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Synthesis of novel pyrazole – 1,2,3-triazole hybrids as anticancer agents

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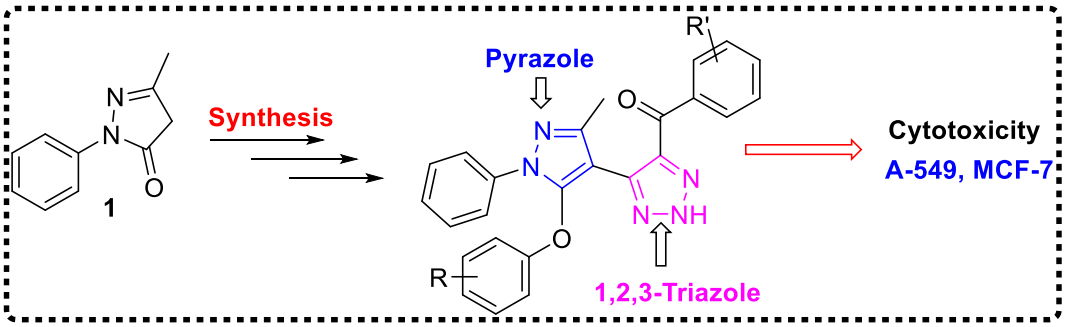
Abstract

A series of pyrazole – 1,2,3-triazole hybrids were synthesized using a metal-free catalyst in a multicomponent reaction. The new compounds were screened for its *in vitro* anticancer activity against A-549 and MCF-7 cell lines. The compound featuring methyl and iodo substitutions demonstrated remarkable activity, exhibiting IC_{50} values of 9.25±0.2 µM and 9.56±0.2 µM against A-549 and MCF-7 cells, respectively. In a similar manner, the compound featuring methoxy and bromo substitutions demonstrated enhanced activity, measuring 11.42±0.2 µM and 10.95±0.2 µM. The compound featuring bromo and iodo substituents exhibited the highest activity, with IC_{50} values of 13.40±0.3 µM and 12.42±0.2 µM. Evaluated their toxicity on normal human embryonic kidney cells (Hek-293) and observed no morphological changes in the cells. Additionally, a molecular docking study was conducted against the proto-oncogene c-Src, revealing a highly potent ligand that demonstrated a remarkable docking score and significant binding interactions.



Keywords: Anticancer, molecular docking, multicomponent reaction, pyrazole, 1,2,3-triazole

Introduction

Pyrazoles are significant constituents of heterocyclic compounds characterized by the adjacency of two nitrogen atoms within a five-membered ring structure.[1] The nature of the two nitrogen atoms is such that one of them is alkaline, while the other is neutral. The flat, conjugated ring topologies of these compounds, which include six delocalized π-electrons, give them aromatic characteristics. The biological activity of pyrazoles is broad and can be found in a number of specialized domains, including but not limited to antibacterial,[2] anticancer,[3] antioxidant,[4] antifungal,[5] anti-HIV,[6] antidiabetic,[7] antiviral[8] and anti-inflammatory[9] properties. A number of medications that include a pyrazole core have been documented. These medications include the pain reliever Antipyrine, the prescription for arthritis known as Phenylbutazone, and a number of non-steroidal anti-inflammatory drugs (NSAIDs) such as Rimonabant,[10] Lonazolac,[11] and Ramifenazone.[12] (Figure 1) In addition, medications such as Axitinib is used for treatment of advanced renal cell carcinoma,[13] Zaleplon is used as sleeping-pill to remedy insomnia,[14] Reversan is extensively used in the treatment of colon cancer, neuroblastoma and renal cell carcinoma,[15] Sildenafil alleviates erectile dysfunction by enhancing blood circulation to the penis during sexual arousal and Tracazolate is a pharmaceutical agent utilized in scientific research, exhibiting anxiolytic and anticonvulsant properties[16] are exhibits of fused pyrazole systems.

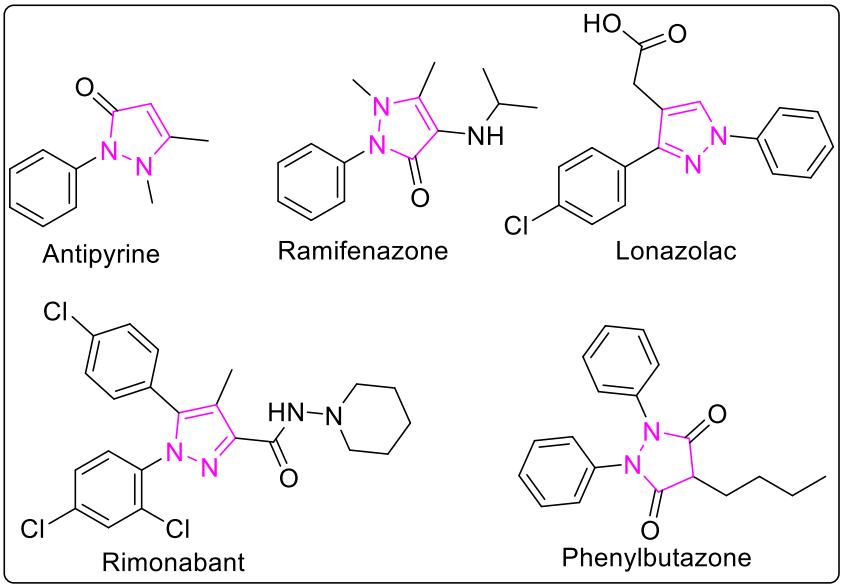


Figure 1. Pyrazole based therapeutic drugs.

It has been discovered that the 1,2,3-triazole moiety possesses a wide variety of biological activities, including antibacterial,[17] antifungal,[18] anti-vitiligo,[19] antiplatelet,[20] antiviral,[21] anti-HIV,[22] antiallergic,[23] anticancer,[24] antitubercular,[25] anticoccidiostats,[26] antioxidant,[27] chemosensors,[28] antivenom,[29] antimalarial,[30] anti-inflammatory,[31] peptidomimetic[32], and versatile scaffolding properties. In addition, several drugs that contain 1,2,3-triazole scaffolds are now being utilized in clinical settings. These treatments include Cefatrizine,[33] which is an antibiotic; Carboxyamidotriazole,[34] which is an anti-cancer agent; TSAO,[35] which is an anti-HIV agent; and Tazobactum,[36] which is an anti-bacterial agent. Synthesis of 1,2,3-triazole is versatile and many reports are available for their green synthesis. A recent sustainable method for synthesizing 1,2,3-triazole has been reported, employing ultrasound-assisted deep eutectic solvent (DES) as a biodegradable medium, yielding a

highly efficient and eco-friendly process with elevated yields.[37,38] Yet in present work a sustainable synthetic procedure employed a multicomponent reaction using a metal-free catalyst.

One method of combining two or more bioactive fragments into a single molecule is referred to as molecular hybridization. This method involves the utilization of the appropriate linkers.[39] It is always the case that these new hybrids are endowed with greater activity or unusual biological traits in comparison to their individual components.[40] The molecular hybridization of the pyrazole ring with 1,2,3-triazole nuclei has been extensively studied in the literature. These hybrids possess extensive medicinal properties including antimicrobial, anti-inflammatory, anticancer, antimycobacterial,[39] antioxidant, antitubercular etc. activities.[41–43] On the other hand, cancer has been a devastating health problem that has been responsible for the deaths of individuals of all ages and from all over the world. In the year 2020, it was responsible for ten million deaths across the globe, and by the year 2030, it is anticipated that this number will rise to thirteen million.[44] In spite of the fact that there are a number of therapies and chemotherapeutics available, it is still a disorder that offers a significant risk to one's life, and it is imperative that research into new anticancer drugs be continued.[45] Hence, inspired by medicinal importance of pyrazole – 1,2,3-Triazole hybrids and dire need for development of new anticancer agents, aimed to design and synthesize novel pyrazole based 1,2,3-triazole scaffolds (Figure 2), evaluated their invitro anticancer activities and performed molecular docking studies.

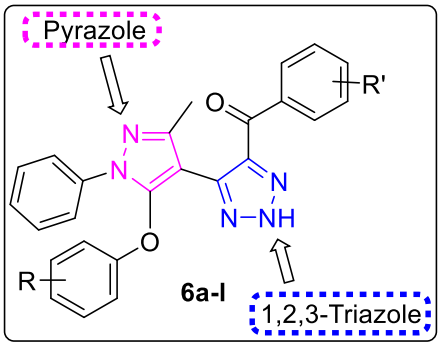


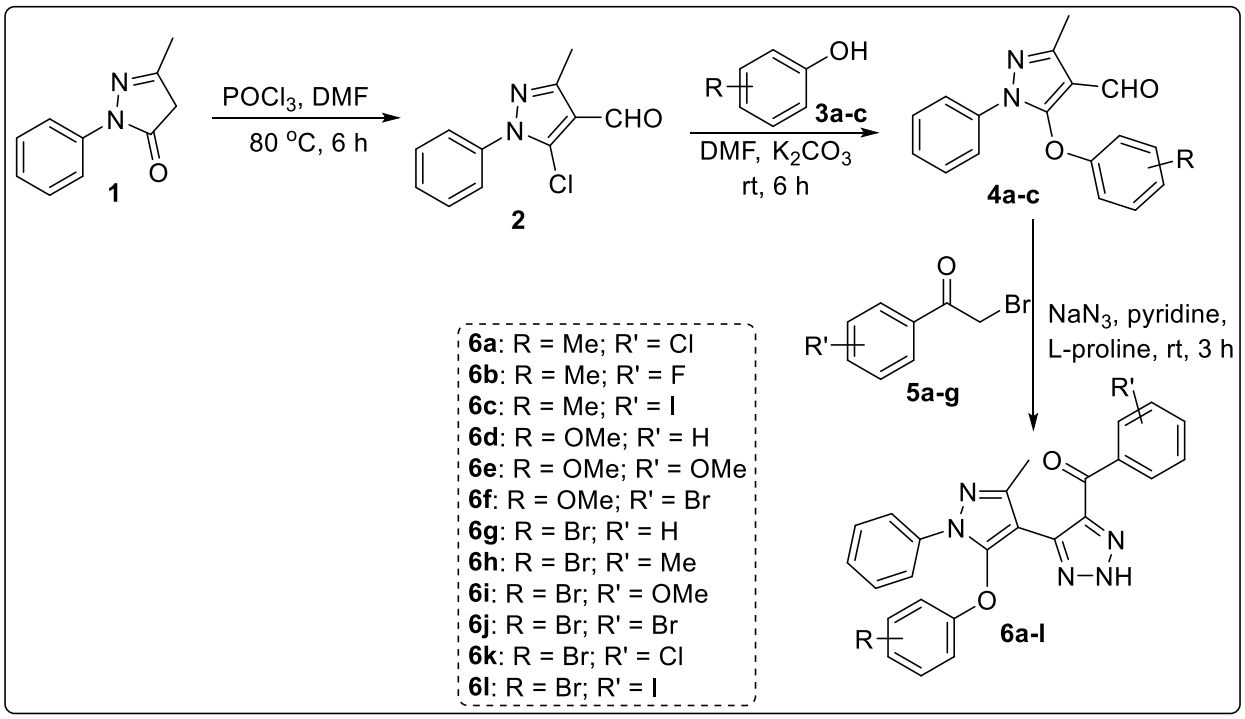
Figure 2. Design rationale of pyrazole – 1,2,3-triazole hybrids.

Results and Discussion

Chemistry

Synthesis of library of new pyrazole based 1,2,3-triazole hybrids achieved as depicted in Scheme 1. 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1** precursor was treated via Vilsmeier-Haack reaction using $POCl_3$ in DMF which introduced -Cl in C5 position and formyl group in C4 to obtain 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **2**.[46] The compound **2** was allowed to react with substituted phenols **3a-c** individually under alkaline conditions using K_2CO_3 to obtain 3-methyl-5-phenoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde intermediates **4a-c**.[47] The metal-free one pot reaction between the individual mixtures of compounds **4a-c**, NaN_3 and various substituted phenacyl bromides **5a-c**, using pyridine yielded the title compounds viz. (5-(3-methyl-5-phenoxy-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(phenyl)methanone **6a-l** derivatives.[48] The characterization of the new title compound was characterized by analysis of ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectral data. For illustration of compound **6a**, in ^{1}H NMR spectrum, The protons of two methyl groups on the pyrazole and phenoxy rings characterized as two singlets at δ 2.23 ppm (3H) and δ 2.08 ppm (3H).

Additionally, there were 14 protons in the aromatic region, integrated in the downfield range of δ 7.90 ppm to δ 6.57 ppm. Among these, the aromatic protons of the p-tolyl group appeared as two sets of doublets at δ 6.85 ppm (2H, J 8.4 Hz) and δ 6.58 ppm (2H, J 8.4 Hz). In the ^{13}C NMR spectrum, the C=O carbon signal appeared at δ 187.0 ppm, the signals of two methyl groups carbons appeared at δ 20.4 ppm and δ 13.8 ppm, there were 17 sets of aromatic carbons appeared ranging from δ 155.2 ppm to δ 115.7 ppm. The *m/z* 470.13 $[M+H]^+$ peak obtained in mass spectrum of compound **6a**.



Scheme 1. Synthesis of pyrazole – 1,2,3-triazole hybrid derivatives.

Anticancer activity

The target compounds **6a-l** were screened for their invitro anticancer activity against human lung cancer (A-549) and human breast cancer (MCF-7) cell lines and presented the IC_{50} values in Table 1. The widely used anticancer drug, *Doxorubicin*, was utilized as the standard reference. All compounds shown activity, particularly compounds **6c**, **6f**, and **6l**, which exhibited significant efficacy against both cell lines in contrast to *Doxorubicin*.

Table 1. IC_{50} value of compounds **6a-l** against A-549, MCF-7 and Hek-293 cell lines

Entry	R	R'	IC_{50} ± Standard Deviation (μM)		
			A-549	MCF-7	Hek-293
6a	Me	Cl	20.58±0.2	19.87±0.2	97.43±1.4
6b	Me	F	24.71±0.3	30.57±0.5	89.53±1.2
6c	Me	I	9.25±0.2	9.56±0.2	>100
6d	OMe	H	46.19±0.4	52.32±0.6	98.22±1.2
6e	OMe	OMe	48.23±0.5	49.37±0.6	93.14±1.0
6f	OMe	Br	11.42±0.2	10.95±0.2	87.89±1.2
6g	Br	H	36.29±0.4	42.33±0.2	88.44±1.1
6h	Br	Me	46.44±0.5	47.39±0.5	>100
6i	Br	OMe	56.11±0.5	57.74±0.7	>100
6j	Br	Br	68.76±0.4	57.27±0.3	94.43±1.2
6k	Br	Cl	42.22±0.5	38.14±0.4	89.12±1.1
6l	Br	I	13.40±0.3	12.42±0.2	91.12±1.1
Doxorubicin	-	-	10.02±0.3	9.74±0.3	97.75±1.2

Conducted a structure-activity relationship (SAR) analysis to elucidate the importance of substituents on molecules. Compound **6c** featuring an electron-donating group in R (-CH_3) position and Iodine in R' position demonstrated outstanding activity with IC_{50} value of 9.25±0.22 μM and 9.56±0.19 μM against A-549 and MCF-7 cells respectively. Replacing R with -OCH_3 and R' with -Br functions in compound **6f** showed an infinitesimally lessened activity with IC_{50} value of 11.42±0.25 μM (A-549) and 10.95±0.22 μM (MCF-7). Presence of R = Br and R' = I bulky functions lead to small decrease in activity with IC_{50} value **of 13.40±0.29 μM (**A-549) and 12.42±0.25 μM (MCF-7). Moderate activity obtained from **6a** and **6b**, which possess methyl, chloro, and fluoro functionalities. The actions of the other substituents were found to be inadequate. The evaluated compounds exhibited no substantial toxicity towards normal human embryonic kidney (HEK-293) cells.

Molecular docking against proto-oncogene c-Src (Src)

The proto-oncogene c-Src (Src) is a nonreceptor tyrosine kinase whose expression and activity correlate with advanced malignancy and worse prognosis in several human malignancies.[49] Src has been significantly associated with the genesis, maintenance, progression, and metastatic dissemination of various human malignancies, including prostate, lung, breast, and colorectal cancers. It is the most targeted kinase by oncological pharmaceuticals. To assess the binding efficacy of the dominant ligand **6c**, molecular docking studies were conducted using the crystal structure of Src (PDB ID: 4U5J).[50] Validated the docking protocol by re-docking the co-crystalized ligand Ruxolitinib which presented an RMSD of 1.12 Å, and docking score of -8.4 kcal/mol.

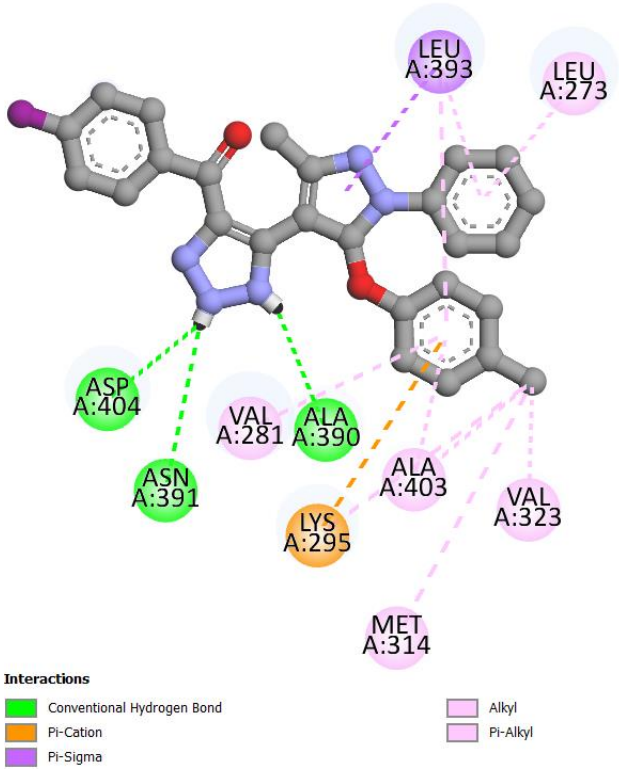


Figure 3. Binding interactions of ligand **6c** against Src.

The ligand **6c** scored a binding affinity value of -9.4 kcal/mol more noteworthy than Ruxolitinib score. The nitrogen atoms of triazole ring of ligand **6c** involved in three H-bond interactions with amino acid sites Ala390, Asn391 and Asp404 of Src, the bond distance of Ala390 found to be moderate with 2.97 Å, and bond distance of Asn391 and Asp404 were 2.49 Å and 2.15 Å respectively indicating strong interactions. The hydrophobic interactions were displayed against Leu273, Val281, Lys295, Met314, Val323, Leu393 and Ala403 of Src, among which a π-cation interaction was shown against Lys295 (**Fig 3**). The reference ligand Ruxotilinib showed a H-bond interaction with Met341 and hydrophobic interactions with Leu273, Val281, Lys295, Leu393 and Ala403 of Src (**Fig 4**). In comparison the hydrophobic interactions of ligand **6c** were coinciding with the reference ligand Ruxolitinib, evidenced the binding efficiency. The dock pose image (**Fig 5**) of ligand **6c** and Ruxolitinib demonstrated optimal fitting within the Src cavity.

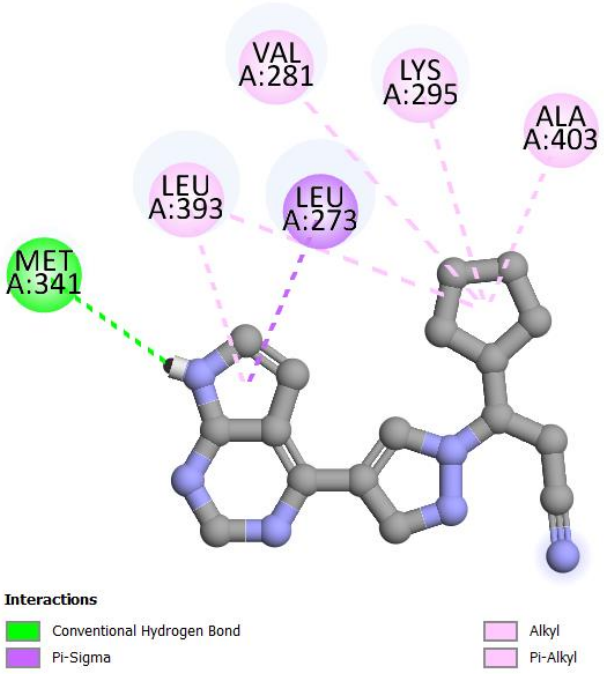


Figure 4. Binding interactions of Ruxolitinib against Src.

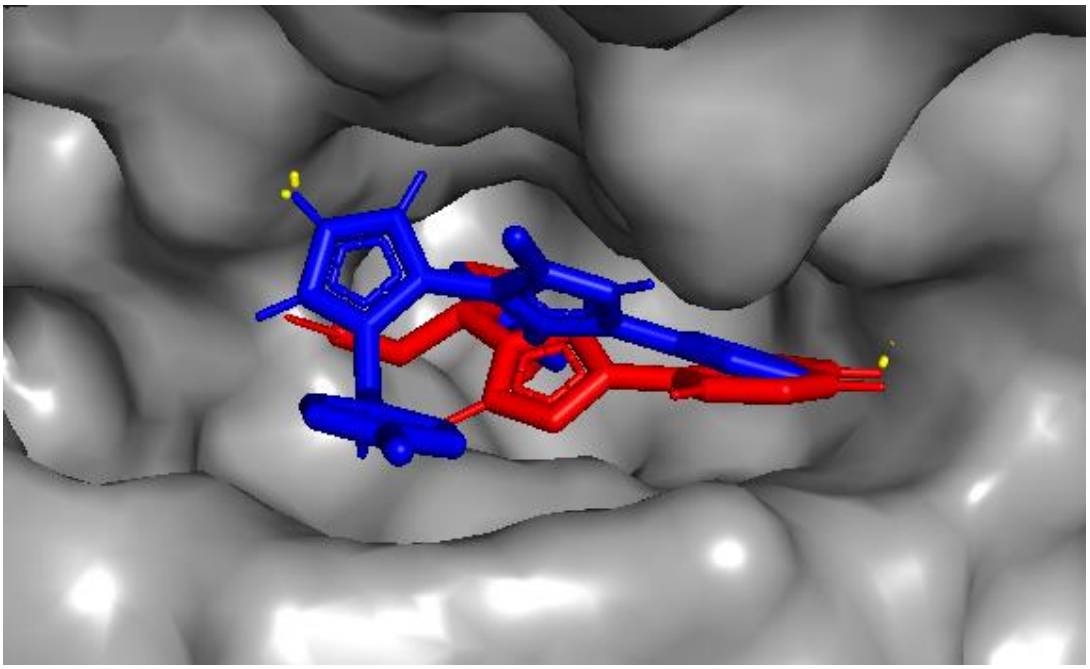


Figure 5. Dock pose of ligand **6c** (blue) and Ruxolitinib (red) in cavity of Src.

Conclusions

A library of pyrazole – 1,2,3-triazole scaffolds synthesized by utilizing a metal-free catalyst multicomponent reaction between aldehyde, phenacyl bromides and sodium azide. Confirmed their structure by interpretation of ^{1}H NMR, ^{13}C NMR and Mass spectral data. Screened for their invitro anticancer activity against A-549 and

MCF-7 cell lines. The compounds **6c** showed outstanding activity with IC_{50} value of 9.25±0.22 µM and 9.56±0.19 µM against A-549 and MCF-7 cells respectively. Similarly, compound **6f** superior activity with 11.42±0.25 µM and 10.95±0.22 µM. Another compound **6l** presented best activity with IC_{50} value of 13.40±0.29 µM and 12.42±0.25 µM. To check the binding efficacy, we performed molecular docking study against proto-oncogene c-Src with potent ligand **6c**, produced noteworthy docking score and important binding interactions. Hence, the newly synthesized pyrazole – 1,2,3-triazole hybrids could be investigate further in development of anticancer agent.

Experimental Section

General. All chemicals and reagents were procured from commercial sources (Spectrochem, TCI and Avra). They were utilized without further purification. Reactions were monitored by Thin Layer Chromatography (TLC), performed on silica gel glass plates containing 60 F-254. Visualization on TLC was achieved either by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER NMR instrument operating at 100/75 MHz or 400/300 MHz. Chemical shifts (δ) are reported in parts per million (ppm) downfield from internal standards. Electrospray Ionization (ESI) spectra were recorded on a Micro mass QuattroLC instrument using ESI+ software. The capillary voltage was set at 3.98 kV, and ESI mode positive ion trap detector was employed. Melting points were determined using an electro-thermal melting point apparatus.

Procedure for the synthesis of 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (2). To a solution of 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one **1** (1 g, 0.011 mol) in DMF (50 mL) added $POCl_3$ (0.011 mol, 1 eq.) and stirred the solution for 6 h by maintaining 80 °C. After completion reaction mixture was poured on crushed ice and collected the obtained solid by filtration. Yield 73%.

General procedure for the synthesis of 3-methyl-5-phenoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde (4a-c) intermediates. To the individual solutions of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **2** (1g, 0.004 mol) in DMF (25 mL), added individual substituted phenols **3a-c** (0.004 mol) and K_2CO_3 (0.5 g, 0.004 mol), and stirred the reaction mixture for 6h at room temperature, after completion, the reaction mass was poured onto ice cold water and quenched wit dil. HCl (10 mL), obtained solid was collected by filtration and obtained in good yields.

General procedure for the synthesis of (5-(3-methyl-5-phenoxy-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(phenyl)methanone (6a-l). To the solution of individual substituted phenacyl bromides **5a-g** (0.001 mol) in DMF (25 mL), added individual 3-methyl-5-phenoxy-1-phenyl-1H-pyrazole-4-carbaldehyde **(4a-c)** intermediates (0.001 mol), NaN_3 (0.65 g, 0.001 mol), L-proline (0.001 mol). The mixture was stirred at an ambient temperature for 3 h. Upon full conversion, the mixture was transferred into ice water and extracted with ethyl acetate (20 mL x 4). The amalgamated organic layers were desiccated using Na_2SO_4 and subsequently concentrated at reduced pressure. The residue was subjected to chromatography on silica gel using an ethyl acetate/petroleum ether eluent, resulting in the target compounds being obtained in substantial yields.

(4-Chlorophenyl)(5-(3-methyl-1-phenyl-5-(*p*-tolyloxy)-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)methanone (6a). Off-white solid. Yield: 77%. mp: 178 – 180 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 7.89 (d, 7.2 Hz, 1H), 7.68 – 7.43 (m, 8H), 7.35 – 7.30 (m, 1H), 6.85 (d, *J* 8.4 Hz, 2H), 6.58 (d, *J* 8.4 Hz, 2H), 2.26 (s, 3H), 2.08 (s, 3H) ppm. ^{13}C NMR (75 MHz, d_6-DMSO) δ 187.1, 155.2, 153.4, 148.3, 147.0, 142.5, 137.8, 137.2, 133.7, 133.3, 130.4, 130.3, 129.8, 128.7, 127.7, 122.3, 121.3, 115.8, 20.4, 13.8 ppm. ESI-MS: *m/z* 470.13 $[M+H]^+$. Elemental analysis calc for $C_{26}H_{20}ClN_5O_2$: C, 66.45; H, 4.29; N, 14.90%. Found: C, 66.40; H, 4.25; N, 14.84%.

(4-Fluorophenyl)(5-(3-methyl-1-phenyl-5-(*p*-tolyloxy)-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)methanone (6b). Off-white solid. Yield: 75%. mp: 176 – 178 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 8.01 – 7.98 (m, 2H), 7.58 (d, *J* 7.6 Hz, 2H), 7.47 – 7.43 (m, 2H), 7.37 – 7.30 (m, 4H), 6.83 (d, J 8.4 Hz, 2H), 6.57 (d, *J* 8.4 Hz, 2H), 2.22 (s, 3H), 2.06 (s, 3H) ppm. ^{13}C NMR (75 MHz, d_6-DMSO) δ 185.4, 166.8, 164.2, 153.3, 148.4, 147.1, 137.7, 133.8, 133.3, 133.2, 130.4, 129.8, 127.7, 122.4, 116.7, 115.9, 115.8, 115.7, 20.4, 13.7 ppm. ESI-MS: *m/z* 454.16 $[M+H]^+$. Elemental analysis calc for $C_{26}H_{20}FN_5O_2$: C, 68.86; H, 4.45; N, 15.44%. Found: C, 68.80; H, 4.40; N, 15.41%.

(4-Iodophenyl)(5-(3-methyl-1-phenyl-5-(*p*-tolyloxy)-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)methanone (6c). Off-white solid. Yield: 80%. mp: 183 – 185 °C. ^{1}H NMR (300 MHz, d_6-DMSO): δ 7.93 (d, J 8.4 Hz, 2H), 7.67 – 7.57 (m, 5H), 7.49 – 7.44 (m, 2H), 7.35 – 7.31 (m, 1H), 6.84 (d, *J* 8.4 Hz, 2H), 6.58 (d, *J* 8.4 Hz, 2H), 2.22 (s, 3H), 2.06 (s, 3H) ppm. ^{13}C NMR (75 MHz, d_6-DMSO) δ 186.2, 153.4, 148.3, 147.1, 142.5, 142.3, 137.7, 136.4, 133.7, 133.3, 131.9, 130.4, 129.8, 127.7, 125.0, 122.4, 115.8, 102.4, 20.4, 13.8 ppm. ESI-MS: *m/z* 562.07 $[M+H]^+$. Elemental analysis calc for $C_{26}H_{20}IN_5O_2$: C, 55.63; H, 3.59; N, 12.48%. Found: C, 55.59; H, 3.55; N, 12.44%.

(5-(5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(phenyl)methanone (6d). Off-white solid. Yield: 74%. mp: 192 – 194 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.92 (d, *J* 7.2 Hz, 2H), 7.68 – 7.60 (m, 3H), 7.54 – 7.45 (m, 5H), 6.67 – 6.58 (m, 4H), 3.56 (s, 3H), 2.21 (s, 3H) ppm. ^{13}C NMR (101 MHz, d_6-DMSO) δ 187.0, 155.8, 149.7, 148.4, 147.7, 142.6, 137.9, 137.3, 136.2, 133.6, 130.3, 129.8, 128.7, 127.6, 122.4, 117.5, 117.4, 114.9, 55.7, 13.8 ppm. ESI-MS: *m/z* 452.17 $[M+H]^+$. Elemental analysis calc for $C_{26}H_{21}IN_5O_3$: C, 69.17; H, 4.69; N, 15.51%. Found: C, 69.14; H, 4.65; N, 15.48%.

(5-(5-(4-Methoxyphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(4-methoxyphenyl)methanone (6e). Off-white solid. Yield: 72%. mp: 197 – 199 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.96 (d, *J* 6.8 Hz, 2H), 7.61 (d, J 7.2 Hz, 2H), 7.49 – 7.45 (m, 2H), 7.35 – 7.31 (m, 1H), 3.86 (s, 3H), 3.56 (s, 3H), 2.20 (s, 3H) ppm. ^{13}C NMR (101 MHz, d_6-DMSO) δ 185.3, 163.80 155.7, 149.2, 148.3, 147.6, 143.2, 137.9, 132.8, 129.9, 129.8, 127.6, 122.6, 122.4, 117.3, 114.9, 114.6, 114.1, 56.0, 55.7, 13.8 ppm. ESI-MS: *m/z* 482.12 $[M+H]^+$. Elemental analysis calc for $C_{27}H_{23}N_5O_4$: C, 67.35; H, 4.81; N, 14.54%. Found: C, 67.30; H, 4.77; N, 14.50%.

(4-Bromophenyl)(5-(5-(4-methoxyphenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)methanone (6f). Off-white solid. Yield: 75%. mp: 201 – 203 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.97 – 7.91 (m, 3H), 7.58 (s, 2H), 7.41 – 7.37 (m, 2H), 7.31 – 7.26 (m, 1H), 7.13 – 7.09 (m, 2H), 6.97 – 6.94 (m, 2H), 6.65 – 6.62 (m, 2H), 3.89 (s, 3H), 2.30 (s, 3H) ppm. ^{13}C NMR (101 MHz, d_6-DMSO) δ 185.8, 155.8, 149.0, 148.4, 146.4, 142.0, 137.8, 136.2, 132.2, 131.9, 129.8, 127.8, 127.7, 122.5, 117.6, 117.4, 117.3, 114.9, 55.7, 13.7 ppm. ESI-MS: *m/z* 530.28 $[M+H]^{2+}$. Elemental analysis calc for $C_{26}H_{20}BrN_5O_3$: C, 58.88; H, 3.80; N, 13.20%. Found: C, 58.83; H, 3.75; N, 13.15%.

(5-(5-(4-Bromophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(phenyl)methanone (6g). Off-white solid. Yield: 79%. mp: 201 – 203 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.95 (d, J 6.8 Hz, 2H), 7.70 – 7.69 (m, 1H), 7.62 – 7.58 (m, 3H), 7.49 – 7.45 (m, 2H), 7.41 – 7.37 (m, 2H), 7.30 – 7.26 (m, 1H), 7.10 (d, *J* 8.4 Hz, 2H), 6.63 (d, *J* 8.4 Hz, 2H), 2.32 (s, 3H) ppm. ^{13}C NMR (101 MHz, d_6-DMSO) δ 191.9, 159.3, 153.4, 144.2, 142.3, 141.7, 141.5, 139.6, 137.9, 137.1, 135.9, 134.9, 133.9, 133.1, 132.9, 131.9, 127.0, 122.4, 18.5 ppm. ESI-MS: *m/z* 501.06 $[M+2H]^+$. Elemental analysis calc for $C_{25}H_{18}BrN_5O_2$: C, 60.01; H, 3.63; N, 14.00%. Found: C, 59.95; H, 3.59; N, 13.96%.

(5-(5-(4-Bromophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(*p*-tolyl)methanone (6h). Off-white solid. Yield: 75%. mp: 206 – 208 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.82 (d, *J* 8.0 Hz, 2H), 7.56 (d, *J* 7.6 Hz, 2H), 7.48 – 7.44 (m, 2H), 7.35 – 7.32 (m, 4H), 7.24 (d, *J* 8.8 Hz, 2H), 6.69 (d, *J* 8.8 Hz, 2H), 2.39 (s, 3H), 2.22 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO) δ 186.6, 154.7, 148.4, 146.3, 144.3, 142.6, 138.0, 137.6, 134.5, 132.9, 130.4, 129.9, 129.4, 127.9, 122.5, 121.9, 118.3, 116.0, 21.7, 13.8 ppm. ESI-MS: *m/z* 514.38 $[M+H]^{2+}$. Elemental analysis calc for $C_{26}H_{20}BrN_5O_2$: C, 60.01; H, 3.92; N, 13.62%. Found: C, 59.98; H, 3.87; N, 13.57%.

(5-(5-(4-Bromophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(4-methoxyphenyl)methanone (6i). Off-white solid. Yield: 70%. mp: 210 – 212 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.97 – 7.81 (m, 3H), 7.58 (s, 2H), 7.41 – 7.27 (m, 3H), 7.13 – 6.94 (m, 4H), 6.64 (s, 2H), 3.89 (s, 3H), 2.30 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO) δ 185.4, 163.7, 154.56 148.5, 146.0, 137.7, 137.5, 132.6, 132.4, 131.4, 129.7, 129.3, 127.3, 122.2, 117.7, 115.9, 114.4, 113.6, 55.7, 13.8 ppm. ESI-MS: *m/z* 531.07 $[M+2H]^+$. Elemental analysis calc for $C_{26}H_{20}BrN_5O_3$: C, 58.88; H, 3.80; N, 13.20%. Found: C, 58.83; H, 3.75; N, 13.16%.

(5-(5-(4-Bromophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(4-bromophenyl)methanone (6j). Off-white solid. Yield: 82%. mp: 222 – 224 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.85 – 7.25 (m, 12H), 6.71 (s, 2H), 2.24 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO) δ 185.9, 154.7, 151.1, 148.4, 146.5, 138.3, 138.1, 137.6, 136.0, 132.9, 132.2, 131.9, 131.9, 129. 9, 128.0, 122.5, 118.4, 116.1, 13.8 ppm. ESI-MS: *m/z* 578.97 $[M+2H]^+$. Elemental analysis calc for $C_{25}H_{17}Br_2N_5O_2$: C, 51.84; H, 2.96; N, 12.09%. Found: C, 51.79; H, 2.91; N, 12.03%.

(5-(5-(4-Bromophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2H-1,2,3-triazol-4-yl)(4-chlorophenyl)methanone (6k). Off-white solid. Yield: 78%. mp: 205 – 207 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.85 – 7.76 (m, 4H), 7.57 – 7.25 (m, 8H), 6.71 (s, 2H), 2.24 (s, 2H) ppm. ^{13}C NMR (101 MHz, DMSO) δ 185.7, 154.6, 148.5, 146.4, 144.9, 140.6, 138.8, 137.6, 135.7, 132.9, 132.1, 130.0, 129.9, 129.0, 127.9, 122.5, 118.4, 116.1, 13.8 ppm. ESI-MS: *m/z* 535.02 $[M+2H]^+$. Elemental analysis calc for $C_{25}H_{17}BrClN_5O_2$: C, 56.15; H, 3.20; N, 13.10%. Found: C, 56.11; H, 3.15; N, 13.04%.

(5-(5-(4-Bromophenoxy)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2*H*-1,2,3-triazol-4-yl)(4-iodophenyl)methanone (6l). Off-white solid. Yield: 78%. mp: 205 – 207 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.93 (d, *J* 8.4 Hz, 2H), 7.68 – 7.56 (m, 5H), 7.49 – 7.45 (m, 2H), 7.36 – 7.33 (m, 1H), 7.24 (d, J 8.4 Hz, 2H), 6.69 (d, *J* 8.4 Hz, 2H), 2.23 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO) δ 186.3, 162.1, 154.7, 148.4, 146.4, 137.8, 137.6, 136.3, 135.3, 132.9, 131. 9, 131.0, 129.9, 127.9, 122.5, 118.3, 116.1, 102.6, 13.8 ppm. ESI-MS: *m/z* 626.96 $[M+2H]^+$. Elemental analysis calc for $C_{25}H_{17}BrIN_5O_2$: C, 47.95; H, 2.74; N, 11.18%. Found: C, 47.90; H, 2.69; N, 11.14%.

MTT Assay

Cell viability was assessed using the MTT Assay with concentrations ranging from 0.5 to 100 µM of the newly synthesized compounds dissolved in DMSO and tested in triplicate. Cells were trypsinized and subjected to the trypan blue assay to determine the viability of cells in the suspension. Cells were enumerated using a hemocytometer and inoculated at a density of 5.0 x 10^3 cells per well in 100 µl of culture medium in a 96-well plate, followed by overnight incubation at 37°C. Following incubation, removed the old media and introduced 100 µl of fresh media with varying concentrations of the substance into the designated wells of the 96-well plates. After 48 hours, discarded the solution and introduced fresh medium containing MTT solution (0.5 mg/mL) to each well, then incubate the plates at 37°C for 3 hours. Upon completion of the incubation period, precipitates are generated due to the conversion of MTT salt to chromophore formazan crystals by cells possessing metabolically active mitochondria. The optical density of dissolved crystals in DMSO was assessed at 570 nm using a microplate reader. The proportion of growth inhibition was determined using the subsequent formula. The IC_{50} value was determined by using linear regression equation i.e. y = mx+c. Here, y = 50, m and c values were derived from the viability graph.

Molecular Docking Procedure

The crystal structure of proto-oncogene c-Src (PDB ID: 4U5J) obtained from Protein Data Bank, removed water and hetero atoms and added polar hydrogens. Ligands were sketched in ChemDraw Professional 16.0 tool. PyRx supported by Autodock Vina program employed for docking simulations. Results were visualized using Pymol and Biovia Studio.[51]

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Supplementary Material

Copies of all ^{1}H and ^{13}C NMR spectra of all products are available in the supplementary material.

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