Asian Journal of Chemistry; Vol. 34, No. 6 (2022), 1451-1456

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23710

Design, Synthesis, Molecular Docking Studies and Biological Evaluation of Indole Fused Novel Pyrazole Derivatives

N. Prasanna Lakshmi[1,*] and M. Ajitha[2]

[1]Department of Pharmacy, Sri Venkateshwara College of Pharmacy, Hyderabad-500081, India
[2]JNTUH-University College of Pharmaceutical Sciences, Sultanpur (Village), Pulkal (Mandal), Sangareddy District-502273, India

*Corresponding author: E-mail: lucky6prasanna@gmail.com

Received: 13 January 2022; *Accepted*: 25 February 2022; *Published online*: 18 May 2022; AJC-20805

The present work deals with the sequence of indole fused novel pyrazole compounds (**5a-l**) synthesized by conventional method and were screened for anthelmintic, anticancer activities and molecular docking studies. All the newly synthesized compounds were characterized by IR, ^{1}H NMR and Mass spectral analysis. Further, all the pyrazole derivatives were screened for anthelmintic activity using albendazole as standard drug and anticancer activity against MCF-7 and SKVO3 cell lines by MTT assay method. The results showed that compounds **5b** and **5j** exhibited good anticancer activity and compounds **5c, 5f, 5h** and **5l** exhibited potential anthelmintic activity. Additionally, the molecular docking studies of novel pyrazole derivatives were carried out to explain putative bonding interaction between the active site of EGFR enzyme and potent inhibitors.

Keywords: Pyrazole, Isatin, Phenyl hydrazine, Anthelmintic activity, Anticancer activities, MCF-7, SKVO3.

INTRODUCTION

One of the most functioning classes of heterocyclic moieties possess a wide spectrum of biological activities. In particular, these have shown promising biological activities [1-3] like antitumor, insecticidal, anti-inflammatory, antidiabetic, antibacterial, antifungal, antiparasitic, antitubercular and analgesic [4-7]. Immune resistance is one of the flaring issues facing clinical practice and detecting new effectual compounds against multi-resistant pathogens is one of the vital goals in present pandemic situations, in ongoing biomedical research [8]. The researches on latest antimicrobial and antiviral compounds is a significant desire in the context of today's COVID-19 pandemic situations [9,10]. The presence of different heterocyclic moieties linked with pyrazole help them to display various pharmacological activities.

Pyrazole and indole ring systems is a beneficial structural element in medicinal chemistry and has wide-ranging application in the drug development process. Among the pyrazole linked with other hetero nucleus compounds known in the literature for their potent biological active compounds with a wide range of therapeutic properties. The *N*-substituted indole elements have been extensively studied for their potentiality and aiming to earmark multiple proteins, necessary at different stages of various infections. On the other hand, the nitrogen atoms in the indole and pyrazole ring might confer bioactivity of molecules and boost the efficiency of the molecules [11-13]. And also the survey gives away that the mixture of two or more bioactive pharmacophores into the same molecule is a salient tool for scheming more active narrative chemical entities.

In view of these surveillance, great significance of these pyrazole and indole nucleus on biologically active heterocycles, an attempt has been made to synthesize newly indole fused pyrazole derivatives, with the expectation to procure new molecules with strengthen biological activity.

EXPERIMENTAL

The innovation of indole fused novel pyrazole derivatives was screened for anthelmintic, anticancer activities. Fourier transform IR spectrometer (model Shimadzu 8700) was used in the range of 400-4000 cm^{-1} using KBr pellets. ^{1}H NMR spectra were recorded on DPX-200 MHz NMR spectrometer exploiting DMSO-d_6 while the mass spectra were catalogued

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

on mass spectrophotometer (Shimadzu). The precoated silica gel G plates were used to detect the progress of the reaction as well as for assessing the purity of the compounds: *n*-hexane: ethyl acetate (7:3). Initial geometrical optimization and strength minimization of molecules have been carried out through the usage of the Ligprep device of Schrödinger suite. Various ionization states have been generated the usage of Ligprep module the use of exceptional software EPIK alongside with quite a number achievable conformers and tautomer's.

General procedures

Step-I: Synthesis of hydrazones (1a-d): A mixture of (0.01 mol) of acetophenone and (0.01 mol) of phenyl hydrazine in 10 mL ethanol was refluxed in water bath for 4 h, then the reaction mixture was cooled. The solid formed was dried and crystallized from diethyl ether.

Step-II: Synthesis of 1,3-diphenyl-pyrazole-4-carbaldehyde (2a-d): A mixture of 2.0 g (0.01 mol) of (**Ia-d**), Vilsmeier reagent and 0.73 g (0.01 mol) of DMF, 1.53 g (0.01 mol) of $POCl_3$ was added dropwise with mechanical stirring for 5 h. The reaction mixture was refluxed for 6 h at 70-80 ºC, then hydrolyzed on ice/water mixture and neutralized by 5% NaOH solution till pH 4, the reaction mixture was cooled. The solid formed was filtered, washed with water, dried and crystallized from isopropanol.

Step-III: Synthesis of *N*-methyl 5-substituted isatin derivative (3a-d): Isatin (5 g) was dissolved in dilute NaOH solution then 0.62 mL of dimethyl sulphate was added. Then whole contents were refluxed in water bath for approximately 50 min. After refluxing, the mixture was poured into beaker and cooled in ice. Then the content was evaporated on water bath and dried.

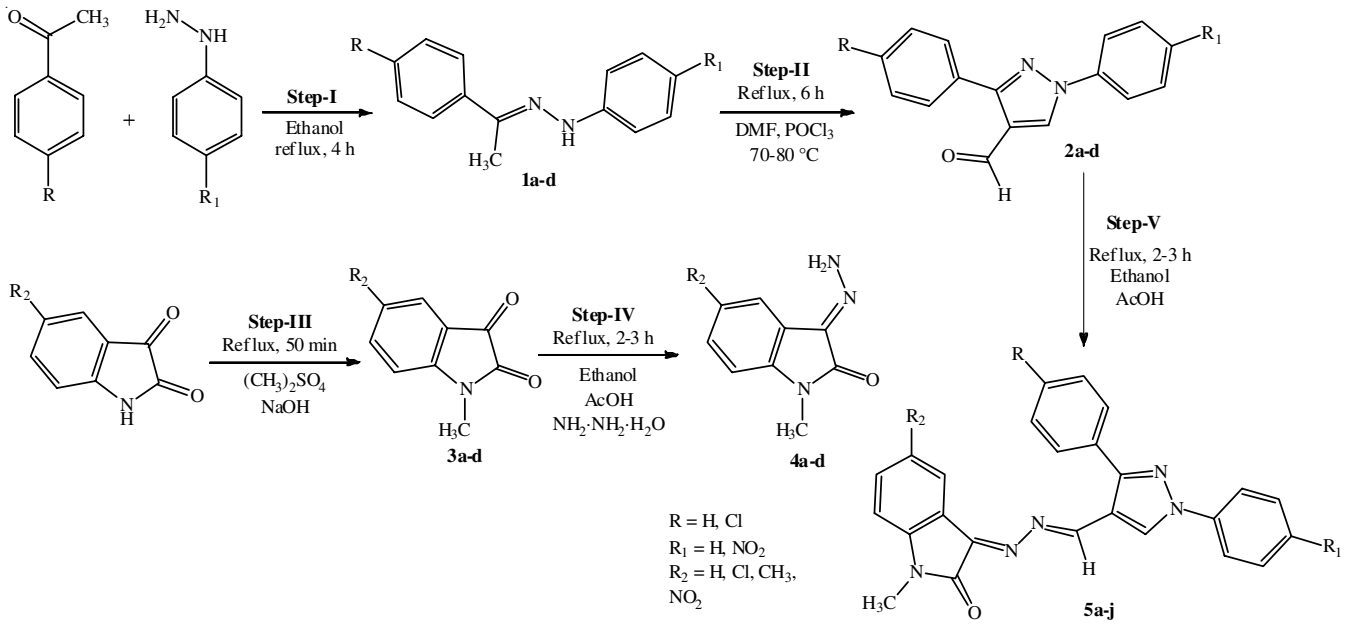
Step-III: Synthesis of Schiff bases (4a-d): A mixture of (0.01 mol) isatin derivative and (0.01 mol)hydrazine hydrate were dissolved in 40 mL of ethanol and 2 mL of glacial acetic acid (as catalyst) in a round bottom flask and then whole of the substance was refluxed for about 2-3 h and then checked for completion by thin layer chromatography. After refluxing, the whole contents were poured into cold water. At last, the precipitated product was recrystallized.

Step-IV: Synthesis of novel pyrazole derivatives (5a-l): A mixture of (0.01 mol) Schiff bases (**4a-d**) derivative and (0.01 mol) 1,3-diphenyl-pyrazole-4-carbaldehyde (**2a-d**) were dissolved in 40 mL of ethanol and 2 mL of glacial acetic acid (as a catalyst) in a round bottom flask and then whole of the substance was refluxed for about 2-3 h and then checked for completion by thin layer chromatography. After refluxing, the whole of the content was poured into cold water. At last, the precipitated product was recrystallized (**Scheme-I**).

3-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)-1-methylindolin-2-one-ethane (5a): FTIR (KBr, ν_{max}, cm^{-1}): 3056 (C-H *str.,* Ar), 2927, 2882, 2799 (C–H *str.*, aliphatic), 1705 (C=O *str.*, indole), 1616 (-C=N *str.*), 1538 (C=CH *str.*), 1310 (C=C *str.*, Ar), 1048 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 8.990 (s, 1H, -CH=N- imine proton), 8.378-8.310 (d, 2H, Ar-H), 8.150-8.113 (t, 2H, Ar-H), 8.093-8.010 (t, 3H, Ar-H), 7.893-7.840 (d, 2H, Ar-H), 7.800-7.789 (t, 3H, Ar-H), 7.949-7.903(s, 1H, Ar-H), 7.893, 7.759-7.678 (d, 2H, Ar-H), 2.106 (s, 3H, $-CH_3$). Mass (LC-MS): *m/z* 405 (M), 406 (M + 1, 100%).

3-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)-1,5-dimethylindolin-2-one-ethene (5b): FTIR (KBr, ν_{max}, cm^{-1}): 3083 (C-H *str.,* Ar), 2914, 2838, 2773 (C–H *str.*, aliphatic), 1712 (C=O *str.*, indole), 1616 (-C=N *str.*), 1548 (C=CH *str.*), 1358 (C=C *str.*, Ar), 1064 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 8.962 (s, 1H, -CH=N- imine proton), 8.367-8.116 (d, 2H, Ar-H), 7.885-7.841 (t, 3H, Ar-H), 7.686-7.675 (t, 2H, Ar-H), 7.545-7.543 (d, 3H, Ar-H), 7.452-7.415 (s, 1H, pyrazole), 7.140-7.102 (s, 2H, Ar-H), 2.376 (s, 3H, $-N-CH_3$), 1.890 (s, 3H, $-Ar-CH_3$). Mass (LC-MS): *m/z* 419 (M), 420 (M + 1, 100%).

5-Chloro-3-(((1,3-diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)-1-methylindolin-2-one-ethene (5c):



Scheme-I

FTIR (KBr, ν_{max}, cm^{-1}): 3069 (C-H *str.,* Ar), 2937, 2814, 2702 (C–H *str.*, aliphatic), 1711 (C=O *str.*, indole), 1615 (-C=N *str.*), 1564 (C=CH *str.*), 1449 (C=C *str.*, Ar), 1153 (C-N *str.*), 759 (C-Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 9.393 (s, 1H, -CH=N- imine proton), 8.297-8.115 (s, 1H, Ar-H), 7.897-7.855 (d, 2H, Ar-H), 7.805-7.797 (t, 3H, Ar-H), 7.793-7.767 (d, 2H, Ar-H), 7.692-7.648 (d, 2H, Ar-H), 7.598-7.565 (t, 3H, Ar-H), 7.546-7.517 (s, 1H, pyrazole), 2.210 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 439 (M), 440 (M + 1, 100%), 441 (M + 2, 30%).

3-(((1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)-1-methyl-5-nitroindolin-2-one-ethene (5d): FTIR (KBr, ν_{max}, cm^{-1}): 3020 (C-H *str.,* Ar), 2990, 2840, 2723 (C–H *str.*, aliphatic), 1715 (C=O *str.*, indole), 1642 (NO_2 *str.*), 1592 (-C=N *str.*), 1542 (C=CH *str.*), 1382 (C=C *str.*, Ar), 1095 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 9.212 (s, 1H, -CH=N- imine proton), 8.168-8.002 (s, 1H, Ar-H), 7.976-7.962 (d, 2H, Ar-H), 7.715-7.695 (t, 3H, Ar-H), 7.588-7.518 (t, 3H, Ar-H), 7.508-7.502 (d, 2H, Ar-H), 7.494-7.475 (s, 1H, pyrazole), 7.440-7.400 (d, 2H, Ar-H), 2.047 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 450 (M), 451 (M + 1, 100%).

1-Methyl-3-(((1-(4-nitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)indolin-2-one-ethene (5e): FTIR (KBr, ν_{max}, cm^{-1}): 3010 (C-H *str.,* Ar), 2991, 2842, 2723 (C–H *str.*, aliphatic), 1713 (C=O *str.*, indole), 1636 (NO_2 *str.*), 1591 (-C=N *str.*), 1542 (C=CH *str.*), 1383 (C=C *str.*, Ar), 1095 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 8.873 (s, 1H, -CH=N- imine proton), 8.106-8.043 (d, 2H, Ar-H), 7.960-7.904 (t, 3H, Ar-H), 7.875-7.851 (d, 2H, Ar-H), 7.791-7.742 (d, 2H, Ar-H), 7.664-7.646 (d, 2H, Ar-H), 7.501-7.205 (d, 2H, Ar-H), 7.169-7.105 (s, 1H, pyrazole), 2.091 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 450 (M), 451 (M + 1, 100%).

1,5-Dimethyl-3-(((1-(4-nitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)indolin-2-one-ethene (5f): FTIR (KBr, ν_{max}, cm^{-1}): 3029 (C-H *str.,* Ar), 2979, 2814, 2712 (C–H *str.*, aliphatic), 1714 (C=O *str.*, indole), 1619 (-C=N *str.*), 1560 (C=CH *str.*), 1469 (C=C *str.*, Ar), 1153 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 8.972 (s, 1H, -CH=N- imine proton), 8.273-8.043 (s, 1H, Ar-H), 7.957-7.906 (d, 2H, Ar-H), 7.869 (d, 2H, Ar-H), 7.806 (s, 1H, pyrazole), 7.791-7.732 (d, 2H, Ar-H), 7.662-7.642 (t, 3H, Ar-H), 7.335-7.264 (d, 2H, Ar-H), 2.162 (s, 3H, -N-CH_3), 1.928 (s, 3H, -Ar-CH_3). Mass (LC-MS): *m/z* 464 (M), 465 (M + 1, 100%).

5-Chloro-3-(((1,3-diphenyl-1*H*-pyrazol-4-yl)methylene)-hydrazineylidene)-1-methylindolin-2-one-ethene (5g): FTIR (KBr, ν_{max}, cm^{-1}): 3029 (C-H *str.,* Ar), 2979, 2814, 2712 (C–H *str.*, aliphatic), 1714 (C=O *str.*, indole), 1619 (-C=N *str.*), 1560 (C=CH *str.*), 1469 (C=C *str.*, Ar), 1153 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 8.905 (s, 1H, -CH=N- imine proton), 7.754-7.612 (d, 2H, Ar-H), 7.567-7.510 (d, 2H, Ar-H), 7.490-7.432 (d, 2H, Ar-H), 7.386-7.264 (t, 3H, Ar-H), 7.141-7.114 (d, 2H, Ar-H), 7.094-7.002 (t, 2H, Ar-H), 6.715-6.704 (s, 1H, (s, 1H, pyrazole), 2.021 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 439 (M), 440 (M + 1, 100%), 441 (M + 2, 30%).

3-(((3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)-1-methylindolin-2-one-ethene (5h): FTIR (KBr, ν_{max}, cm^{-1}): 3003 (C-H *str.,* Ar), 2996, 2802 (C–H *str.*, aliphatic), 1710 (C=O *str.*, indole), 1636 (NO_2 *str.*), 1599 (-C=N *str.*), 1537 (C=CH *str.*), 1370 (C=C *str.*, Ar), 1041 (C-N *str.*), 780 (C-Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 8.812 (s, 1H, -CH=N- imine proton), 8.561 (d, 2H, Ar-H), 8.254-8.127 (d, 2H, Ar-H), 8.054-8.038 (d, 2H, Ar-H), 7.852-7.836 (d, 2H, Ar-H), 7.785-7.723 (d, 2H, Ar-H), 7.670-7.540 (t, 2H, Ar-H), 7.388-7.357 (s, 1H, pyrazole), 2.515 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 484 (M), 485 (M + 1, 100%), 486 (M + 2, 30%).

5-Chloro-1-methyl-3-(((1-(4-nitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)indolin-2-one-ethene (5i): FTIR (KBr, ν_{max}, cm^{-1}): 3034 (C-H *str.,* Ar), 2939, 2802, 2702 (C–H *str.*, aliphatic), 1715 (C=O *str.*, indole), 1638 (-C=N *str.*), 1531 (C=CH *str.*), 1344 (C=C *str.*, Ar), 1046 (C-N *str.*), 768 (C-Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 8.787 (s, 1H, -CH=N- imine proton), 8.377 (s, 2H, Ar-H), 8.476 (d, 2H, Ar-H), 7.888-7.841 (d, 3H, Ar-H), 7.796 (t, 1H, Ar-H), 7.148 (d, 2H, Ar-H), 6.804-6.763 (d, 2H, Ar-H), 2.297 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 484 (M), 485 (M + 1, 100%), 486 (M + 2, 30%).

1-Methyl-5-nitro-3-(((1-(4-nitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)indolin-2-one-ethene (5j): FTIR (KBr, ν_{max}, cm^{-1}): 3058 (C-H *str.,* Ar), 2955, 2890 (C–H *str.*, aliphatic), 1713 (C=O *str.*, indole), 1632 (NO_2, *str.*), 1590 (-C=N *str.*), 1501 (C=CH *str.*), 1451 (C=C *str.*, Ar), 1056 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 8.986 (s, 1H, -CH=N- imine proton), 8.488-8.451 (s, 1H, Ar-H), 8.388-8.380 (d, 2H, Ar-H), 8.176-8.119 (d, 2H, Ar-H), 8.098-8.019 (d, 2H, Ar-H), 7.947-7.902 (d, 2H, Ar-H), 7.888-7.686 (t, 3H, Ar-H), 7.089-7.140 (s, 1H, pyrazole), 2.145 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 495 (M), 496 (M + 1, 100%).

1,5-Dimethyl-3-(((1-phenyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)indolin-2-one-ethane (5k): FTIR (KBr, ν_{max}, cm^{-1}): 3082 (C-H *str.,* Ar), 2952, 2821, 2737 (C–H *str.*, aliphatic), 1700 (C=O *str.*, indole), 1614 (-C=N *str.*), 1532 (C=CH *str.*), 1405 (C=C *str.*, Ar), 11053 (C-N *str.*), 794 (C-Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 9.393 (s, 1H, -CH=N- imine proton), 8.509 (s, 1H, Ar-H), 8.160-8.053 (d, 2H, Ar-H), 7.978-7.949 (d, 2H, Ar-H), 7.710-7.699 (d, 2H, Ar-H), 7.590-7.508 (d, 2H, Ar-H), 7.499-7.438 (t, 3H, Ar-H), 7.416-7.400 (s, 1H, pyrazole), 2.293 (s, 3H, -N-CH_3), 2.004 (s, 3H, -Ar-CH_3). Mass (LC-MS): *m/z* 453 (M), 454 (M + 1, 100%), 455 (M + 2, 30%).

5-Chloro-3-(((3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazineylidene)-1-methylindolin-2-one-ethene (5l): FTIR (KBr, ν_{max}, cm^{-1}): 3060 (C-H *str.,* Ar), 2984, 2874 (C–H *str.*, aliphatic), 1705 (C=O *str.*, indole), 1612 (-C=N *str.*), 1564 (C=CH *str.*), 1450 (C=C *str.*, Ar), 1056 (C-N *str.*), 851 (C-Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 9.081 (s, 1H, -CH=N- imine proton), 8.463 (s, 1H, Ar-H), 8.402-8.114 (d, 2H, Ar-H), 7.884-7.841 (d, 2H, Ar-H), 7.795-7.780 (d, 2H, Ar-H), 7.682-7.675 (d, 2H, Ar-H), 7.593-7.498 (t, 3H, Ar-H), 7.141-7.100 (s, 1H, pyrazole), 2.176 (s, 3H, -N-CH_3). Mass (LC-MS): *m/z* 473 (M), 474 (M + 1, 100%), 475 (M + 2, 30%).

Pharmacological activity

Anthelmintic activity: All the synthesized indole fused novel pyrazole derivatives were screened for *in vitro* the

anthelminthic activity by utilizing the Indian Earth worms at concentrations of 0.1%, 0.2% and 0.5%. Albendazole was used as the standard drug to compare anthelmintic activity [14,15]. Almost equal size of six earthworms had been positioned in preferred drug answer and take a look at compound's options at room temperature. Normal saline was used as control.

Anticancer activity: Evaluation of the viability was carried out by the MTT assay with three independent experiments with six concentrations of indole fused novel pyrazole in triplicates [16]. Cells were trypsinized and perform the trypan blue assay to know viable cells in cell suspension and cells were counted by haemocytometer and seeded at density of 5.0×10^3 cells/well in 100 µL media in 96 well plate culture medium and incubated overnight at 37 ºC. Take off the old media and add fresh media 100 µL with different concentrations of test compound in labelled wells in 96 plates after the incubation. After 48 h, discard the drug solution and add the fresh media with MTT solution (0.5 mg/mL) was added to each well and plates were incubated at 37 ºC for 3 h. At the end of incubation time, precipitates were formed as a result of the reduction of the MTT salt to chromophore Formosan crystals by the cells with metabolically active mitochondria. The optical density of the solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using by standard formula and concentration of test drug needed to inhibit cell growth by 50% values (IC_{50}).

Molecular docking studies: The synthesized pyrazole compounds were docked into the active site of the Tyrosine kinases (RTKs) by using the Ligprep tool of Schrödinger suite and consequently to rationalize the obtained pharmacological data. Furthermore, structurally optimized protein shape was used to observe protein-ligand interactions of the dataset ligands the use of Glide Xp docking protocol. Initially, a 3D grid used to be set up to the binding pocket (active site) of the protein, into which all the dataset ligands had been docked into. The binding interactions and efficiency of the binding were calculated in phrases of Glide score and it is a mixture of hydrophilic, hydrophobic, metal binding groups, van der Waals energy, freezing rotatable bonds and polar interactions with receptor [17].

RESULTS AND DISCUSSION

A series of synthesized novel pyrazole derivatives showed a satisfactory analysis for the proposed structures, which were confirmed on the basis of their FT-IR, LC-Mass and ^{1}H NMR spectral data. This synthetic process is based on Schiff base mechanism between substituted acetophenone with substituted phenyl hydrazine in the presence of glacial acetic acid followed by Vilsmeier reaction to give 1,3-di-phenyl-pyrazole-4-carbaldehyde (**2a-d**). Further, it reacts with substituted *n*-methyl isatin derivative (**4a-d**) by Schiff base to give the title compounds (**5a-l**). All the newly synthesized compounds structures were characterized as **5a-l** on the basis of satisfactory physical and spectral data including IR, LC-Mass and ^{1}H NMR data.

In all the compounds, the IR spectra show the aromatic and aliphatic C-H stretching frequency, as expected at around 3070-3000 and 2960-2800 cm^{-1}. All the compounds showed a strong absorption in the region of 1730-1680 cm^{-1} is due to be presence of C=O stretching frequency and in most of the compounds the C=C stretching of the aromatic ring is around 1540-1500 cm^{-1}, respectively. The Ar-Cl stretching shows a strong absorption at 820-780 cm^{-1} and few compounds containing -OCH_3 group shows peaks due to asymmetric and symmetric bending of –OCH_3 group is observed at around 1220 cm^{-1} and 1045 cm^{-1}, respectively. Similarly, the ^{1}H NMR (DMSO-d_6) spectra of indole fused novel pyrazole derivatives showing a singlet at δ 8.890-9.890 ppm for N=CH protons. All these compounds having aromatic protons were found between δ 8.501-6.902 ppm as singlet, doublet and triplet protons. All the derivatives display a singlet at δ 2.320-1.980 ppm for methyl protons (Ar-CH_3). The carbon atoms of compounds **4a-o**, which were the most most affected by substitution.

Anthelmintic activity: Anthelmintic activity screening of the synthesized indole fused novel pyrazole derivatives was done at 0.1%, 0.2%, 0.5% by using Indian earth warms. Albendazole was used as the standard drug and the time taken for complete paralysis and death of earthworms were recorded (Table-1). The results indicated that the synthesized compound **5c**, **5f**, **5h** and **5l** showed good anthelmintic activities whereas others showed a significant activities.

TABLE-1
ANTHELMINTIC ACTIVITY OF COMPOUNDS (**5a-l**)

Compound	Time (min)					
	For paralysis % concentration			For death % concentration		
Conc. →	0.1	0.2	0.5	0.1	0.2	0.5
Control	–	–	–	–	–	–
Albendazole	18	13	9	42	31	25
5a	29	30	36	50	47	34
5b	30	25	20	54	48	35
5c	23	20	18	48	39	30
5d	25	21	19	47	37	32
5e	30	27	23	58	44	36
5f	22	17	14	47	36	29
5g	34	30	21	55	49	32
5h	21	18	16	58	45	33
5i	32	26	23	60	51	39
5j	24	19	15	40	34	28
5k	30	23	19	54	49	35
5l	20	18	14	45	34	30

Anticancer activity: Indole fused novel pyrazole derivatives were screened for cytotoxic activity against two cancer cell like human breast cancer cells (MCF7) and ovarian cancer cells (SKVO3) by using MTT assay method, with doxorubicin as a standard drug. All the results (Table-2) proposed that both cell lines were susceptible to the evaluated compounds showed IC_{50}values in the range of 30.84 µM to 76.71 µg against MCF7 cell line and 44.02 to 84.32 µg against SKVO3 cell line. Compounds **5b**, **5f** and **5h** showed good activity against both cell lines, whereas, remaining compounds showed the moderate activity against both cell lines.

Molecular docking studies: Molecular docking studies had been carried out by using the Ligprep tool of Schrödinger suite. Various ionization states were generated using Ligprep

TABLE-2
CYTOTOXIC ACTIVITY OF INDOLE FUSED NOVEL PYRAZOLE DERIVATIVES ON MCF-7 AND SKVO3 CELLS

Sample description	Test parameters IC_{50} (μg)	
	MCF 7	SKVO3
5b	37.39	44.02
5c	48.51	53.32
5f	40.91	53.02
5i	76.71	84.32
5j	30.84	46.21
5j	50.56	69.21
Doxorubicin	12.04	15.4

module using a special program EPIK along with various possible conformers and tautomer's. The protein ligand complex conformations for every docked complex was once generated *via* Glide XP module. Based on the E Model energy, solely one was once displayed in the result. Glide dock sores of the dataset ligands are displayed in Table-3. Among the docked ligands, compound **5b** reported highest dock score of -6.131 with Glide binding energy of -47.434 Kcal/mol. Dock scores of all the compounds ranged from -6.131 (compound **5b**) to -1.476 (compound **5k**). Compounds with pyrazole had shown at least one hydrogen bond with LYS 721 and/or MET 769 amino

TABLE-3
In silico EGFR INHIBITION OF INDOLE FUSED NOVEL PYRAZOLE DERIVATIVES-GLIDE DOCK SORES OF THE DATASET LIGANDS

Compd. No.	Dock score XP GScore	No. of H-bonds	Interacting amino acids	H-bond lengths (Å)	E model energy	Glide energy
5b	-6.131	2	LYS 721	2.54	-70.211	-47.434
			MET 769	1.97		
5c	-6.103	2	LYS 721	2.72	-73.015	-47.433
			MET 769	2.07		
5f	-6.012	2	LYS 721	2.74	-72.277	-49.278
			MET 769	2.10		
5j	-5.928	1	Met 769	2.13	-59.528	-46.55
5i	-5.696	2	LYS 721	2.60	-73.467	-45.886
			MET 769	2.13		
5k	-1.476	1	CYS 773	1.90	-79.647	-49.132

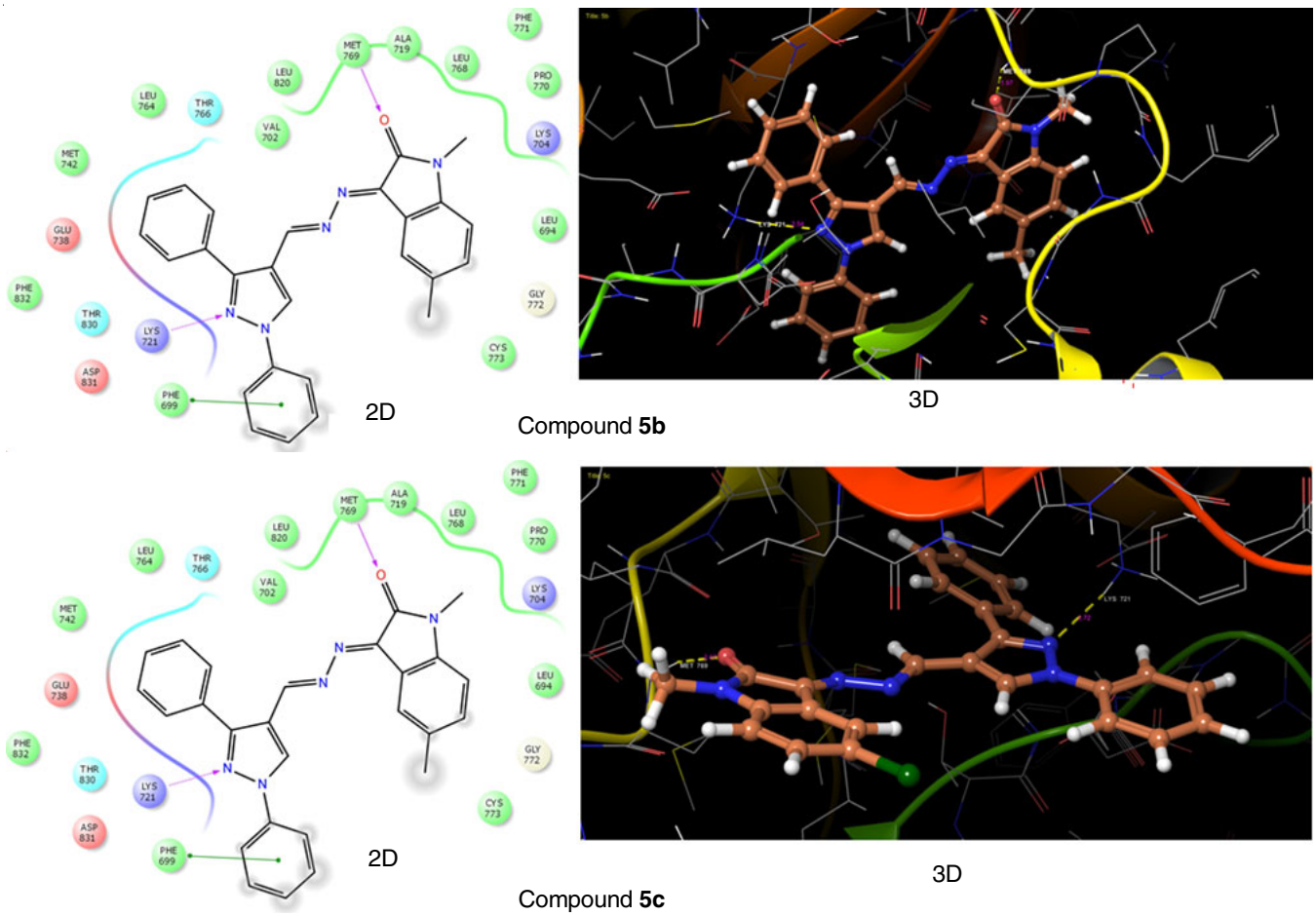


Fig. 1. Docking pose between the ligand and the protein (Dock-1 and Dock-2)

acids (compound **5k** with CYS 773). The PHE 699 residue in compound **5b**, **5c**, **5f** and **5j** were observed by hydrophobic interactions (Fig. 1).

Conclusion

The indole fused novel pyrazole derivatives **(5a-j)** were synthesized and characterized by analytical and spectral techniques. All these compounds exhibited significant biological activities like anthelmintic and anticancer activities.

ACKNOWLEDGEMENTS

The authors are grateful to Department of Chemistry, Sri Venkateshwara College of Pharmacy and JNTU, Hyderabad, Telangana, India for providing the laboratory facilities.

CONFLICT OF INTEREST

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

REFERENCES

1. W.A. Ali, W.A. Wani, A. Khan, A. Haque, A. Ahmad, K. Saleem and N. Manzoor, *Microb. Pathog.*, **53**, 66 (2012); https://doi.org/10.1016/j.micpath.2012.04.005
2. R.V. Ragavan, V. Vijayakumar and N.S. Kumari, *Eur. J. Med. Chem.*, **45**, 1173 (2010); https://doi.org/10.1016/j.ejmech.2009.12.042
3. N.C. Desai, A.H. Makwana and K.M. Rajpara, *J. Saudi Chem. Soc.*, **20**, S334 (2016); https://doi.org/10.1016/j.jscs.2012.12.004
4. I. Ali, M.N. Lone, Z.A. Al-Othman, A. Al-Warthan and M.M. Sanagi, *Curr. Drug Targets*, **16**, 711 (2015); https://doi.org/10.2174/1389450116666150309115922
5. B. Tiperciuc, V. Zaharia, I. Colosi, C. Moldovan, O. Crisan, A. Pirnau, L. Vlase, M. Duma and O. Oniga, *J. Heterocycl. Chem.*, **49**, 1407 (2012); https://doi.org/10.1002/jhet.1060
6. N. Kerru, L. Gummidi, S. Maddila, K.K. Gangu and S.B. Jonnalagadda, *Molecules*, **25**, 1909 (2020); https://doi.org/10.3390/molecules25081909
7. C.D. Pawar, A.P. Sarkate, K.S. Karnik, S.S. Bahekar, D.N. Pansare, R.N. Shelke, C.S. Jawale and D.B. Shinde, *Bioorg. Med. Chem. Lett.*, **26**, 3525 (2016); https://doi.org/10.1016/j.bmcl.2016.06.030
8. G. Chen, Y. Wang, X. Hao, S. Mu and Q. Sun, *Chem. Cent. J.*, **5**, 37 (2011); https://doi.org/10.1186/1752-153X-5-37
9. K.R. Ethiraj, P. Nithya, V. Krishnakumar, A.J. Mathew and F.N. Khan, *Res. Chem. Intermed.*, **39**, 1833 (2012); https://doi.org/10.1007/s11164-012-0718-3
10. O. Abdel Hafez, K. Ahmed and E. Haggag, *Molecules*, **6**, 396 (2011); https://doi.org/10.3390/60400396
11. R.F. Costa, L.C. Turones, K.V.N. Cavalcante, I.A. Rosa Jr., C.H. Xavier, L.P. Rosseto, H.B. Napolitano, P.F. da Silva Castro, M.L.F. Neto, G.M. Galvão, R. Menegatti, G.R. Pedrino, E.A. Costa, J.L.R Martins and J.O. Fajemiroye, *Front. Pharmacol*., **12**, 666725 (2021); https://doi.org/10.3389/fphar.2021.666725
12. L. De la Cruz-Martínez, C. Duran-Becerra, M. González-Andrade, J.C. Páez-Franco, J.M. Germán-Acacio, J. Espinosa-Chávez, J.M. Torres-Valencia, J. Pérez-Villanueva, J.F. Palacios-Espinosa, O. Soria-Arteche and F. Cortés-Benítez, *Molecules*, **26**, 4375 (2021); https://doi.org/10.3390/molecules26144375
13. J.J. Matilda and T.F.A. Fen Reji, *Indian J. Chem.*, **60B**, 1121 (2021).
14. M. Himaja, B. Sirisha, D. Moonjit and D. Munirasekhar, *J. Indian Chem. Soc.*, **92**, 908 (2015).
15. A. Venkanna, B. Siva, B. Poornima, P.R. Rao Vadaparthi, K.R. Prasad, K.A. Reddy, G.B.P. Reddy and K.S. Babu, *Fitoterapia*, **95**, 102 (2014); https://doi.org/10.1016/j.fitote.2014.03.003
16. K.P. Leena, M.Z. Subin and C.H. Deepthy, *Asian J. Pharm. Clin. Res.*, **10**, 50 (2017).
17. X.Y. Meng, H.X. Zhang, M. Mezei and M. Cui, *Curr. Computer-Aided Drug Design*, **17**, 146 (2014); https://doi.org/10.2174/1573409117956776602