

## RESEARCH ARTICLE

# Novel Pyrazolyl Benzoxazole Conjugates: Design, Synthesis, Molecular Docking Studies and *in vitro* Anticancer Activities

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**Abstract: Background:** Nowadays, hybrid drugs have gained a significant role in the treatment of different health problems. Most of the hybrid molecules with different heterocyclic moieties were proved to be potent anti-tumor agents in cancer chemotherapy. Hence, the present study is aimed at the evaluation of *in vitro* anticancer activity of novel hybrid molecules (pyrazolyl benzoxazole conjugates) and to investigate their anticancer activity by molecular docking studies.

**Methods:** Designed, synthesized and characterized the novel pyrazolyl benzoxazole conjugates. Anticancer activity of these compounds was determined by SRB assay. Then molecular docking studies were carried out against proto-oncogene tyrosine-protein kinase (ATP-Src, PDB: 2BDF), a putative target for cancer.

**Results:** All the synthesized compound derivatives were evaluated against MCF-7, KB, Hop62 and A549 cancer cell lines. Compounds **9b** and **9c** exhibited excellent anticancer activities with GI<sub>50</sub> values of <0.1 μM against **MCF-7** and **A549** cell lines. Compound **9e** exhibited good antitumor activity on MCF-7 and A-549 with GI<sub>50</sub> values of 0.12 μM and 0.19 μM respectively. Compound **9g** showed better anticancer activity on A-549 cancer cell line with GI<sub>50</sub> value of 0.34 μM.

**Conclusion:** The two-hybrid molecules **9b** and **9c** are found to be comparably potent with the standard drug doxorubicin and may act as drug lead compounds in medicinal chemistry aspect. The present docking investigation proved that having benzoxazole of compound **9c** at benzofuran of reference compound N-acetyl pyrazoline derivative might be valid for contributing to anti-cancer activity.

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## 1. INTRODUCTION

Worldwide, cancer is a very dangerous disease and the number of deaths caused due to cancer are more than malaria, AIDS and tuberculosis. Among the seven deaths, one is due to cancer. In high-income countries, cancer-causing deaths occupied second place, whereas third place in low and middle-income countries [1]. 14.1 million new cancer cases were registered across the world in 2012, leading to 14.6% or 8.2 million deaths [1, 2]. The total number of cancer registrations is expected to enhance from 979,786 to 1,148,757 over ten years of the time period (2010-2020) in India [3]. Over the past few decades, extensive research led to the development of anticancer agents [4]. The limitation of current

anticancer drugs and the rapid development of drug resistance [5, 6] have highlighted the need for the discovery of new anticancer agents, preferably with a novel mechanism of action.

Recent literature collection shows that the model of "hybrid drugs" has attained appreciation in the medication field. Combination of therapies used in this concept is mostly functional to treat unresponsive patients [7]. This is a proved concept to cure various health issues like anti-tumor reagents [8], systemic heart diseases [9] and antiparasitic activities [10]. Chemical hybridization is used to prepare these hybrid molecules by incorporating two pharmacophores in a single molecule in order to present twin drug action. For instance, if tumor vessels are targeted by one of the pharmacophores, the active agent role is played by the other [11]. Therefore, conjugated or multifunctional drugs are the other names of hybrid molecules. In comparison to the individual pharmacophores, the synergetic effect is exhibited by

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hybrid molecules [12]. Two active reagents are released simultaneously due to the action of specific enzymes on these hybrid drugs. It helps for a decrease in systemic toxicity and also optimized the delivery of the drug [13]. The other plausible prominent benefits of hybrids are evading potential drug resistance, enhanced bioavailability of drug and hence, transportation through cell organelles membranes, shielding the active compound from enzymatic degradation, improved efficacy, reduced hazard of drug-drug interactions, compensating one pharmacophore's side effects by another and lessening of side effects [8]. A number of hybrid molecules possessing various heterocyclic moieties exhibited decent anticancer activities against an array of human cancer cell lines [14-16] and hence proved to be effective antitumor agents in cancer chemotherapy.

Most of the advanced materials [17-22] and heterocyclic compounds [23-26] play a vital role in medicinal chemistry as well as industrial field and exhibited potent antitumor activities against different human cancer cell lines. The pyrazole scaffolds belong to a highly important class of heterocyclic compounds which have attracted much attention for their significant bioactivity. It has been well established that

pyrazole derivatives possess a wide spectrum of chemotherapeutic activities including anticancer [27], antitumor [28], anti-inflammatory [29], antimicrobial [30], antiviral [31], and antituberculosis [32, 33]. Some pyrazole containing drugs were approved by FDA such as Ruxolitinib (**1**, Fig. 1), a selective JAK1 and JAK2 inhibitor approved for the treatment of myelofibrosis [34] and Crizotinib (**2**), a c-Met and ALK inhibitor used in non-small cell lung carcinoma cancer treatment [35]. It gave great information about the search of potent anticancer scaffolds. In addition, benzoxazole derivatives attracted the attention of researchers because of their potential applications in the medicinal field. Some of their biological activities include antitumor [36, 37], topoisomerase-I and II inhibitory [38], antineoplastic [39], anti-inflammatory [40], treatment of metabolic disorders [41] irritable bowel syndrome (IBS) [42], antiviral [43], thrombolytic [44] and sleep disorders [45].

In view of the above information, in the present study, we have synthesized a novel series of benzoxazole-pyrazole conjugates (**9a-j**). Further, these derivatives were checked for their anticancer activity against four human cancer cell lines.

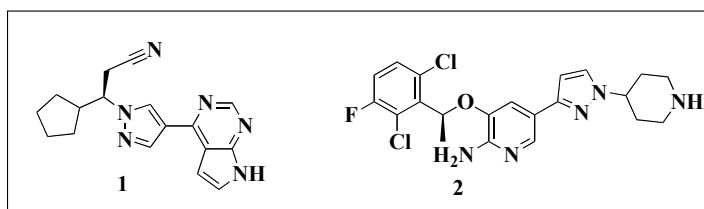
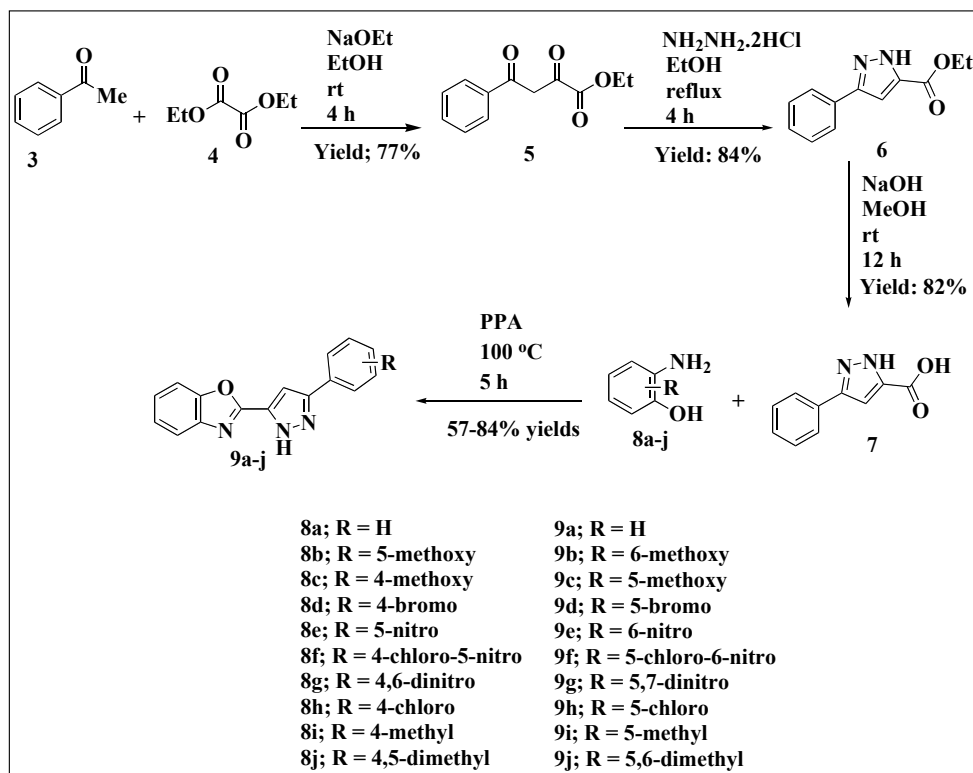


Fig. (1). Structures of Ruxolitinib (**1**) and Crizotinib (**2**).



Scheme (1). Synthesis of pyrazolyl benzoxazole derivatives.

## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

The synthesis of pyrazolyl benzoxazole derivatives (**9a-j**) was successfully achieved by carrying out the reaction between 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) and substituted 2-amino phenols (**8a-j**) in the presence of polyphosphoric acid (PPA) as shown in Scheme 1. Initially, acetophenone (**3**) reacted with diethyl oxalate in basic atmosphere such as sodium ethoxide at room temperature for 4 hours to afford the corresponding phenyl diketo ester (**5**). Subsequently, this phenyl diketo ester (**5**) underwent dehydrative cyclization with hydrazine dihydrochloride in refluxing ethanol for 4 hours to give ethyl 3-phenyl-1H-pyrazole-5-carboxylate (**6**) in 84% yield. This ester intermediate (**6**) was hydrolyzed with aq. NaOH (2M) in methanol as a solvent medium under room temperature over 12 hours to give 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**). The acid intermediate (**7**) underwent cyclisation with different substituted 2-amino phenols (**8a-j**) in PPA at 100°C for 5 hours to afford pure benzoxazole-pyrazole conjugates (**9a-j**). The final target derivatives were characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectral data.

### 2.2. Biological Evaluation

#### 2.2.1. In vitro Cytotoxicity

Initially, the benzoxazole-pyrazole conjugates (**9a-j**) were screened for their *in vitro* anticancer activity against a panel of four different cancer cell lines such as MCF-7, KB, Hop 62 and A549, using “sulphorhodamine B assay” [46] by taking Doxorubicin as a positive control. The GI<sub>50</sub> values ranged between <0.1 μM to 45.7 μM and are summarized in Table 1. Compound **9c** showed excellent anticancer activity against all the tested cancer cell lines with GI<sub>50</sub> values of <0.1 μM. Compound **9b** also showed potent cytotoxic activity against A549 and MCF-7 cancer cell lines with GI<sub>50</sub> val-

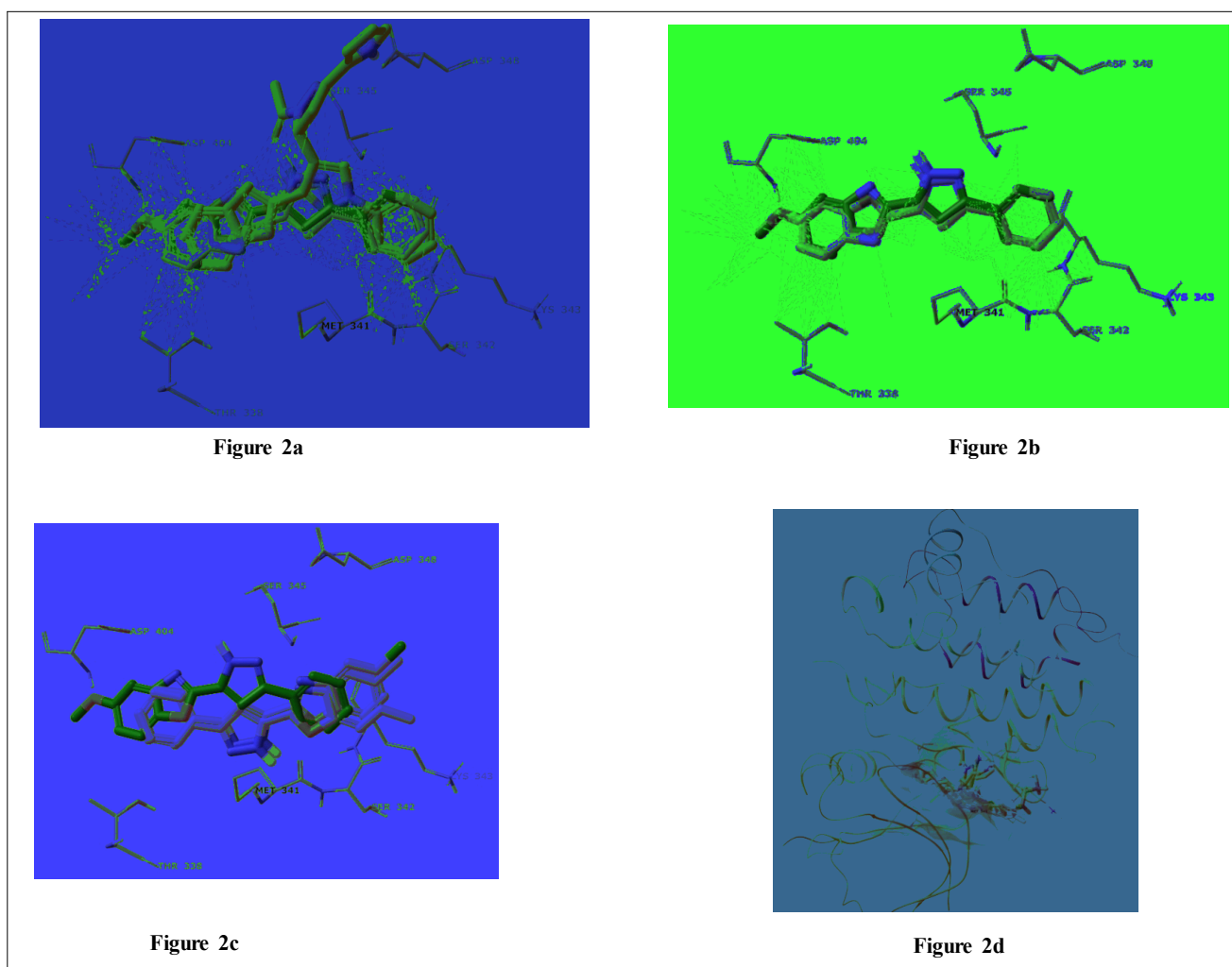
ues of <0.1 μM. These values show that these two compounds are comparably potent to the standard drug, “Doxorubicin”. Hence, these two compounds may act as drug lead compounds in medicinal chemistry. The structure-activity relationship studies concluded that the presence of electron donating group like methoxy on benzene ring may cause an enhancement of the anticancer activity. Compound **9e** exhibited good antitumor activity against MCF-7 and A-549 with GI<sub>50</sub> values of 0.12 μM and 0.19 μM respectively. Compound **9g** showed better anticancer activity on A-549 cancer cell line with GI<sub>50</sub> values of 0.34 μM. Compounds **9a**, **9d** and **9g** showed average anticancer activities on particular cancer cell lines. The remaining compounds showed less anticancer activities on these cancer cell lines.

### 2.3. Molecular Docking Studies

The validated molecular docking studies against proto-oncogene tyrosine-protein kinase (ATP-Src, PDB: 2BDF), a putative target for cancer has been carried out. The inhibitory activity of ATP-Src with benzofuran linked pyrazole derivatives has been investigated previously [47]. Here we attempted the potentials of benzoxazole linked pyrazoles to impede the role of ATP-Src by aligning with previously reported active compound N-acetyl pyrazoline derivative [47]. The salient features of the present molecular docking studies are alignment, overlap of compounds **9b** and **9c** (Fig. 2a-2d), and overlap of ligands in ATP-Src active (Fig. 2d). The molecular docking simulations of benzoxazole derivatives provided the support for the most active compound **9c** with superior binding efficiency than others. It displayed the hydrogen bond with Asp404, the orientation of benzoxazole is found to be similar to that benzofuran and despite phenyl positioned on pyrazole, its binding pattern is comparable when aligned with compound N-acetyl pyrazoline derivative. The compound **9b** displayed parallel interactions with that of **9c**. Whereas, the lowest active compounds failed to exhibit

Table 1. Anticancer activities of compounds (9a-9j).

Compound	GI <sub>50</sub> (μM)				Docking Score K.cal/mol
	MCF-7 (Breast)	KB (Oral)	Hop62 (Lung)	A-549 (Lung)	
<b>9a</b>	2.89	-	-	3.56	-4.781
<b>9b</b>	<0.1	1.23	2.10	<0.1	-5.011
<b>9c</b>	<0.1	<0.1	<0.1	<0.1	-5.813
<b>9d</b>	1.20	1.30	2.34	1.78	-4.719
<b>9e</b>	0.12	1.45	-	0.19	-5.006
<b>9f</b>	-	45.7	-	38.1	-3.785
<b>9g</b>	2.78	-	1.56	0.34	-3.622
<b>9h</b>	24.3	-	6.28	7.01	-4.835
<b>9i</b>	8.51	5.46	-	-	-4.678
<b>9j</b>	12.4	-	45.1	-	-4.366
<b>Doxorubicin</b>	0.13	0.13	0.15	<0.1	-



**Fig. (2).** Schematic representation of molecular docking studies of compounds (9a-j).

**a)** Alignment of Compound **9b** and **9c** (green and macro model thick residues) with reference compound N-acetyl pyrazoline derivative (Orange thick residue), thin stick indicates active site residues and green dotted line indicates interactions; **b)** Alignment of Compound **9b** and **9c** (green and macro model thick residues), thin stick indicates active site residues and green dotted line indicates interactions; **c)** Overlap of Compound **9b** and **9c** (green and macro model thick residues) with **9h** and **9j** (plum color thick sticks) and thin stick indicates active site residues and **d)** Surface diagram of ATP-Src active with overlap of all ligands along with co-crystal ligand.

the interactions which are made by active compounds **9c** and **9b**. The present docking investigation proved that having benzoxazole of compound **9c** at benzofuran of reference compound N-acetyl pyrazoline derivative might be valid for contributing to the anti-cancer activity. Additionally, these compounds occupied the ATP-Src active, which might be planar. Docking score values further reinforce the earlier conclusion of excellent anticancer activities of compounds **9c** and **9d** as reported from  $GI_{50}$  values (Table 1).

### 3. EXPERIMENTAL

#### 3.1. General

All the reagents, fine chemicals, salts, TLC plates, silica gel, and solvents were purchased from Delta Scientific PVT Limited (dealer for chemicals supply). 500 MHz and 400 MHz Bruker NMR spectrometers were employed to record the proton NMR spectra of our synthesized stepwise com-

pounds. 100 MHz Gemini NMR was employed for  $^{13}C$  NMR spectral recording.  $\delta$  values were taken in ppm units with respect to the signal of TMS, an internal standard.  $J$  remains coupling constant value of the particular signal and mentioned in Hz. The purity of aromatic compounds was determined by TLC and UV cabinet. Multiplet, quartet, triplet, double doublet, doublet and singlet were denoted by the corresponding symbols like m, q, t, dd, d and s. Perkin Elmer mass spectrophotometer was used to record ESIMS mass spectra.

#### 3.2. Ethyl 2,4-dioxo-4-phenylbutanoate (5)

Initially, sodium ethanolate was synthesized *in situ* and it was cooled to 0 °C followed by the addition of diethyl oxalate (**4**) (16.9 ml, 124.8 mmol) very slowly. This reaction mixture was allowed for stirring over a time period of 15 minutes at 0 °C. A small portion of acetophenone (**3**) (15 g, 124.8 mmol) was also added to the above reaction mass at

the same temperature (0 °C). The total reaction mass was allowed for stirring over a period of 4 hours by maintaining at room temperature. The completion of the reaction was confirmed by TLC using a mixture of EtOAc and hexane (2:8 v/v) mobile phase.

After completion of the reaction, a sufficient quantity of dilute H<sub>2</sub>SO<sub>4</sub> was added to the above reaction mass for neutralization purpose. Further, ethyl acetate solvent was used to extract the reaction mass. This solvent was evaporated by using rotavapor under reduced pressure to afford pure pale yellow color compound **5**, with 21 g, in 77% yield. This compound was taken as such for the further next step without any purification. M.P 36-41°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.38 (t, 3H), 4.36 (q, 2H), 7.06 (s, 1H), 7.46 (dd, 2H, *J* = 7.8, *J* = 1.2 Hz), 7.58 (m, 1H, *J* = 7.2, *J* = 1.2 Hz), 7.95 (d, 2H, *J* = 7.8 Hz), 15.31 (bs, 1H); MS (ESI): 221 [M + H]<sup>+</sup>.

### 3.3. Ethyl 3-phenyl-1H-pyrazole-5-carboxylate (6)

Hydrazine dihydrochloride (N<sub>2</sub>H<sub>4</sub>·2HCl) (11.45 g, 109.0 mmol) dissolved in ethanol was added to ethyl 2,4-dioxo-4-phenylbutanoate (**5**) (16 g, 72.7 mmol) produced in the previous step and refluxed for 4 hours. After the completion of the reaction, ethanol was evaporated under high vacuum conditions. 150-200 mL water was mixed to the above reaction mass, followed by extraction with 4x50 mL of ethyl acetate. The four fractions of organic layer were mixed with each other and dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>. The ethyl acetate, organic solvent was evaporated by rotavapor under reduced pressure to give crude compound. Finally, a silica gel column chromatography technique with 7:3 v/v hexane and ethyl acetate solvent system was applied to the above crude to afford pure pale yellow color compound **6**, with 13.2 g in 84% yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.34-1.39 (t, 3H), 4.33-4.41 (q, 2H), 7.05 (s, 1H), 7.36 (d, 2H, *J* = 7.9 Hz), 7.45-7.49 (m, 3H), 9.31 (brs, 1H); MS (ESI): 217 [M + H]<sup>+</sup>.

### 3.4. 3-Phenyl-1H-pyrazole-5-carboxylic acid (7)

50 mL of 2M NaOH was mixed with a solution of ester linkers of ethyl 3-phenyl-1H-pyrazole-5-carboxylate (**6**) (12 g, 55.5 mmol) in MeOH (60 ml) and the reaction mass was stirred for over a 12 hour time period at room temperature. After evaporation of most of the methanol, the aqueous phase was adjusted to pH level at 7 and extracted with 4x50 mL of ethyl acetate solvent. The four fractions of organic layer were layers mixed with each other and dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>. The ethyl acetate organic solvent was evaporated by rotavapor under reduced pressure to give crude compound. Finally, silica gel column chromatography technique with 7:3 v/v hexane and ethyl acetate solvent system was applied to the above crude compound to afford pure compound **7**, with 8.5 g in 82% yield, M.p: 238-241°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.08 (s, 1H), 7.37 (d, 2H, *J* = 7.9 Hz), 7.46-7.50 (m, 3H), 9.33 (brs, 1H), 10.13 (brs, 1H); MS (ESI): 189 [M + H]<sup>+</sup>.

### 3.5. 2-(3-Phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9a)

A mixture of the 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and the 2-aminophenol (**8a**) (290 mg, 2.65 mmol) was dissolved in sufficient amount of PPA (Polyphosphoric acid). The reaction mass was heated at 100

°C over a time period of 5 hours. The reaction mass was allowed to rest at room temperature and added with an excess of 10% Na<sub>2</sub>CO<sub>3</sub> solution for quenching purpose. To this, 4x50 mL of ethyl acetate was added. The four fractions of organic layer were mixed with each other and dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>. Finally, column chromatography with 1:1 v/v EtOAc/hexane was applied to crude compound and then pure compound **9a** was achieved, with 418 mg in 60% yield. M.p: 254-256°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.05 (s, 1H), 7.12 (d, 1H, *J* = 7.6 Hz), 7.35 (d, 2H, *J* = 8.0 Hz), 7.45-7.49 (m, 3H), 7.53-7.58 (m, 2H), 7.62 (d, 1H, *J* = 8.3 Hz), 9.34 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 111.8, 112.5, 119.5, 124.7, 126.3, 128.2, 129.5, 129.8, 132.6, 136.9, 144.5, 151.3, 156.3, 157.4; MS (ESI): 262 [M+H]<sup>+</sup>.

### 3.6. 6-Methoxy-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9b)

Compound **9b** was synthesized using the same method described for the compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-5-methoxyphenol hydrochloride (**8b**) (465 mg, 2.65 mmol), and the crude product was subjected to column chromatography with 3:7 v/v ethyl acetate/hexane to purify and then afforded pure compound **9b**, with 492 mg in 64% yield. Mp: 259-261°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.86 (s, 3H), 6.78 (s, 1H), 7.04 (s, 1H), 7.17 (d, 1H, *J* = 8.23 Hz), 7.36 (d, 2H, *J* = 7.90 Hz), 7.47-7.50 (m, 3H), 7.54 (d, 1H, *J* = 8.23 Hz), 9.35 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 57.3, 96.2, 112.4, 112.8, 121.7, 128.5, 129.2, 130.4, 132.4, 136.7, 138.2, 154.6, 155.4, 156.5, 157.5; MS (ESI): 292 [M+H]<sup>+</sup>.

### 3.7. 5-Methoxy-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9c)

Compound **9c** was synthesized using the same method described for compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4-methoxyphenol (**8c**) (369 mg, 2.65 mmol), and the crude product was subjected to column chromatography with 1:1 v/v ethyl acetate/hexane to purify and then afforded pure compound **9c**, with 476 mg in 62% yield. Mp: 260-262°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.85 (s, 3H), 6.82-6.87 (m, 2H), 7.07 (s, 1H), 7.14-7.16 (m, 1H), 7.36 (d, 2H, *J* = 8.12 Hz), 7.46-7.50 (m, 3H), 9.35 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 57.4, 104.3, 106.5, 109.3, 112.6, 128.4, 128.7, 130.5, 132.6, 136.4, 146.3, 146.9, 155.6, 156.8, 157.6; MS (ESI): 292 [M+H]<sup>+</sup>.

### 3.8. 5-Bromo-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9d)

Compound **9d** was synthesized using the same method described for compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4-bromophenol (**8d**) (498 mg, 2.65 mmol), and the crude product was subjected to column chromatography with 1:1 v/v ethyl acetate/hexane to purify and then afforded pure compound **9d**, with 512 mg in 57% yield. Mp: 265-267°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.08-7.15 (m, 2H), 7.37 (d, 2H, *J* = 8.10 Hz), 7.47-7.51 (m, 3H), 7.57-7.59 (q, 1H), 7.83 (d, 1H, *J* = 8.10 Hz), 9.34 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CD-

Cl<sub>3</sub>):  $\delta$  110.6, 112.8, 113.8, 128.5, 128.7, 128.9, 129.7, 130.5, 132.2, 136.8, 144.8, 146.7, 156.5, 157.8; MS (ESI): 341 [M+H]<sup>+</sup>.

### 3.9. 6-Nitro-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9e)

Compound **9e** was synthesized using the same method described for compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-5-nitrophenol (**8e**) (408 mg, 2.65 mmol), and the crude product was allowed for column chromatography with 4:6 v/v ethyl acetate/hexane to purify and then afforded pure compound **9e**, with 561 mg in 69% yield. Mp: 274-276°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.07 (s, 1H), 7.38 (d, 2H, *J* = 8.20 Hz), 7.48-7.53 (m, 3H), 7.56 (d, 1H, *J* = 8.11 Hz), 7.63 (d, 1H, *J* = 8.11 Hz), 7.78 (s, 1H), 9.38 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  97.8, 112.6, 120.6, 121.8, 128.5, 128.9, 132.7, 136.8, 142.6, 149.7, 152.4, 156.8, 157.6; MS (ESI): 307 [M+H]<sup>+</sup>.

### 3.10. 5-Chloro-6-nitro-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9f)

Compound **9f** was synthesized using the same method described for the compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4-chloro-5-nitrophenol (**8f**) (500 mg, 2.65 mmol), and the crude product was allowed for column chromatography with 4:6 v/v ethyl acetate/hexane to purify and then afforded pure compound **9f**, with 562 mg in 50% yield. Mp: 285-287°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.09 (s, 1H), 7.39 (d, 2H, *J* = 8.20 Hz), 7.49-7.54 (m, 3H), 7.69 (s, 1H), 7.83 (s, 1H), 9.39 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  106.4, 112.8, 122.8, 128.7, 128.4, 130.5, 132.4, 132.8, 136.7, 143.8, 147.6, 149.8, 156.8, 157.9; MS (ESI): 341 [M+H]<sup>+</sup>.

### 3.11. 5,7-dinitro-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9g)

Compound **9g** was synthesized using the same method described for the compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4,6-dinitrophenol (**8g**) (528 mg, 2.65 mmol), and the crude product was allowed for column chromatography with 4:6 v/v ethyl acetate/hexane to purify and then afforded pure compound **9g**, with 618 mg in 66% yield. Mp: 293-295°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.12 (s, 1H), 7.40 (d, 2H, *J* = 8.20 Hz), 7.49-7.55 (m, 3H), 8.87 (s, 1H), 8.96 (s, 1H), 9.40 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  112.9, 114.6, 119.6, 128.7, 128.9, 130.8, 132.5, 136.5, 136.8, 140.6, 145.6, 145.9, 156.8, 157.7; MS (ESI): 352 [M+H]<sup>+</sup>.

### 3.12. 5-Chloro-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9h)

The compound **9h** was synthesized using the same method described for compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4-chlorophenol (**8h**) (380 mg, 2.65 mmol), and the crude product was allowed for column chromatography with 4:6 v/v ethyl acetate/hexane to purify and then afforded pure compound **9h**, with 641 mg in 82% yield.

Mp: 287-289°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.05 (s, 1H), 7.36-7.40 (m, 3H), 7.47-7.51 (m, 4H), 7.66 (d, 1H, *J* = 8.0 Hz), 9.37 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  110.4, 112.8, 119.6, 125.4, 128.6, 128.9, 129.2, 130.7, 132.5, 136.8, 141.6, 148.5, 156.7, 157.8; MS (ESI): 296 [M+H]<sup>+</sup>.

### 3.13. 5-Methyl-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9i)

Compound **9i** was synthesized using the same method described for the compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4-methylphenol (**8i**) (326 mg, 2.65 mmol), and the crude product was allowed for column chromatography with 3:7 v/v ethyl acetate/hexane to purify and then afforded pure compound **9i**, with 614 mg in 84% yield. Mp: 264-266°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.33 (s, 3H), 7.04 (s, 1H), 7.12-7.18 (m, 2H), 7.36 (d, 2H, *J* = 8.03 Hz), 7.46-7.50 (m, 3H), 7.64-7.67 (m, 1H), 9.34 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  22.4, 109.6, 112.6, 122.7, 128.6, 128.9, 129.2, 130.5, 132.4, 132.8, 136.7, 143.6, 149.6, 156.5, 157.6; MS (ESI): 276 [M+H]<sup>+</sup>.

### 3.14. 5,6-Dimethyl-2-(3-phenyl-1H-pyrazol-5-yl)benzo[d]oxazole (9j)

Compound **9j** was synthesized using the same method described for the compound **9a** synthesis, employing 3-phenyl-1H-pyrazole-5-carboxylic acid (**7**) (500 mg, 2.65 mmol) and 2-amino-4,5-dimethylphenol (**8j**) (364 mg, 2.65 mmol), and the crude product was allowed for column chromatography with 3:7 v/v ethyl acetate/hexane to purify and then afforded pure compound **9j**, with 620 mg in 81% yield. Mp: 277-279°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.29 (s, 3H), 2.32 (s, 3H), 6.68 (s, 1H), 7.03 (s, 1H), 7.35 (d, 2H, *J* = 7.9 Hz), 7.44-7.49 (m, 3H), 9.35 (brs, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  22.5, 22.7, 110.5, 112.7, 125.8, 128.6, 128.9, 130.6, 132.5, 134.2, 136.7, 140.4, 142.5, 148.5, 156.5, 157.7; MS (ESI): 290 [M+H]<sup>+</sup>.

### 3.15. Procedure of the SRB-assay

All the synthesized derivatives (**9a-j**) were screened for their *in vitro* anticancer activity against specified human cancer cell lines. In this protocol, drug exposure takes over a continuous period of 48 hours and the estimation of cell growth or cell viability was done by a sulforhodamine B (SRB) protein assay. The cancer cell lines were grown in 2 mM L-glutamine and 10% fetal bovine serum *i.e.*, DMEM medium. These were inoculated into 96 well microtiter plates. These microtiter plates undergo incubation at 37 °C, 100% relative humidity, 5% CO<sub>2</sub> and 95% air over a period of 24 hours prior to the mixing of different experimental drugs. 10 mL of aliquots of diluted drug solutions was mixed to the particular microtiter wells, which already contain 90 mL of cells giving the final drug concentrations (0.1  $\mu$ M, 1  $\mu$ M, 10  $\mu$ M and 100  $\mu$ M). Each compound with four different concentrations was evaluated and each test was done in triplicate. The plates were incubated over a period of 48 hours and were removed by adding 50 mL of cold CCl<sub>3</sub>COOH (TCA) (10% TCA, final concentration) and incubated at 4 °C over 60 minutes. These plates were cleaned with tap water for five times and dried by using air. 50 mL of

SRB solution (at 0.4% w/v in 1% AcOH) was added to each cell and the plates undergo incubation over 20 minutes time period at room temperature. The residual dye was eliminated by washing with 1% AcOH over five times and the plates were dried by air. The bound stain was eluted with the help of 10 mM trizma base, and its absorbance was recorded at 540 nm wavelength on ELISA plate reader with a reference of 690 nm wavelengths.

The percent growth was determined on a plate through plate basis to test the relative wells with respect to control wells and these calculations were repeated for three times. The percentage growth was expressed by using the below formula.

$$\text{Percentage Growth} = \frac{\text{Average absorbance of the test well}}{\text{Average absorbance of the control wells}} * 100$$

The growth inhibition of 50% (GI<sub>50</sub>) was calculated by using the below formula,

$$[(Ti-Tz)/(C-Tz)] * 100 = 50$$

Where, Tz = Optical density at time zero,

C = OD of control

and Ti = OD of test growth in the presence of drug

### 3.16. Molecular Docking Protocol

The X-ray crystallographic structure of Src tyrosine kinase protein was obtained from the protein data bank (PDB: 2BDF) [48]. The chain A was chosen for current docking experiment among the homodimers. The protein was prepared and energy was minimized by protein preparation wizard. The receptor grid around the co-crystal ligand was generated by Glide. All the ligands were sketched using Maestro 11.0 and the ligands were prepared by ligand preparation. The extra precision docking mode of Glide was employed for current docking methods [49]. All the results were carefully analyzed.

### CONCLUSION

In summary, we have designed and synthesized a new series pyrazole core structures conjugated with benzoxazole moieties (9a-j) and evaluated their *in vitro* anticancer activity against a panel of four cancer cell lines such as MCF-7, KB, Hop62 and A549. Among them, compounds 9b and 9c exhibited excellent anticancer activities with GI<sub>50</sub> values of <0.1 μM against MCF-7 and A549 cell lines. These two compounds are comparably potent to the standard drug doxorubicin and may act as drug lead compounds in cancer chemotherapy. The molecular docking studies proved these compounds to be able to interfere with the role of ATP-Src tyrosine kinase protein. The potent molecules of the present study may be further studied for clinical trials.

### CONSENT FOR PUBLICATION

Not applicable.

### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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