DESIGN SYNTHESIS OF NOVEL ACRIDINE TAGGED PYRAZOLE DERIVATIVES AS AURORA KINASE INHIBITORS

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ABSTRACT

Objective: A series of novel synthesis of 5-Substituted-3-phenyl-4,5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]-amide (IV) were synthesized using standard procedures and evaluated for cytotoxic studies.

Methods: 9-(4-Chloro-phenyl)-9 and 10-dihydro-acridine (I) were formed by cyclization of diphenylamine with substituted acids in the presence of zinc chloride and synthesis of 5-substituted-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid amide (3) by the cyclization of different chalcones (II) and final compounds were synthesized by fusion of 5-substituted-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid amide (III) with 9-(4-Chloro-phenyl)-9, 10-dihydro-acridine (I) by microwave irradiation method. Characterization of synthesized compounds by infrared, 1H nuclear magnetic resonance (NMR), 13C NMR, and mass spectroscopic methods. Obtained compounds were evaluated for their cytotoxicity against human breast cancer cell lines (MCF/wt) by sulforhodamine-B assay. Docking studies with Aurora kinase protein were performed to elucidate the possible mechanistic insights of these novel acridine tagged pyrazole derivatives.

Results: Moderate to good in vitro cytotoxic potentials of the newly synthesized molecules were reported against selected human breast cancer cell lines. Among the tested molecules, compound C6 showed good cytotoxic activity against MCF/wt (08.2±0.4 μM). The dock scores of the tested compounds were ranged between −8.926 and −5.139. Compound C6 which has been reported as the most effective cytotoxic agent among the series also reported the highest dock score of -8.926 and showed hydrogen bond interaction with GLU-211, LYS-162, and LYS-143. Ligand binding energy with protein suggested compound C6 has shown the highest binding energy of −86.32133 kcal/mol.

Conclusion: The in vitro studies of the newly synthesized acridine tagged pyrazole derivatives reported considerable cytotoxic potentials against human breast cancer cell lines and structure-activity relationship studies to suggest that acridine tagged pyrazole derivatives with hydroxy group present on phenyl ring at fifth position of pyrazole ring could probably increase the cytotoxic potentials. With the reported bioactivities of these derivatives, further studies on the derivatization could elucidate the broader cytotoxic potentials.

Keywords: Microwave irradiation, Acridine, Pyrazoles, Chalcones, Aurora kinase.

INTRODUCTION

Across the world, cancer is being considered as a major cause for elevated mortality rate in humans. Among women, breast cancer is the most prevalent cancer with an estimate of 255,180 new cases, respectively [1]. Elevated levels of Aurora kinases detected in wide variety of human cancers strongly indicate that high expression of these kinases plays an important role in the development of anticancer molecules as aurora kinase inhibitors [2].

Acridine C(H₂)N is a nitrogen heterocycle, which is structurally related to anthracene with one of the central CH groups replaced by nitrogen. The acridines were first developed as dyes and during the early 20th century their pharmacological properties were evaluated. At this time, proflavine was used as a topical antibacterial and antifungal agent [3]. In the 1940’s and to the present day, the acridines (e.g., quinacrine, pyronaridine, and aracrinil) have been used as antimalarial drugs [4]. The first acridine-based therapeutic agents specifically designed for cancer treatment were developed during the 1970’s. These efforts led to the development of m-amsacrine, a 9-aminoacridine introduced into clinical use in 1976 [5]. Accordingly, this acridine has been clinically utilized as a single agent or in combination with other anti-neoplastic drugs in the treatment of acute nonlymphocytic, lymphocytic [6,7] and acute myeloid [8], leukemias [9]. However, m-Amsacrine has not generally been effective in the treatment of solid tumors [10]. Acridine has an irritating odor. It crystallizes in colorless to light yellow needles with melting point of 110°C and boiling point of 346°C. It is characterized by its irritating action on skin and by the blue fluorescence showed by solutions of its salts.

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position, and are also called as azoles [11]. [1-1-pyrazolyl] alanine was isolated from the seeds of water melons (Citrullus lanatus). The best described property of almost every group of pyrazoles is in the treatment of inflammation and inflammation associated disorders, such as arthritis [12]. Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial [13], antihistaminic [14], antidespresant [15], 5-a-reductase inhibitor [16], antiproliferative [17], and herbicides [18]. During the years, pyrazoles are proved to be potent Aurora kinase inhibitors (IC₅₀ = 0.16±0.03 μM) [19].

METHODS

Chemistry

All the chemicals were obtained from Sigma-Aldrich chemical company (USA), Lancaster (USA) and S.D. Fine chem. Limited (Mumbai). All the
glassware is of borosilicate grade. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was ascertained by thin-layer chromatography (TLC) on silica gel-G plate.

Fourier transform infrared (IR) spectra were taken in KBr on a Thermo Nicolet Nexus 670 spectrophotometer. H nuclear magnetic resonance (NMR) spectra were recorded on AVANCE 300 MHz spectrophotometer in CDCl3 with tetramethylsilane (TMS) as internal standard. 13CNMR spectra were recorded on BRUKER AVANCE 300 MHz spectrometer in CDCl3 with TMS as an internal standard. The chemical shift values are in ppm. Mass spectra were recorded on Polaris Q apparatus (Thermo Electron) and the fragmentations were obtained by electronic impact (EI). The data are given as mass to charge ratio (m/z) and nominal masses were used for the calculation of molecular weights of the prepared products.

**Synthetic procedure**

**Step 1:** General procedure for synthesis of 9-(4-chlorophenyl)-9, 10-dihydroacridine (1)
A mixture of diphenylamine (0.01 mol) and 4-Chloro benzoic acid (0.01 mol) was taken in a conical flask. To this zinc chloride (0.01 mol) was added as solvent. Then, the reaction mixture was irradiated under microwave irradiation at 450 W for 11 min. The precipitate obtained after cooling was filtered and recrystallized with methanol.

**Step 2:** General procedure for synthesis of chalcones (II)
Equimolar quantity of different aldehydes (0.01 mol) and acetoephone (0.01 mol) (1:2 mL) was placed in 30 mL of ethanol. The mixture was allowed to stir for 15 min. A 0.1 M 40% aqueous potassium hydroxide solution was slowly added dropwise to the reaction flask through a dropping funnel. The reaction solution was allowed to stir at room temperature for 1.5 h. The solid separated and was collected by suction filtration.

**Step 3:** General procedure for synthesis of 5-substituted-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid amide (III)
A mixture of chalcones (0.01 mol), thiosemicarbazide (0.01 mol, 0.91 g), and KOH (0.0025 mol, 1.4 g) was refluxed in ethanol (30 mL) for 6 h. The solution was poured into ice-water. The precipitate was filtered and recrystallized from methanol.

**Step 4:** General procedure for the synthesis of 5-substituted-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (IV)
In a 250 mL flask, a mixture of (0.03 mol) of 5-substituted-3-phenyl-4, 5-dihydro-pyrazole-1-carboxamide acid (III), (0.0256 mol) of 9-(4-Chloro-phenyl)-9, 10-dihydro-acridine (1), and 80 mL of 2-butanol was taken and subjected to microwave irradiation for 3 min at 65% intensity (455 W). Completion of the reaction was monitored by TLC using n-hexane and ethyl acetate in 6:4 ratio. After completion, the reaction mixture was allowed to cool at room temperature then it was poured into 150 mL of ice water. A precipitate formed was filtered by suction, washed with water and dried recrystallized from ethanol.

The spectral data for all the newly synthesized compounds were completely in agreement with the proposed structures.

N-(4-(9, 10-dihydroacridin-9-yl)phenyl)-5-phenoxy-3-phenyl-4, 5-dihydro-1H-pyrazole-1-carbothioamide (C1)
Light yellow color solid, yield-52%, m.p. 310–312°C, IR (KBr): 1597 cm

1(-NH-), 1487 cm

(-CH3), and 1680 cm

(-OCH3), 1'H NMR (CDCl3) δ ppm: 8.5 (s, 1H-NH proton), 8 ppm 2.5 (s, 2H-CH proton), 8 ppm 4.0 (s, 1H-NH proton), 8 ppm 4.7 (s, CH3), 8 ppm 4.7 (s, 3H-CH3 protons), and 6 ppm 6.9–8.0 (m, 21H-Aromatic protons), M.W-552.69 g/mol, EI-MS: m/z 556(M). 13CNMR: δ ppm 117.92–143.24 represents Pyrazole CH protons, δ ppm 63.08 represents pyrazole carbon, δ ppm 156.09 represents C=N carbon, δ ppm 30 aromatic carbons, δ ppm 117.92–143.24 represents Pyrazole CH proton, 6 ppm 63.08 represents pyrazole CH proton, 6 ppm 176.91 represents C=S carbon.

5-Phenyl-5-styryl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (C3)
White color solid, yield-70%, m.p. 327–330°C, IR (KBr): 1575 cm

(-NH-), 1400 cm

(-CH=CH), 1'H NMR (CDCl3) δ ppm: 2.1 (s, 2H-CH protons), 2.7 (s-6H-NCH3), 4.0 (s, 1H-NH protons), 3.4 (s, 1H-CH protons) and 6.8–7.8 (m, 21H-Ar-H) M.W-581.69 g/mol, EI-MS: m/z 581 (M)+, 583 (M-1).

5-(4-Methylamino-phenyl)-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (C4)
Cream color solid, yield-70%, m.p. 317–320°C, IR (KBr): 1540 cm

(-NH-), 1400 cm

(-CH=CH), 1'H NMR (CDCl3) δ ppm: 1.3 (s, 2H-CH protons), 4.0 (s, 1H-NH proton), 3.4 (s, 1H-CH proton) and 6.8–7.8 (m, 21H-Ar-H) M.W-579.76 g/mol, EI-MS: m/z 579 (M-1).

5-(4-Nitro-phenyl)-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (C5)
Yellow color solid, yield-52%, m.p. 314–317°C, IR (KBr): 1595 cm

(-NH-), 1450 cm

(-C=S), 1575 cm

(-CH=CH), 1'H NMR (CDCl3) δ ppm: 8 ppm 1.1 (s, 2H-CH proton), 2.7 (s-6H-NCH3), 4.0 (s, 1H-NH proton), 5.0 (s, 1H-CH proton) and 6.9–8.0 (m, 22H-Ar-H) M.W-579.76 g/mol, EI-MS: m/z 579 (M-1).

5-(4-Hydroxy-phenyl)-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (C6)
White color solid, yield-70%, m.p. 237–239°C, IR (KBr): 1575 cm

(-NH-), 1469 cm

(-C=S), and 3520 cm

(-OH, Phenolic), 1'H NMR (CDCl3) δ ppm: 2.1 (s, 2H-CH proton), 3.9 (s, 1H-NH proton), 3.2 (s, 1H-CH proton), 5.0 (s, 1H-OH, Phenolic protons) and 7.3–7.9 (m, 21H-Ar-H) M.W-552.69 g/mol, EI-MS: m/z 553(M).

5-Methyl-3-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (C7)
Cream color solid, yield-72%, m.p. 295–297°C, IR (KBr): 1510 cm

(-NH-), 1444 cm

(-C=S), and 1461 cm

(-CH=CH), 1'H NMR (CDCl3) δ ppm: 2.1 (s, 2H-CH proton), 4.8 (s, 1H-NH proton), 3.2 (s, 1H-CH proton), 1.4 (s, 3H-CH3 proton) and 7.0–7.8 (m, 17H-Ar-H) M.W-474.62 g/mol, EI-MS: m/z 475(M).

5-Methyl-3-nitro-phenyl-4, 5-dihydro-pyrazole-1-carbothioic acid [4-(9, 10-dihydro-acridin-9-yl)-phenyl]amide (C8)
White color solid, yield-59%, m.p. 300–302°C, IR (KBr): 1581 cm

(-NH-), 1508 cm

(-C=S), 1469 cm

(-CH=CH), and 1342 cm

(-CH=CH), 1'H NMR (CDCl3) δ ppm: 2.0 (s, 2H-CH proton), 4.0 (s, 1H-NH proton), 3.2 (s, 1H-CH proton), 5.6 (s, 2H Vinylic protons) and 7.3–7.7 (m, 17H-Ar-H) M.W-500.66 g/mol, EI-MS: m/z 500(M)-1.

**In vitro cytotoxic activity**

In vitro cytotoxic studies of synthesized compounds of acridine tagged pyrazole derivatives compounds in comparison with reference drugs Doxorubicin (Dx) mitoxantrone (Mr) were studied by sulforhodamine-B (SRB) assay [20]. In brief, cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, and cultures were passage once or twice a week using trypsin EDTA to detach the cells from their culture flasks. The fast-growing cells were harvested, counted, and plated at suitable concentrations in 96-well microplates. Cells were allowed to adhere for 24 h. Thereafter, one plate was fixed to determine the initial absorbance (T). To the other plates compounds dissolved in the culture medium were added to the culture wells in triplicate and incubated further for

79
72 h at 37°C under 5% CO₂ atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After dissolving the bound stain with 150 μL of 10 mM unbuffered Tris-base (Tris(hydroxymethyl)aminomethane) solution using gyratory shaker, absorbance was measured at 540 nm using a microplate reader (Tecan). Growth was calculated by correcting the control plate (without drugs) cultured for 72 h (T₀ control) with the T₀ plate. Growth inhibition was calculated as: (T₀ - drug - T₀)/(T₀ - control - T₀)*100%. The efficacy of the compounds was compared by estimating the concentration required to inhibit cellular growth by 50% (i.e. IC₅₀) from each separate growth inhibition curve. Each value represents the mean of triplicate experiments.

**Molecular docking and binding energy calculations**

Dataset ligands and ligand optimization cytotoxic activity possessing acridine tagged pyrazole derivatives which were earlier developed in our laboratory were selected (Scheme 1) [21]. 2D structures of the compounds were converted to 3D using potential algorithms and application of high efficient force fields. Initial geometrical optimization and energy minimization of molecules were performed using the LigPrep tool of Schrodinger suite [22]. Various ionization states were generated using LigPrep module using a special program EPIK along with various possible conformers and tautomers.

Molecular properties of the processed ligands were studied using Qikprop module. Qikprop module also predicts ADME profiles such as blockage of Human Ether-a-go-go-related Gene (hERG) K⁺ channels, apparent Caco-2 cell permeability, brain/blood partition coefficient, apparent Madin-Darby canine kidney cell permeability, skin permeability, binding to human serum albumin, and human oral absorption of the given set of ligands [23].

![Scheme 1: Synthesis of acridine tagged pyrazole derivatives](image_url)
### Table 1: Physical characterization data of acridine tagged pyrazole derivatives

| Molecule | R | MW   | log P | Yield (%) | MP (°C)  |
|----------|---|------|-------|-----------|----------|
| C1       |   | 552.69 | 8.18  | 70        | 310–312  |
| C2       |   | 536.69 | 8.3   | 63        | 323–325  |
| C3       |   | 562.73 | 8.82  | 55        | 310–312  |
| C4       |   | 579.76 | 8.59  | 58        | 307–310  |
| C5       |   | 589.61 | 7.99  | 52        | 314–317  |
| C6       |   | 552.69 | 7.91  | 70        | 327–330  |
| C7       | CH₃ | 474.62 | 6.91  | 72        | 295–297  |
| C8       | CH₃ | 500.66 | 7.63  | 59        | 300–302  |
| C9       | Cl  | 571.13 | 8.86  | 69        | 280–283  |
| C10      | O₂N | 581.69 | 7.92  | 58        | 278–281  |
| C11      |     | 526.65 | 6.92  | 72        | 261–263  |
| C12      | NO₂ | 616   | 7.4   | 70        | 265–268  |
| C13      | O₂N | 616   | 7.92  | 69        | 263–265  |

(Contd...)
| Molecule | R | MW  | log P | Yield (%) | MP (°C) |
|----------|---|-----|-------|-----------|---------|
| C14      |  | 581.69 | 7.65  | 63        | 252–255 |
| C15      |  | 593.74 | 7.21  | 68        | 301–303 |
| C16      |  | 594.72 | 7.89  | 71        | 283–285 |
| C17      |  | 582.71 | 7.79  | 68        | 275–278 |
| C18      |  | 630.60 | 8.33  | 69        | 286–289 |
| C19      |  | 566.71 | 8.18  | 71        | 291–293 |
| C20      |  | 645.61 | 9.01  | 65        | 310–312 |
| C21      |  | 615.58 | 9.13  | 68        | 291–293 |
| C22      |  | 587.13 | 8.47  | 60        | 287–289 |
| C23      |  | 642.81 | 9.91  | 64        | 278–281 |
| C24      |  | 550.72 | 8.79  | 72        | 282–286 |
| C25      |  | 616.13 | 8.89  | 66        | 271–274 |

(Contd...)
The digital structure of the Aurora-A kinase protein was retrieved from the protein databank website with PDB Id: 1MQ4 and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using OPLS-2005 force field using Protein Preparation Wizard tool of Schrodinger Suite [24]. Thus, structurally optimized protein structure was used to examine protein-ligand interactions of the dataset ligands using Glide XP docking protocol. Initially, a 3D grid was established to the binding pocket (active site) of the protein, into which all the dataset ligands were docked into. Binding interactions and efficiency of the binding were calculated in terms of Glide Score, which is a combination of hydrophilic, hydrophobic, metal binding, BuryP (Penalty for buried polar groups), RotB (Penalty for freezing rotatable bonds), Site (Polar interactions in the active site) 

GScore = 0.065× Van der Waals energy+0.130× Coulomb energy+Lipophilic term (Hydrophobic interactions)+H bonding+Metal binding+BuryP (Penalty for buried polar groups)+RotB (Penalty for freezing rotatable bonds)+Site (Polar interactions in the active site) 

Post docking calculations Prime MM/GBSA (molecular mechanics based generalized Born/surface area), module of Schrodinger suite was used to calculate the binding energies of the docked complexes, which is a combination of OPLS molecular mechanics energies (EMM), an SGB solvation model for polar solvation (GSGB), and a non-polar solvation term (GNP) containing non-polar solvent accessible surface area and Van der Waals interactions. In this, docking results were rescoring through an energy function with a well-defined description of binding contributions. The total free energy of binding is then expressed in the form below mentioned Equation [22]:

\[ \Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}}) \]

Where, \( \Delta G_{\text{bind}} \) is ligand binding energy.

RESULTS

Chemistry

Acridine tagged pyrazole derivatives have been synthesized and the reaction sequence for the synthesis of compounds is outlined in Scheme 1. Physical Characterization data of newly synthesized compounds are outlined in Table 1. The protein ligand interactions and post docking calculations of acridine tagged pyrazole derivatives with aurora kinase are shown in Table 2. Cytotoxic activity of acridine tagged pyrazole derivatives is shown in Table 3.

Cytotoxic activity

All the synthesized compounds were evaluated for their cytotoxic activity against human breast cancer MCF/wt cell line by SRB assay using standard drug doxorubicin in mitoxantrone.

For each cell line, the IC\textsubscript{50} values (response parameter) were calculated and outlined in Table 3.

Table 1: (Continued)

| Molecule | R     | MW     | log P | Yield (%) | MP (°C) |
|----------|-------|--------|-------|-----------|---------|
| C26      |       | 578.77 | 9.54  | 69        | 267–269 |
| C27      | Cl    | 605.58 | 9.42  | 68        | 301–303 |
| C28      | OC\textsubscript{2}H\textsubscript{5} | 580.74 | 8.51  | 71        | 288–301 |
| C29      | CH\textsubscript{3} | 564.74 | 9.28  | 66        | 283–285 |
| C30      | OCH\textsubscript{3} | 566.71 | 8.18  | 69        | 305–308 |

Table 2: Cytotoxic activity of acridine tagged pyrazole derivatives

| Cell lines/IC\textsubscript{50} (µM) ± SEM* | MCF7/Wt |
|-------------------------------------------|---------|
| Compound                                  |         |
| IIIa                                      | 23.2±0.7|
| IIIb                                      | 25.2±0.5|
| IIIb                                      | 10.2±0.2|
| C5                                        | 15.2±0.4|
| C6                                        | 08.2±0.4|
| C7                                        | 09.2±0.2|
| C11                                       | 10.2±0.5|
| C13                                       | 09.2±0.4|
| C14                                       | 12.2±0.5|
| C17                                       | 21.2±0.3|
| C20                                       | 17.2±0.4|
| C23                                       | 11.2±0.7|
| C27                                       | 18.2±0.8|
| Mitoxantrone (Mr)                         | 0.090   |
| Doxorubicin (Dx)                          | 0.098   |

The digital structure of the Aurora-A kinase protein was retrieved from the protein databank website with PDB Id: 1MQ4 and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using OPLS-2005 force field using Protein Preparation Wizard tool of Schrodinger Suite [24]. Thus, structurally optimized protein structure was used to examine protein-ligand interactions of the dataset ligands using Glide XP docking protocol. Initially, a 3D grid was established to the binding pocket (active site) of the protein, into which all the dataset ligands were docked into. Binding interactions and efficiency of the binding were calculated in terms of Glide Score, which is a combination of hydrophilic, hydrophobic, metal binding groups, Van der Waals energy, freezing rotatable bonds, and polar interactions with receptor [25]. GScore = 0.065× Van der Waals energy+0.130× Coulomb energy+Lipophilic term (Hydrophobic interactions)+H bonding+Metal binding+BuryP (Penalty for buried polar groups)+RotB (Penalty for freezing rotatable bonds)+Site (Polar interactions in the active site)
.binding energy with protein suggested that compound C6 has shown the highest binding energy of −86.32133 kcal/mol, protein-ligand interactions along with binding energies of acridine tagged pyrazole derivatives with aurora kinase are shown in Table 3.

The majority of interactions are observed with acridone N-atom with LEU 139. Substituted halogens also show substantial interactions.

### Table 3: Protein ligand interactions and post docking calculations of acridine tagged pyrazole derivatives with aurora kinase

| Compound | Dock score | No of H-bonds | Interacting amino acids | H-bond distance | Bond energy |
|----------|------------|---------------|-------------------------|-----------------|-------------|
| C6       | −8.926     | 3             | GLU 211, LYS 162, LYS 143 | 2.63, 2.51, 2.29 | −86.32133  |
| c5       | −8.773     | 3             | ASN 261, ASP 256, LYS 258 | 2.29, 2.01, 2.17 | −73.793     |
| c20      | −6.972     | 2             | LEU 139               | 2.61, 2.03      | −68.989     |
| c19      | −6.617     | 1             | LEU 139               | 2.66            | −69.017     |
| c18      | −6.545     | 1             | LEU 139               | 2.66            | −69.696     |
| c27      | −6.418     | 1             | LYS 258               | 2.74, 2.75      | −72.608     |
| c16      | −6.405     | 1             | LEU 139               | 2.74, 2.75      | −74.62      |
| c30      | −6.36      | 0             | -                      |                | −73.519     |
| c23      | −6.357     | 1             | LEU 139               | 2.70            | −77.467     |
| c25      | −6.344     | 1             | LEU 139               | 2.32            | −68.847     |
| c17      | −6.281     | 1             | LEU 139               | 2.66            | −72.458     |
| c9       | −6.266     | 0             | -                      |                | −67.57      |
| c4       | −6.266     | 1             | LEU 139               | 2.67            | −70.836     |
| c21      | −6.225     | 2             | LEU 139, LYS 258      | 2.60, 2.78      | −68.579     |
| c28      | −6.216     | 0             | -                      |                | −71.688     |
| c26      | −6.171     | 0             | -                      |                | −65.321     |
| c3       | −6.154     | 0             | -                      |                | −73.544     |
| c22      | −6.117     | 1             | LEU 139               | 2.79            | −67.504     |
| c29      | −6.112     | 0             | -                      |                | −72.295     |
| c13      | −6.091     | 1             | LEU 139               | 2.51            | −64.777     |
| c14      | −6.077     | 0             | -                      |                | −71.29      |
| c12      | −6.011     | 2             | LEU 139, LYS 258      | 2.56, 2.78      | −63.903     |
| c1       | −6.001     | 0             | -                      |                | −64.898     |
| c10      | −5.996     | 1             | TRP 277               | 1.91            | −62.737     |
| c2        | −5.924     | 2             | LEU 139, LYS 258      | 2.64, 2.79      | −65.869     |
| c15      | −5.741     | 2             | LYS 258, CYS 290      | 2.33, 1.98      | −71.744     |
| c7        | −5.67      | 0             | -                      |                | −64.912     |
| c11       | −5.669     | 1             | LEU 139               | 2.50            | −70.09      |
| c8        | −5.468     | 0             | -                      |                | −61.574     |
| c24      | −5.139     | 1             | LYS 258               | 2.25            | −65.201     |

**Molecular docking and binding energy calculations**

The association of Aurora kinase in the human breast cancers was observed from the previous reports. The dock scores of the tested compounds were ranged between −8.926 and −5.139. Compound C6 has been reported as the most effective cytotoxic agent among the series also reported the highest dock score of −8.926 and showed hydrogen bond interaction with GLU-211, LYS-162, and LYS-143 (Fig 1). Ligand binding energy with protein suggested that compound C6 has shown the highest binding energy of −86.32133 kcal/mol, protein-ligand interactions along with binding energies of acridine tagged pyrazole derivatives with aurora kinase are shown in Table 3.

The majority of interactions are observed with acridone N-atom with LEU 139. Substituted halogens also show substantial interactions.
DISCUSSION

We have designed a convenient method for the synthesis of acridine tagged pyrazole derivatives and screened for in vitro cytotoxic activity against human breast cancer cell lines MCF/wt. By observing the above results, these synthesized compounds showed good cytotoxic activity of IC50 values in the range of 0.2±0.4–21.2±0.3. The electron releasing OH group in compound C6 is responsible for good binding interaction at kinase domain of aurora kinase protein.

CONCLUSION

In the present study, novel acridine tagged pyrazole derivatives were synthesized. The synthesized compounds were characterized by IR, 1H NMR, 13C NMR, and mass spectrum. All the synthesized compounds showed characteristic absorption peaks in IR, 1H NMR, and mass spectrum. Synthesized compounds were evaluated for in vitro cytotoxic activity by SRB assay against human breast cancer cell lines MCF/ wt.

The cytotoxic activity of the synthesized compounds revealed that the maximum activity was obtained when R was substituted by a hydroxyl group in the phenyl ring of pyrazole moiety. It was found that the compounds possessing electron releasing groups considerably enhance the cytotoxic activity when compared to the electron withdrawing groups on the phenyl ring.

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AUTHOR’S CONTRIBUTIONS

Harathi Perka and Vivekananda Boya contributed to the experimental work and preparation of the manuscript. Deepak Reddy Gade and Dr. Satyavati supervised the manuscript preparation and Dr. V.V.S. Rajendra Prasad organized and reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts.

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