBr, cm^{-1},): 3433.29(N-H), 2829.57(C-H), 1502.55(C=N), 1404.18(C=C), 1325.10(C-N), 1139.93(C-F), 1099.43(C=S), 1043.49(C-O); ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.20(d, 2H), 2.33(s, 2H), 3.62(s, 6H), 3.94(m, 1H), 6.94-7.03(m, 4H), 7.05-7.38(m, 4H); GCMS (EI,*m/z*): 359(M+).

3-(2,4-dimethoxyphenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P14) FTIR (KBr, cm^{-1},): 3471.87(N-H), 2829.57(C-H), 1576.76(C=N), 1495.34(C=C), 1332.81(C-N), 1178.51(C-F), 1099.43(C=S), 1062.78(C-O); GCMS (EI,*m/z*): 359(M+).

3-(2-fluorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P15) FTIR (KBr, cm^{-1},): 3473.80(N-H), 3047.53(C-H), 1577.77(C=N), 1537.27(C=C), 1303.88(C-N), 1134.14(C-F), 1049.28(C=S); GCMS (EI,*m/z*): 317(M+).

3-(2,3-dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P16) FTIR (KBr, cm^{-1},): 3435.22(N-H), 2933.45(C-H), 1597.06(C=N), 1539.20(C=C), 1317.38(C-N), 1139.93(C=S), 1066.64(C-O), 813.96(C-Cl); GCMS (EI,*m/z*): 380(M+).

3-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P17) FTIR (KBr, cm^{-1},): 3437.15(N-H), 2989.66(C-H), 1597.06(C=N), 1582.54(C=C), 1359.44(C-N), 1155.36(C=S), 1068.56(C-O), 742.56(C-Cl); GCMS (EI,*m/z*): 380(M+).

3-(2,3-dimethoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P18) FTIR (KBr, cm^{-1},): 3435.22(N-H), 2827.64(C-H), 1597.06(C=N), 1512.19(C=C), 1294.24(C-N), 1139.93(C=S), 1018.41(C-O); GCMS (EI,*m/z*): 371(M+).

3-(2,4-dimethoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P19) FTIR (KBr, cm^{-1},): 3435.22(N-H), 2833.43(C-H), 1597.06(C=N), 1568.31(C=C), 1230.58(C-N), 1093.64(C=S), 1058.92(C-O), 833.25(C-Cl); ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.06(d, 2H), 2.17(s, 2H), 3.59(s, 9H), 4.02(m, 1H), 6.90-7.16(m, 4H), 7.14-7.68(m, 4H); GCMS (EI,*m/z*): 371(M+).

3-(4-fluorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P20) FTIR (KBr, cm^{-1},): 3475.73(N-H), 2831.50(C-H), 1573.91(C=N), 1473.62(C=C), 1284.59(C-N), 1192.01(C-F), 1138.00(C=S), 1060.85(C-O); GCMS (EI,*m/z*): 329(M+).

3-(2-chloro-4-fluorophenyl)-5-(2,3-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P21) FTIR (KBr, cm^{-1},): 3457.23 (N-H), 2855.60(C-H), 1569.08(C=N), 1488.26(C=C), 1311.29(C-N), 1124.10(C-F), 1105.73(C=S), 793.61(C-Cl); GCMS (EI,*m/z*): 403(M+).

3-(2-chloro-4-fluorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P22) FTIR (KBr, cm^{-1},): 3394.22(N-H), 2853.48(C-H), 1578.22(C=N), 1488.22(C=C), 1324.77(C-N), 1131.87(C-F), 1121.49(C=S), 785.51(C-Cl); GCMS (EI,*m/z*): 403(M+).

3-(2-chloro-4-fluorophenyl)-5-(2,3-dmethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P23) FTIR (KBr, cm^{-1},): 3398.30(N-H), 2826.45(C-H), 1578.28(C=N), 1497.11(C=C), 1324.77(C-N), 1124.10(C-F), 1120.53(C=S), 1048.51(C-O), 796.26(C-Cl); GCMS (EI,*m/z*): 394(M+).

3-(2-chloro-4-fluorophenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P24) FTIR (KBr, cm^{-1},): 3395.36(N-H), 2926.58(C-H), 1588.82(C=N), 1508.49(C=C), 1311.26(C-N), 1155.92(C-F), 1095.27(C=S), 1066.28(C-O), 763.75(C-Cl); GCMS (EI,*m/z*): 394(M+).

3-(2-chloro-4-fluorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P25) FTIR (KBr, cm^{-1},): 3398.30(N-H), 2794.58(C-H), 1563.21(C=N), 1488.39(C=C), 1309.47(C-N), 1155.36(C-F), 1149.82(C=S), 793.64(C-Cl); ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.10(d, 2H), 2.26(s, 2H), 3.99(m, 1H), 6.94-7.09(m, 4H), 7.09-7.36(m, 4H); GCMS (EI,*m/z*): 352(M+).

BIOLOGICAL ACTIVITY

Antioxidant Activity

Antioxidant activity of the test compounds was determined by diphenylpicrylhydrazyl (DPPH) radical scavenging method [16]. The assay was carried out in a 96 well microtitre plate. 100µl of test sample and standard solution were added to each well separately and in triplicates. 100µl of DPPH solution was added to each well. Control wells wereloaded with 100µl each of DMSO and DPPH. Sample blank and control blank were also performed. The plates were incubated at 37^{o}C for 30 minutes without exposing to light and the absorbance of each solution was measured with ELISA reader using 540 nm filters. The percentage scavenging of test samples at each concentration were calculated using the following formula

[(Control absorbance – Test absorbance) / Control absorbance] X 100

The IC_{50} was calculated using the Microsoft excel.

Table 3: Results of Antioxidant activity of Pyrazole derivatives

Compound	% scavenging				IC_{50}(µg/ml)
	25µg/ml	50µg/ml	100µg/ml	200µg/ml	
P1	58.09	60.08	61.41	69.50	76.03
P2	67.24	70.42	70.69	71.88	48.22
P3	53.32	55.44	62.33	69.23	83.17
P4	60.74	63.53	66.98	69.63	65.61
P5	54.11	56.50	58.09	58.89	99.31
P6	62.60	68.70	77.19	81.57	47.53
P7	59.09	63.53	68.04	77.19	61.99
P8	53.98	60.08	69.10	75.20	68.99
P9	75.20	82.10	84.88	91.38	21.00
P10	51.33	56.76	58.09	59.28	100.21
P11	56.76	58.75	66.98	78.65	67.26
P12	54.78	55.04	60.35	67.91	85.28
P13	64.19	66.98	74.40	84.88	48.42
P14	66.58	70.29	75.73	83.95	43.15
P15	54.51	54.64	56.76	57.43	104.78
P16	64.06	68.30	71.62	76.92	51.39
P17	52.65	54.24	55.97	57.43	107.71
P18	54.38	56.76	63.66	70.29	79.16
P19	56.37	57.69	63.66	70.69	76.50
P20	53.85	55.57	56.10	58.89	102.51
P21	69.50	80.24	85.54	88.59	27.25
P22	71.09	76.66	84.75	89.26	29.07
P23	57.56	63.79	74.14	84.62	56.16
P24	56.37	56.76	68.83	72.55	71.60
P25	55.97	59.42	68.30	80.64	65.50
Ascorbic acid	90.19	92.71	93.10	94.43	7.43

Table 4: Results of Anticancer activity of Pyrazole derivatives on MCF-7 cell line

Compound	% Cell Viability				IC_{50} (µM/ml)
	25µM/ml	50µM/ml	100µM/ml	200µM/ml	
P1	41.7	32.9	23.6	13.7	16.1
P2	61.2	47.0	35.8	19.5	44.0
P3	61.5	46.5	34.1	18.1	43.1
P4	62.8	53.9	31.7	19.9	48.8
P5	83.9	72.3	68.1	54.3	>200
P6	37.7	24.7	15.8	14.8	11.2
P7	33.7	20.8	13.8	14.1	7.9
P8	66.5	58.3	37.9	32.2	65.6
P9	72.3	66.3	48.0	35.8	96.8
P10	75.8	71.0	66.6	51.6	>200
P11	38.7	22.1	17.8	15.1	10.9
P12	28.8	18.5	13.9	14.3	3.5
P13	82.3	71.2	39.8	33.0	91.3
P14	93.2	84.1	63.9	36.5	142.0
P15	103.2	97.0	97.2	82.0	>200
P16	47.0	25.3	15.9	14.9	19.8
P17	43.6	31.5	18.2	16.6	17.2
P18	81.8	68.5	41.8	35.4	94.3
P19	83.2	69.3	42.6	33.9	94.6
P20	89.3	66.6	50.5	46.2	133.4
P21	49.9	41.0	36.0	20.3	26.9
P22	48.6	45.0	35.8	28.4	25.2
P23	102.7	104.7	79.2	67.5	>200
P24	79.7	71.9	67.4	60.1	>200
P25	114.3	85.0	54.8	51.8	>200
Doxorubicin	73.3	55.2	40.0	24.7	1.4

Anticancer Activity

The cytotoxic effect of drugs on cancer cells (MCF-7 and A549) was assessed by MTT assay [17]. In brief, exponentially growing cells (1×10^4 cells/well) were plated in 96-well plates and allowed to adhere for 24 hr. prior to extract addition. The drugs were dissolved in 0.1% DMSO and then diluted with the medium. The cells were then exposed to different concentrations of drug for 24 hr. The cells in the control wells received medium containing the same volume of DMSO (0.1%). After the incubation, 100 µL of MTT reagent (1 mg/ml in PBS) was added, and

cells were incubated for an additional 4 hr. The formazan produced by the viable cells was solubilized by addition of 100 µL DMSO. The suspension was placed on a microvibrator for 5 min, and absorbance was recorded at 540 nm by the plate reader (ELx800; BioTek, Winooski, VT, USA). The experiment was performed in triplicate. Doxorubicin was used as positive control. The percentage of growth inhibition was calculated with respect to vehicle control using the formula:

% Inhibition = [(Control absorbance – Blank absorbance) –(Test absorbance– Blank absorbance) ⁄ (Control absorbance– Blank absorbance)] × 100.

Table 5: Results of Anticancer activity of Pyrazole derivatives on A549 cell line

Compound	% Cell Viability				
	25µM/ml	50µM/ml	100µM/ml	200µM/ml	IC_{50} (µM/ml)
P1	78.1	61.4	34.3	11.5	63.5
P2	81.7	81.1	68.0	32.2	137.4
P3	67.7	60.3	43.5	34.3	77.3
P4	78.1	71.2	46.5	34.1	99.7
P5	71.8	69.1	58.3	41.3	139.4
P6	62.8	46.6	19.4	13.5	39.8
P7	44.5	35.9	16.0	14.3	20.6
P8	80.4	76.4	61.5	39.5	143.8
P9	81.1	81.7	58.5	44.1	157.1
P10	71.1	67.0	60.8	51.8	>200
P11	27.9	20.4	13.9	13.1	3.7
P12	42.1	16.8	11.9	10.4	17.6
P13	101.9	95.9	67.7	52.3	>200
P14	96.4	96.7	76.1	55.9	>200
P15	93.2	95.2	85.9	75.2	>200
P16	58.1	42.3	17.4	14.0	34.2
P17	68.6	55.7	26.1	22.0	52.5
P18	93.0	79.5	62.7	51.1	>200
P19	89.2	74.9	60.5	43.6	152.6
P20	76.4	62.9	50.0	46.9	131.5
P21	62.6	56.6	37.1	25.4	55.5
P22	64.6	58.9	46.0	31.3	72.2
P23	84.9	74.6	62.9	53.0	>200
P24	55.3	43.9	29.0	21.4	33.8
P25	94.5	86.6	52.6	40.2	134.5
Doxorubicin	89.2	52.4	38.1	22.5	1.0

RESULTS AND DISCUSSION

In docking studies compound P12 was found with highest negative dock score (Table 1), indicating most stable drug-receptor complex amongst all the compounds. It was found to form hydrogen bonding with Asp 831 and Gly 833 of the receptor (Fig. 1). It also formed hydrophobic bond with Glu734, Gly 833 and Leu 834 (Fig. 2). It exhibited large number of van der Waal's interaction with wide range of residues (Fig. 3). Based on the docking results, novel pyrazole derivatives were synthesized and these compounds were screened for their antioxidant and anticancer activity. Among the tested compounds 3-(4-methoxyphenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydropyrazole-1-carbothioamide (P9), 3-(2-chloro-4-fluorophenyl)-5-(2,3-dichlorophenyl)-4,5-dihydro pyrazole-1-carbothioamide (P21) and 3-(2-chloro-4-fluorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P22) showed good antioxidant activity by DPPH method with IC_{50} value of 21µg, 27.25µg and 29.07µg, which was comparable with that of IC_{50} of Ascorbic acid 7.43 µg (Table 3). Compound 3-(4-fluorophenyl)-5-(2,3-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P1) showed IC_{50}16.1µm/ml against MCF-7 and 63.5µm/ml against A549, 3-(4-methoxyphenyl)-5-(2,3-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P6)showed IC_{50}11.2µm/ml against MCF-7 and 39.8µm/ml against A549, 3-(4-methoxyphenyl)-5-(2,4-dichlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P7) showed IC_{50}7.9µm/ml against MCF-7 and 20.6µm/ml against A549, 3-(2,3-dichlorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P11) showed IC_{50}10.9µm/ml against MCF-7 and 3.7µm/ml against A549, 3-(2,4-dichlorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazole-1-carbothioamide (P12) showed IC_{50}3.5µm/ml against MCF-7 and 17.6µm/ml against A549, which were comparable with that of IC_{50} of Doxorubicin 1.4µM/ml against MCF-7 and 1.0µM/ml against A549 (Table 4 and Table 5).

From docking as well as biological results it was clear that compounds having electron withdrawing groups like chlorine, fluorine and electron donating group like methoxy on the phenyl rings attached to pyrazole exhibit good antioxidant and anticancer activity.

CONCLUSION

Based on the docking results, we have synthesized novel pyrazole derivatives. As a preliminary study, these compounds were screened for their antioxidant activity. Further, all these compounds were tested for their anticancer activity. The compounds which have shown highest negative dock score has exhibited good anticancer activity. Docking studies and anticancer activity revealed that the compounds can be evaluated further for their Epidermal Growth Factor Receptor Tyrosine Kinase (EGFR-TK) inhibitory activity.

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