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Synthesis and Characterization of Novel Indole Containing 1,2,3-Triazole Linked 1,3,4-Thiadiazole Hybrids: Evaluation of Anticancer Activity and Molecular Docking Studies

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Novel scaffolds containing indole, 1,2,3-triazole linked thiadiazole derivatives (**9a-l**) were synthesized and confirmed with analytical techniques like ^{1}H NMR, ^{13}C NMR and LC-MS. All the compounds were further screened for their anticancer activity against two cancer cell lines A-549 and MCF-7 by making use of MTT assay and doxorubicin as standard drug. Among the compounds **9c**, **9d** and **9e** demonstrated prominent activity against the tested cancer cell lines A-549 and MCF-7 cell lines compared to doxorubicin. Auto dock Vina of PyRx tool was employed in order to perform molecular docking studies on ERK2. Interestingly, the binding energies and interactions acquired from docking results of compounds are in accordance with the investigatory data.

Keywords: Indole, 1,2,3-Triazole, 2-Aminothiadiazole, Anticancer, Molecular docking.

INTRODUCTION

Heterocyclic compounds are exigent sources of bioactive compounds that could be synthesized in the laboratory as well as from the natural sources [1,2]. In particular, nitrogen bearing heterocyclic compounds like triazole and indole have attained considerable interest in previous decade owing to their multiple bioactivity profile. Indole (1*H*-benzo[*b*]pyrrole) is a planar bicyclic molecule, which has wide distribution among the bioactive molecules along with natural products [3]. Indole has proved to demonstrate versatile pharmacophoric activity against breast oncogenic cells by inhibiting NFkB/mTOR/PI3K/AkT and regulatingthe estrogen-mediated activity [4,5]. Natural products like vinblastine, vincristine and apaziquone bearing indole moiety are made use in treatment of different types of cancers [6,7]. The indole compounds are as well extensively explored in the advancement of therapeutic agents with diverse properties like antibacterial [8], anticancer [9,10], antidiabetic [11], antiviral [12], *etc*.

1,2,3-Triazoles has the ability to act as 'linker' as they could form different non-covalent interactions, like hydrogen bonds, dipole-dipole bonds and van der Waals force with diverse proteins, enzymes and receptors [13]. Their potential spans broad spectrum including anticancer [14,15], antifungal [16], anti-diabetic [17], antiviral [18], antioxidant [19] and antiobesity [20]. Similarly, the thiadiazole amines are reported to be having diverse biological activities like antifungal [21], antibacterial [22], CA inhibition [23], anticonvulsant [24], antileishmanial [25], anticancer [26,27], *etc*.

Similarly, 1,2,3-triazoles has the potential to demonstrate anticancer activity *via* cell cycle arrest induction and apoptosis of cancer cells [28]. Receptor tyrosine kinases (RTKs) like VEGFR (vascular endothelial growth factor receptor), EGFR (epidermal growth factor receptor), PDGFRs (platelet-derived growth factor receptors) and MET (hepatocyte growth factor receptor or *c*-MET proto-oncogene) are the few crucial regulatory signaling proteins which by their deviant activation mediate the development and advancement of different types of cancers, hence making these receptors prominent therapeutic targets [29]. Several drugs, either under investigation or approved have been progressed to pharmacologically modulate the oncogenic RTKs activity profile.

The methodologies of 1,2,3-triazole derivatives synthesis have been through tremendous elaboration and innovation, with

the primary focus being improving the yield, efficiency and sustainability. In addition, the bioactivities of the target compounds are also enhanced by the presence of an electron-rich sulfur atom in the thiadiazole ring [30]. The sulfur atom improves lipophilicity and modulates electron density in the molecule, which increases transmembrane diffusion and macromolecular target contact [31].

Recent advances in the synthetic approaches *via* green chemistry as well as click chemistry bestowed tremendous impact over the advancement of diverse scaffolds with enhanced biological efficacy. Further, this knowledge has sophisticated several researches to design and optimize novel derivatives with better pharmacological efficiency [32]. Taking into the account abovesaid features, it is of our interest to synthesize certain indole-based 1,2,4-triazole hybrid with 1,3,4-thiadiazoles (**9a-l**) with different substituent on the triazole nucleus. Subsequently, the molecular docking studies on the synthesized hybrids **9a-l** were also performed to evaluate their anticancer activities.

EXPERIMENTAL

Unless specified all chemicals and solvents were purchased from the reputed commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO by using 500 MHz spectrometers (Bruker Avance III 500 MHz). Distilled hexane and ethyl acetate solvents were used to perform column chromatography over silica gel (60-120 mesh). Mass and Infrared spectra were recorded on QSTAR XL GCMS and Perkin-Elmer spectrum 2 mass spectrometer. Melting points were recorded in open glass capillary tube and are uncorrected.

Synthesis of 5-(1*H*-indol-3-yl)-1,3,4-thiadiazol-2-amine (3): In the first step, 1*H*-indole-3-carbaldehyde (**1**, 1 mmol) was allowed to react with thiosemicarbazide (**2**, 1.2 mmol) in presence of *tert*-butyl hydroperoxide (TBHP) in ethanol at 0-5 ºC for 4 h to yield 5-(1*H*-indol-3-yl)-1,3,4-thiadiazol-2-amine (**3**).

Synthesis of 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1*H*-indol-3-yl)-1,3,4-thiadiazole (5): The amine protection of compound **3** (1 mmol) with 2,5-hexadione (**4**, 1.2 mmol) in presence of toluene and catalytical amount of PTSA reflux for 12 h to yield 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1*H*-indol-3-yl)-1,3,4-thiadiazole (**5**).

Synthesis of 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (7): The intermediate compound **5** (1 mmol) was then propargylated using propargyl bromide (**6**, 1.2 mmol) in the presence of K_2CO_3 in DMF at room temperature for 3-4 h to yield 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (**7**).

Synthesis of 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1-((substituted-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9a-l): Terminal alkyne of compound **7** (1 mmol) on further reaction with various substituted aryl azides (**8a-l**) (1.2 mmol) in click reaction resulted respective1,2,3-triazole containing title compounds (**9a-l**), the products were obtained in good yields.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9a): Yield: 60%, m.p.: 134-136 ºC; R_f = 0.32 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.326 (m, 2H), 7.446 (t, *J* = 7.366, 7.366 Hz, 1H), 7.479-7.556 (m, 3H), 7.672-7.726 (m, 2H), 7.816 (s, 1H), 8.051 (s, 1H), 8.127 (d, *J* =7.098 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 143.03, 136.89, 136.15, 132.53, 129.84, 129.64, 127.32, 126.08, 124.07, 122.05, 122.01, 121.75, 120.67, 110.26, 109.54, 109.51, 42.86, 39.53, 13.11. MS-ESI $C_{25}H_{21}N_7S$: *m/z* 452 $[M+H]^+$. Calcd. %: C, 66.50; H, 4.69; N, 21.71; found %: C, 66.47; H, 4.67; N, 21.69.

2-(1-((1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1*H*-indol-3-yl)-5-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1,3,4-thiadiazole (9b): Yield: 67%, m.p.: 131-133 ºC; R_f = 0.39 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.326 (m, 2H), 7.506 (d, *J* = 1.916, 6.994 Hz, 1H), 7.670-7.706 (m, 4H), 7.816 (s, 1H), 8.051 (s, 1H), 8.127 (d, *J* = 2.061, 7.105 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 143.04, 136.89, 134.94, 133.52, 132.53, 129.64, 126.08, 124.07, 122.05, 121.82, 121.75, 121.33, 119.59, 110.26, 109.54, 109.51, 42.86, 39.53, 13.11. MS-ESI $C_{25}H_{20}N_7Br$: *m/z* 530 $[M+H]^+$. Calcd. %: C, 56.61; H, 3.80; N, 18.48; found %: C, 56.59; H, 3.78; N, 18.45.

2-(1-((1-(2-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-5-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1,3,4-thiadiazole (9c): Yield: 62%, m.p.: 139-141 ºC; R_f = 0.38 (EtOAc: *n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 7.261 (dd, *J* = 3.747, 7.290, 4H), 7.371 (dd, *J* = 2.031, 6.926 Hz, 1H), 7.506 (dd, *J* = 1.887, 7.038 Hz, 1H), 7.563 (dd, *J* = 1.995, 6.924 Hz, 1H), 7.816 (s, 1H), 8.124 (s, 1H), 8.099-8.154 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 142.85, 136.89, 136.17, 132.53, 131.41, 129.64, 127.89, 127.77, 127.13, 126.08, 124.07, 123.01, 122.05, 121.75, 120.78, 110.26, 109.54, 109.51, 42.86, 39.53, 13.11. MS-ESI $C_{25}H_{20}N_7SCl$: *m/z* 486 $[M+H]^+$. Calcd. %: C, 61.79; H, 4.15; N, 20.17; found %: 61.76; H, 4.13; N, 20.14.

2-(1-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-5-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1,3,4-thiadiazole (9d): Yield: 64%, m.p.: 143-145 ºC; R_f = 0.38 (EtOAc: *n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.326 (m, 2H), 7.505 (d, *J* = 7.493 Hz, 3H), 7.847 (d, *J* = 7.492 Hz, 2H), 7.930 (s, 1H), 8.051 (s, 1H), 8.127 (d, *J* = 2.061, 7.101 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 143.04, 136.89, 134.70, 134.54, 132.53, 130.24, 129.64, 126.08, 124.07, 122.07, 122.05, 121.79, 121.75, 110.26, 109.54, 109.51, 42.86, 39.56, 13.11. MS-ESI, $C_{25}H_{20}N_7SCl$: *m/z* 486 $[M+H]^+$. Calcd. %: C, 61.79; H, 4.15; N, 20.17; found %: C, 61.76; H, 4.13; N, 20.14.

4-(4-((3-(5-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1,3,4-thiadiazol-2-yl)-1*H*-indol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-phenol (9e): Yield: 60%, m.p.: 133-135 ºC; R_f = 0.28 (EtOAc: *n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 6.914 (d, *J* = 7.396 Hz, 2H), 7.225-7.326 (m, 2H), 7.506 (d, *J* = 6.993 Hz, 1H), 7.587

(d, *J* = 7.314 Hz, 2H), 7.816 (s, 1H), 8.051 (s, 1H), 8.127 (d, *J* = 7.089 Hz, 1H), 10.091 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.35, 156.27, 155.92, 143.11, 136.89, 132.53, 130.11, 129.64, 126.08, 124.07, 122.05, 121.83, 121.75, 121.74, 117.17, 110.26, 109.54, 109.51, 42.86, 39.53, 13.11. MS-ESI, $C_{25}H_{21}N_7OS$: *m/z* 468 $[M+H]^+$. Calcd. %: C, 64.22; H, 4.53; N, 20.97; found %: C, 64.218; H, 4.50; N, 20.5.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9f): Yield: 63%, m.p.: 135-137 ºC; R_f = 0.40 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 3.873 (s, 3H), 5.339 (s, 2H), 5.950 (s, 2H), 7.040 (d, *J* = 7.533 Hz, 1H), 7.168-7.326 (m, 3H), 7.369 (td, *J* = 1.479, 7.327, 7.441 Hz, 1H), 7.506 (dd, *J* = 1.916, 7.001 Hz, 1H), 7.715 (dd, *J* = 1.572, 7.585 Hz, 1H), 7.816 (s, 1H), 8.107 (s, 1H), 8.265 (dd, *J* = 1.923, 7.038 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 153.54, 142.59, 136.89, 132.53, 129.64, 129.07, 126.63, 126.08, 124.33, 124.07, 122.94, 122.05, 121.75, 119.85, 113.54, 110.26, 109.54, 109.51, 55.68, 42.48, 39.57, 13.11. MS-ESI, $C_{26}H_{23}N_7OS$: *m/z* 482 $[M+H]^+$. Calcd. %: C, 64.85; H, 4.81; N, 20.36; found %: C, 64.82; H, 4.78; N, 20.33.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9g): Yield: 65%, m.p.: 138-140 ºC; R_f = 0.40 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 3.790 (s, 3H), 5.339 (s, 2H), 5.950 (s, 2H), 6.999 (d, *J* = 7.550 Hz, 2H), 7.276 (d, *J* = 7.452 Hz, 2H), 7.506 (d, *J* = 6.997 Hz, 1H), 7.601 (d, *J* = 7.492 Hz, 2H), 7.816 (s, 1H), 8.051 (s, 1H), 8.127 (d, *J* = 7.103 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 159.75, 156.27, 155.92, 143.11, 136.89, 132.53, 131.05, 129.64, 126.08, 124.07, 122.13, 122.05, 121.75, 115.20, 110.26, 109.54, 109.51, 55.34, 42.86, 39.51, 13.11. MS-ESI, $C_{26}H_{23}N_7OS$: *m/z* 482 $[M+H]^+$. Calcd %: C, 64.85; H, 4.81; N, 20.36; found %: C, 64.82; H, 4.78; N, 20.33.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((1-(*o*-tolyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9h): Yield: 62%, m.p.: 129-131 ºC; R_f = 0.40 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.050 (s, 3H), 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.413 (m, 4H), 7.479-7.553 (m, 3H), 7.816 (s, 1H), 8.056 (s, 1H), 8.127 (d, *J* = 2.050, 7.087 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 142.65, 136.93, 136.89, 132.55, 132.53, 130.72, 129.64, 128.36, 126.76, 126.08, 124.07, 122.62, 122.05, 121.75, 120.25, 110.26, 109.54, 109.51, 42.89, 39.51, 17.49, 13.11. MS-ESI, $C_{26}H_{23}N_7S$: *m/z* 466 $[M+H]^+$. Calcd %: C, 67.07; H, 4.98; N, 21.06; found %: C, 67.03; H, 4.95; N, 21.03.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9i): Yield: 64%, m.p.: 133-135 ºC; R_f = 0.40 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 2.383 (s, 3H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.297 (m, 2H), 7.272-7.363 (m, 2H), 7.506 (d, 1H), 7.667 (d, *J* = 7.399 Hz, 2H), 7.816 (s, 1H), 8.051 (s, 1H), 8.127 (d, *J* = 2.062, 7.099 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 143.03, 137.06, 136.89, 134.36, 132.53, 130.26, 129.64, 126.08, 124.07, 122.05, 122.01, 121.75, 120.01, 110.26, 109.54, 109.51, 42.86, 39.58, 21.07, 13.11. MS-ESI, $C_{26}H_{23}N_7S$: *m/z* 466 $[M+H]^+$. Calcd. %: C, 67.07; H, 4.98; N, 21.06; found %: C, 67.03; H, 4.95; N, 21.03.

1-(3-(4-((3-(5-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1,3,4-thiadiazol-2-yl)-1*H*-indol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-phenyl)ethan-1-one (9j): Yield: 67%, m.p.: 137-139 ºC; R_f = 0.36 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 2.572 (s, 3H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.326 (m, 2H), 7.506 (d, *J* = 1.927, 7.004 Hz, 1H), 7.686 (d, *J* = 7.505, 7.505 Hz, 1H), 7.759 (d, *J* =7.326 Hz, 1H), 7.798-7.873 (m, 2H), 7.995 (s, 1H), 8.050 (s, 1H), 8.127 (d, *J* = 2.063, 7.100 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 197.44, 156.27, 155.92, 143.17, 137.73, 137.64, 136.89, 132.53, 129.64, 129.52, 126.08, 126.02, 124.07, 122.46, 122.05, 121.93, 121.75, 118.86, 110.26, 109.54, 109.51, 42.86, 39.53, 26.69, 13.11. MS-ESI, $C_{27}H_{23}N_7OS$: *m/z*: 494 $[M+H]^+$. Calcd. %: C, 65.70; H, 4.70; N, 19.86; found %: C, 65.67; H, 4.68; N, 19.83.

1-(4-(4-((3-(5-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1,3,4-thiadiazol-2-yl)-1*H*-indol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)ethan-1-one (9k): Yield: 69%, m.p.: 143-145 ºC; R_f = 0.36 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 2.527 (s, 3H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.326 (m, 2H), 7.506 (d, *J* = 1.918, 6.997 Hz, 1H), 7.626 (d, *J* = 7.349 Hz, 2H), 7.816 (s, 1H), 7.864 (d, *J* = 7.360 Hz, 2H), 8.051 (s, 1H), 8.127 (d, *J* = 2.063, 7.099 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 196.74, 156.27, 155.92, 143.04, 138.01, 136.89, 135.35, 132.53, 130.34, 129.64, 126.08, 124.07, 122.05, 121.83, 121.75, 119.93, 110.26, 109.54, 109.51, 42.86, 39.58, 26.40, 13.11. MS-ESI, $C_{27}H_{23}N_7OS$: *m/z* 494 $[M+H]^+$. Calcd. %: C, 65.70; H, 4.70; N, 19.86; found %: C, 65.67; H, 4.68; N, 19.83.

2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (9l): Yield: 70%, m.p.: 145-147 ºC; R_f = 0.35 (EtOAc:*n*-hexane 2:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.196 (s, 6H), 5.339 (s, 2H), 5.950 (s, 2H), 7.225-7.326 (m, 2H), 7.506 (dd, *J* = 1.901, 6.997 Hz, 1H), 7.816 (s, 1H), 7.991 (d, *J* = 7.455 Hz, 2H), 8.051 (s, 1H), 8.127 (dd, *J* = 2.051, 7.087 Hz, 1H), 8.361 (d, *J* = 7.497 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 156.27, 155.92, 149.44, 143.12, 139.27, 136.89, 132.53, 129.64, 126.08, 125.98, 124.07, 122.05, 121.75, 121.74, 121.02, 110.26, 109.54, 109.51, 42.86, 39.53, 13.11. MS-ESI, $C_{25}H_{20}N_8O_2S$: *m/z* 497 $[M+H]^+$. Calcd. %: C, 60.47; H, 4.06; N, 22.57; found %: C, 60.43; H, 4.02; N, 22.54.

MTT assay: The cancer cells were appropriately plated and cultured (100 µL per well) in a clear bottom 96-well tissue culture plates with a concentration of 10^5 cells per well. The test samples were added to the well plates with concentrations ranging from 5 to 100 µM (5, 10, 20, 40, 60, 80 and 100 µM) in triplicate after 24 h seeding the cells were incubated for 72 h. Phosphate buffer solution was used to wash the cells in well for twice followed by adding 20 µL of MTT staining solution (5 mg/mL in phosphate buffer solution) to each well. The place was then incubated at 37 ºC. Subsequently, after 4 h, 100 µL of DMSO was carefully added to each well in order to dissolve the formazan crystals and micro plate reader was used to record

the absorbance at 570 nm. Pad Prism Version 5.1. graph is employed to calculate the IC_{50} values.

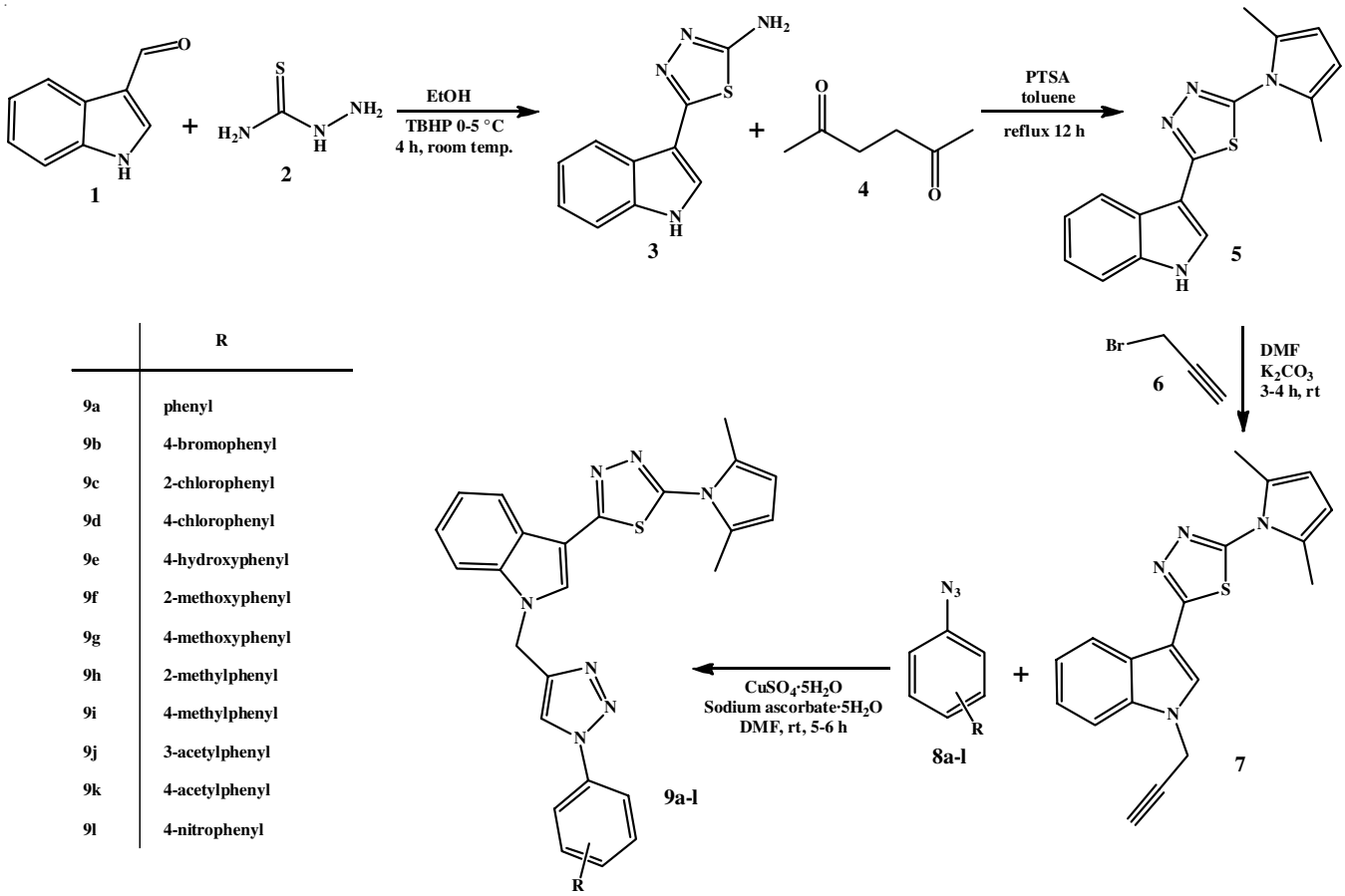
Docking studies: The protein structure was obtained from RCSB PDB database [33]. Water molecules and other entities were cleaned from the protein using Discovery Studio Visualizer [34]. ChemDraw software was used to draw the molecular structures of synthesized compounds **9a-l**, the standard drug doxorubicin and the co-crystallized drug sorafenib. The docking experiments were performed using PyRx, a virtual screening software [35]. Visualization of the interactions of the ligands with the target protein was done using Pymol [36] and Discovery Studio Visualizer.

RESULTS AND DISCUSSION

The synthetic route for desired 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1-((substituted-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (**9a-l**) is shown in **Scheme-I**. The synthesis started with 1*H*-indole-3-carbaldehyde (**1**) was allowed to react with thiosemicarbazide (**2**) in presence of *tert*.-butyl hydroperodixe (TBHP) in ethanol at 0-5 ºC for 4 h, yielded 5-(1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**3**), the amine protection of compound **3** with 2,5-hexadione (**4**) in presence of toluene and catalytical amount of PTSA reflux for 12 h to yield 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1*H*-indol-3-yl)-1,3,4-thiadiazole (**5**). The intermediate compound **5** was then propargylated using propargyl bromide (**6**) in the presence of K_2CO_3 in DMF at room temperature for 3-4 h to obtain 2-(2,5-dimethyl-1H-pyrrol-1-yl)-5-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (**7**).The terminal alkyne of compound **7** on further reaction with various substituted aryl azides (**8a-l**) in click reaction resulted respective 1,2,3-triazole containing title compounds (**9a-l**), the products were obtained in good yields.

Anticancer activity: The novel series of 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-5-(1-((substituted-phenyl-1*H*-1,2,3-triazol-4-yl)-methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (**9a-l**) were selected to carry out the cytotoxicity study against two cancer cell lines A-549 and MCF-7 using MTT assay. Among all the tested compounds **9c**, **9e** and **9d** showed superior anticancer activity against both cancer cell lines A-549 and MCF-7 cell lines. The calculated IC_{50} values of compound **9a-l** are shown in Table-1. Compounds **9c** (o-chloro), **9e** (*p*-hydroxy) and **9d** (*p*-chloro) exhibited superior activity compared with standard drug doxorubicin with IC_{50} ranging from 20.09 ± 0.87, 24.39 ± 1.21 and 21.09 ± 0.23, 25.06 ± 2.11 and 22.16 ± 0.79, 26.32 ± 2.19 M against A-549 and MCF-7 cancer cell lines, respectively. The activity of compound **9c**, **9e** and **9d** may be attributed to electron with drawing and electron donating effect of *o*-chloro, *p*-chloro *p*-hydroxy groups are *ortho*- and *para*-directing nature which activates triazole ring. The other compounds substituted with electron donating and withdrawing group indicated good to poor activity.

Molecular docking studies: In molecular docking studies, the binding interactions between novel derivatives (**9a-l**) and the Crystal structure of Vascular Endothelial Growth Factor



	R
9a	phenyl
9b	4-bromophenyl
9c	2-chlorophenyl
9d	4-chlorophenyl
9e	4-hydroxyphenyl
9f	2-methoxyphenyl
9g	4-methoxyphenyl
9h	2-methylphenyl
9i	4-methylphenyl
9j	3-acetylphenyl
9k	4-acetylphenyl
9l	4-nitrophenyl

Scheme-I: 2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-(1-((substituted-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-1,3,4-thiadiazole (**9a-l**)

TABLE-1
ANTICANCER ACTIVITY OF THE SYNTHESIZED COMPOUNDS (**9a-l**)

Compound	A-549	MCF-7
9a	29.74 ± 1.23	33.47 ± 2.78
9b	53.63 ± 1.31	61.21 ± 4.1
9c	20.09 ± 0.87	24.39 ± 1.21
9d	22.16 ± 0.79	26.32 ± 2.19
9e	21.09 ± 0.23	25.06 ± 2.11
9f	33.69 ± 0.87	39.47 ± 2.98
9g	27.59 ± 0.51	29.23 ± 2.43
9h	59.69 ± 1.43	61.32 ± 3.89
9i	51.63 ± 1.19	54.38 ± 379
9j	47.69 ± 1.19	50.33 ± 3.47
9k	41.49 ± 113	45.37 ± 2.76
9l	32.57 ± 0.97	37.76 ± 2.67
Doxorubicin	21.33 ± 0.47	25.19 ± 2.54

Receptor *i.e.*,VEGFR2 (Juxtamembrane and kinase Domains) (PDB ID: 4ASD), along with the standard drug doxorubicin. The VEGFR2 plays a pivotal role in tumor angiogenesis [37], progression and metastasis [38]. Inhibition of VEGFR2 leads to tumor suppression. The findings of the docking study revealed diverse binding affinities and modes for the derivatives compared to the reference compounds (Fig. 1). Derivatives **9c** and **9e** exhibited significantly superior binding energies (-11.1 and -11 Kcal/mol, respectively) in comparison to all the derivatives and the reference compound doxorubicin (B.E. -8.6 Kcal/mol). To validate the results sorafenib, the co-crystallised drug with the VEGFR2 was redocked. Surprisingly, all the novel derivatives scored good binding scores even in comparison to sorafenib (B.E. -9.2 Kcal/mol) (Table-2). The good binding affinities can be attributed to hydrogen bonding interactions and other interactions including pi-sigma, amide-pi stacked, alkyl, pi-alkyl, pi-sulfur, pi-anion and pi-cation interactions of the compounds with the target protein VEGFR2.

Conclusion

A novel series of 1,2,3-triazole and thiadiazole molecular hybrids (**9a-l**) were synthesized and investigated for their anticancer activity against A-549 and MCF-7 cell lines. Among the tested series, compounds **9a**, **9g** containing phenyl and 4-methoxy phenyl substitutions, respectively have suppressed the A-549 and MCF-7cells with IC_{50} values being relatively closer to those of standard drug doxorubicin. However, rest of the compounds in the series demonstrated mild to moderate activity against the tested cell lines. In conclusion, the hybridization strategy assisted in the synthesis of compounds has been manifested to generate superior lead hybrids for intending novel anticancer medicines that could target VEGFR2.

CONFLICT OF INTEREST

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

TABLE-2
MOLECULAR DOCKING OF THE SYNTHESIZED COMPOUNDS (**9a-l**)

Molecule	Binding energy	Interacting residues	
		H-Bonding	Hydrophobic interactions
9a	-10.5	Ala 881, Asp 1046	Asp 814, Cys 817, Val 848, Ala 866, Lys 868, Leu 882, Ser 884, Glu 885, Leu 889, Val 899, Val 914, Val 916, Cys 1024, Ile 1025, His 1026, Leu 1035, Ile 1044, Cys 1045
9b	-10.2	Glu 885, Asp 1046	Asp 814, Leu 840, Val 848, Ala 866, Ly 868, Glu 885, Ile 888, Leu 889, Val 899, Val 916, Leu 1035, Asp 1046, The 1047,
9c	-11.1	Glu885, Asp1046	Cys 817, Val 848, Ala 866, ly 868, Ala 881, Ser 884, Ile 888, Leu 889, Val 914, Val 916, Leu 1019, Ile 1025, His 1026, Leu 1035, Asp 1046, Gly 1048, Leu 1049
9d	-10.7	Glu 885, Asp 1046	Asp 814, Leu 840, Val 848, Ala 866, Lys 868, Ala 881, Glu 885, Leu 889, Val 899, Val 914, Cys 1045, Leu 1049
9e	-11	Ala881, Asp1046	Asp 814, Cys 817, Val 848, Ala 866, Lys 868, Ala 881, Leu 882, Ser 884, Glu 885, Ile 888, Ile 892, Val 898, Val 899, Val 914, Val 916, Leu 1019, Cys 1024, His 1026, Leu 1035, Cys 1045
9f	-10.3	Ile 1025, His 1026	Asp 814, Glu 885, Ile 888, Leu 889, Ile 892, Val 898, Leu 1019, Cys 1024, His 1026, Arg 1027, Asp 1028, Asp 1046, Gly 1048, Tyr 1059, Leu 1067
9g	-10.6	Glu 885	Asp 814, Val 848, Ala 866, Ala 881, Leu 882, Glu 885, Leu 889, Val 899, Val 916, Leu 1035, Cys 1045, Asp 1046, Phe 1047, Gly 1048, Leu 1049,
9h	-10.2	Ile 1025, His 1026	Asp 814, Ile 888, Leu 889, Ile 892, Val 898, Val 899, Leu 1019, His 1026, Arg 1027, Asp 1028, Asp 1046, Tyr 1059, Pro 1068, Tyr 1082
9i	-10.1	Nil	Val 848, Ala 866, Lys 868, Ala 881, Ser 884, Glu 885, Ile 888, Leu 889, Val 899, Val 916, Cys 1045, Asp 1046, Phe 1047
9j	-10.3	Asp1046	Asp814, Ala881, Glu885, Ile888, Leu889, Ile892, Arg1027, Asp1028, Leu1049, Ile1053, Tyr 1059, Pro1068, Tyr1082
9k	-10.3	Asp 814, Asp 1046	Ala 881, Glu 885, Leu 889, Ile 892, Val 899, Arg 1027, Asp 1028, Asp 1046, Leu 1049, Ile 1053
9l	-10.5	Glu 885	Asp 814, Val 848, Ala 866, Lys 868, Glu 885, Leu 889, Val 999, Val 914, Val 916, Cys 1045, Asp 1046
Doxorubicin	-8.6	Asp814, Ala881, Asp1046	Cys 817, Lys 868, Ser 884, Glu 885, Ile 888, Leu 889, Ile 892, Leu 1019, Arg 1027, Asp 1028, Ile 1044, Cys 1045, Gly 1048, Leu 1049
Sorafenib	-9.2	Lys868, Asp1046	Asp 814, Val 848, Ala 866, Lys 868, Glu 885, Ile 888, Leu 889, Val 899, Val 916, His 1026, Arg 1027, Asp 1028, Leu 1035, Cys 1045, Asp 1046, Phe 1047, Glu 1048

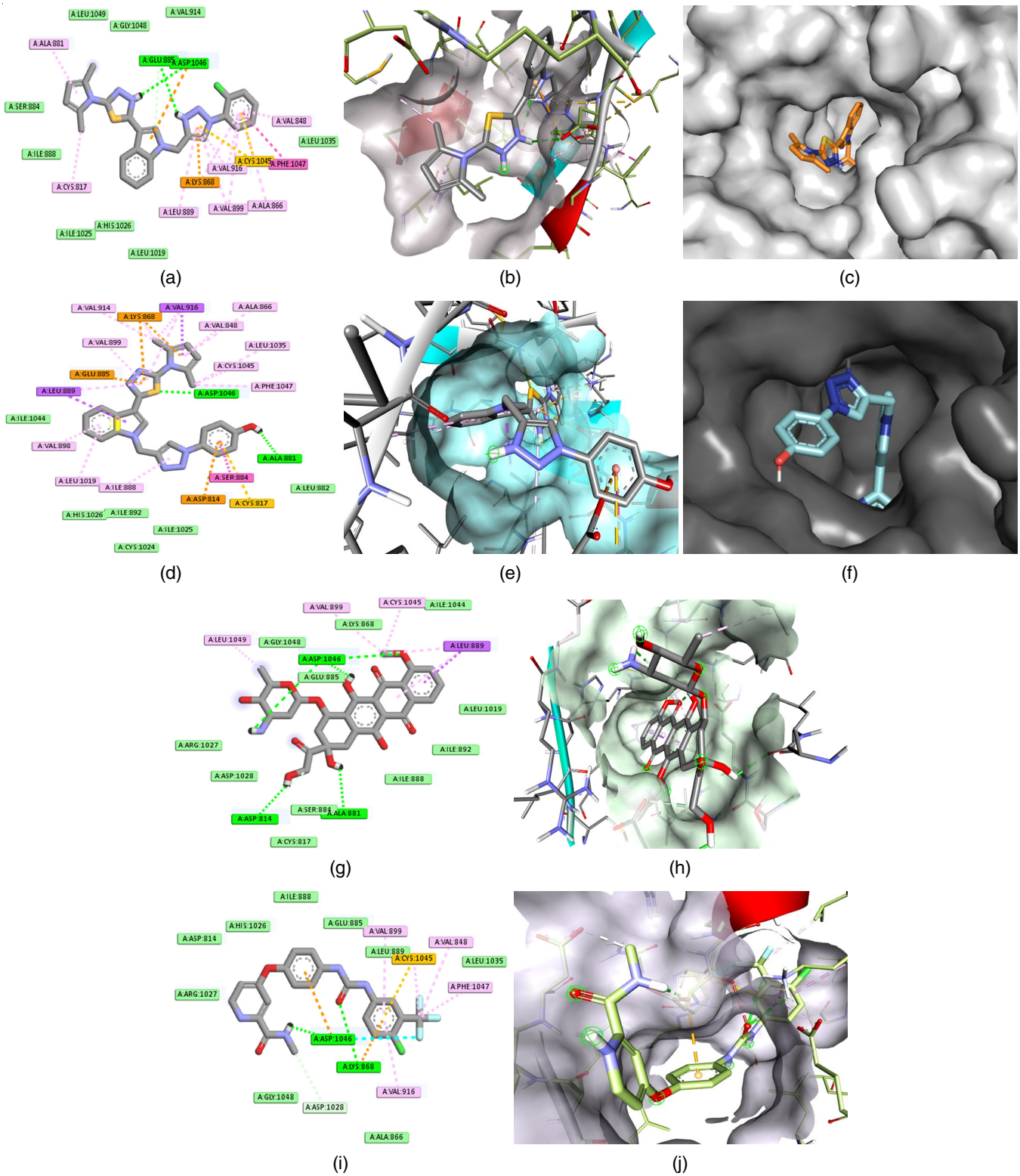


Fig. 1.

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