Synthesis, Evaluation and Molecular Docking of Bis-pyrazole for the Treatment of Pancreatic Cancer

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Abstract:

The pyrazole heterocycle displayed versatile pharmacological activity ranging from anti-inflammatory to antiviral. Herein, we report the synthesis of bis-pyrazole as receptor- interacting protein 1 kinase inhibitor and evaluated for antiproliferative activity against pancreatic cancer line, PANC-1. Among twelve compounds, three candidates displayed the cytotoxicity IC50 value in single digit micromolar concentration. From the preliminary evaluation, the compound 31 emerged as a potential inhibitor with an IC50 = 4.1 µM. The lead compound showed similar inhibitor IC50 value in comparison to the positive control, doxorubicin. The docking studies confirm that these analogs may require further investigation to improve the potency.

Keywords: Pyrazole; Molecular docking; RIP 1 kinase inhibitor; Pancreatic cancer

1. Introduction

Pancreatic cancer is more lethal and accounts 1.8 percent death of various cancer related mortality. The recent survey concludes that in last two decades there was sharp increase in mortality rate (around 50 percent) and it is expected to increase by two fold in coming decades. [1] Pancreatic ductal adenocarcinoma (PDAC) is an exocrine tumor and it is highly responsible for pancreatic cancer. [2] The cancer reached advance stage at the time of detection and it is incurable due to its asymptomatic pathological conditions. [3] For the treatment of PDAC, gemcitabine alone or in combination of 5-fluorouracil or paclitaxel has been used. However, the currently available drugs developed resistance to PDAC and it is most common for all types of cancer. [4, 5] In addition to chemotherapy, targeted therapy such as small molecule kinase inhibitors and immune checkpoint inhibitors are attractive methods in the treatment of PDAC. Over a decade, an increasing number of small molecule inhibitors against pancreatic cancer have been reported and currently ten small molecules reached phase I clinical trials. [6,7] In recent years, immune checkpoint inhibitors (ICIs) are developed and approved by FDA for the broad range of cancer types. In 2011, ipilimumab is a first ICI and approved for the treatment of melanoma cancer. In comparison to traditional chemotherapeutic agents, ICIs are lower toxicity profile and work by strengthening the immune system of the host and weaken the tumour cells. To date, no ICIs are clinically approved for the treatment of pancreatic cancer. So far, clinically less effective results were obtained when immune checkpoint inhibitors has deployed as single agents. Currently, some ICIs are tested in combination to check their efficacy along with small molecule kinase inhibitors. [8, 9] Hence, it is an urgent and important to develop new and potent anticancer drugs with suppressed toxicity. Pyrazole heterocycle were often utilized as a versatile scaffold in the development of new medicinal compounds for various therapeutic categories. [10, 11] Furthermore, pyrazole analogs isolated from natural sources are familiar for diverse pharmacological action. Withasomnine a pyrazole alkaloid isolated from *Withania somnifera* and display anticancer and COX-2 inhibitory activity. [12] Formycin a natural antibiotic isolated from *Nocardia interforma* and display cytotoxic property against leukemic cancer cell lines. [13] Pyrazofurin structurally related to ribavirin, isolated from *Streptomyces* species and possess broad spectrum of biological applications like antibacterial, antiviral and antiproliferative activity. [14] Fluviols are isolated Pseudomonas fluorescens and showed significant antibacterial and antitumor activity. [15] In addition, pyrazole containing compounds are able to display favorable pharmacokinetic profile owing to presence of two adjacent nitrogen atoms in the ring and act as hydrogen bond donor and hydrogen bond acceptor. [16] The presence of nitrogen heteroatom in the heterocyclic compounds facilitate a strong affinity between the enzyme targets or receptors through dipole–dipole interactions, van der Waals forces and π-π stacking interactions. Also, the heterocyclic nitrogen-H bonding improves their solubility in the

biological system. [17–19]. One of our research interests is to synthesize library of pyrazole analogs and investigate for diverse pharmacological action. In recent past years, USFDA has approved nearly forty compounds for various remedial categories. [20] Eradafitinib, crizotinib, ruxolitinib, darolutamide are some of the familiar pyrazole analogs approved by USFDA for different malignancy treatment. In literature, some of the pyrazole compounds were found effective against receptor interacting protein kinase 1 (RIP 1). [21, 22] RIP 1 plays vital role in the pathophysiology of various disorders such as psoriasis, rheumatoid arthritis, ulcerative colitis, neurodegenerative and oncogenic diseases. [23, 24] Recently, the inhibition of RIP 1 has been reported for pancreatic cancer treatment and the combination of RIP 1 inhibitor with other anticancer therapies shows more beneficial for pancreatic cancer treatment. [25, 26] Herein, we report the synthesis of bis-pyrazole analogs and its antiproliferative activity against pancreatic cancer cell line, PANC-1.

2. Materials and Methods

All chemicals and solvents were purchased from SD Fine Chemicals (India), Spectrochem (India) and Loba Chemicals (India) and used without any further purification. All reactions were monitored by thinlayer chromatography (TLC). TLC plates were visualized by illuminating with UV light (254 nm) or exposure to iodine vapors. 1H NMR was recorded using Bruker AVANCE 500 MHz spectrometers. All NMR spectra were recorded in CDCl3 or DMSO-*d*6 using tetramethylsilane (TMS) as internal standard and the chemical shifts were expressed in ppm. Mass spectra were recorded on AB Sciex API 4000 LC-MS/MS instrument. IR spectra (KBr pellet) were recorded by on Agilent Cary 630 FTIR instrument.

3. Result and Discussion

The intermediate and was synthesized by refluxing the mixture of β-ketoester and substituted phenylhydrazine hydrochloride in acetic acid. The mixture of corresponding intermediate and one equivalent of appropriate aromatic aldehyde in presence of piperidine in ethanol to obtain bis-pyrazole analogs as target compounds in 70–87 % yield. [27] The IR spectrum of bis-pyrazole displays prominent broad peaks at 3200–3450 cm- 1 for –OH group. The 1H NMR exhibit a singlet at δ 4.5 to 5.1 ppm for –CH proton of benzyl group and broad peak around 12–13 ppm for –OH proton, respectively. In the search for new antiproliferative agents the newly synthesized compounds were screened against pancreatic cancer line, PANC- 1, using standard MTT protocol. [28] The IC50 results is displayed in Table 1. Among twelve compounds, three compounds emerged as single-digit micro molar inhibitors. The compound found to be equally potent (4.1 μM) as compared to the standard drug, doxorubicin (IC50 = 4.32 ± 1.06 μM). Among the various substitution patterns, substitution in benzyl ring plays an important role in the cytotoxicity. The trifluoromethyl substituent at para position in compound showed maximum cytotoxicity. Replacement of trifluoromethyl with other substituents like hydroxyl, nitro and dimethylamino reduce the

antiproliferative activity. The second active potential compound was found and the potency is reduced by 50 percent when methyl was replaced with an aryl ring. In conclusion, the presence of highly electron withdrawing substituents like trifluromethyl group in central aryl ring plays a critical role in cytotoxicity. To understand the interaction pattern between bis-pyrazole and RIP1 kinase, we docked bis-pyrazole into the binding pocket of the RIP1 kinase crystal structure (PDB ID: 6HHO) with the help of PyRx. [29] The predicted binding conformation and the interaction pattern of bis-pyrazole and RIP 1 kinase domain are shown in Fig. 5a and 5b. The RIP 1 kinase active binding has three major domains such as (i) catalytic triad residues Lys45-Glu63-Asp156, (ii) P-loop residues 24–31 and (iii) catalytic loop residues 136—143. The analysis of our docking results shows that amino acid residues at catayltic loop such as Phe28, Gly29 and Val31 forms hydrogen bonding with OH group of bispyrazole. The trifluromethyl group has shown strong interaction with Leu145 and Leu157 which are located near to the catalytic triad. The aromatic rings showed hydrobhopic interactions with Lys30 and Ala183. From the analysis of molecular docking, further structural optimization is required to improve the biological activity and its possible mode of its action.

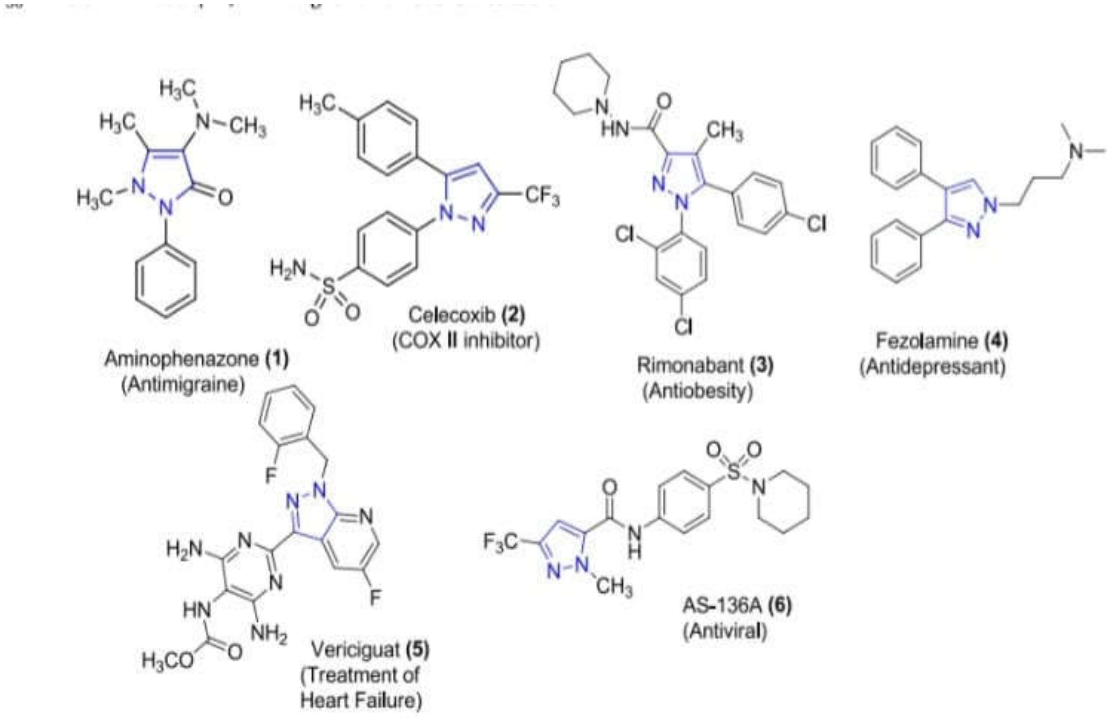

Fig.1. Marketed drugs containing pyrazole motif.

General procedure for synthesis of pyrazolone

An equimolar solution of ethyl benzoylacetate/ethylacetoacetate and substituted phenylhydrazine hydrochloride in acetic acid was treated with triethylamine. The mixture was stirred at reflux temperature

for 16–20 hrs. TLC was monitored at regular intervals to examine the progress of the reaction. The solvent was removed by evaporation, and the residue was extracted with AcOEt. The organic phase was dried over anhydrous Na2SO4 and evaporated under reduced pressure to get crude solid. The crude product was sufficiently pure and utilized to complete the synthesis of title compounds.

General procedure for synthesis of bis-pyrazole analogs

To a solution of pyrazolone (2 equivalents) and aldehyde (1 equivalent) in ethanol (20 ml) sodium acetate (1 equivalent) was added. The resultant mixture was allowed to stir at 30 ◦C for 6 to 8 h. The formation of product and complete consumption of starting materials was checked by TLC at regular intervals. On completion of reaction the resultant precipitate was filtered, washed with aqueous ethanol and dried. The purification of crude product was effected by recrystallization from aqueous ethanol. 4,4′-(Phenylmethylene)bis(1,3-diphenyl-1*H*-pyrazol-5-ol) Yield, 83 %; white solid; IR (KBr): 3214, 1603, 1499, 1461, 1372, 769 cm- 1; 1H NMR (CDCl3): δ 5.01(1H, s, CH), 6.86–6.88 (2H, d, ArH), 6.90–6.92 (4H, d, ArH), 6.95–6.98 (4H, t, ArH), 7.09–7.19 (8H, d, ArH), 7.26–7.27 (3H, d, ArH), 7.49–7.51 (4H, d, ArH), 13.48 (1H, br, OH); MS: 561.1(M + 1). 4,4′-(Phenylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) Yield, 87 %; white solid; IR (KBr): 3224, 1626, 1506, 1424, 1380, 762 cm- 1; 1H NMR (CDCl3): δ 2.12 (6H, s, CH3), 4.77 (1H, s, CH), 7.06–7.14 (3H, m, ArH), 7.21–7.25 (8H, m, ArH), 7.52–7.54 (4H, m, ArH), 13.32 (1H, br, OH); MS: 437.2 (M + 1). 4,4′-((4-Nitrophenyl)methylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol)

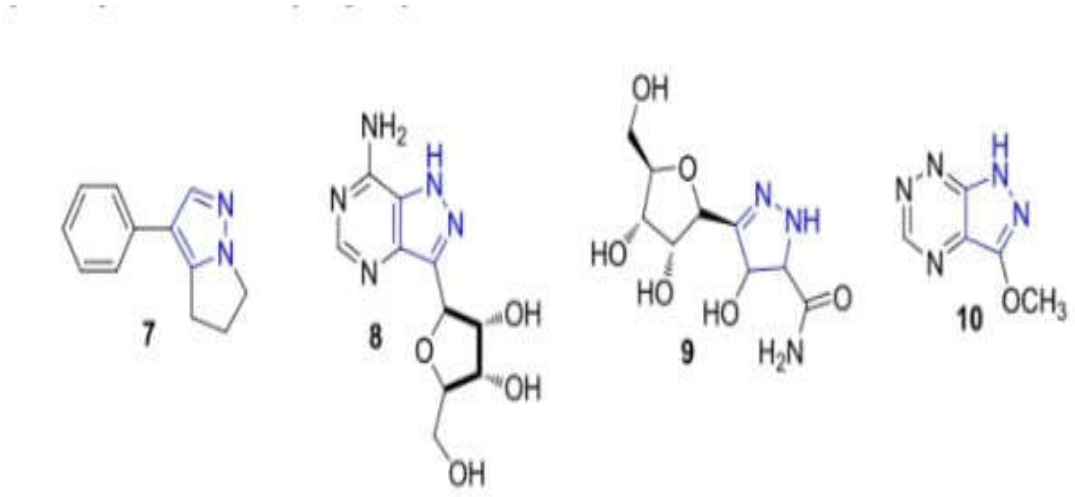

Fig.2. Pyrazole analogs from natural resources.

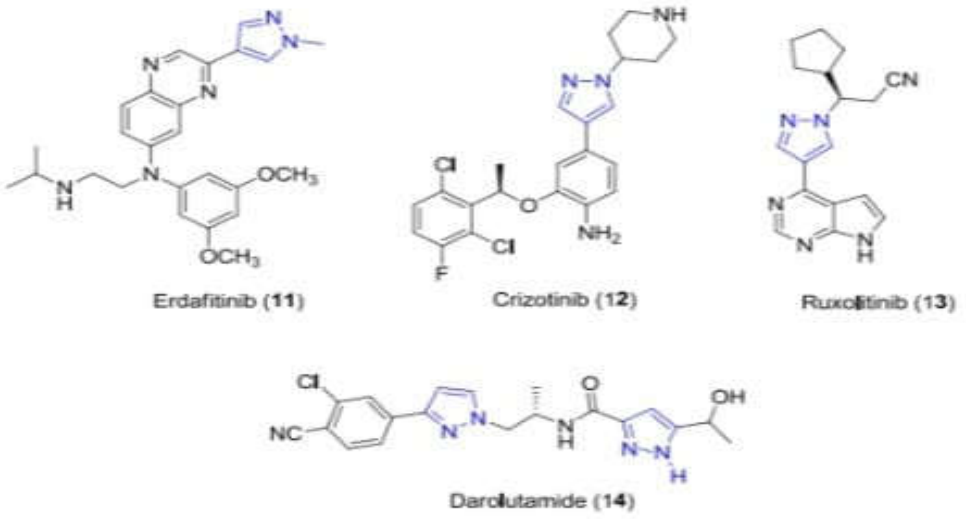

Fig. 3. Pyrazole analogs as Antiproliferative agents.

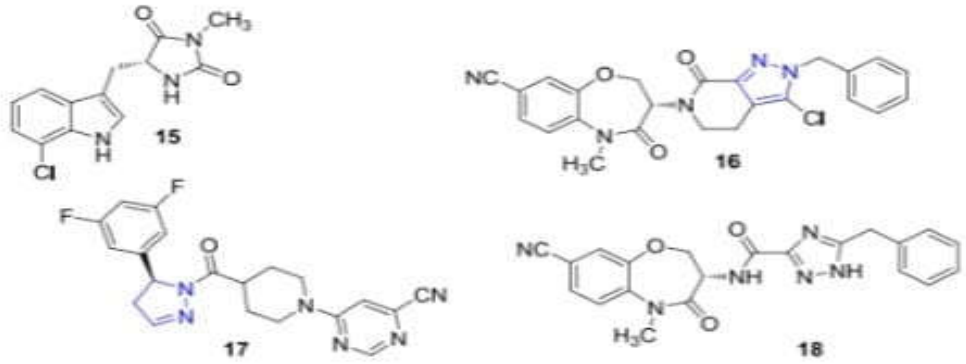

Fig. 4. RIP 1 kinase inhibitors.

Yield, 81 %; white solid; IR (KBr): 3258, 1603, 1506, 1350, 747 cm- 1; 1H NMR (CDCl3): δ 2.14 (6H, s, CH3), 4.82 (1H, s, CH), 7.28–7.36 (6H, m, ArH), 6.79–6.81 (2H, d, ArH), 8.05–8.07 (4H, m, ArH), 8.37–8.39 (2H, d, ArH),13.81 (1H, br, OH); MS: 482.2 (M + 1). 4,4′-((4-Methoxyphenyl)methylene)bis(3-methyl-1-phenyl-1*H*pyrazol-5-ol) Yield, 89 %; white solid; IR (KBr): 3257, 1616, 1514, 1261, 1185, 1045, 762 cm- 1; 1H NMR (DMSO-*d*6): δ 1.63 (6H, s, CH3), 3.70 (3H, s, OCH3), 5.16 (1H, s, CH), 6.79–6.81 (2H, d, ArH), 7.11–7.17 (4H, m, ArH), 7.27–7.29 (2H, d, ArH), 7.37–7.40 (4H, t, ArH), 8.05–8.06 (2H, d, ArH),18.12 (1H, br, OH); MS: 467.3 (M + 1). 4,4′-((4-Hydroxyphenyl)methylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) Yield, 79 %; yellow solid; IR (KBr): 3205, 1603, 1506, 1424, 762 cm- 1; 1H NMR (DMSO-*d*6): δ 2.45 (6H, s, CH3), 4.78 (1H, s, CH), 6.59–6.61 (2H, d, ArH), 6.98–7.00 (2H, m, ArH), 7.37–7.39(6H, m, ArH), 7.65–7.67 (4H, m, ArH), 12.32 (1H, br, OH), 14.01 (1H, br, OH); MS: 453.2(M + 1). 4,4′-((4-(Dimethylamino)phenyl)methylene)bis(3-methyl-1-phenyl- 1*H*-pyrazol-5-ol) Yield, 84 %; orange solid; IR (KBr): 3254, 1603, 1514, 1380, 1208, 1052, 853, 754 cm- 1; 1H NMR

(DMSO-*d*6): *δ* 2.18 (6H, s, CH3), 2.78 (6H, s, N(CH3)2), 4.8 (1H, s, CH), 6.59–6.61 (2H, d, ArH), 7.00–7.02 (2H, m, ArH), 7.19–7.21 (2H, t, ArH), 7.37–7.41 (4H, t, ArH), 7.65–7.67 (4H, d, ArH), 12.30 (1H, br, OH),13.93 (1H, br, OH); MS: 480.2 (M + 1). 4,4′-((4-Nitrophenyl)methylene)bis(1,3-diphenyl-1*H*-pyrazol-5-ol).

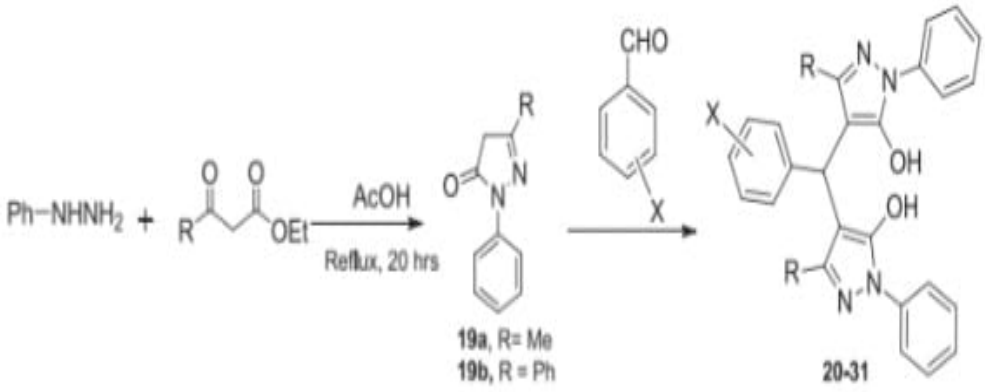

Fig.5. Synthesis of Bis-pyrazole analogs.

Yield, 82 %; yellow solid; IR (KBr): 3392, 1595, 1401, 1115, 740 cm- 1; 1H NMR (CDCl3): *δ* 5.08 (1H, s, CH), 6.95–7.06 (5H, m, ArH), 7.17–7.22 (5H, m, ArH), 7.33–7.36 (6H, m, ArH), 7.59–7.61 (4H, m, ArH), 8.03–8.05 (2H, d, ArH), 8.37–8.39 (2H, d, ArH); 13.49 (1H, br, OH); MS: 606.0 (M + 1). 4,4′-((4-Hydroxyphenyl)methylene)bis(1,3-diphenyl-1*H*-pyrazol-5-ol) Yield, 86 %; light brown solid; IR (KBr): 3175, 1593, 1401, 1113, 1069, 764 cm- 1; 1H NMR (DMSO-*d*6): *δ* 5.12 (1H, s, CH), 6.62–6.64 (2H,s, ArH), 6.92–6.94 (2H, d, ArH), 7.14–7.25 (10*H*, m, ArH), 7.41–7.45 (5H, t, ArH), 7.79–7.81 (5H, d, ArH), 9.17 (1H, br, OH), 13.49 (1H, br, OH); MS: 576.9 (M + 1). 4,4′-((4-Methoxyphenyl)methylene)bis(1,3-diphenyl-1*H*-pyrazol-5-ol) Yield, 89 %; orange solid; IR (KBr): 3398, 1593, 1503, 1401, 699 cm- 1; 1H NMR (CDCl3): *δ* 3.88 (3H, s, OCH3), 5.11 (1H, s, CH), 6.72–6.74 (2H, d, ArH), 6.96–6.98 (2H, d, ArH), 7.16–7.21 (4H, m, ArH), 7.31–7.35 (4H, m, ArH), 7.51–7.54 (4H, m, ArH), 7.65–7.67 (4H, t, ArH), 8.01–8.03 (2H, d, ArH), 8.51–8.53 (2H, d, ArH), 13.14 (1H, br, OH); MS: 591.1 (M + 1). 4,4′-((4-(Dimethylamino)phenyl)methylene)bis(1,3-diphenyl-1*H*pyrazol-5-ol) Yield, 91 %; orange solid; IR (KBr): 3068, 1603, 1521, 1499, 1372, 1171, 762 cm- 1; 1H NMR (CDCl3): *δ* 3.12 (6H, s, N(CH3)2), 3.8 (1H, s, CH), 6.69–6.71 (2H, d, ArH), 7.46–7.51 (8H, m, ArH), 7.64–7.77 (8H, m, ArH), 7.95–7.97 (2H, d, ArH), 8.07–8.09 (2H, d, ArH), 8.52–8.54 (2H, d, ArH), 12.30 (1H, br, OH); MS: 604.5 (M + 1). 4,4′-((4-(Trifluoromethyl)phenyl)methylene)bis(3-methyl-1-phenyl- 1*H*-pyrazol-5-ol) Yield, 79 %; light brown solid; IR (KBr): 3126, 1603, 1506, 1376, 1298, 1115, 821, 754 cm- 1; 1H NMR (DMSO-*d*6): *δ* 2.41 (6H, s, CH3), 5.02 (1H, s, CH), 7.18–7.21 (2H, m, ArH), 7.38–7.42 (5H, m, ArH), 7.65–7.67 (5H, m, ArH),

12.56 (1H, br, OH), 13.87 (1H, br, OH); MS:505.9 (M + 1). 4,4′-((4-(Trifluoromethyl)phenyl)methylene)bis(1,3-diphenyl-1*H*pyrazol-5-ol) Yield, 89 %; light brown solid; IR (KBr): 3268, 1458, 1328, 1123, 1071, 829, 758 cm- 1; 1H NMR (CDCl3): δ 5.13(1H, s, CH), 7.00–7.08 (10*H*, m, ArH), 7.17–7.22 (5H, m, ArH), 7.33–7.37 (5H, t, ArH), 7.43–7.45 (2H, d, ArH), 7.62–7.64 (2H, d, ArH), 13.34 (1H, br, OH); MS:630.2 (M + 2).

Antiproliferative activity

The antiproliferative effect of bis-pyrazole analogs against pancreatic cancer cell line, PANC-1, was determined by colorimetric assay using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The cells were trypsinized and 4 × 103 cells were seeded in 100 µl medium in each well of a 96-well plate. The cells were incubated at 37 ◦C in a CO2 incubator for 24 h. After 24 hrs, the cells were treated with increasing concentrations of 1 µM, 10 µM and 100 µM of bis-pyrazole analogs. Doxorubicin used as positive control. After 72 h, 20 µl of MTT (Sigma-Aldrich) was added to each well and incubated at 37 ◦C in a CO2 incubator for 2 h. The formazan crystals formed were dissolved in 100 µl of stock solution, dimethyl sulphoxide (DMSO). The absorbance was measured at 570 nm using a Multiskan GO microplate spectrophotometer. Percentage survival curves were plotted to calculate half-maximal inhibitory concentrations (IC50).

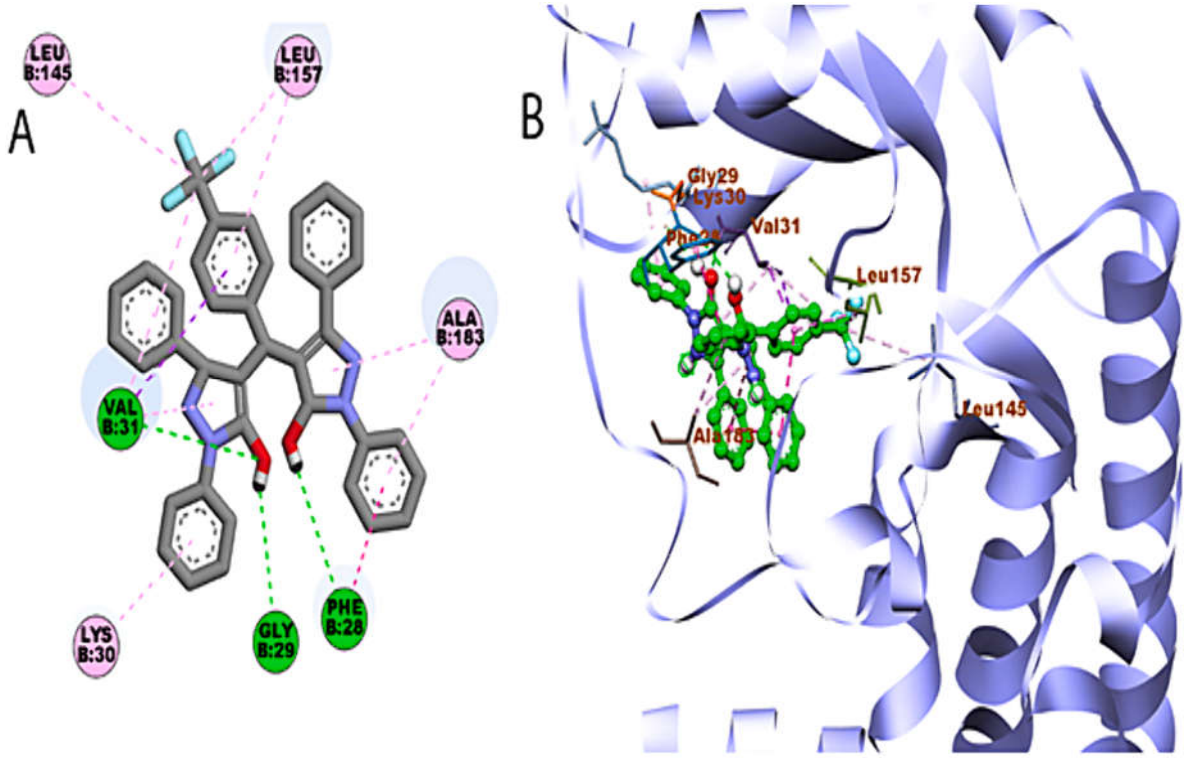

Fig.6. A and 5B: 2D and 3D interaction of compound with RIP 1 kinase, respectively.

Docking studies

Receptor-interacting serine/threonine protein kinase 1 (RIPK 1) is highly expressed in pancreatic ductal adenocarcinoma. Docking studies was performed in the RIPK 1 active site (PDB code: 6HHO) in order to understand the molecular insight and mode of inhibition of the active compound. The docking analysis was carried out using PyRx with set of parameters to systematize the genetic algorithm operations. The standard settings were adopted for conditions like 'grid resolution' of 5 Å, 'site opening' of 12 Å, and 'binding site' selected for defining the active site cavity.

4. Conclusion

In conclusion, a new series of bis-pyrazole were synthesized and screened against pancreatic cancer line. In addition, docking studies were carried to understand the binding mode at the active site of RIP 1 kinase. Three out of twelve synthesized compounds showed significant anti-proliferative activity with lower micromolar concentration. Among them had displayed equally inhibitory potential in comparison to standard drug doxorubicin, which reveals that bis-pyrazole is a promising scaffold for receptor interacting protein 1 kinase inhibitor drug development. Further structural modification of the lead compound and additional biological studies are needed to improve the potency and elucidate its possible mode of action, respectively.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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