Design, Synthesis, and Docking Studies of Novel Dimethyl Triazene Incorporated Thiazolyl Pyrazolines for Anticancer Activity

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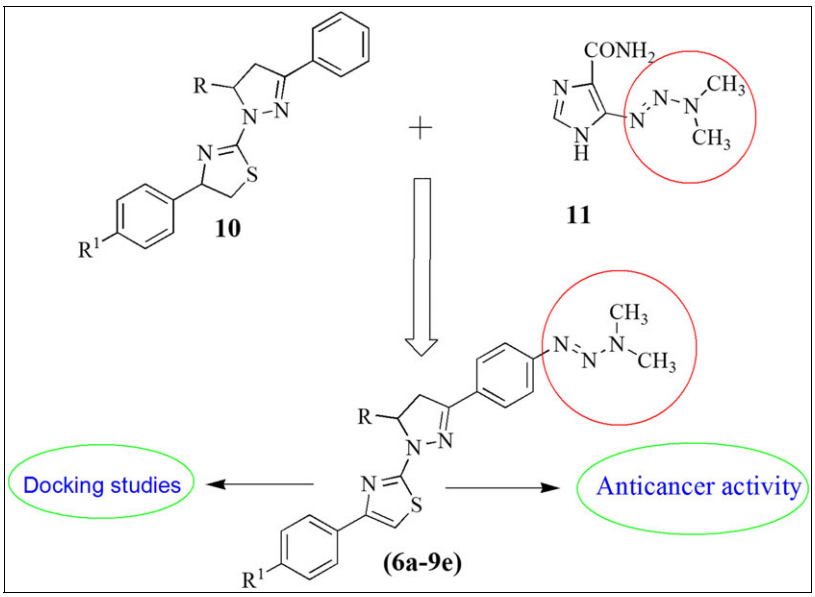


A novel series of dimethyl triazene incorporated thiazolyl pyrazolines have been designed on the basis of hybridization and also in support with combi-targeting approach. The designed compounds were synthesized through facile synthetic methods, and the compounds were confirmed by ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. Further, compounds were screened for *in vitro* anticancer activity against human breast cancer (MCF-7) and human colon cancer (HT-29) cell lines by MTT assay. Among all the tested compounds, compound **9b** showed highest activity against both the cell lines in comparison with reference drug, Cisplatin. In addition, the synthesized compounds were docked into VEGFR-2 kinase (PDB code: 2XIR) to explore their binding interactions at the active site. The compounds showed essential key interactions as that of known VEGFR-2 inhibitors, and hence, the synthesized compounds may be considered as molecular scaffolds for anticancer activity.

INTRODUCTION

Cancer is a second leading cause of fatality worldwide [1], and currently available anticancer drugs entail severe side effects with drug resistance that lead to a severe medical problem [2]. Therefore, there is an utmost need to discover and develop novel anticancer agents with improved tumor selectivity, safety, and efficiency. In recent years, thiazole and their derivatives have been paid greater attention because of their varied biological activities such as anticancer [3–6], antibacterial [7], anti-inflammatory [8], and antiviral [9]. On the other hand, pyrazoline is found to be a core structure in many of the biological active compounds [10–13], and in recent years, there have been several reports showing them as vascular endothelial growth factor receptor-2 (VEGFR-2), B-raf, cyclin dependent, and tyrosine kinase inhibitors for anticancer activity [14–17]. In addition, thiazolyl pyrazolines **10** also have drawn a great attention due to their potent anticancer activity [18–20].

Dacarbazine **11** is an imidazole derivative possessing dimethyl triazene moiety at 5th position, and the drug is proved to show anticancer activity by spontaneous decomposition in the presence of cytochrome P450 enzyme generating methyl cation, which alkylates the N7 position of guanine [21]. In recent years, an interesting novel strategy involving combi-targeting design of molecules links triazene pharmacophore with two molecules, namely, Gleevec and 4-anilinoquinazoline, with improved cytotoxic activity by a dual mode of action interacting with two biological targets, that is, DNA and tyrosine kinase [22,23].

In view of the aforementioned facts and in continuation of our research on pyrazoline derivatives [24,25], in the present study, it is planned to design target compounds by incorporating dimethyl triazene pharmacophore into thiazolyl pyrazoline motif with view to produce hybrid molecules (Fig. 1). Furthermore, on the molecular design level, various substituents were introduced on the terminal phenyl ring with the purpose of exploring the influence of substituents on the anticancer activity by regulating the electronic and steric effects. Further modifications were also performed at 5th position of pyrazoline ring by using different substituted aldehydes, in order to explain the structure activity relationships. The synthesized compounds were screened for their *in vitro* anticancer activity against two cancer cell lines, namely, human colon cancer (HT-29) and human breast adenocarcinoma (MCF-7) by MTT assay method. In addition, a molecular docking study was performed for synthesized compounds against VEGFR-2 kinase to explore their binding interactions at the active site. VEGFR-2 is pathologically involved in various processes of angiogenesis, which include increased vascular permeability, endothelial cell migration, survival, and proliferation [26,27]. Consequently, in recent years, this receptor has gained much scientific focus as a target for the design of novel anticancer agents [28].

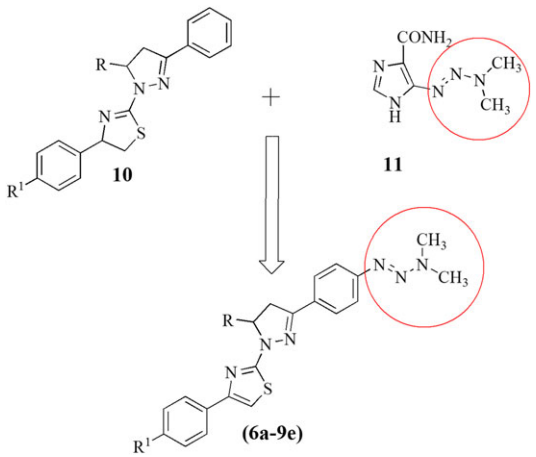
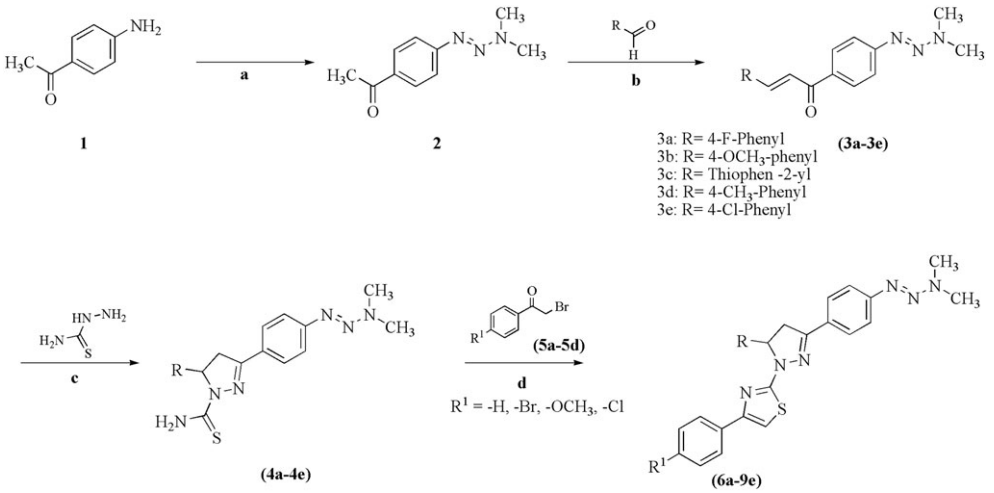


Figure 1. A design strategy of dimethyl triazene incorporated thiazolyl pyrazolines (**6a**-**9e**). [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS AND DISCUSSION

According to Scheme 1, (E)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)ethanone **2** was prepared by diazotization of 4-aminoacetophenone **1** with sodium nitrite in 6*N* HCl followed by coupling with dimethylamine hydrochloride as per the previously reported method [29]. Further, the compound **2** was reacted with different aromatic aldehydes in the presence of aq KOH solution (10%) as a

Scheme 1. Synthesis of the title compounds (**6a**-**9e**). Reagents and conditions: (a) $NaNO_2$ (1.0 equiv), 6*N* HCl (10 vol), 5°C, 1 h; dimethylamine hydrochloride (1.0 equiv), 15 to 20°C, 1 h; (b) aryl aldehydes (1.0 equiv), KOH solution (10%, 10 vol), rt, 16 h; (c) thiosemicarbazide (1.0 equiv), KOH (1.0 equiv), ethanol, 80°C, 2 h (d) substituted phenacylbromide (1.0 equiv), DMF, 25°C, 2 h.



base to give different chalcones (**3a**-**3e**). ^{1}H NMR spectrum of (E)-1-(4-((E)-3,3-dimethyltriaz-1-enyl) phenyl)-3-(4-fluorophenyl)prop-2-en-1-one **3a** in $CDCl_3$ showed two broad singlets at δ 3.57 and δ 3.26 corresponding to two methyl protons of triazene moiety and the peaks for aromatic and –CH═CH– protons appeared in the range of δ 7.11–8.03. Further, the structure was confirmed from mass spectrum, which showed $[M+H]^+$ peak at *m/z* 298.1. The cyclization of chalcones (**3a**-**3e**) with thiosemicarbazide under basic conditions in ethanol afforded pyrazoline derivatives with carbothioamide moiety (**4a**-**4e**). The resultant product **4a** was confirmed by two broad singlets at δ 8.02 and δ 7.84, which corresponds to NH_2 protons of thioamide group, and the aromatic protons appeared in the range of δ 7.83–7.13. The two diastereotopic methylene protons at position 4 and methine proton at position 5 of the pyrazoline ring displayed three doublet of doublet following ABX pattern of splitting, which clearly indicated the formation of pyrazoline ring. The signals for two methylene protons (H_A and H_B) appeared as two doublet of doublet in the region of δ 3.84–3.92 (J = 29.6 Hz, 8.7 Hz) and δ 3.11–3.16 (clubbed with methyl protons), respectively. The methine proton (H_X) also exhibited a doublet of doublet in the region of δ 5.89–5.93 (J = 14.8 Hz, 3.2 Hz), and two methyl proton peaks of triazene moiety appeared at δ 3.52 and δ 3.16 in ^{1}H NMR spectrum (400 MHz, DMSO-d_6). The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **4a** showed a signal at δ 40.1 for CH_3 carbon linked to nitrogen. The C-4 and C-5 carbons of the pyrazoline ring showed signals at δ 42.3 and δ 62.1, respectively, a peak at δ 175.8 due to C═S carbon, a peak at δ 154.7 for C═N of pyrazoline carbon, and aromatic carbons appeared in the range of δ 115.1–162.2. In addition, the mass spectrum of compound **4a** showed $[M+H]^+$ peak at *m/z* 371.1, which confirmed the proposed structure.

Finally, the compounds (**4a**-**4e**) were reacted with different phenacyl bromides (**5a**-**5d**) in DMF at 25°C by stirring, which afforded the target compounds (**6a**-**9e**). The compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, mass, and elemental analysis. The disappearance of thioamide protons at δ 8.02 and δ 7.84 and the appearance of singlet signal at δ 6.81 due to lone proton of thiazole ring in the ^{1}H NMR spectrum of compound **6a** clearly indicated the formation of thiazole ring. The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **6a** showed two signals at δ 104.2 and 151.6, which could be attributed to the C-5 and C-4 carbons of the thiazole ring, the signals at δ 152.6 and δ 164.3 represented C═N of pyrazoline and thiazole ring, respectively. Aromatic carbons appeared in the range of δ 115.1–150.4 and the absence of the characteristic signal of C═S at δ 175.8 indicating the formation of thiazole ring. Moreover, the mass spectrum of compound **6a** showed $[M+H]^+$ peak at *m/z* 471.1, which confirmed the structure.

Anticancer activity. The synthesized compounds (**6a**-**9e**) were evaluated for their anticancer activity by *in vitro* method using MTT assay against MCF-7 and HT-29 cell lines, and their IC_{50} (μg/mL) values were presented in

Table 1

Physical data and *in vitro* anticancer activity of test compounds (**6a**-**9e**).

Compound	R	R^1	Yield (%)	mp (°C)	IC_{50} (μg/mL)	
					MCF-7	HT-29
6a	4-F-Phenyl	H	66	180–182	55.75	57.97
6b	4-OCH_3-Phenyl	H	62	175–176	28.37	31.81
6c	Thiophen-2-yl	H	59	140–143	67.38	55.23
6d	4-CH_3-Phenyl	H	75	174–175	39.12	42.25
6e	4-Cl-Phenyl	H	72	185–186	44.29	48.53
7a	4-F-Phenyl	Br	69	176–178	27.87	26.09
7b	4-OCH_3-Phenyl	Br	76	168–169	18.56	20.17
7c	Thiophen-2-yl	Br	72	185–186	30.32	37.20
7d	4-CH_3-Phenyl	Br	68	174–176	19.65	22.13
7e	4-Cl-Phenyl	Br	79	182–183	26.21	24.75
8a	4-F-Phenyl	OCH_3	85	181–182	36.34	38.65
8b	4-OCH_3-Phenyl	OCH_3	64	171–173	21.25	24.52
8c	Thiophen-2-yl	OCH_3	77	153–154	39.16	43.64
8d	4-CH_3-Phenyl	OCH_3	67	162–164	27.11	26.08
8e	4-Cl-Phenyl	OCH_3	79	168–169	33.46	30.23
9a	4-F-Phenyl	Cl	81	173–174	17.44	16.25
9b	4-OCH_3-Phenyl	Cl	82	178–180	9.04	10.65
9c	Thiophen-2-yl	Cl	74	135–136	19.39	21.20
9d	4-CH_3-Phenyl	Cl	82	185–187	12.23	13.22
9e	4-Cl-Phenyl	Cl	78	181–183	16.34	15.42
Cisplatin					5.68	6.05

Table 2

Interaction patterns of thiazolyl pyrazolines (**6a**-**9e**) with active site amino acids of VEGFR-2 in molecular docking studies.

Compound	Active site amino acid interactions	Percentage binding	Distance between ligand and active site amino acid in Å	Type of interaction
6a	LYS 868	25.7	2.62	Hydrogen
	ILE 888, LEU 889, ASP 1046, VAL 848	—	—	Hydrophobic
6b	ASP 1046	40.1	2.87	Hydrogen
	ARG 1027	84.0	2.32	Hydrogen
	ARG 1027	—	—	Stacking
	ILE 888, LEU 889, VAL 916	—	—	Hydrophobic
6c	ASP 1046	29.4	3.00	Hydrogen
	HIS 1026	51.0	2.94	Hydrogen
	ARG 1027	—	—	Stacking
	ILE 888, LEU 889, VAL 916, VAL 899	—	—	Hydrophobic
6d	ASP 1046	14.1	2.64	Hydrogen
	HIS 1026	—	—	Stacking
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
6e	ASP 1046	12.2	2.77	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 848, VAL 916	—	—	Hydrophobic
7a	ASP 1046	10.4	2.65	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 1035, VAL 916, VAL 848	—	—	Hydrophobic
7b	ASP 1046	79.9	2.87	Hydrogen
	ARG 1027	80.5	2.75	Hydrogen
	HIS 1026	29.6	2.73	Hydrogen
	ILE 888, LEU 889, VAL 916	—	—	Hydrophobic
7c	ASP 1046	10.2	2.75	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
7d	ASP 1046	20.1	2.71	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
7e	ASP 1046	10.3	2.65	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
8a	ASP 1046	14.5	3.16	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 848, PHE 1047	—	—	Hydrophobic
8b	ASP 1046	10.0	2.65	Hydrogen
	ASP 1046	61.4	2.77	Hydrogen
	HIS 1026	24.7	3.14	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, VAL 899, VAL 848, VAL 916	—	—	Hydrophobic
8c	ARG 1027	23.6	3.08	Hydrogen
	ARG 1027	—	—	Stacking
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, ILE 1025	—	—	Hydrophobic
8d	ARG 1027	—	—	Stacking
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916	—	—	Hydrophobic
8e	ARG 1027	—	—	Stacking
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, VAL 899	—	—	Hydrophobic
9a	ASP 1046	20.3	2.72	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
9b	ASP 1046	36.2	2.63	Hydrogen
	LYS 868	—	—	Stacking
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
9c	ASP 1046	38.8	2.86	Hydrogen
	ARG 1027	—	—	Stacking
	ILE 888, LEU 889, VAL 916	—	—	Hydrophobic
9d	ASP 1046	20.0	2.71	Hydrogen

(*Continues*)

Table 2

(*Continued*)

Compound	Active site amino acid interactions	Percentage binding	Distance between ligand and active site amino acid in Å	Type of interaction
	ILE 888, LEU 889, VAL 916, LEU 1035	—	—	Hydrophobic
9e	ASP 1046	20.8	3.09	Hydrogen
	HIS 1026	62.9	2.87	Hydrogen
	ARG 1027	—	—	Stacking
	ILE 888, LEU 889, VAL 916, VAL 899	—	—	Hydrophobic

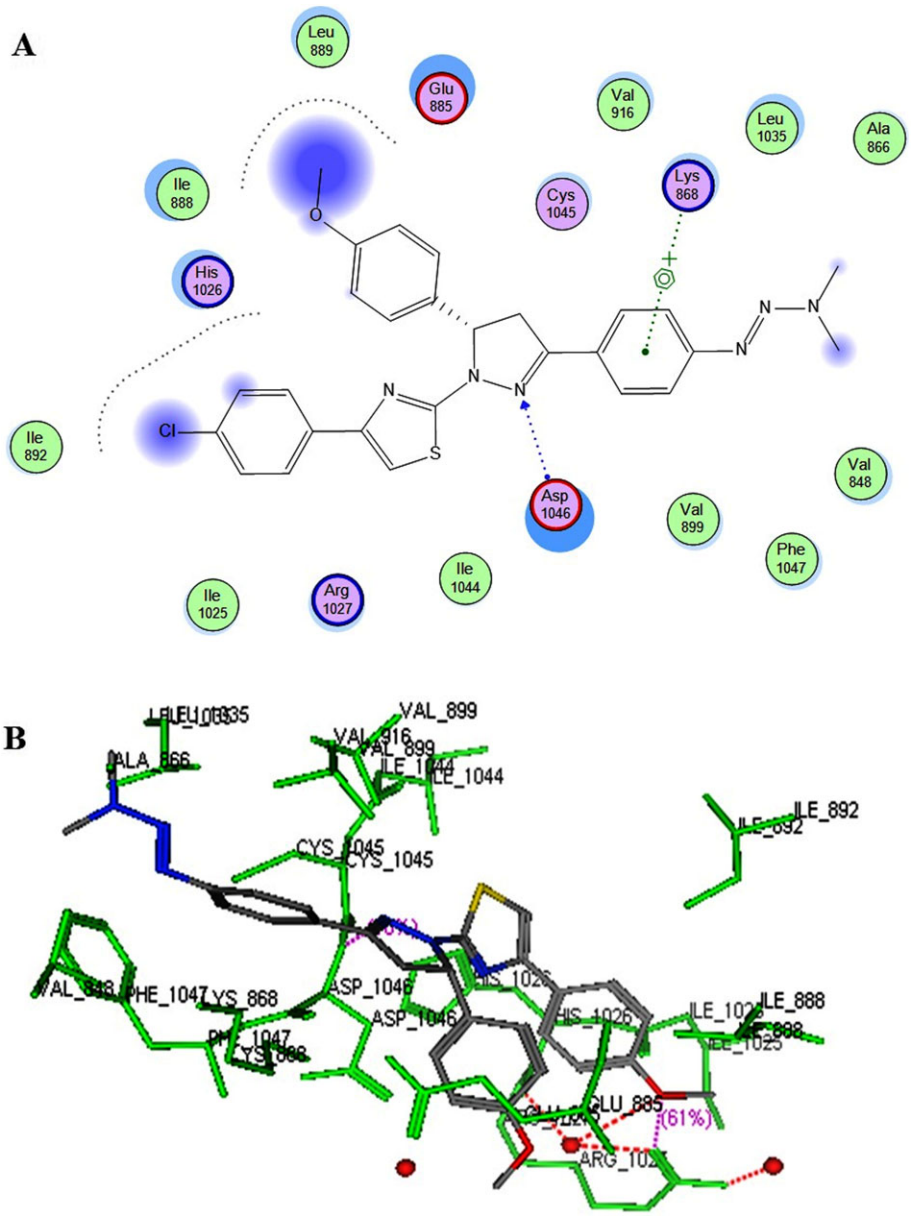


Figure 2. (a) Two-dimensional representation of the interacting mode of **9b** with VEGFR-2 kinase. (b) Three-dimensional structural model of compound **9b** into VEGFR-2 kinase. [Color figure can be viewed at wileyonlinelibrary.com]

Table 1. Among the tested compounds, **9b** showed highest activity against both the cell lines in comparison with reference drug, Cisplatin. Compound **9b** (R = 4-OCH_3-Phenyl, R^1 = Cl) elicited potent inhibitory activity against MCF-7 and HT-29 cell lines with IC_{50} values of 9.04 and 10.65 μg/mL, respectively. Additionally, compound **8b** (IC_{50} = 21.25 μg/mL) owning a substitution at R^1 position is an electron-donating group (R^1 = OCH_3), but when it is replaced by electron-withdrawing group (R^1 = Cl, Br) in compounds **9b** (IC_{50} = 9.04 μg/mL) and **7b** (IC_{50} = 18.56 μg/mL), the activity increases against MCF-7 cell line. The aryl ring, when replaced with a hetero cyclic ring (**6c**, **7c**, **8c**, and **9c**), led to the reduction in anticancer activity. The activity in increasing order for R^1 substitution is H < OCH_3 < Br < Cl and for R substitution is thiophen-2-yl < 4-F-Phenyl < 4-Cl-Phenyl < 4-CH_3-Phenyl < 4-OCH_3-Phenyl against MCF-7 and HT-29 cell lines. In the present study, MTT assay was used only as a preliminary tool for the selection of the compounds for anticancer activity. These selected compounds may prove to be more potent in the *in vivo* screening as dimethyl triazene moiety is expected to undergo decomposition in the presence of cytochrome P450 to release CH_3 cations that alkylate DNA. Further studies are being conducted to elucidate the mechanism of action of these compounds and optimization of their anticancer activity.

Molecular docking. In the present study, the synthesized compounds were docked into VEGFR-2 kinase (PDB code: 2XIR) to explore their binding interactions at the active site. Docking studies revealed that compounds (**6a**-**9e**) interacted with amino acids such ASP 1046, LYS 868, ARG 1027, and HIS 1026, which are also found to interact with known VEGFR-2 inhibitors [30–33]. Further, N-2 of the pyrazoline ring or N-2 of trizene interacts with H-atom of amino acid backbone of ASP 1046 *via* a hydrogen bond, and phenyl ring of test compounds showed stacking interaction with LYS 868. Moreover, thiazolyl pyrazoline is surrounded by the hydrophobic residues such as ILE 888, LEU 889, VAL 899, VAL 916, and LEU 1035 indicating its role in hydrophobic interactions in the active site of VEGFR-2. These interactions underscore the importance of inhibitory capacity against VEGFR-2. Ligand **9b** exhibited effective interaction fit with ASP 1046 of 36.2% binding at a distance of 2.63 Å; triazenyl phenyl ring formed stacking interaction with LYS 868. Apart from this, the compound was surrounded by GLU 885, ILE 888, VAL 916, LEU 889, and LEU 1035 and also showed potent *in vitro* anticancer activity in their series. Data pertaining to the interaction of thiazolyl pyrazolines (**6a**-**9e**) with amino acids on VEGFR-2 active site were given in Table 2 indicating that all the tested compounds have shown akin and essential key interactions as that of known VEGFR-2 inhibitors and hence, the synthesized compounds considered as promising inhibitors for proliferation. The two-dimensional and three-dimensional representations of compound **9b** were shown in Figure 2, and the remaining compounds docking representations were given in the Supporting Information.

CONCLUSIONS

In summary, a series of dimethyl triazene incorporated thiazolyl pyrazolines (**6a**-**9e**) were designed and synthesized through facile synthetic methods. The compounds were confirmed by ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. They were subjected to *in vitro* anticancer activity against two cancer cell lines by MTT assay. Among the tested compounds, **9b** showed highest activity against HT-29 and MCF-7 cell lines in comparison with reference drug, Cisplatin. From the activity data, it was observed that the presence of electron-withdrawing groups at R^1 position showed increases in activity, while electron-donating groups at R^1 have shown a decrease in activity. Moreover, the synthesized compounds were docked into VEGFR-2 kinase to explore their binding interactions at the active site. The compounds showed similar and essential key interactions as that of known VEGFR-2 inhibitors. Further studies are being conducted to elucidate the mechanism of action of these compounds and optimization of their anticancer activity.

EXPERIMENTAL

All chemicals were purchased from Sigma-Aldrich, Merck, and were used without further purification. All melting points were uncorrected and determined in one end open capillary tubes using Guna Digital Melting Point apparatus. Elemental analyses were performed on a PerkinElmer 240 CHN elemental analyzer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer operating at 400/300 MHz for ^{1}H and 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts were expressed in parts per million (ppm) with reference to tetramethylsilane (TMS). Mass spectra were recorded on the Agilent Technologies mass spectrometer. TLC was performed using E. Merck 0.25 mm silica gel plates, and visualization of spots was accomplished with UV light.

Synthesis of (E)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl) ethanone 2. 4-Aminoacetophenone **1** (5.00 g, 37.0 mmol) was dissolved in 6*N* hydrochloric acid (50.0 mL). The solution was cooled to 5°C in an ice bath, and a solution of sodium nitrite (2.58 g, 37.0 mmol) in

water (10 mL) was added at such a rate that the temperature did not rise above 10°C. To the resulting solution of the diazonium salt was added dimethylamine hydrochloride (3.01 g, 37.0 mmol) portion wise and stirred at 15–20°C for 1 h. Then the reaction mixture was neutralized with aq NaOH (10%) solution. The obtained solid was filtered and washed thoroughly with water to remove any alkaline impurities. The crude product was recrystallized from toluene to afford (E)-1-(4-(3,3-dimethyltriaz-1-en-1-yl) phenyl)ethanone (**2**) as an off white solid. (6.1 g, 86%), mp 125–126°C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, 2H, *J* = 8.4 Hz), 7.47 (d, 2H, *J* = 8.8 Hz), 3.55 (bs, 3H), 3.24 (bs, 3H), 2.59 (s, 3H). MS *m/z*: 192.1 $[M+H]^+$; *Anal*. Calcd $C_{10}H_{13}N_3O$: C, 62.81; H, 6.85; N, 21.97; Found: C, 62.84; H, 6.82; N, 21.99.

General procedure for the synthesis of chalcones (3a-3e). To a stirred solution of the (E)-1-(4-(3,3-dimethyltriaz-1-en-1-yl)phenyl)ethanone **2** (1.00 g, 5.23 mmol) and the appropriate aryl aldehyde (5.23 mmol) in 10.0 mL of ethanol, 10% KOH solution (10 mL) was added. The reaction mixture was stirred for 16 h at room temperature. The solid obtained was filtered, washed with water, and finally recrystallized from ethanol to afford the chalcones (**3a**-**3e**).

(E)-1-(4-((E)-3,3-Dimethyltriaz-1-enyl)phenyl)-3-(4-fluorophenyl)prop-2-en-1-one 3a. Brown solid, mp 130–133°C, yield: 80%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (dd, 2H, *J* = 6.8 Hz, 1.6 Hz), 7.78 (d, 1H, *J* = 15.6 Hz), 7.65–7.62 (m, 2H), 7.54–7.49 (m, 3H), 7.11 (t, 2H, *J* = 17.2 Hz), 3.57 (bs, 3H), 3.26 (bs, 3H). MS *m/z*: 298.1 $[M+H]^+$; *Anal*. Calcd $C_{17}H_{16}FN_3O$: C, 68.67; H, 5.42; N, 14.13; Found: C, 68.65; H, 5.45; N, 14.17.

(E)-1-(4-((E)-3,3-Dimethyltriaz-1-enyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one 3b. Off white solid, mp 133–134°C, yield: 78%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (dd, 2H, *J* = 8.4 Hz, 1.6 Hz), 7.79 (d, 1H, *J* = 15.6 Hz), 7.62–7.60 (m, 2H), 7.52–7.45 (m, 3H), 6.94 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 3.86 (s, 3H), 3.56 (bs, 3H), 3.26 (bs, 3H). MS *m/z*: 310.1 $[M+H]^+$; *Anal*. Calcd $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58; Found: C, 69.85; H, 6.17; N, 13.61.

(E)-1-(4-((E)-3,3-Dimethyltriaz-1-enyl)phenyl)-3-(thiophen-2-yl)prop-2-en-1-one 3c. Off white solid, mp 124–126°C, yield: 79%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, 2H, *J* = 8.4 Hz), 7.90 (d, 1H, *J* = 15.2 Hz), 7.79 (d, 1H, *J* = 5.2 Hz), 7.68 (d, 1H, *J* = 3.6 Hz), 7.59 (d, 1H, *J* = 15.6 Hz), 7.46 (d, 1H, *J* = 8.4 Hz), 7.20 (dd, 2H, *J* = 8.8 Hz, 4.0 Hz), 3.56 (s, 3H), 3.21 (s, 3H). MS *m/z*: 286.1 $[M+H]^+$; *Anal*. Calcd $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73; Found: C, 63.10; H, 5.33; N, 14.77.

(E)-1-(4-((E)-3,3-Dimethyltriaz-1-enyl)phenyl)-3-p-tolylprop-2-en-1-one 3d. Brown solid, mp 122–123°C, yield: 75%, ^{1}H NMR (400 MHz, DMSO-d_6): 8.16 (d, 2H, *J* = 8.8 Hz), 7.92 (d, 1H, *J* = 15.6 Hz), 7.79 (d, 2H, *J* = 8.4 Hz), 7.70 (d, 1H, *J* = 15.2 Hz), 7.47 (d, 2H, *J* = 8.4 Hz), 7.28 (d, 2H, *J* = 8.0 Hz), 3.56 (s, 3H), 3.21 (s, 3H), 2.35 (s, 3H). MS *m/z*: 294.1 $[M+H]^+$; *Anal*. Calcd $C_{18}H_{19}N_3OS$: C, 73.69; H, 6.53; N, 14.32; Found: C, 73.66; H, 6.57; N, 14.35.

(E)-3-(4-Chlorophenyl)-1-(4-((E)-3,3-dimethyltriaz-1-enyl) phenyl)prop-2-en-1-one 3e. White solid, mp 135–137°C, yield: 78%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (dd, 2H, *J* = 8.4 Hz, 1.6 Hz), 7.79 (d, 1H, *J* = 15.6 Hz), 7.62–7.60 (m, 2H), 7.52–7.45 (m, 3H), 6.94 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 3.86 (s, 3H), 3.56 (bs, 3H), 3.26 (bs, 3H). MS *m/z*: 314.1 $[M+H]^+$; *Anal*. Calcd $C_{17}H_{16}ClN_3O$: C, 65.07; H, 5.14; N, 13.39; Found: C, 65.10; H, 5.17; N, 13.37.

General procedure for the synthesis of pyrazoline carbothioamides (4a-4e). A mixture of chalcone (**3a**-**3e**) (3.5 mmol), thiosemicarbazide (3.5 mmol), and potassium hydroxide (3.5 mmol) in ethanol (10 mL) was heated to reflux for 2 h at 80°C. After completion of reaction (monitored by TLC), the reaction mixture was cooled and the product obtained was filtered, washed with water, and recrystallized from ethanol to afford pyrazoline carbothioamides (**4a**-**4e**).

(E)-3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide 4a. Brown solid, mp 150–152°C, yield: 79%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (bs, 1H), 7.84 (bs, 1H), 7.83 (d, 2H, *J* = 8.4 Hz), 7.38 (d, 2H, *J* = 8.8 Hz), 7.16–7.13 (m, 4H), 5.91 (dd, 1H, *J* = 14.8 Hz, 3.2 Hz), 3.88 (dd, 1H, *J* = 29.6 Hz, 8.7 Hz), 3.52 (bs, 3H), 3.16–3.11 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.8, 162.2, 159.8, 154.7, 152.1, 139.1, 128.0, 127.3, 120.0, 115.2, 115.0, 62.1, 42.3, 40.1. MS *m/z*: 371.1 $[M+H]^+$; *Anal*. Calcd $C_{18}H_{19}FN_6S$: C, 58.36; H, 5.17; N, 22.69; Found: C, 58.39; H, 5.14; N, 22.66.

(E)-3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide 4b. Off white solid, mp 137–140°C, yield: 77%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, 2H, *J* = 8.4 Hz), 7.46 (d, 2H, *J* = 8.4 Hz), 7.17 (d, 2H, *J* = 8.8 Hz), 6.85 (d, 2H, *J* = 8.4 Hz), 5.98 (dd, 1H, *J* = 14.8 Hz, 3.6 Hz), 3.86–3.81 (m, 1H), 3.77 (s, 3H), 3.54 (bs, 3H), 3.23–3.17 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.7, 158.1, 154.8, 152.1, 135.0, 128.0, 127.3, 126.6, 120.0, 113.7, 62.2, 55.0, 42.3, 40.1. MS *m/z*: 383.1 $[M+H]^+$; *Anal*. Calcd $C_{19}H_{22}N_6OS$: C, 59.66; H, 5.80; N, 21.97; Found: C, 59.68; H, 5.77; N, 21.99.

(E)-3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide 4c. Off white solid, mp 136–137°C, yield: 76%, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (bs, 1H), 7.86 (d, 2H, *J* = 11.2 Hz), 7.83 (bs, 1H), 7.41–7.35 (m, 3H), 6.98–6.91 (m, 2H), 6.22 (d, 1H, *J* = 12.4 Hz), 3.86 (dd, 1H, *J* = 38.0 Hz, 15.2 Hz), 3.52 (bs, 3H), 3.37–3.18 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.6, 155.1, 152.2, 145.3, 128.1, 127.1, 126.4, 124.5, 124.4, 120.1, 58.5, 42.0, 40.1. MS *m/z*: 359.1 $[M+H]^+$; *Anal*.

Calcd $C_{16}H_{18}N_6S_2$: C, 53.61; H, 5.06; N, 23.44; Found: C, 53.64; H, 5.04; N, 23.47.

(E)-3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-p-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide 4d. Brown solid, mp 133–135°C, yield: 75%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, 2H, *J* = 8.4 Hz), 7.45 (d, 2H, *J* = 8.8 Hz), 7.13 (s, 4H), 5.99 (dd, 1H, *J* = 14.8 Hz, 3.2 Hz), 3.82 (dd, 1H, *J* = 28.8 Hz, 11.6 Hz), 3.54 (bs, 3H), 3.29–3.17 (m, 4H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.7, 154.7, 152.1, 140.0, 135.9, 128.9, 128.0, 127.3, 125.2, 120.0, 62.5, 42.4, 40.1, 20.5. MS *m/z*: 367.1 $[M+H]^+$; *Anal*. Calcd $C_{19}H_{22}N_6S$: C, 62.27; H, 6.05; N, 22.93; Found: C, 62.25; H, 6.09; N, 22.95.

(E)-5-(4-Chlorophenyl)-3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide 4e. Brown solid, mp 144–146°C, yield: 78%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, 2H, *J* = 8.4 Hz), 7.46 (d, 2H, *J* = 8.7 Hz), 7.30 (d, 2H, *J* = 8.7 Hz), 7.18 (d, 2H, *J* = 8.4 Hz), 6.02 (dd, 1H, *J* = 15.0 Hz, 3.6 Hz), 3.84 (dd, 1H, *J* = 29.4 Hz, 11.4 Hz), 3.51–3.14 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.7, 154.7, 152.1, 141.9, 131.3, 128.4, 128.1, 127.3, 127.1, 120.0, 62.2, 42.1, 40.1. MS *m/z*: 387.1 $[M+H]^+$; *Anal*. Calcd $C_{18}H_{19}ClN_6S$: C, 55.88; H, 4.95; N, 21.72; Found: C, 55.85; H, 4.97; N, 21.75.

General procedure for the synthesis of the title compounds (6a-9e). A mixture of pyrazoline carbothioamide (**4a**-**4e**) (0.5 mmol) and appropriate substituted phenacyl bromide (**5a**-**5d**) (0.5 mmol) in DMF (5.0 mL) was stirred for 2 h at 25°C. After completion of reaction, the reaction mixture was diluted with a saturated sodium chloride solution. The obtained solid was filtered and washed thoroughly with water. The crude product was purified by column chromatography (ethyl acetate/hexane) to afford the title compounds (**6a**-**9e**).

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole 6a. Brown solid, mp 180–182°C, yield: 66%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, 2H, *J* = 8.4 Hz), 7.66 (d, 2H, *J* = 7.2 Hz), 7.47 (d, 2H, *J* = 8.8 Hz), 7.42 (dd, 2H, *J* = 13.6 Hz, 5.2 Hz), 7.31 (t, 2H, *J* = 15.6 Hz), 7.22 (d, 1H, *J* = 7.6 Hz), 7.04 (t, 2H, *J* = 17.2 Hz), 6.81 (s, 1H), 5.64 (dd, 1H, *J* = 18.8 Hz, 7.2 Hz), 3.91 (dd, 1H, *J* = 29.6 Hz, 12.0 Hz), 3.62–3.19 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 152.6, 151.6, 150.4, 134.4, 128.8, 128.7, 128.4, 127.4, 127.3, 125.4, 120.2, 115.3, 115.1, 104.2, 63.5, 43.0, 40.1. MS *m/z*: 471.1 $[M+H]^+$; *Anal*. Calcd $C_{26}H_{23}FN_6S$: C, 66.36; H, 4.93; N, 17.86; Found: C, 66.39; H, 4.96; N, 17.88.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole 6b. White solid, mp 175–176°C, yield: 62%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74–7.68 (m, 4H), 7.47 (d, 2H, *J* = 8.4 Hz), 7.38–7.29 (m, 4H), 7.23 (t, 1H, *J* = 14.8 Hz), 6.87 (d, 1H, *J* = 8.8 Hz), 6.79 (s, 1H), 5.62 (dd, 1H, *J* = 18.4 Hz, 6.4 Hz), 3.87 (dd, 1H, *J* = 29.2 Hz, 12.0 Hz), 3.78 (s, 3H), 3.37–3.30 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.7, 158.3, 158.1, 152.0, 151.1, 149.9, 133.4, 127.5, 127.1, 127.0, 126.8, 126.4, 119.9, 113.4, 101.4, 63.2, 54.6, 42.6, 39.7. MS *m/z*: 483.1 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{26}N_6OS$: C, 67.20; H, 5.43; N, 17.41; Found: C, 67.24; H, 5.47; N, 17.43.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole 6c. White solid, mp 140–143°C, yield: 59%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80–7.73 (m, 4H), 7.48 (d, 2H, *J* = 8.4 Hz), 7.35 (t, 2H, *J* = 15.2 Hz), 7.27–7.20 (m, 3H), 6.97–6.95 (m, 1H), 6.85 (s, 1H), 5.96 (dd, 1H, *J* = 17.6 Hz, 6.0 Hz), 3.90 (dd, 1H, *J* = 28.8 Hz, 11.6 Hz), 3.62–3.40 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.9, 158.3, 152.3, 151.3, 149.9, 143.6, 126.99, 126.90, 126.4, 126.2, 125.3, 125.0, 119.9, 113.4, 101.9, 59.1, 42.3, 39.7. MS *m/z*: 459.2 $[M+H]^+$; *Anal*. Calcd $C_{24}H_{22}N_6S_2$: C, 62.86; H, 4.84; N, 18.33; Found: C, 62.83; H, 4.81; N, 18.36.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole 6d. Off white solid, mp 174–175°C, yield: 75%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74–7.68 (m, 4H), 7.46 (d, 2H, *J* = 8.8 Hz), 7.33–7.29 (m, 4H), 7.23–7.14 (m, 3H), 6.79 (s, 1H), 5.62 (dd, 1H, *J* = 18.4 Hz, 6.4 Hz), 3.88 (dd, 1H, *J* = 29.6 Hz, 12.0 Hz), 3.48–3.30 (m, 7H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.6, 159.2, 159.0, 152.9, 152.0, 150.8, 134.3, 128.4, 128.0, 127.9, 127.7, 127.3, 120.8, 114.3, 102.3, 64.0, 43.5, 40.6, 20.5. MS *m/z*: 467.2 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{26}N_6S$: C, 69.50; H, 5.62; N, 18.01; Found: C, 69.52; H, 5.60; N, 18.04.

(E)-2-(5-(4-Chlorophenyl)-3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole 6e. Brown solid, mp 185–186°C, yield: 72%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72–7.66 (m, 4H), 7.45 (d, 2H, *J* = 8.4 Hz), 7.35–7.27 (m, 4H), 7.21–7.18 (m, 1H), 6.85 (d, 2H, *J* = 8.8 Hz), 6.77 (s, 1H), 5.60 (dd, 1H, *J* = 18.4 Hz, 6.4 Hz), 3.87 (dd, 1H, *J* = 29.6 Hz, 12.0 Hz), 3.35–3.27 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.5, 158.1, 151.9, 151.0, 149.6, 140.2, 131.3, 127.9, 127.8, 126.6, 126.1, 119.6, 113.2, 101.4, 62.8, 42.3, 39.5. MS *m/z*: 487.0 $[M+H]^+$; *Anal*. Calcd $C_{26}H_{23}ClN_6S$: C, 64.12; H, 4.76; N, 17.26; Found: C, 64.10; H, 4.79; N, 17.29.

(E)-4-(4-Bromophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) thiazole 7a. Off white solid, mp 176–178°C, yield: 69%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, 2H, *J* = 8.8 Hz), 7.66 (dd, 2H, *J* = 8.4 Hz, 1.6 Hz), 7.47 (d, 2H, *J* = 6.0 Hz), 7.48–7.38 (m, 4H), 7.03 (t, 2H, *J* = 17.6 Hz), 6.81 (s, 1H), 5.62 (dd, 1H, *J* = 18.8 Hz, 7.2 Hz), 3.91 (dd, 1H, *J* = 29.2 Hz, 12.0 Hz), 3.34–3.28 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ

163.9, 152.3, 151.3, 150.0, 134.0, 128.4, 128.3, 128.1, 127.1, 126.9, 125.0, 119.9, 115.0, 114.8, 103.8, 63.1, 42.7, 39.7. MS *m/z*: 549.1 $[M+H]^+$; *Anal*. Calcd $C_{26}H_{22}BrFN_6S$: C, 56.83; H, 4.04; N, 15.30; Found: C, 56.87; H, 4.01; N, 15.34.

(E)-4-(4-Bromophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) thiazole 7b. White solid, mp 168–169°C, yield: 76%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (dd, 2H, *J* = 8.4 Hz, 1.6 Hz), 7.56 (dd, 2H, *J* = 8.8 Hz, 4.8 Hz), 7.48–7.42 (m, 4H), 7.35 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 6.87 (d, 1H, *J* = 8.8 Hz), 6.79 (s, 1H), 5.59 (dd, 1H, *J* = 18.4 Hz, 6.4 Hz), 3.87 (dd, 1H, *J* = 29.2 Hz, 12.0 Hz), 3.78 (s, 3H), 3.36–3.30 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 157.8, 157.7, 151.5, 150.7, 149.4, 133.0, 127.1, 126.6, 126.5, 126.3, 126.3, 125.9, 119.4, 112.9, 101.1, 62.7, 54.1, 42.1, 39.2. MS *m/z*: 561.0 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{25}BrN_6OS$: C, 57.75; H, 4.49; N, 14.97; Found: C, 57.78; H, 4.46; N, 14.99.

(E)-4-(4-Bromophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazole 7c. White solid, mp 185–186°C, yield: 72%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75–7.73 (m, 2H), 7.65 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 7.49–7.46 (m, 4H), 7.22–7.18 (m, 2H), 6.97–6.94 (m, 1H), 6.84 (s, 1H), 5.94 (dd, 1H, *J* = 17.6 Hz, 6.0 Hz), 3.91 (dd, 1H, *J* = 28.8 Hz, 11.6 Hz), 3.55–3.40 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 158.7, 152.6, 151.6, 150.2, 143.9, 127.3, 127.2, 126.8, 126.6, 125.7, 125.4, 120.2, 113.8, 102.3, 59.5, 42.7, 40.0. MS *m/z*: 537.0 $[M+H]^+$; *Anal*. Calcd $C_{24}H_{21}BrN_6S_2$: C, 53.63; H, 3.94; N, 15.64; Found: C, 53.60; H, 3.97; N, 15.66.

(E)-4-(4-Bromophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-*p*-tolyl-4,5-dihydro-1H-pyrazol-1-yl)thiazole 7d. Brown solid, mp 174–176°C, yield: 68%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73–7.71 (m, 2H), 7.54 (d, 2H, *J* = 8.8 Hz), 7.48–7.41 (m, 4H), 7.31 (d, 2H, *J* = 8.0 Hz), 7.15 (d, 2H, *J* = 8.0 Hz), 6.79 (s, 1H), 5.60 (dd, 1H, *J* = 18.8 Hz, 6.8 Hz), 3.89 (dd, 1H, *J* = 29.2 Hz, 12.0 Hz), 3.58–3.25 (m, 7H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 158.8, 158.7, 152.5, 151.7, 150.4, 134.0, 128.1, 127.7, 127.5, 127.3, 126.9, 120.4, 113.9, 102.0, 63.7, 43.1, 40.3, 20.1. MS *m/z*: 545.0 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{25}BrN_6S$: C, 59.45; H, 4.62; N, 15.41; Found: C, 59.48; H, 4.60; N, 15.45.

(E)-4-(4-Bromophenyl)-2-(5-(4-chlorophenyl)-3-(4-(3,3-dimethyltriaz-1-enyl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl) thiazole 7e. Brown solid, mp 182–183°C, yield: 79%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, 2H, *J* = 8.4 Hz), 7.53–7.42 (m, 6H), 7.37–7.31 (m, 4H), 6.81 (s, 1H), 5.59 (dd, 1H, *J* = 18.8 Hz, 6.8 Hz), 3.90 (dd, 1H, *J* = 29.2 Hz, 12.0 Hz), 3.59–3.26 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 158.4, 152.2, 151.3, 149.9, 140.5, 131.6, 128.3, 128.1, 126.9, 126.4, 119.9, 113.5, 101.8, 63.2, 42.6, 39.8. MS *m/z*: 564.9 $[M+H]^+$; *Anal*. Calcd $C_{26}H_{22}BrClN_6S$: C, 55.18; H, 3.92; N, 14.85; Found: C, 55.21; H, 3.90; N, 14.82.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-methoxyphenyl)thiazole 8a. Off white solid, mp 181–182°C, yield: 85%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, 2H, *J* = 8.4 Hz), 7.59 (dd, 2H, *J* = 10.0 Hz, 1.6 Hz), 7.47 (dd, 2H, *J* = 9.6 Hz, 1.6 Hz), 7.42–7.39 (m, 2H), 7.06–7.01 (m, 2H), 6.86–6.67 (m, 2H), 6.67 (s, 1H), 5.63 (dd, 1H, *J* = 18.0 Hz, 6.0 Hz), 3.98–3.81 (m, 1H), 3.80 (s, 3H) 3.59–3.27 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.9, 153.3, 152.2, 151.0, 135.0, 129.4, 129.3, 129.0, 128.1, 127.9, 126.0, 120.9, 115.9, 115.7, 104.8, 64.1, 55.0, 43.6, 40.7. MS *m/z*: 501.2 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{25}FN_6OS$: C, 64.78; H, 5.03; N, 16.79; Found: C, 64.75; H, 5.04; N, 16.76.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-methoxyphenyl)thiazole 8b. Brown solid, mp 171–173°C, yield: 64%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, 2H, *J* = 8.4 Hz), 7.62 (d, 2H, *J* = 8.4 Hz), 7.47 (d, 2H, *J* = 8.4 Hz), 7.36 (d, 2H, *J* = 8.0 Hz), 6.89–6.84 (m, 4H), 6.66 (s, 1H), 5.62 (dd, 1H, *J* = 18.4 Hz, 6.4 Hz), 3.91–3.87 (m, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 3.49–3.09 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 158.7, 158.5, 152.4, 151.5, 150.2, 133.8, 127.9, 127.5, 127.4, 127.2, 126.8, 120.2, 113.8, 101.8, 63.5, 55.0, 55.0, 43.0, 40.1. MS *m/z*: 513.0 $[M+H]^+$; *Anal*. Calcd $C_{28}H_{28}N_6O_2S$: C, 65.60; H, 5.51; N, 16.39; Found: C, 65.64; H, 5.54; N, 16.36.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-methoxyphenyl)thiazole 8c. Brown solid, mp 153–154°C, yield: 77%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, 2H, *J* = 8.8 Hz), 7.64 (d, 2H, *J* = 8.8 Hz), 7.41 (d, 2H, *J* = 8.8 Hz), 7.15–7.13 (m, 2H), 6.90–6.88 (m, 1H), 6.87–6.81 (m, H), 6.81 (s, 1H), 5.89 (dd, 1H, *J* = 18.0 Hz, 6.4 Hz), 3.85–3.81 (m, 1H), 3.75 (s, 3H), 3.59–3.25 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 158.7, 152.7, 151.7, 150.3, 144.0, 127.4, 127.3, 126.8, 126.6, 125.7, 125.5, 120.3, 113.8, 102.3, 59.6, 55.0, 42.7, 40.1. MS *m/z*: 489.0 $[M+H]^+$; *Anal*. Calcd $C_{25}H_{24}N_6OS_2$: C, 61.45; H, 4.95; N, 17.20; Found: C, 61.41; H, 4.98; N, 17.23.

(E)-2-(3-(4-(3,3-Dimethyltriaz-1-enyl)phenyl)-5-*p*-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-methoxyphenyl)thiazole 8d. Brown solid, mp 162–164°C, yield: 67%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, 2H, *J* = 8.4 Hz), 7.62 (d, 2H, *J* = 8.8 Hz), 7.47 (d, 2H, *J* = 8.8 Hz), 7.15 (d, 2H, *J* = 8.0 Hz), 6.85 (d, 2H, *J* = 9.2 Hz), 6.65 (s, 1H), 5.62 (dd, 1H, *J* = 18.8 Hz, 6.8 Hz), 3.92–3.88 (m, 1H), 3.81 (s, 3H), 3.49–3.29 (m, 7H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.0, 159.5, 159.4, 153.2, 152.4, 151.1, 134.7, 128.8, 128.3, 128.2, 128.0, 127.6, 121.1, 114.6, 102.7, 64.4, 55.9, 43.8, 40.9, 20.4. MS *m/z*:

497.2 $[M+H]^+$; *Anal*. Calcd $C_{28}H_{28}N_6OS$: C, 67.72; H, 5.68; N, 16.92; Found: C, 67.70; H, 5.71; N, 16.96.

(E)-2-(5-(4-Chlorophenyl)-3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-methoxyphenyl) thiazole 8e. Brown solid, mp 168–169°C, yield: 79%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, 2H, *J* = 8.8 Hz), 7.59 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 7.47 (d, 2H, *J* = 8.4 Hz), 7.38–7.31 (m, 4H), 6.85 (d, 2H, *J* = 8.8 Hz), 6.68 (s, 1H), 5.61 (dd, 1H, *J* = 18.8 Hz, 6.8 Hz), 3.94–3.90 (m, 1H), 3.81 (s, 3H), 3.40–3.12 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 158.7, 152.5, 151.6, 150.2, 140.9, 131.9, 128.6, 128.4, 127.3, 126.7, 120.2, 113.8, 102.1, 63.5, 55.0, 42.9, 40.1. MS *m/z*: 517.2 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{25}ClN_6OS$: C, 62.72; H, 4.87; N, 16.25; Found: C, 62.75; H, 4.83; N, 16.28.

(E)-4-(4-Chlorophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) thiazole 9a. Off white solid, mp 173–174°C, yield: 81%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (dd, 2H, *J* = 8.4 Hz, 1.6 Hz), 7.51–7.36 (m, 8H), 7.02 (t, 2H, *J* = 17.2 Hz), 6.79 (s, 1H), 5.59 (dd, 1H, *J* = 18.8 Hz, 7.2 Hz), 3.89 (dd, 1H, *J* = 29.6 Hz, 12.0 Hz), 3.32–3.26 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 152.5, 151.5, 150.2, 134.2, 128.6, 128.5, 128.3, 127.3, 127.1, 125.2, 120.1, 115.2, 115.0, 104.0, 63.3, 42.9, 39.9. MS *m/z*: 505.0 $[M+H]^+$; *Anal*. Calcd $C_{26}H_{22}ClFN_6S$: C, 61.84; H, 4.39; N, 16.64; Found: C, 61.81; H, 4.36; N, 16.67.

(E)-4-(4-Chlorophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) thiazole 9b. White solid, mp 178–180°C, yield: 82%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, 2H, *J* = 8.4 Hz), 7.62 (d, 2H, *J* = 8.8 Hz), 7.47 (d, 2H, *J* = 8.8 Hz), 7.36 (d, 2H, *J* = 8.8 Hz), 7.29–7.27 (m, 2H), 6.88 (d, 1H, *J* = 8.8 Hz), 6.78 (s, 1H), 5.62 (dd, 1H, *J* = 20.8 Hz, 9.6 Hz), 3.93–3.85 (m, 2H), 3.78 (s, 3H), 3.49–3.30 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.5, 158.1, 157.9, 151.8, 150.9, 149.6, 133.2, 127.3, 126.9, 126.8, 126.6, 126.2, 119.6, 113.2, 101.2, 62.9, 54.4, 42.3, 39.5. MS *m/z*: 517.1 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{25}ClN_6OS$: C, 62.72; H, 4.87; N, 16.25; Found: C, 62.75; H, 4.84; N, 16.28.

(E)-4-(4-Chlorophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazole 9c. White solid, mp 135–136°C, yield: 74%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, 2H, *J* = 8.8 Hz), 7.56 (d, 2H, *J* = 8.8 Hz), 7.41–7.37 (m, 4H), 7.14–7.10 (m, 2H), 6.87 (dd, 1H, *J* = 8.8 Hz, 3.6 Hz), 6.76 (s, 1H), 5.86 (dd, 1H, *J* = 17.6 Hz, 6.0 Hz), 3.91 (dd, 1H, *J* = 28.8 Hz, 11.6 Hz), 3.46–3.32 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.0, 158.5, 152.4, 151.4, 150.0, 143.7, 127.1, 127.0, 126.6, 126.4, 125.5, 125.2, 120.0, 113.6, 102.0, 59.3, 42.5, 39.8. MS *m/z*: 493.1 $[M+H]^+$; *Anal*. Calcd $C_{24}H_{21}ClN_6S_2$: C, 58.46; H, 4.29; N, 17.05; Found: C, 58.48; H, 4.26; N, 17.08.

(E)-4-(4-Chlorophenyl)-2-(3-(4-(3,3-dimethyltriaz-1-enyl) phenyl)-5-*p*-tolyl-4,5-dihydro-1H-pyrazol-1-yl)thiazole 9d. Brown solid, mp 185–187°C, yield: 82%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 7.61 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 7.46 (dd, 2H, *J* = 8.4 Hz, 2.0 Hz), 7.32–7.28 (m, 4H), 7.15 (d, 2H, *J* = 8.0 Hz), 6.78 (s, 1H), 5.61 (dd, 1H, *J* = 18.8 Hz, 6.8 Hz), 3.89 (dd, 1H, *J* = 29.2 Hz, 12.0 Hz), 3.65–3.30 (m, 7H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 158.8, 158.6, 152.5, 151.6, 150.4, 133.9, 128.0, 127.6, 127.5, 127.3, 126.9, 120.4, 113.9, 101.9, 63.7, 43.1, 40.2, 20.5. MS *m/z*: 501.1 $[M+H]^+$; *Anal*. Calcd $C_{27}H_{25}ClN_6S$: C, 64.72; H, 5.03; N, 16.77; Found: C, 64.75; H, 5.07; N, 16.74.

(E)-4-(4-Chlorophenyl)-2-(5-(4-chlorophenyl)-3-(4-(3,3-dimethyltriaz-1-enyl)phenyl)-4,5-dihydro-1H-pyrazol-1-yl) thiazole 9e. Brown solid, mp 181–183°C, yield: 78%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 7.58 (dd, 2H, *J* = 8.8 Hz, 2.0 Hz), 7.48 (d, 2H, *J* = 8.8 Hz), 7.37–7.28 (m, 6H), 6.80 (s, 1H), 5.60 (dd, 1H, *J* = 18.8 Hz, 6.8 Hz), 3.90 (dd, 1H, *J* = 29.6 Hz, 12.0 Hz), 3.67–3.26 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.0, 158.6, 152.4, 151.5, 150.1, 140.7, 131.8, 128.5, 128.3, 127.1, 126.6, 120.1, 113.7, 102.0, 63.4, 42.8, 40.0. MS *m/z*: 521.0 $[M+H]^+$; *Anal*. Calcd $C_{26}H_{22}Cl_2N_6S$: C, 59.88; H, 4.25; N, 16.12; Found: C, 59.85; H, 4.28; N, 16.16.

In vitro anticancer activity. The anticancer activity of all the 20 compounds was evaluated against two cancer cell lines, namely, HT-29 and MCF-7. The cell lines were procured from National Center for Cell Sciences, Pune, India. All tumor cells were grown in DMEM media supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 mg/mL streptomycin, 100 IU/mL penicillin, and 2 m*M* glutamine. All cell lines were maintained under humidified atmosphere with 5% CO_2 at 37°C for 48 h.After incubation, cells (5×10^3 cells/well) were inoculated in 96-well plate and cultured with or without compounds at different concentrations in triplicates for 24 h in a final volume of 200 μL. Afterwards, the medium was removed, and 20 μL of MTT (5 mg/mL in PBS) was added. After 3 h incubation at 37 °C, 100 μL of DMSO was added to each well, and the plate was agitated for 1 min. The absorbance was read at 570 nm with a multi-well plate reader (Victor3, PerkinElmer), and IC_{50} values were calculated [34,35].

Molecular docking. Molecular docking for test compounds (**6a**-**9e)** was performed on Windows 2002 operating system with the help of the MOE software 2008.10 version. VEGFR-2 kinase was obtained from the Swedish protein data bank (PDB code: 2XIR), and the target receptor was visualized using sequence option, and further co-factors were deleted. The partial charge of VEGFR-2 receptor was calculated and readjusted, using the force field method AMBER 99. Later, the receptor

was allowed for 3D protonation at cutoff 12.0, and subsequently, hydrogens were added as required for standard geometry and the VEGFR-2 kinase was subjected to energy minimization by using the force field MMFF94x method at 0.01 kJ/mol gradients. The target structures were drawn using a builder module of MOE software and balanced the partial charges using Hamilton MMFF94 force field method, and subsequently, 3D protonated and hydrogen were added according to standard geometry. Thiazolyl pyrazoline structures (**6a-9e)** were energy minimized using force field MMFF94x application of MOE software at cutoff 12 at 0.01 kJ/mol gradient, and 6.0 Å grid was generated on the active site of the enzyme. Molecular docking analysis was carried out by opting simulation mode following the dock module on the selected active site amino acids with the help of sequence option, and eventually docked by selecting options, such as receptor and solvent, selected residues, alpha triangle, affinity dG, force field refinement, and best 30 pose. Finally, molecular docking results were obtained, out of the 30 best posed conformers resulted for each chemical structure. Among these, one of the best conformers of the ligand was retained. The resultant best pose score computed values in the series were used for analysis of molecular docking and interaction [24].

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