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Synthesis and Characterization of 2-Phenylpyrazoline Derivatives and Evaluation of their Activities against Antimicrobial and Breast Cancer Cell Line *in vitro* and *in silico* Studies

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The new series of 2-phenylpyrazoline derivatives (**2a-j**) were synthesized and evaluated for their antimicrobial, *in silico* and *in vitro* anticancer activity was performed by MTT assay using MDA-MB-231 (human breast adenocarcinoma) cell line. The 2-phenylpyrazoline derivatives (**2a-j**) were obtained by the cyclization of chalcones with phenylhydrazine hydrochloride. Synthesized compounds were confirmed using FT-IR, ^{1}H NMR and ^{13}C NMR spectral data. Molecular docking studies were carried out using Auto Dock Tool version 1.5.6 and Auto dock version 4.2.5.1 docking program. *in silico* Docking study, compound **2d** showed good binding score and good binding interaction with selected bacterial proteins and breast cancer protein. Based on this result, compound **2d** was performed the anticancer activity by MTT assay method. From this result, compound **2d** shown the LC_{50} value is 185.30 ± 1. 469 µg/mL. From the antibacterial activity compound **2i** (2,3-dichloro substituted 2-pyrazoline derivative) showed a good zone of inhibition at high concentration (100 mg/mL) as compared to other derivatives (2**a-j**) and compound **2c** (fluoro substituted 2-phenylpyrazoline derivative) showed a good zone of inhibition at low concentration (25 mg/mL) compared to other derivative (**2a-j**).

Keywords: Biphenyl Chalcone, Phenylhydrazine hydrochloride, Antimicrobial activity, Anticancer activity.

INTRODUCTION

In 20th century the most prevailing disease is cancer. The spreading of the disease is rising rapidly day by day. Surgery, radiation and chemotherapy are the most common treatment for this disease. Despite these treatments, cancer is uncontrollable and medical field needs a new approach to treat this dreadful disease. One of the most commonly used treatment methods is chemotherapy. In this method, it spreads throughout the body because the medicine and radiation used in this method travel to an entire part of the body and have some affect on the human being [1]. Therefore, we have to develop and pay more attention to update, modify and recreate the drugs and treatment methods for cancer. For medicinal chemistry and drug discovery, it is one of the urgent requirements to introduce new drugs and treatment methods for the disease [2]. The breast cancer as the second leading cause of death throughout the world is still the most frequently identified cancer in woman [3]. The literature clearly indicated that more than 90 % of cancer patients die due to chronic tumor metastases. Despite the presence of a large number of anticancer drugs, no currently available agents can eradicate cancer cells without harming normal tissues. Thus, the development of newer chemotherapeutic scaffolds which with selectively act on the target without side effects has become a primary objective of medicinal chemists [4].

The chalcones are the convenient intermediate for the synthesis of pyrazoline derivatives. It has been reported various biological activities, such as muscle relaxant, tranquilizing, psychoanalytic, anticonvulsant, antidepressant and antihypertensive activities [5,6]. Pyrazolines are very useful synthone in organic synthesis. Among various pyrazoline derivatives, 2-pyrazolines derivative of compounds is mostly studied [7]. Pyrazoline compounds have adjacent two nitrogen atom occur from saturated and partially saturated pyrazoles in biologically active compounds and natural products [8]. Pharmacologically interesting heterocyclic systems like pyrazoline derivatives have been widely studied to their pharmacological activities such as antitumor, anti-inflammatory, antiparasitary, anticonvulsant, antimicrobial, antinociceptives, antimalarial,

Alzheimer, Huntington and inflammatory arthritis, anticancer, antiviral, antioxidant, antiamoebic, antifungal, antidiabetic, cytotoxic and pesticidal properties [9]. In our previous studies, we synthesized several 1-thiocarbamoyl derivatives and tested them for antimicrobial activity and *in silico* activity. We reported that *in vitro* antimicrobial activity and *in silico* activity of 1-thiocarbamoyl substituted pyrazole derivatives [10].

A new series of 2-phenylpyrazoline derivatives are formed by cyclization of chalcones with phenylhydrazine hydrochloride. The chemical structures of 2-phenylpyrazoline derivatives was confirmed using FT-IR, ^{1}H NMR and ^{13}C NMR spectral data. *in silico* Studies were carried out for synthesized 2-phenyl - pyrazoline compounds (**2a-j**) using bacterial proteins (1UAG, 3UDI and 2X5O) and breast cancer protein (1OQA). Based on the high binding score, the best compound was performed to *in vitro* anticancer activity by MTT assay. Furthermore, antimicrobial activity was carried out using different strains (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Candida albicans*) by agar disc diffusion method.

EXPERIMENTAL

General procedure for the synthesis of *E*-3-(phenyl)-1-(diphenyl-2-yl)-3-arylprop-2-en-1-one derivatives (1a-j): Various substituted aldehydes (**1a-j**, 0.1 mol) and 4-acetyl biphenyl (0.1 mol) were taken in a 250 mL Erlenmeyer flask and to this approximately added nearly 30-35 mL of ethanol containing 20 % of NaOH solution. This mixture was stirred well for 3 h, then this mixture was transferred to 500 mL beaker containing pieces of ice cubes and kept overnight at room temperature. The solid obtained is a chalcone, which was filtered, dried and recrystallized using ethanol. Finally, the purity of the compound was checked by TLC using $CHCl_3$ as a solvent [10].

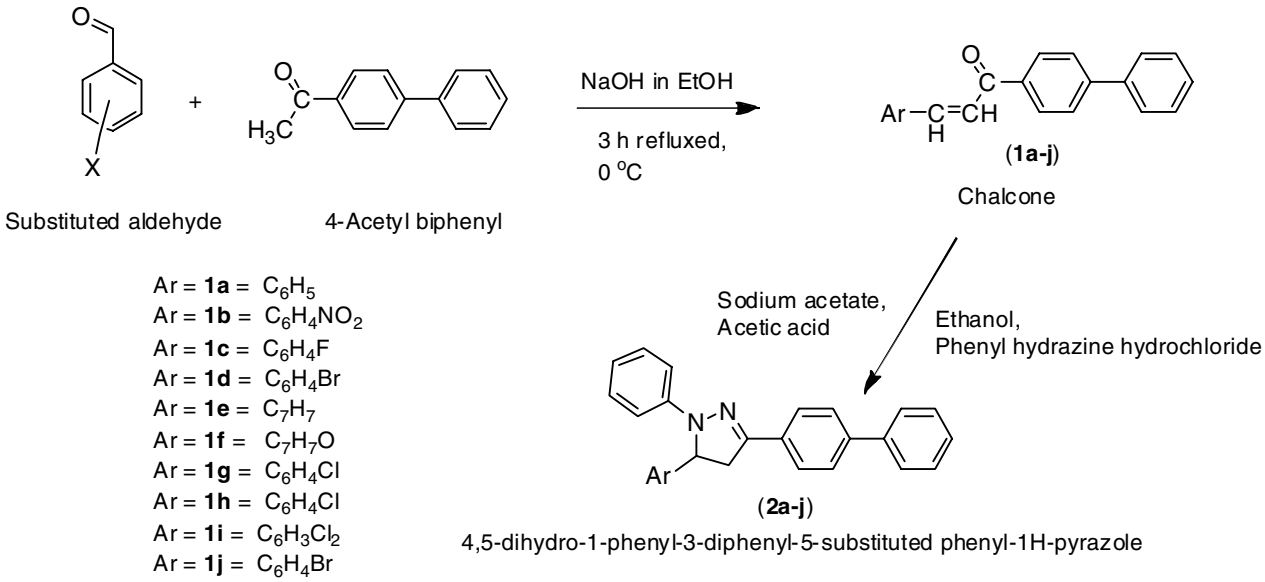
General procedure for the synthesis of 4,5-dihydro-1-phenyl-3-diphenyl-5-phenyl-1*H*-pyrazole derivatives (2a-j): Substituted chalcone (1 mol) and phenylhydrazine hydrochloride (1 mol) was taken in a 250 mL round bottom flask containing 40 mL ethanol. After, 1 mol of sodium acetate was dissolved in 10 mL acetic acid in a 100 mL beaker. Then, the solution was added to the round bottom flask. After that the reaction mixtures was refluxed for 14 -16 h. Completion of the reaction was monitored by TLC using $CHCl_3$ used as a solvent. This reaction mixture was poured into crushed ice and kept in overnight at room temperature. Then these precipitate was filtered, dried and recrystallized using ethanol. The purity of the compound was checked by TLC using $CHCl_3$ as a solvent (**Scheme-I**).

Spectral data

4,5-Dihydro-1-phenyl-3-diphenyl-5-phenyl-1*H*-pyrazole (2a): Yield 81%; m.p: 181 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1598.71 (C=N), 1513.85 (C=C), 1426.01 (C-N) 3067.13 (Ar-CH); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz): 3.06 (1H, dd, H_{4a}, $J_{4a,4b}$ 17.4 Hz, $J_{4a,5a}$ 6.2 Hz); 3.91 (1H, dd, H_{4b}, $J_{4b,4a}$ 16.4 Hz, $J_{4b,5a}$ 12.8 Hz); 5.67 (1H, dd, H_{5a}, $J_{5a,4a}$, 12 Hz, $J_{5a,4b}$ 6 Hz); 6.92-7.87 (Ar-H). ^{13}C NMR δ: 149.72 (C3 of pyrazole ring), 43.14 (C4 of pyrazole ring), 62.51 (C5 of pyrazole ring); 145.71, 144.52, 143.87, 143.21 (IPSO carbon); 113.21, 118.78, 122.90, 124.35, 124.90, 126.35, 126.86, 126.97, 127.12, 127.83, 1278.49, 128.55, 129.30, 129.73, 129.91, 131.16 (Ar-C). Elemental analysis of $C_{27}H_{22}N_2$ calcd. (found) %: C, 86.59 (86.60); H, 5.87 (5.92); N, 7.47 (7.48).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(4-nitrophenyl)-1*H*-pyrazole (2b): Yield 84 %; m.p: 186 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1595.81 (C=N), 1521 (C=C), 1410 (C-N), 3053.73 (Ar-CH); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz): 3.14 (1H, dd,H_{4a}, $J_{4a,4b}$ 17 Hz, $J_{4a,5a}$ 7 Hz); 3.93 (1H, dd, H_{4b}, $J_{4b,4a}$ 16.8 Hz, $J_{4b,5a}$ 12.8 Hz); 5.38 (1H, dd, H_{5a}, $J_{5a,4a}$ 12.2 Hz, $J_{5a,4b}$ 7 Hz); 6.81-8.21 (Ar-H). ^{13} C NMR δ: 149.81 (C3 of pyrazole ring), 43.25 (C4 of pyrazole ring), 63.81 (C5 of pyrazole ring); 147.46, 146.58, 144.35, 141.58 (IPSO carbon); 113.48, 119.85, 123.88, 124.25, 124.61, 125.50, 126.35, 126.66, 126.97, 127.00, 127.31, 127.49, 127.75, 129.00, 129.13, 129.24, 129.43, 131.18, 140.31 (Ar-C). Elemental analysis of $C_{27}H_{21}N_3O_2$ calcd. (found) %: C, 77.30 (77.31); H, 5.00 (5.05); N, 10.01 (10.02); O, 7.62 (7.63).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(4-fluorophenyl)-1*H*-pyrazole (2c): Yield 74 %;; m.p: 169 ºC; solid yellow



Scheme-I: Synthetic pathway for 2-phenyl pyrazoline derivatives (**2a-j**)

colour; IR (KBr, ν_{max}, cm^{-1}): 1599.66 (C=N), 1513.85 (C=C), 1401.03 (C-N), 3034.44 (Ar-CH); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz): 3.19 (1H, dd, H_{4a}, $J_{4a,4b}$ 17.3 Hz, $J_{4a,5a}$ 6.9 Hz); 3.91 (1H, dd, H_{4b}, $J_{4b,4a}$16.9 Hz, $J_{4b,5a}$ 12.2 Hz); 5.28 (1H, H_{5a}, $J_{5a,4a}$ 12.6 Hz, $J_{5a,4b}$ 6.7 Hz); 6.79-8.18 (Ar-H). ^{13}C NMR δ: 149.34 (C3 of pyrazole ring), 45.87 (C4 of pyrazole ring), 61.53 (C5 of pyrazole ring);145.24, 145.09, 144.23, 141.58 (IPSO carbon); 115.28, 117.32, 122.88, 124.25, 124.31, 125.67, 125.95, 126.13, 127.97, 128.00, 128.18, 128.77, 129.20, 129.63, 129.74, 130.43, 141.18, 141.73 (Ar-C). Elemental analysis of $C_{27}H_{21}N_2F$ calcd. (found) %: C, 82.62 (82.63); H, 5.35 (5.39); N, 7.13 (7.14); F, 4.83 (4.84).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(4-bromophenyl)-1*H*-pyrazole (2d): Yield 86 %; m.p.: 168 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1593 (C=N), 1544 (C=C), 1405 (C-N), 3057.58 (Ar-CH); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz): 3.13(1H, dd, H_{4a}, $J_{4a,4b}$ 17 Hz, $J_{4a,5a}$ 7 Hz); 3.87 (1H, dd, H_{4b}, $J_{4b,4a}$ 14.8 Hz, $J_{4b,5a}$ 12.6 Hz); 5.38 (1H, H_{5a}, $J_{5a,4a}$ 12.4 Hz, $J_{5a,4b}$ 7.2 Hz); 6.79-8.11 (Ar-H). ^{13}C NMR δ: 146.39 (C3 of pyrazole ring), 43.42 (C4 of pyrazole ring), 63.93 (C5 of pyrazole ring);144.53, 143.38, 141.58, 141.39 (IPSO carbon); 113.41, 119.42, 122.75, 125.42, 126.19, 126.27, 126.98, 127.02, 127.25, 127.40, 127.57, 127.69, 127.86, 128.81, 128.87, 129.14, 129.32, 130.24, 131.79, 140.46, 140.73 (Ar-C). Elemental analysis of $C_{27}H_{21}N_2Br$ calcd. (found) %: C, 71.52 (71.53); H, 4.63 (4.67); N, 6.17 (6.18); Br, 17.62 (17.62).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(*p*-tolyl)-1*H*-pyrazole (2e): Yield 84 %; m.p:. 174 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1589.32 (C=N), 1523.46 (C=C), 1414.61 (C-N), 3073.98 (Ar-CH); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz) : 3.14 (1H, dd, H_{4a}, $J_{4a,4b}$ 16.8 Hz, $J_{4a,5a}$ 7.2 Hz); 3.84 (1H, dd, H_{4b}, $J_{4b,4a}$ 16.8 Hz, $J_{4b,5a}$ 12.4 Hz); 5.26 (1H, H_{5a}, $J_{5a,4a}$ 12 Hz, $J_{5a,4b}$ 7.2 Hz); 2.36 (S, 3H, CH_3); 6.76-7.79 (Ar-H). ^{13}C NMR δ: 146.41 (C3 of pyrazole ring), 43.64 (C4 of pyrazole ring), 64.34 (C5 of pyrazole ring); 21.35 (S, 3H , CH_3); 144.86, 141.16, 140.56, 139.67 (IPSO carbon); 113.45, 119.12, 125.85, 126.19, 127.00, 127.05, 127.21, 127.43, 127.54, 128.68, 128.85, 128.89, 128.95, 129.93, 129.86, 131.84, 137.28, (Ar-C). Elemental analysis of $C_{28}H_{24}N_2$ calcd. (found) %: C, 86.55 (86.56); H, 6.17 (6.18); N, 7.20 (7.21).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(4-methoxyphenyl) -1*H*-pyrazole (2f): Yield 71 %; m.p.:167 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1596.77 (C=N), 1544.77 (C=C), 1407.78 (C-N), 3061.44 (Ar-CH); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz): 3.14 (1H, dd, H_{4a}, $J_{4a,4b}$ 17.14 Hz, $J_{4a,5a}$ 7 Hz); 3.84 (1H, dd, H_{4b}, $J_{4b,4a}$ 13.4 Hz, $J_{4b,5a}$ 9 Hz); 5.26 (1H, H_{5a}, $J_{5a,4a}$ 12 Hz, $J_{5a,4b}$ 7.2 Hz); 3.77 (s, 3H, OCH_3); 6.76-7.77 (Ar-H); ^{13}C NMR δ: 146.43 (C3 of pyrazole ring), 43.63 (C4 of pyrazole ring), 64.04 (C5 of pyrazole ring); 55.31 (s, 3H, OCH_3); 144.84, 141.15, 140.54, 134.67 (IPSO carbon); 113.49, 114.02, 114.54, 119.14, 125.47, 126.20, 126.35, 127.00, 127.05, 127.11, 127.22, 127.40, 127.56, 128.86, 128.97, 129.02, 130.12, 131.85, 159.02 (Ar-C). Elemental analysis of $C_{28}H_{24}N_2O$ calcd. (found) %: C, 83.13 (83.14); H, 5.93 (5.98); N, 6.92 (6.93); O, 3.95 (3.96).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(2-chlorophenyl)-1*H*-pyrazole (2g): Yield 77 %; m.p.: 177 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1596.77 (C=N), 1521.56 (C=C), 1447.31 (C-N), 3055.66 (Ar-CH); ^{1}H NMR ($CDCl_3$): 400 MHz, δ, ppm (*J*, Hz): 3.15 (1H, dd, H_{4a}, $J_{4a,4b}$ 17.1 Hz, $J_{4a,5a}$ 7.2 Hz); 3.81 (1H, dd, H_{4b}, $J_{4b,4a}$ 16.4 Hz, $J_{4b,5a}$ 12.4 Hz); 5.22 (1H, H_{5a}, $J_{5a,4a}$ 11.8 Hz, $J_{5a,4b}$ 7.4Hz); 6.68-7.79 (Ar-H). ^{13}C NMR δ: 150.00 (C3 of pyrazole ring), 43.65 (C4 of pyrazole ring), 64.23 (C5 of pyrazole ring); 146.44, 145.04, 141.01, 140.61 (IPSO carbon); 113.04, 113.52, 118.96, 126.18, 126.81, 127.21, 127.52, 128.91, 128.93, 130.31, 132.06 (Ar-C). Elemental analysis of $C_{27}H_{21}N_2Cl$ calcd. (found) %: C, 79.29 (79.30); H, 5.13 (5.18); N, 6.84 (6.85); Cl, 8.66 (8.67).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(4-chlorophenyl)-1*H*-pyrazole (2h): Yield 81 %; m.p.: 181 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1599.66 (C=N), 1542.77 (C=C), 1407.78 (C-N), 3032.51 (Ar-CH); ^{1}H NMR ($CDCl_3$): 400 MHz, δ, ppm (*J*, Hz): 3.12 (1H, dd, H_{4a}, $J_{4a,4b}$ 16.8 Hz, $J_{4a,5a}$ 6.8 Hz); 3.86 (1H, dd, H_{4b}, $J_{4b,4a}$ 16.4 Hz, $J_{4b,5a}$ 12.8 Hz); 5.27 (1H, H_{5a}, $J_{5a,4a}$ 11.8 Hz, $J_{5a,4b}$ 7 Hz); 3.65 (S, 3H, OCH_3); 6.80-8.11 (Ar-H). ^{13}C NMR δ: 149.89 (C3 of pyrazole ring), 43.78 (C4 of pyrazole ring), 64.36 (C5 of pyrazole ring); 145.72, 144.58, 143.34, 143.27 (IPSO carbon); 113.45, 119.43, 122.40, 125.42, 126.99, 127.03, 127.25, 127.34, 127.37, 127.42, 128.32, 128.86, 128.91, 129.04, 129.15, 129.18, 129.39, 130.01, 131.52, 133.37, 136.47, 139.89, 140.46, 141.07 (Ar-C). Elemental analysis of $C_{27}H_{21}N_2Cl$ calcd. (found) %: C, 79.29 (79.30); H, 5.13 (5.18); N, 6.86 (6.85); Cl, 8.66 (8.67).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(2,3-dichloro-phenyl)-1*H*-pyrazole (2i): Yield 61 %; m.p; 183 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}): 1597.74 (C=N), 1515.67 (C=C), 1427.04 (C-N), 3031.86 (Ar-CH); ^{1}H NMR ($CDCl_3$): 400 MHz, δ, ppm (*J*, Hz): 3.19 (1H, dd, H_{4a}, $J_{4a,4b}$ 16.6 Hz, $J_{4a,5a}$ 6.4 Hz); 3.81 (1H, dd, H_{4b}, $J_{4b,4a}$ 16.9 Hz, $J_{4b,5a}$ 13.4 Hz); 5.38 (1H, H_{5a}, $J_{5a,4a}$ 13.8 Hz, $J_{5a,4b}$ 6.8 Hz); 6.73- 8.21 (Ar-H). ^{13}C NMR δ: 146.63 (C3 of pyrazole ring), 43.47 (C4 of pyrazole ring), 63.88 (C5 of pyrazole ring);56.17 (s, 3H, OCH_3); 145.23, 144.61, 143.14, 143.27 (IPSO carbon); 113.75, 115.43, 119.40, 125.42, 126.00, 127.61, 127.85, 128.01, 128.86, 128.97, 129.00, 129.34, 129.77, 129.89, 130.01, 131.32, 132.37, 133.51, 134.89, 138.46, 139.31,139.79, 140.56, 141.07, 159.03 (Ar-C). Elemental analysis of $C_{27}H_{20}N_2Cl_2$ calcd. (found) %: C, 73.13 (73.14); H, 4.51 (4.55); N, 6.32 (6.31); Cl, 15.99 (15.99).

4,5-Dihydro-1-phenyl-3-diphenyl-5-(3-bromophenyl)-1*H*-pyrazole (2j): Yield 86 %; m.p.: 172 ºC; solid yellow colour; IR (KBr, ν_{max}, cm^{-1}); ^{1}H NMR ($CDCl_3$); 400 MHz, δ, ppm (*J*, Hz): 3.12 (1H, dd, H_{4a}, $J_{4a,4b}$ 16.8 Hz, $J_{4a,5a}$ 6.8 Hz); 3.83 (1H, dd, H_{4b}, $J_{4b,4a}$ 14.4 Hz, $J_{4b,5a}$ 12.8 Hz); 5.34 (1H, H_{5a}, $J_{5a,4a}$ 12.8 Hz, $J_{5a,4b}$ 7.2 Hz); 6.78-8.14 (Ar-H). ^{13}C NMR δ: 146.47 (C3 of pyrazole ring), 43.76 (C4 of pyrazole ring), 63.45 (C5 of pyrazole ring); 143.17, 143.38, 142.78, 141.03 (IPSO carbon); 113.17, 119.65, 122.14, 123.19, 124.39, 125.37, 126.98, 127.00, 127.25, 127.47, 127.98, 128.00, 128.66, 128.81, 128.87, 129.13, 129.54, 130.01, 131.43, 138.81, 139.67 (Ar-C). Elemental analysis of $C_{27}H_{21}N_2Br$ calcd. (found) %: C, 71.52 (71.53); H, 4.63 (4.67); N, 6.17 (6.18); Br, 17.62 (17.62).

***in silico* Studies:** *in silico* Study has been carried out for synthesized new 2-phenylpyrazoline derivatives (**2a-j**) using bacterial proteins (1UAG, 3UDI, and 2X5O) and breast cancer protein (1OQA). Different software is used in molecular docking studies, they are Chem draw, Pyrx, Chimera and Discovery studies.

Molecular docking studies: Molecular docking studies have been carried out using ADT (Auto Dock Tool) version 1.5.6. and Auto Dock version 4.2.5.1 docking program.

Preparation of protein: The bacterial proteins and breast cancer protein were downloaded from Protein Data Bank (PDB id: 1UAG, 3UDI, 2X50 and 10QA).

Ligand preparation: 2D structure of 2-phenylpyrazoline derivatives (**2a-j**) is drawn using ChemDraw Ultra 8.0 (Chemoffice 2002). After that Chem 3D Ultra 8.0 was used to convert the 2D structure into the 3D structure and the energy is minimized using semi-empirical AM1 method. All the structures are saved as PDB file format for input to ADT. Finally, all the ligand structures are saved as PDB file format to carry out molecular docking in Auto dock Vina.

Grid formation: A grid box with a dimension of 40 × 40 × 40 $Å^3$ in 0.375 Å spacing and centered on 30.473, 47.997, 9.563 has created around the binding site of protein using ADT. The center of the box was set at ligand center and grid energy calculations have been carried out.

Docking protocol: The auto dock calculation is such as default parameters have been used and 10 docked confirmations are generated for each compound. The energy calculation is done using genetic algorithms. The outputs are exported to Chimera 1.10 and discovery studio 4.5 for visual inspection of the binding modes and interaction of the compounds with amino acid residues in the active site [11].

Anticancer activity

MTT assay: MDA-MB-231 (human breast adenocarcinoma) cell was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco′s modified Eagles medium, DMEM (Sigma Aldrich, USA).

The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with 10 % FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and the antibiotic solution containing penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL). Cultured cell lines were kept at 37 ºC in a humidified 5 % CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate: A two-day old confluent monolayer of cells trypsinized and the cells were suspended in 10 % growth medium, 100 µL cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37 ºC in a humidified 5 % CO_2 incubator.

Preparation of compound stock: 1 mg of sample was weighed and dissolved in 1 mL of DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Anticancer evaluation: After 24 h the growth medium was removed, freshly prepared each compounds in 5 % DMEM was five times serially diluted by two-fold dilution (100, 50, 25, 12.5 and 6.25 µg) in 500 µL of 5 % DMEM and each concentration of 100 µL were added in triplicates to the respective wells and incubated at 37 ºC in a humidified 5 % CO_2 incubator. Non-treated control cells were also maintained.

Anticancer assay by MTT method: 15 mg of MTT (Sigma, M-5655) was reconstituted in 3 mL PBS until completely dissolved and sterilized by filter sterilization. After 24 h of incubation period, the sample content in wells was removed and 30 µL of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well and incubated at 37 ºC in a humidified 5 % CO_2 incubator for 4 h. After the incubation period, the supernatant was removed and 100 µL of MTT solubilization solution (DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm [12]. The percentage of growth inhibition was calculated using the following formula:

$$\text{Viability (\%)} = \frac{\text{Mean OD samples}}{\text{Mean OD of control group}} \times 100$$

Antimicrobial activity: The synthesis of 2-phenylpyrazoline derivatives was subjected to antimicrobial activity by agar disk diffusion method. In these studies, four different strains were used are *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli* and *Klebsiella*.

Antifungal activity: The synthesized new 2-phenylpyrazoline derivatives were screened for antifungal activity using *Candida albicans* by agar disc diffusion method.

RESULTS AND DISCUSSION

In this work, 2-phenylpyrazoline derivatives (**2a-j**) were prepared by the cyclization of phenylhydrazine hydrochloride with chalcones (**1a-j**) in turn generated as intermediates by Aldol condensation reaction between the corresponding 4-acetyl biphenyl and substituted aldehydes in ethanolic NaOH solution.

Molecular docking studies for 4,5-dihydro-1-phenyl-3-diphenyl-5-substituted phenyl-1*H*-pyrazole derivatives (2a-j): In the present study, *in silico* studies were carried out by Auto Dock Tool version 1.5.6 and Auto dock version 4.2.5.1 docking program using bacterial proteins (1UAG, 3UDI and 2X50) and breast cancer protein (1OQA) (Figs. 1 and 2). The bacterial proteins (1UAG, 3UDI and 2X5O) which is involved in cell wall synthesis mechanism.

The docking studies which are reported in terms of binding affinity value, which mean the lower, the score, the better and the interactions. In ligand 2-phenylpyrazoline derivatives (**2a-j**) were individually docked with the bacterial proteins and breast cancer protein. The molecular docking results are shown in Table-1, which showed that 2-phenylpyrazoline derivatives (**2a-j**) exhibited good binding affinity score as compared to the standard drug. Based on binding affinity score, hydrophobic and hydrophilic interactions and 2-phenylpyrazoline derivatives showed better binding affinity as compared to standard drug.

The molecular docking studies show that these 2-phenylpyrazoline derivatives bind well in the active site pocket of bacterial proteins and interact with the active site of amino acid residues. The best compound of these derivatives (**2a-j**) have been explained as follows:

Binding affinity score: The synthesized 2-phenylpyrazoline derivatives (**2a-j**) were docked with bacterial protein 1UAG, 2X5O and 3UDI. From this result, 2-phenylpyrazoline derivative

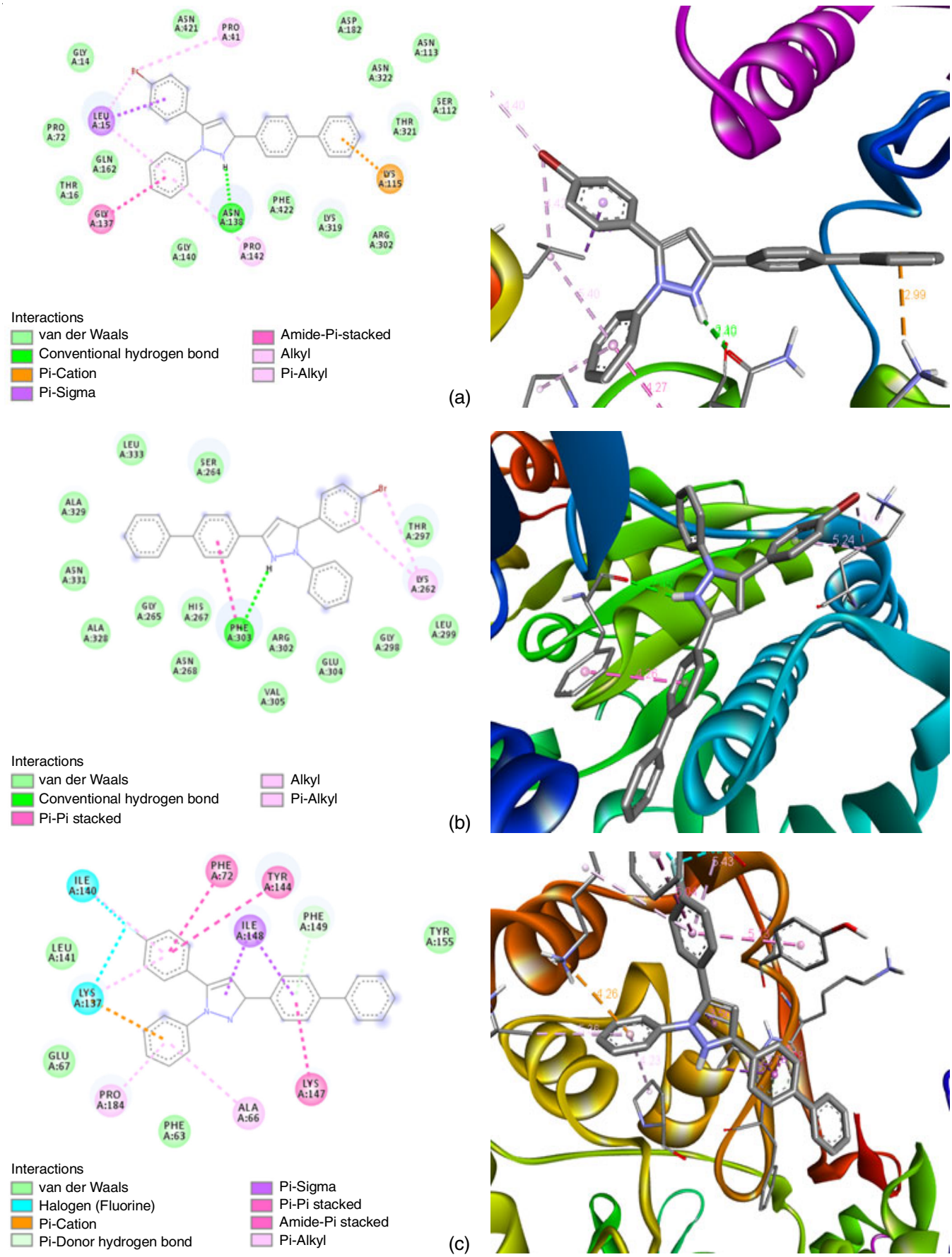


Fig. 1. (a) 2D and 3D image of compound **2d** docked with 1UAG protein; (b) 2D and 3D image of compound **2d** docked with 2X5O protein, (c) compound **2d** and 3D image of compound **2d** docked with 3UDI protein

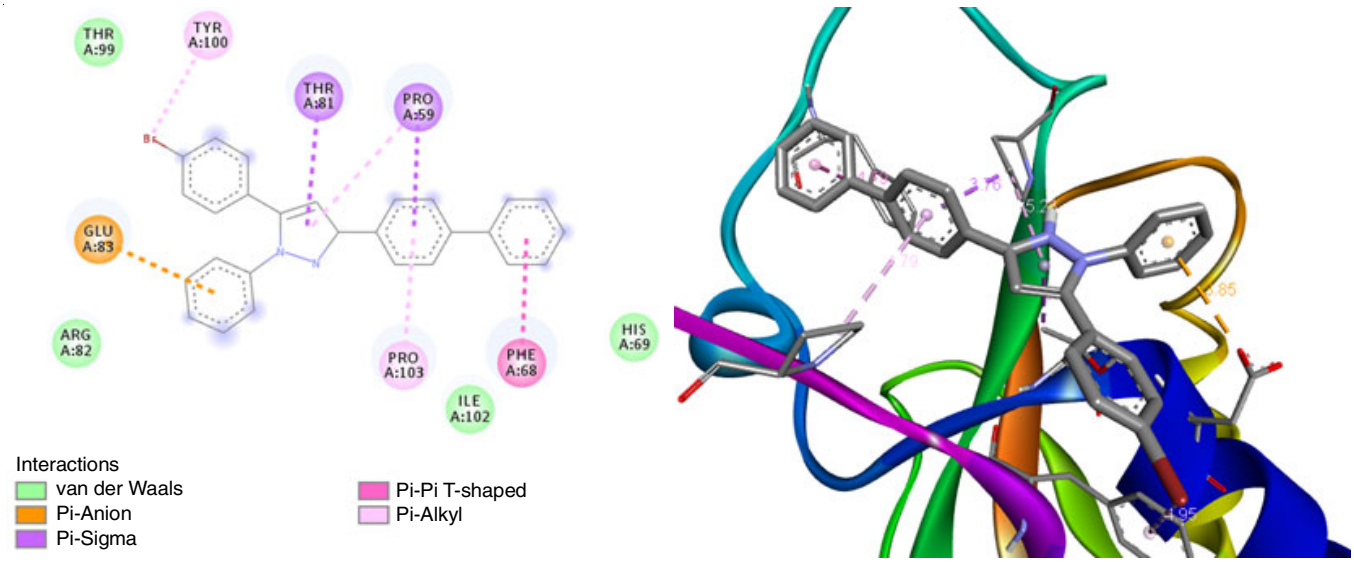
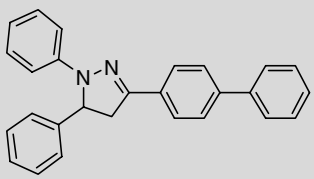
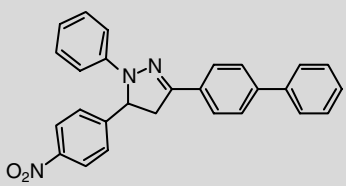
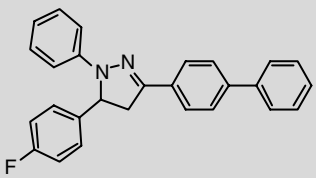
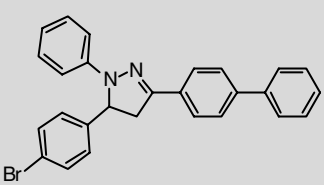
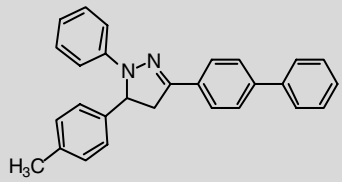
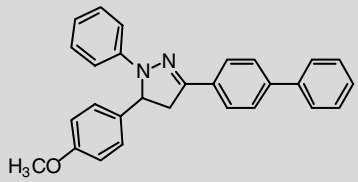
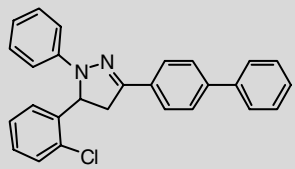
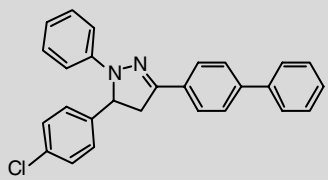
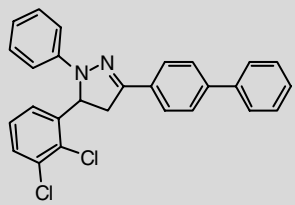
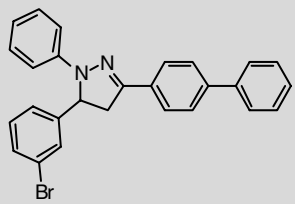
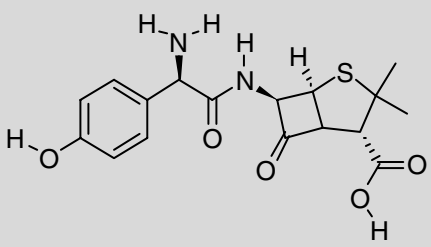


Fig. 2. 2D and 3D image of compound 4,5-dihydro-1-phenyl-3-diphenyl-5-(4-bromophenyl)-1*H*-pyrazole (**2d**) docked with breast cancer protein (1OQA)

TABLE-1
MOLECULAR DOCKING RESULTS FOR 2- PHENYL PYRAZOLINE DERIVATIVES (**2a-j**) DOCKED WITH DIFFERENT BACTERIAL PROTEINS

Compound	Protein	Binding affinity value (kcal/mol)	Conventional H-Bond interaction	H-Bond length	Hydro-phobic interaction	Other interactions
	1UAG	-8.7	Nil	Nil	ALA A: 414, LYS A: 348, LEU A: 416	LYS A: 319, LYS A: 115
	2X5O	-8.6	PHE A: 303	2.40	LYS A: 262	PHE A: 303
	3UDI	-10.7	Nil	Nil	ALA A: 66, LYS A: 137, ILE A: 140, PRO A: 184	PHE A: 72, ILE A: 140, TYR A: 144, LYS A: 147
	1UAG	-9.3	LYS A: 115, ARG A: 302, LYS A: 319	2.75, 2.42, 2.53	LYS A: 348, ALA A: 414, LEU A: 416	PHE A: 422, ASP A: 185, LEU A: 416
	2X50	-8.6	ASN A: 271	2.62	ALA A: 328, LEU A: 216, ALA A: 215	ASP A: 214, GLU A: 327
	3UDI	-9.2	LYS A: 137, ARG A: 202	2.93, 1.90	PRO A: 184	PHE A: 72, LYS A: 147, ILE A: 148TYR A: 144
	1UAG	-8.8	Nil	Nil	LEU A: 416, ALA A: 414	LEU A: 416, LYS A: 319, PHE A: 422, ASP A: 185
	2X5O	-8.7	Nil	Nil	LYS A: 262	PHE A: 303, GLU A: 304, HIS A: 267
	3UDI	-9.0	Nil	Nil	ALA A: 66, PRO A: 184	PHE A: 72, LYS A: 137, ILE A: 140, TYR A: 144, LYS A: 147, ILE A: 148
	1UAG	-9.6	ASN A: 138	2.40	LEU A: 15, PRO A: 41, PRO A: 142	LYS A: 115, GLYA: 137, LEU A: 15
	2X5O	-8.7	PHE A:303,	2.45	LYS A: 262	PHE A: 303
	3UDI	-11.1	Nil	Nil	ALA A: 66, PRO A: 184, LYS A: 137, ILE A**:** 140	PHE A: 72, TYR A: 144, LYS A: 147, LYS A: 137, ILE A: 140, ILE A: 148

	1UAG	-8.9	Nil	Nil	LYS A: 319, AL A: 414, LEU A: 416	LEU A: 416, ASP A: 185, PHE A: 422
	2X5O	-8.6	Nil	Nil	LYS A: 262	HIS A: 267, PHE A: 303
	3UDI	-10.5	Nil	Nil	LYS A: 137, ILE A: 140, PRO A: 184	PHE A: 72, LYS A: 137, TYR A: 144, LYS A: 147, ILE A: 148
	1UAG	-9.1	LYS A: 319	2.86	LEU A: 416	ASP A: 185, LEU A: 416, ALA A: 414, PHE A: 422
	2X5O	-8.4	Nil	Nil	LYS A: 262	HIS A: 267, PHE A: 303, GLU A: 304
	3UDI	-8.1	GLU A: 252, TYR A: 567	1.98, 2.13	LEU A: 426, ILE A: 534	HIS A: 256, PHE A: 554
	1UAG	-8.8	Nil	Nil	LYS A: 348, ALA A: 414, LEU A: 416	LYS A: 115, LYS A: 319
	2X50	-8.5	PHE A: 303	2.42	LYS A: 262	PHE A: 303
	3UDI	-11.0	Nil	Nil	ALA A: 66, PHE A: 72, TYR A: 144, ILE A: 140, LYS A: 137,PRO A: 184	PHE A: 72, TYR A: 144, LYS A: 147, ILE A: 148,LYS A: 137
	1UAG	-8.9	Nil	Nil	LYS A: 348, LEU A: 416	LEU A: 416, ALA A: 414, PHE A: 422, ASP A: 185
	2X5O	-8.4	Nil	Nil	ILE A: 140, PRO A: 184	LYS A: 147,PHE A: 72, TYR A: 144, ILE A: 148
	3UDI	-8.9	Nil	Nil	ILE A: 140, PRO A: 184	TYR A: 144, ILE A: 148, LYS A: 147
	1UAG	-8.7	Nil	Nil	LEU A: 15, LYS A: 420, ALA A: 414	LYS A: 420
	2X5O	-8.7	Nil	Nil	LYS A: 262	HIS A: 267, PHE A: 303, GLU A: 304
	3UDI	-10.4	Nil	Nil	ALA A: 66, PRO A: 184, PHEA: 63, ILE A: 148	LYS A: 147, ILE A: 148, PHE A: 72
	1UAG	-9.1	Nil	Nil	LEU A: 15, PRO A: 142	LEU A: 15, LYS A: 115, GLY A: 137
	2X5O	-8.6	PHE A: 303	2.41	LYS A: 262, LEU A: 333	PHE A: 303
	3UDI	-10.8	Nil	Nil	ILE A: 140, PHE A: 72, ALA A: 66, PRO A: 184	LYS A: 147, ILE A: 148, PHE A: 72, TYR A: 144
	1UAG	-7.8	LEU A: 416, SER A: 415, HIS A: 183, LYS A: 115, LYS A: 319	2.78, 2.68, 2.29, 2.42, 2.10	LYS A: 319,PHE A: 422	ALA A: 414
	3UDI	-7.6	ASN A: 315, TYR A: 317, ALA A: 314, ASN A: 416, GLU A: 281	2.84, 1.78, 2.33, 2.16, 2.74	Nil	GLU A: 281
	2X5O	-8.3	VAL A: 305, PHE A: 303, SER A: 264, ALA A: 328, ALA A: 329	2.66, 2.44, 2.26, 2.81, 1.88	Nil	HIS A: 267, PHE A: 303

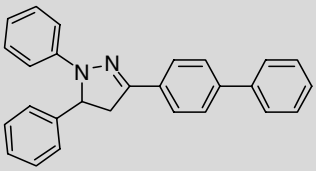
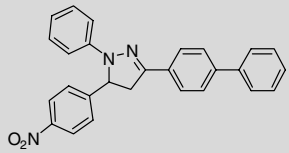
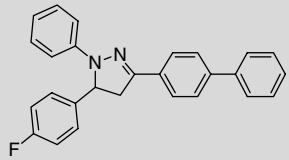
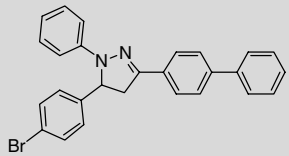
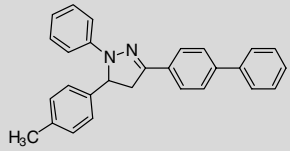
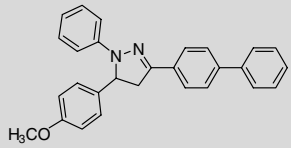
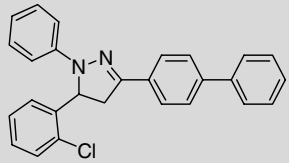
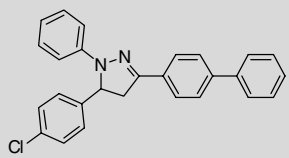
compound (**2d**) showed good binding affinity score (-9.6 kcal/mol, -8.7 kcal/mol and -11.1 kcal/mol) compared to other synthesized 2-phenylpyrazoline derivatives (**2a-j** except **2d**) and a standard drug (amoxicillin). Other derivatives binding affinity scores are given in Table-1.

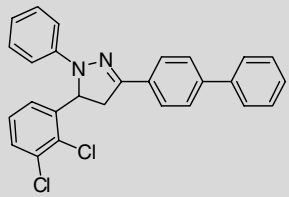
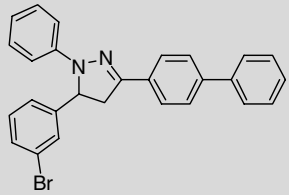
Conventional hydrogen bond interaction: Based on high binding affinity score, compound **2d** has one conventional hydrogen bond interaction (ASN A: 138) formed with the pyrazole moiety (1UAG). The hydrogen bond distance is 2.40 Å. Compound **2d** has one hydrogen bond interaction (PHE A: 303) with the pyrazole moiety (2X5O). The hydrogen bond distance is 2.45 Å. Compound **2d** has no hydrogen bond interaction with the 3UDI protein.

Hydrophobic interaction: Based on high binding affinity score, compound **2d** have three hydrophobic interaction (LEU A: 15, PRO A: 41, PRO A: 142) formed with the benzene ring and bromo moiety (1UAG). Compound **2d** have one hydrophobic interaction (LYS A: 262) formed with the benzene ring and bromo moiety. Compound **2d** have four hydrophobic interaction (ALA A: 66, LYS A: 137, ILE A: 140, PRO A: 184) formed with 2-phenylpyrazoline benzene ring and substituted bromo-benzene ring.

Molecular docking study with breast cancer protein (1OQA): The synthesized new 2-phenylpyrazoline derivatives (**2a-j**) was docked with breast cancer protein (1OQA). The results are shown in Table-2. Thus, compound **2d** have high binding

TABLE -2
MOLECULAR DATA FOR 2- PHENYL PYRAZOLINE DERIVATIVES (**2a-j**) DOCKED WITH BREAST CANCER PROTEIN

Compound	Protein	Binding affinity score (kcal/mol)	Conventional hydrogen bond	H-Bond length (Å)	Hydrophobic interaction	Other interactions
	1OQA	-7.8	Nil	Nil	PRO A: 59, ILE A: 102, PRO A: 103	ASN A: 66, GLY A: 67, PHE A: 68
	1OQA	-8.1	Nil	Nil	PRO A: 103, PRO A: 59	THR A: 81, PHE A: 68
	1OQA	-7.9	Nil	Nil	PRO A: 59, PRO A: 103	PHE A: 68, THR A: 81
	1OQA	-8.8	Nil	Nil	PRO A: 103, PRO A: 59, TYR A: 100	GLU A: 83, PHE A: 68, THR A: 81, PRO A: 59
	1OQA	-8.2	Nil	Nil	PRO A: 59, PHE A: 68, PRO A: 103, ILE A: 102	Nil
	1OQA	-7.7	Nil	Nil	PRO A: 59, ILE A: 102, PRO A: 103	GLU A: 83, THR A: 81
	1OQA	-7.8	Nil	Nil	PRO A: 59, ILE A: 102, PRO A: 103	ASN A: 66, GLY A: 67
	1OQA	-7.8	Nil	Nil	PRO A: 103, PRO A: 59	PHE A: 68, HIS A: 69, THR A:81

	1OQA	-8.0	Nil	Nil	ILE A: 102, PRO A: 59, PRO A: 103	GLY A: 67, PHE A: 68
	1OQA	-7.9	Nil	Nil	PRO A: 103, PRO A: 59, ILE A: 102	PHE A: 68, GLY A: 67, ILE A: 102

affinity score (-8.8 kcal/mol) compared to other compounds (**2a-j**) of this series. Other compounds binding affinity scores are shown in Table-2. Based on the high binding affinity score, compound **2d** have three hydrophobic interactions (PRO A: 59, PRO A: 103, TYR A:100) formed with pyrazole moiety. Other compounds hydrophobic interactions are shown in Table-2. Based on this result, compound **2d** wasperformed *in vitro* anticancer activity against MDA-MB-231 cell line.

Among the compounds (**2a-j**) subjected to *in silico* analysis against the bacterial cell wall proteins 1UAG, 2X5O and 3UDI. Compound **2d** showed good binding affinity score -9.6, -8.7 and -11.1 kcal/mol when docked with these three proteins, indicating a very good affinity. In a similar way, these synthesized compounds (**2a-j**) were docked with the human breast cancer protein (1OQA). Compound **2d** showed a binding score of -8.8 kcal/mol and thereby indicated it is a good candidate for further studies. So *in vitro* studies (MTT assay of antimicrobial studies) were carried out to confirm its activities.

MTT assay: Based on high binding affinity score, compound **2d** (4,5-dihydro-1-phenyl-3-diphenyl-5-(4-bromophenyl)-1*H*-pyrazole) were screened for *in vitro* anticancer studies (human breast cancer). The *in vitro* anticancer activity was performed by MTT assay method. It was done by various concentration (100, 50, 25, 12.5, 6.25 µg/mL) of compound **2d** against MDA-MB-231 cell line. From this result, compound **2d** showed moderate activity in all concentration except 6.25 µg/mL. The LC_{50} value for this compound is -185.309 ± 1.469 µg/mL.

Antimicrobial activity: Antibacterial activity were screened for 2-phenylpyrazoline derivatives (**2a-j**) using different strains (*S. aureus*, *Klebsiella*, *E. coli* and *Streptococcus*) at different concentrations (100, 50 and 25 mg/mL). The results are shown in Table-3. It is seen that all 2-phenylpyrazoline compounds showed a good zone of inhibition against these four strains.

At high concentration (100 mg/mL), compound **2i** showed a good zone of inhibition (34 mm) against *Streptococcus*; Compounds **2b**, **2d** and **2i** showed a good zone of inhibition (13 mm) against *E. coli*; Compound **2j** showed good zone of inhibition against *Klebsiella* strain (28 mm); Compounds **2b**, **2d** and **2i** have shown a good zone of inhibition (13 mm) against *S. aureus*.

At low concentration (25 mg/mL), compounds **2b**, **2c**, **2d** and **2i** shown good zone of inhibition (10 mm) against *S. aureus*; Compound **2a** showed a good zone of inhibition (15 mm) against *Klebsiella*; Compounds **2b**, **2c** and **2d** (10 mm) showed a good zone of inhibition against *E. coli*, while compound **2c** have shown a good zone of inhibition against *Streptococcus*.

Finally from these results, compound **2i** (2,3-dichloro) substitution shown a good zone of inhibition at high concentration compared to other derivatives of this series (**2a-j**). At low concentration, compound **2c** (fluoro substitution) shown a good zone of inhibition compared to other derivatives of this series (**2a-j**).

Antifungal activity: 2-Phenylpyrazoline derivatives were screened for antifungal activity at different concentrations (100,

TABLE-3
ANTIBACTERIAL ACTIVITY TEST FOR 2-PHENYL PYRAZOLINE DERIVATIVES WITH DIFFERENT STRAINS BY AGAR DISK DIFFUSION METHOD

Comd.	*S. aureus*			*Klebsiella*			*E. coli*			*Streptococcus*		
	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL
2a	12	10	5	18	15	12	12	10	5	21	16	14
2b	13	11	10	17	>10	–	13	11	10	23	13	–
2c	12	11	10	13	>10	>10	12	11	10	24	22	20
2d	13	12	10	16	11	>10	13	12	10	20	13	11
2e	–	–	–	12	11	>10	–	–	–	20	18	17
2f	10	–	–	24	11	10	10	–	–	25	13	12
2g	–	–	–	24	14	14	–	–	–	30	20	18
2h	11	8	5	20	15	13	11	8	5	32	27	16
2i	13	12	10	23	16	13	13	12	0	34	19	–
2j	10	5	–	28	16	14	10	5	–	22	13	12

– which means there is no zone of inhibition.

50 and 25 mg/mL), the results are shown in Table-4. It is found that 2-phenylpyrazoline derivatives did not showed any zone of inhibition against *Candida albicans*.

TABLE-4
ANTIFUNGAL ACTIVITY TEST FOR 2-PHENYL PYRAZOLINE DERIVATIVES (**2a-j**)

Antimicrobial susceptibility test against *Candida albicans*			
	Zone of inhibition (diameter in mm)		
Compounds	100 mg/mL	50 mg/mL	25 mg/mL
2a	–	–	–
2b	–	–	–
2c	–	–	–
2d	–	–	–
2e	–	–	–
2f	–	–	–
2g	–	–	–
2h	–	–	–
2i	–	–	–
2j	–	–	–

–which means there is no zone of inhibition.

Conclusion

A new series of 2-phenylpyrazoline derivatives were synthesized and characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectral data. The synthesized compounds were screened for molecular docking studies using bacterial proteins and breast cancer protein. Among the synthesized compounds, compound **2d** (4-bromo substitution) showed a good binding score and good binding interaction as compared to other derivative compounds. Thus, compound **2d** was selected for the anticancer activity using MTT assay method, which showed a moderate activity of LC_{50} value (185.30 ± 1.469 µg/mL). The antimicrobial activity was screened for synthesized 2-phenylpyrazoline derivatives (**2a-j**). The fluoro substituted compound (**2c**) have shown a good zone of inhibition at low concentration against *S. aureus* and 2,3-dichloro substitution showed a good zone of inhibition at high concentration (100 mg/mL) against *Streptococcus*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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