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Ultrasound assisted synthesis of tetrazole based pyrazolines and isoxazolines as potent anticancer agents via inhibition of tubulin polymerization

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ABSTRACT

In search of new active molecules against MCF-7, A549 and HepG2, tetrazole based pyrazoline and isoxazoline derivatives under both conventional and ultrasonic irradiation method were designed and efficiently synthesized. Structures of newly synthesized compounds **5a-h** and **6a-h** were characterized by ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Several derivatives were found to be excellent cytotoxic against MCF-7, A549 and HepG2 cell lines characterized by lower IC_{50} values (0.78–3.12 μg/mL). Compounds **5b** and **5c** demonstrated an antiproliferative effect comparable to that of CA-4. Western blot analysis revealed that, reported compounds accumulate more tubulin in the soluble fraction. Docking studies suggested that, binding of these compounds mimics at the colchicine site of tubulin. *In vitro* study revealed that the tetrazole based pyrazolines and isoxazolines may possess ideal structural requirements for further development of novel therapeutic agents.

Microtubules composed of α, β-tubulin heterodimers are essential in a variety of fundamental cellular processes like cell division and maintenance of cell shape, regulation of motility, cell signalling, intracellular transport, and segregation of chromosomes during mitosis.[1] Dynamic equilibrium between microtubule polymerization and depolymerization is central to most of microtubule mediated functions including cell division.[2] Microtubules possess three ligand-binding sites as vinca, colchicines and taxol domains. Colchicine **(A)** and Combretastatin A-4 **(CA-4) (B)** exhibited cytotoxicity against a broad range of human cancer cell lines as well as multidrug resistant cell lines by inhibiting tubulin polymerization and binding to the colchicine binding site.[3] Their distinctive structural features inspired many researchers worldwide to develop antimitotic agents, as these scaffolds structurally possess two and three rings with a trimethoxyphenyl group (ring-A), *cis*-configuration at the olefinic bridge and (ring-B) modifications. Five membered heterocyclic compounds as pyrazole,[4] imidazole,[5] thiazole,[6] 1,3,4-oxadiazole,[7] isoxazole,[8] 1,2,3-thiadiazole,[9] triazole,[10] tetrazole,[11] oxazole[12] and pyrazoline[13] are considered as a good surrogate for the double bond of **CA-4** to retain *cis*-alkene configuration, known for potent tubulin inhibition with enhanced cytotoxicity. Small molecules disrupting the microtubule/tubulin dynamics are used widely in cancer treatment. Thus, to retain the appropriate configuration of the two adjacent aryl groups required for bioactivity, heterocombretastatin derivatives with general structure **(C)** were obtained by replacing the stilbene core of **CA-4** with tetrazole ring.[14] Hence, discovery and development of newer small molecules with antitubulin activity have attracted medicinal chemists for past few years.[15] Researchers have reported anticancer, antibacterial and anti-HIV activity of compounds possessing isoxazoline nucleus.[16–18] Isoxazoline linked to tetrazole **(D)** significantly reduced the growth of cancer cell lines, and disrupted tubulin polymerization.[19] Molecules containing isoxazolines have been found to elicit anticancer activities with improved pharmacokinetics profile like diaryl analogues **(E)** demonstrated potent cytotoxic activity by blocking most of the cancer cells in G2 phase.[20] Pyrazoline analogues are well known in the area of pharmaceutical research for wide range of biological potential like cytotoxic, [21–23] CNS depressant, [24] antimicrobial [25] and antimalarial activity.[26–27] Pyrazole-oxadiazole conjugates **(F, G)** resulted in promising antitumor activity that

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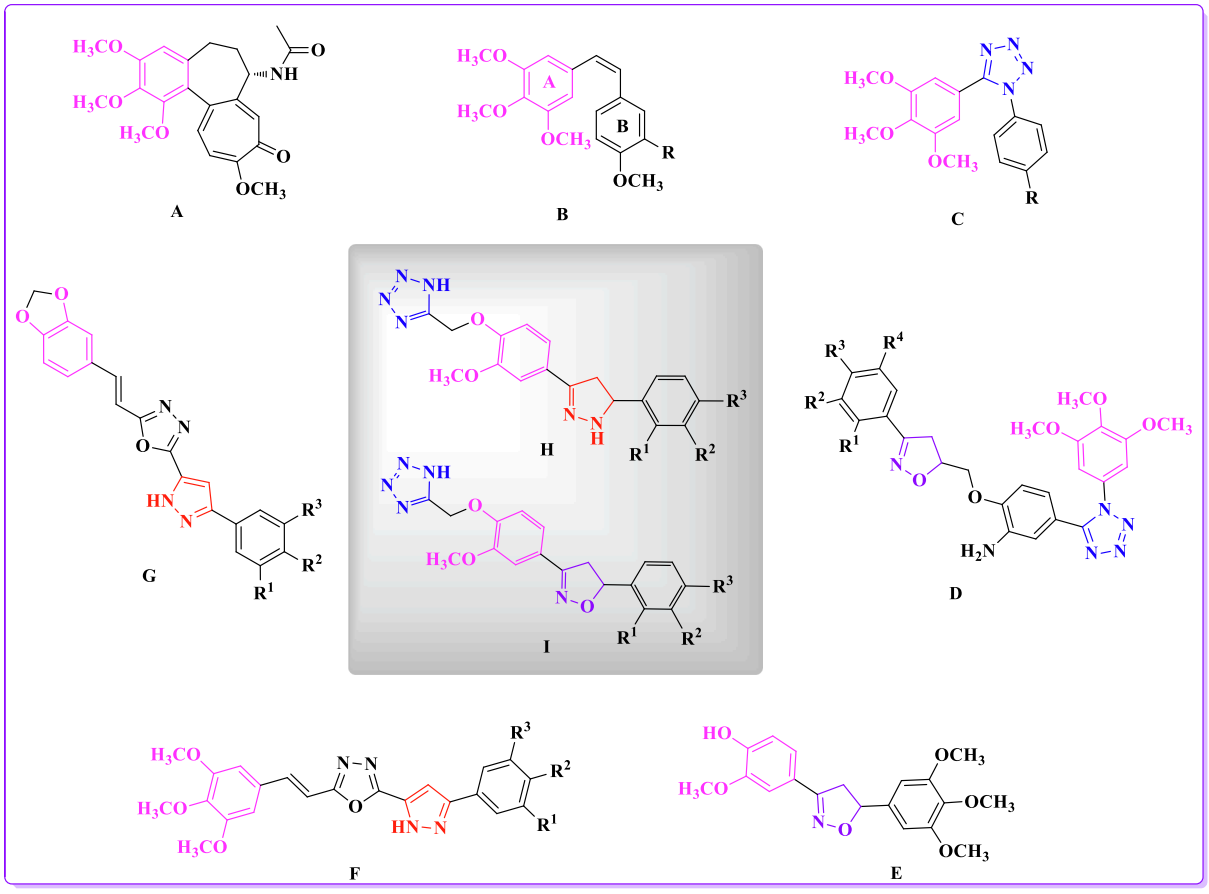


Fig. 1. Chemical structures of microtubule targeting agents: Colchicine (A), CA-4 (B), Tetrazole (C), Tetrazole-Isoxazoline hybrids (D), Isoxazoline (E), Pyrazole-Oxadiazole conjugates (F, G), Tetrazole incorporated Pyrazoline and isoxazoline (H, I).

significantly inhibited tubulin polymerization.[28] Tetrazole scaffold is a unique structure, possessing antihypertensive, anti-allergic, anti-microbial, anti-inflammatory, anticonvulsant, antiamoebic and anticancer activity. [29–36] Tetrazole moiety is generally accepted to exhibit stronger resistance to *in vivo* metabolization than the carboxylate group, thus conferring the bioavailability in blood (see Fig. 1).[37]

Considering the reported literature and in view of need for new anticancer agents, it was of interest to synthesize and evaluate tetrazole based pyrazoline and isoxazoline (**H, I**) for the most common types of cancer found in males (lung cancer) and females (breast cancer). In our previous studies, a series of quinoline based oxadiazole and thiazolidinone derivatives were synthesized for targeting MCF-7 cell line.[38]

Ultrasound irradiation, which accelerates chemical reactions via formation and adiabatic collapse of transient cavitation bubbles, has gained popularity in organic synthesis over traditional methods, because of efficiency and convenience.[39] In continuation of our earlier work of tetrazole derivatives,[40–41] we would like to report synthesis of novel tetrazole based pyrazoline and isoxazoline derivatives using ultrasonic irradiation, evaluation of anticancer activity and molecular docking studies.

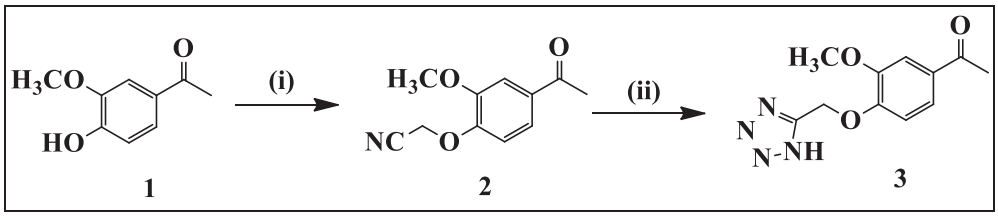
Starting material 1-(4-((1*H*-tetrazol-5-yl)methoxy)-3-methoxyphenyl)ethanone (**3**) was synthesized from 1-(4-hydroxy-3-methoxyphenyl)ethanone (**1**) alkylated with chloroacetonitrile in presence of K_2CO_3 in *N,N'*-dimethyl formamide (DMF) afforded 1-(4-phenoxyacetonitrile-3-methoxyphenyl)ethanone (**2**) in 90% yield, which on further reaction with sodium azide and zinc bromide in water at 100 °C give desired compound (**3**) in 87% yield (Scheme 1).[42]

Target compounds 5-((4-(4,5-dihydro-5- phenyl-1*H*-pyrazol −3-yl) −2-methoxyphenoxy) methyl)-1*H*-tetrazole derivatives (**5a-h**) and 5-((4-(4,5-dihydro-5-phenylisoxazol-3-yl)-2-methoxyphenoxy)methyl)-1*H*-tetrazole derivatives (**6a-h**) were synthesized from Claisen-Schmidt condensation of 1-(4-((1*H*-tetrazol-5-yl)methoxy)-3-methoxyphenyl) ethanone (**3**) with substituted benzaldehydes in presence of a base KOH gives substituted (*E*)-1-(4-((1*H*-tetrazol-5-yl)methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one (**4a-h**) in good yield which on further reacted with hydrazine hydrate in ethanol under ultrasound irradiation led to novel pyrazolines (**5a-h**) with excellent yield. Moreover, substituted (*E*)-1-(4-((1*H*-tetrazol-5-yl)methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one (**4a-h**) reacted with hydroxyl ammonium hydrochloride in ethanol under ultrasound irradiation afforded substituted isoxazolines (**6a-h**) with excellent yield (Scheme 2).

Compounds **4a-h**, **5a-h** and **6a-h** were synthesized with conventional method and ultrasonic irradiation in shorter time, high yield and simple work-up procedure (Table 1). All newly synthesized compounds **4a-h**, **5a-h** and **6a-h** were characterized by spectroscopic techniques viz. ^{1}H NMR, ^{13}C NMR, MS and elemental analysis.[43]

MTT assay[44] was performed to evaluate the cytotoxic effects of all newly synthesized analogues of 5-((4-(4,5-dihydro-5-phenyl-1*H*-pyrazol-3-yl)-2-methoxyphenoxy)methyl)-1*H*-tetrazoles and 5-((4-(4,5-dihydro-5-phenylisoxazol-3-yl)-2-methoxyphenoxy)methyl)-1*H*-tetrazoles against selected human cancer cell lines viz., lung adenocarcinoma (A549), liver (HepG2) and breast (MCF-7), using CA-4 as reference compound. IC_{50} (μM) values are presented in Table 2.

Most of the compounds from these series displayed potent broad spectrum IC_{50} values against all the tested cancer cell lines. In structure activity relationship (SAR) studies, it was considered to investigate the presence of pyrazolines and isoxazolines influence on activity along with tetrazole ring. It was observed that compounds **5a-h** containing pyrazoline ring showed relatively excellent IC_{50} values than isoxazolines with IC_{50} values in range of 0.85–3.16 μM against some

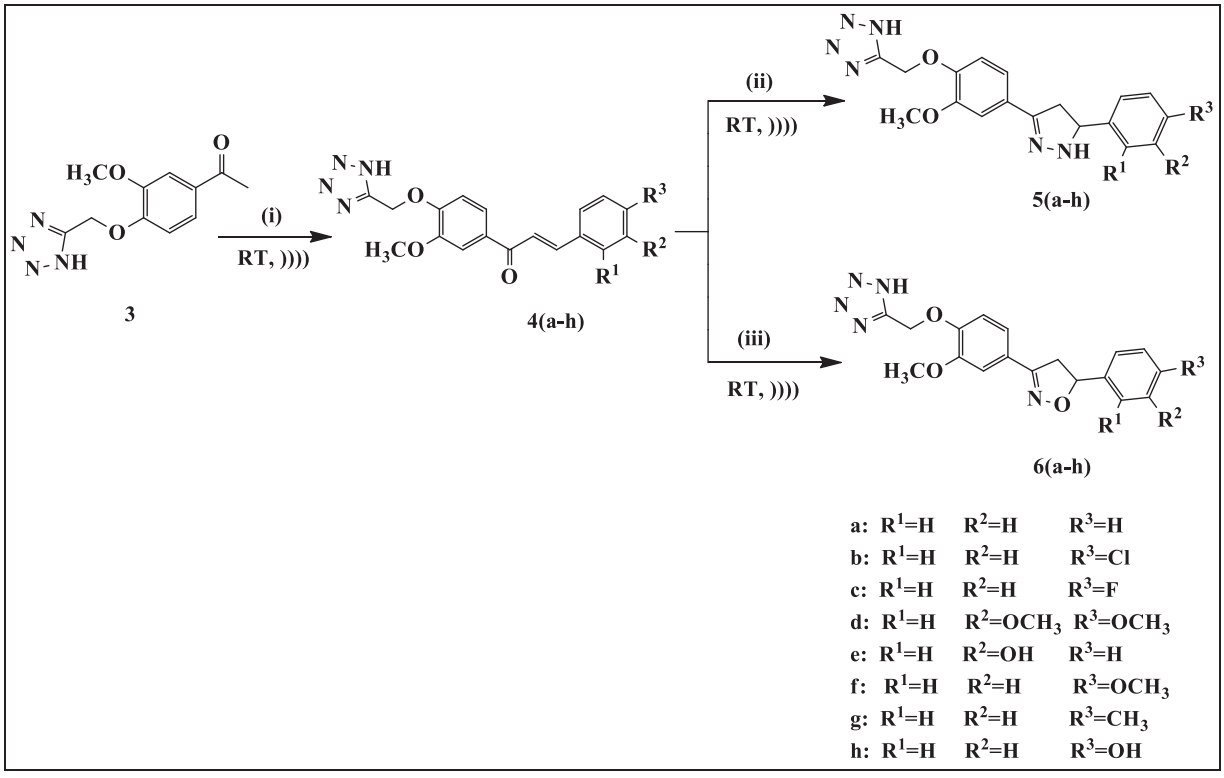


Scheme 1. Reagents and conditions: (i) chloroacetonitrile, DMF, K_2CO_3 (ii) sodium azide, $ZnBr_2$, H_2O, 100 °C.

representative cancer cell lines. Among synthesized derivatives, compound **5c** having fluoro group and **5g** having methyl group exhibited excellent activity with IC_{50} value of 0.94 and 0.99 μM against the lung cancer cell line. Moreover compounds **5b** having chloro and **5c** having fluoro group showed significant activity with IC_{50} value of 0.96 and 0.85 μM against the HepG2 and compound **5c** also exhibited excellent activity with IC_{50} value of 0.92 μM against the MCF-7. Tetrazole based isoxazoline compounds **6a-h** resulted in moderate activity and compound **6g** having electron donating methyl group were the most potent one among synthesized derivatives exhibiting IC_{50} values of 0.78 μM against A549. We then proceeded to explore the influence of substituents on phenyl ring with respect to antiproliferative activity.

Further, all synthesized compounds allowed to investigate SAR effects of electron-withdrawing (F, Cl) as well as electron-releasing substituents like (OMe, Me), on phenyl ring attached to pyrazoline and isoxzoline ring. It was observed that among different substituents studied on phenyl ring attached to pyrazoline ring, fluoro group at para position exhibited significant increase in activity compared to other substituents (H, Me, OMe, OH) irrespective of pyrazoline and isoxazoline rings. Based on these findings, some other substituents were introduced on phenyl ring attached to pyrazoline and isoxazoline to study the effect on antiproliferative activity. Initially, a fluoro group introduced at para position and resulting compound **5c** showed pronounced cytotoxic activity on HepG2 cell line compared to MCF-7 and A549 cell line with IC_{50} value of 0.85 μM having tetrazole incorporated pyrazoline ring. Compound **5g** with IC_{50} values of 0.99 μM and **6g** with IC_{50} values of 0.78 μM having electron donating methyl group at para position exhibited more significant activity against A549 cell line. Introduction of methoxy group in pyrazoline **5f** show moderate activity with IC_{50} values of 2.98–3.12 μM as compared to isoxazoline derivatives **6f** against all cell lines. It is important to notice that, an additional methoxy group viz. disubstitution at meta and para position **5d** having pyrazoline ring, produced better antiproliferative activity with IC_{50} values of 2.82–3.06 μM against all cancer cell lines. Surprisingly, when electron withdrawing substituents fluoro and chloro are incorporated at para position on the phenyl attached to pyrazoline **5c** and **5b** and isoxazoline ring **6b** and **6c**, a substantial increase in cytotoxic potency was observed in all derivatives. On other hand, unsubstituted phenyl ring (R = H) of pyrazoline ring has relatively a stronger influence on activity compared to compounds having isoxazoline moiety. Moreover, introduction of electron–releasing hydroxyl group at meta position on phenyl ring of pyrazoline **5e** showed significant activity with IC_{50} values in range of 1.16–2.88 μM against all cell lines except HepG2 cell line. Compound having hydroxyl group at para position of phenyl ring of pyrazoline **5h** shown excellent activity with IC_{50} values in the range of 1.16–2.02 μM against all cell lines. Interestingly, compounds **5b** and **5c** exhibited promising antiproliferative activity against HepG2 cancer cell line. Effect of heterocyclic rings revealed that, replacement of pyrazoline by isoxazoline ring resulted in lowering the antiproliferative activity.

Overall, SAR studies of these tetrazole based pyrazolines and isoxazolines suggest that, superior activity of pyrazolines compared to



Scheme 2. Reagents and conditions: (i) substituted benzaldehyde, EtOH, NaOH, 15–25 min (ii) $NH_2NH_2{\cdot}H_2O$, EtOH, 10–15 min (iii) $NH_2OH.HCl$, EtOH, 20–25 min.

Table 1
Synthesis of substituted chalcones **4a-h**, pyrazolines **5a-h** and isoxazolines **6a-h** using conventional and ultrasonic irradiation methods.

Entry	Conventional		Ultrasonic Irradiation	
	Time (h)	Yield[a] (%)	Time (min)	Yield (%)
4a	4	69	17	88
4b	3	75	23	90
4c	3	73	20	84
4d	4	64	22	89
4e	4	68	18	81
4f	4	70	21	76
4g	3	60	25	81
4h	4	72	23	85
5a	5	92	10	98
5b	5	95	12	97
5c	5	79	12	96
5d	4	82	14	95
5e	5	78	12	96
5f	5	82	14	95
5g	5	95	12	93
5h	4	83	14	94
6a	3	85	22	98
6b	3	94	20	97
6c	3	92	22	98
6d	4	81	24	96
6e	3	47	20	70
6f	4	52	24	67
6g	3	80	22	97
6h	3	71	22	96

[a] Isolated yield.

Table 2
[a]IC_{50} (μM) values of pyrazoline and isoxazoline derivatives.

Cytotoxicity of synthesized compounds Entry	MCF-7[b]	A549[c]	HepG2[d]
5a	3.16	2.98	3.15
5b	1.04	1.11	0.96
5c	0.92	0.94	0.85
5d	2.94	2.82	3.06
5e	2.16	2.24	2.88
5f	3.10	3.12	2.98
5g	1.76	0.99	1.08
5h	1.94	1.16	2.02
6a	12.5	6.25	6.25
6b	6.25	6.25	12.5
6c	3.12	3.12	3.12
6d	25	12.5	3.12
6e	6.25	25	1.56
6f	12.5	6.25	12.5
6g	12.5	0.78	6.25
6h	25	3.12	6.25
CA-4	0.04	0.05	0.009

[a]50% Inhibitory concentration; [b]Breast cancer cell line; [c]Lung cancer cell line; [d]Liver cancer cell line.

isoxazolines is in agreement with exhibited excellent activity.

Microtubules exhibit dynamic equilibrium with free tubulin monomers in cells. Pharmacological agents exploit this property of microtubules to exert their anticancer effects.[45] Since these inhibit tubulin polymerization and disturbs microtubule dynamics, we elucidated levels of soluble versus polymerized forms of tubulin in A549 cells following treatment with 1 μM of **5b** and **5c** for 48 h. In addition, cells were treated with Combretastatin A-4 (**CA-4**, 1 μM) as positive controls and DMSO as negative in parallel experiments. Subsequently, these soluble and polymerized fractions were collected and subjected to western blot analysis. This analysis reveals that, amount of tubulin protein in both soluble and polymerized fractions were approximately same in DMSO treated cells. Combretastatin A-4 treated cells exhibited a shift of tubulin from the polymerized fraction into the soluble fraction. As expected, cells treated with **5b** and **5c** significantly increased the tubulin content in soluble fraction. Specifically, **5c** treated cells showed a more distinct shift in tubulin balance, with almost all tubulin present in the soluble fraction similar to that of positive control. Therefore, increased tubulin in soluble fraction of cells treated by these hybrids corroborated with inhibition of tubulin assembly as shown in Table 3 and Fig. 2.

Table 3
Inhibition of tubulin polymerization (IC_{50}) of **5b**, **5c** and **CA-4**.

Compound	IC_{50}[a] ± SD (in μM)	% of Tubulin inhibition
5b	2.94 ± 0.08	62.56
5c	2.16 ± 0.12	65.44
CA-4	1.62 ± 0.18	74.24

[a] Concentration of drug to inhibit 50% of tubulin assembly. Values indicated are the mean ± SD of two different experiments performed in triplicates.

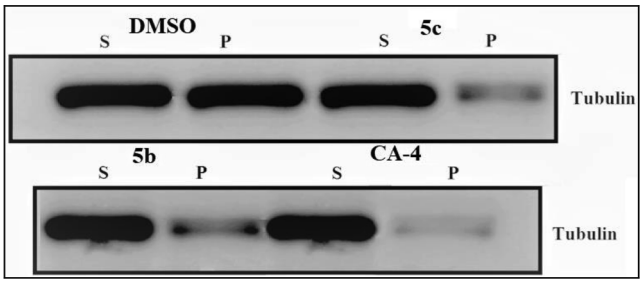


Fig. 2. A549 cells were treated with 1 μM of 5b and 5c for 48 h. CA-4 was used as reference standard. Levels of tubulin were detected by western blot analysis (S: Solubilised fraction; P: Polymerised fraction).

To investigate and clarify binding mode of tetrazole based pyrazolines and isoxazoline analogues, molecular docking studies were performed to colchicine binding site of α,β-tubulin (PDB: 1SA0) by using Glide XP docking mode.[46] The result was shown in (Fig. 3 and Fig. 4). Superimposition of all compounds over colchicine in tubulin structure showed that all compounds firmly occupied the colchicine binding site of α,β-tubulin mostly buried in the β subunit (Fig. 3). Docking scores of compounds varied from −9.66 to −6.38. Relative binding free energies of docked compounds were also calculated, varied from −72.95 to −48.14. Tetrazole ring is seen as coplanar to amino acid residue Val 238 and thus could act as hydrogen bond donor in all compounds. 2-methoxy group of centre phenyl ring is a promising hydrogen bond acceptor of the thiol proton of Cys 241 (Fig. 4). Docking results also revealed that pyrazoline/isoxazoline and substituted phenyl group of all compound was bound into the hydrophobic pocket of tubulin consisting of the amino acid residues; Asn 258, Met 259, Val 315, Ala316, Val318, Asn 350, Val 351 and Lys 352. In some compounds, substituted phenyl group showed aromatic hydrogen bonding with Asn 258 and Asn 350 (Fig. 4A).

In conclusion, an efficient and convenient route for synthesis of new tetrazole based pyrazoline and isoxazoline derivatives has been reported. The process also exhibits significant functional group tolerance and allows for the preparation of number of substituted tetrazole analogues in good to excellent yields. Importance of substituted tetrazole analogues would render this protocol attractive for both synthetic and medicinal chemistry. Among synthesized derivatives, compounds **5b**, **5c** and **6g** were significantly cytotoxic against MCF-7, A549 and HepG2 cancer cell lines at micromolar concentrations. Compound **5c** inhibited tubulin polymerization with IC_{50} value of 2.16. In silico docking studies of synthesized compounds showed binding affinity toward colchicine binding site of α,β-tubulin with highest score of −9.66. Consequently, reported derivatives may serve as lead scaffold for development of novel anticancer agents.

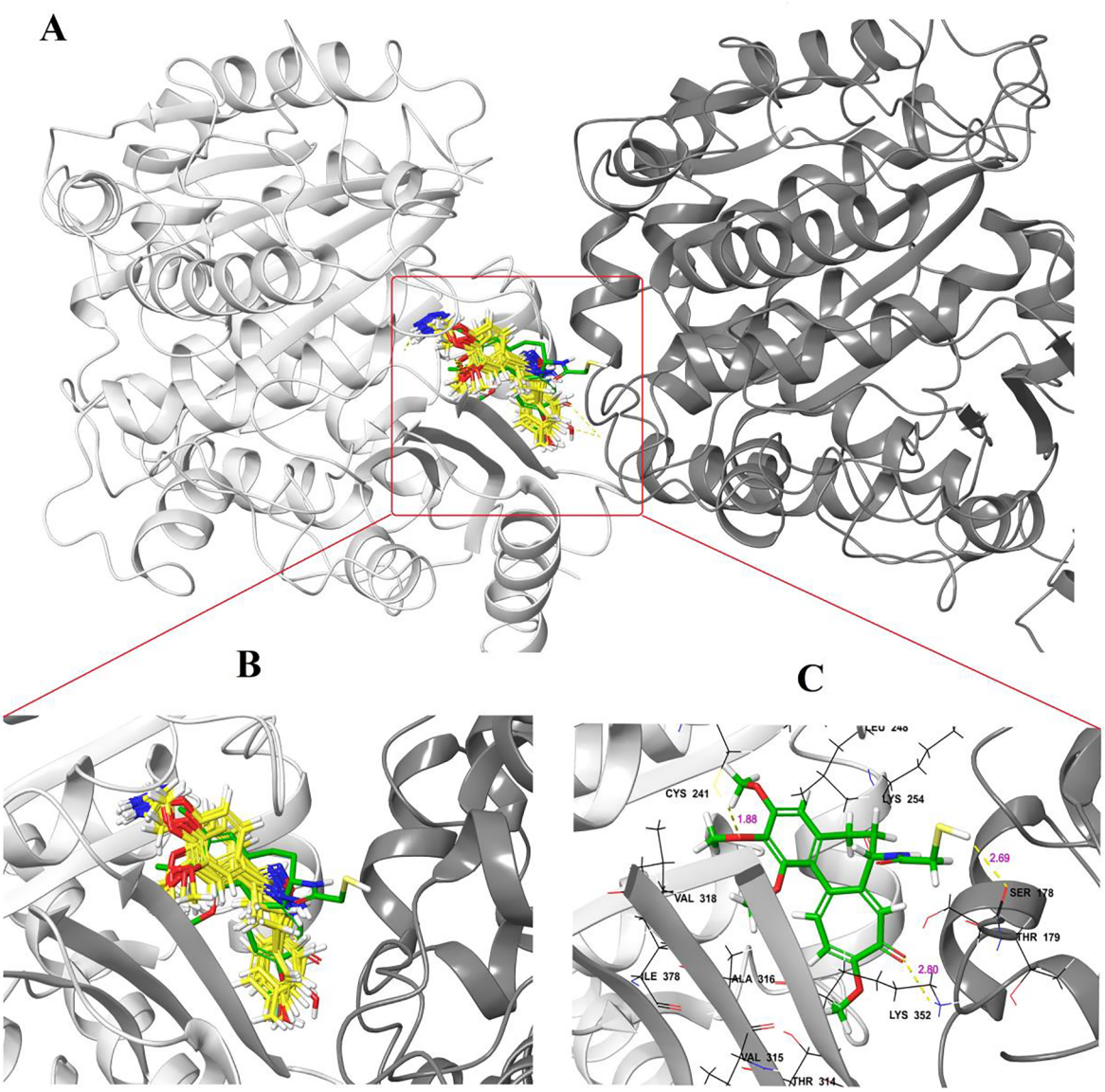


Fig. 3. Structure of α,β-tubulin–ligand complex. (A) Pictorial representation of the overall α,β-tubulin along with superimposition of all compounds over Colchicine at the Colchicine binding Site. α subunit of tubulin is colored in grey and the β subunit is coloured in white. (B) Close-up view of superimposition of all compounds (shown in yellow sticks) over Colchicine (shown in green sticks) at the Colchicine binding Site. (C) The interaction mode of Colchicine at active site of α,β-tubulin Yellow colored dotted lines indicate H-bond interaction.

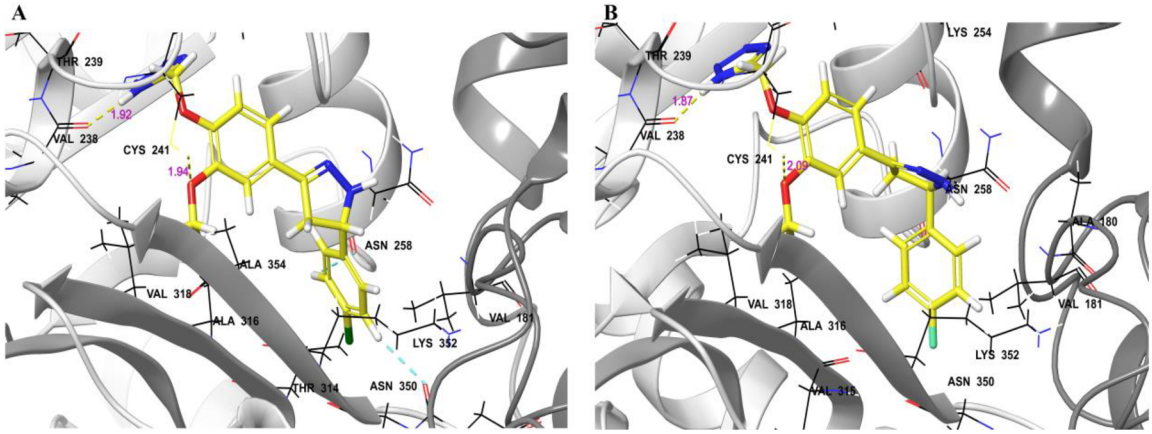


Fig. 4. The interaction mode of (A) Compound **5b** and (B) Compound **5c** with α,β-Tubulin at the Colchicine Binding Site. Yellow coloured dotted lines indicate H-bond interaction and Cyan coloured dotted lines indicate aromatic H-bond interaction between ligand and enzyme.

Declaration of Competing Interest

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

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127592.

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42. General procedure for the synthesis of 1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)ethanone 3: A solution of 1-(4-phenoxyacetonitrile-3-methoxyphenyl)ethanone 2 (1 mmol), sodium azide (1.5 mmol) and zinc bromide (1.5 mmol) in water was stirred at 100°C for 4 h, mixture was poured into aqueous hydrochloric acid. Precipitate was recovered by filtration and recrystallised from ethanol to give desired

product as white crystals. Yield: 84 %; mp 146-148 °C; 1H NMR (500 MHz, DMSO-d6, δH ppm): 2.60 (s, 3H, COCH3), 3.89 (s, 3H, OCH3), 5.62 (s, 2H, OCH2), 7.27 (d, 1H, ArH), 7.54 (s, 1H, ArH), 7.68 (d, 1H, ArH). 13C NMR (125 MHz, DMSO-d6, δC ppm): 26.9, 56.1, 60.2, 111.2, 113.4, 113.5, 123.2, 131.6, 149.4, 151.4, 197.0. HRMS Anal. calcd. for C11H12N4O3 (M+H)+: 249.0988. Found: 249.0982. Anal. calcd. for C11H12N4O3: C, 53.22; H, 4.87; N, 22.57. Found: C, 53.19; H, 4.81; N, 22.62.

43. General procedure for the synthesis of substituted (E)-1-(4-((1H-tetrazol-5-yl) methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one 4a-h: Conventional method: To a solution of KOH (0.03 mol) in ethanol (15 mL) was added 1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)ethanone 3 (0.01 mol) and substituted benzaldehyde (0.01 mol) at 0-5°C. The reaction mixture was stirred 3-4 h at room temperature. Then, reaction mixture was poured over crushed ice and neutralize carefully with 2N HCl and precipitate so obtained was filtered, washed with water and dried. The crude product was crystallized with ethanol to afford desired compound. Sonochemical method: To a solution of KOH (0.03 mol) in ethanol (15 mL) was added 1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)ethanone 3 (0.01 mol) and substituted benzaldehyde (0.01 mol), and resulting mixture subjected to ultrasonic irradiation for 20-25 min. After completion, resulting mixture was poured into ice-cold water and then neutralized with 2N HCl. Solid obtained was filtered off, dried and purified by recrystallization from ethanol. Compound (4a): white solid; mp 166-168°C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 7. 13 (d, 1H, olefinic proton), 7.36–7.38 (m, 3H, ArH), 7.55–7.59 (m, 4H, ArH), 7.67 (d, 1H, olefinic proton), 7.95 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 56.0, 61.5, 112.6, 114.0, 122.9, 123.7, 127.2, 128.8, 129.1, 130.2, 131.4, 132.4, 135.1, 144.3, 150.2, 152.8, 155.7, 190.0. HRMS Anal. calcd. for C18H16N4O3 (M+H)+: 337.1301. Found: 337.1292. Anal. calcd. for C18H16N4O3: C, 64.28; H, 4. 79; N, 16.66. Found: C, 64.26; H, 4.83; N, 16.71. Compound (4b): white solid; mp 132-134 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 7.13 (d, 1H, olefinic proton), 7.42-7.44 (m, 2H, ArH), 7.54-7.57 (m, 4H, ArH), 7.67 (d, 1H, olefinic proton), 7.77 (dd, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 56.0, 61.5, 112.6, 114.0, 123.3, 123.7, 126.3, 129.4, 129.9, 132.4, 134.0, 134.8, 140.3, 144.0, 150.2, 152.8, 155.7, 190.0. HRMS Anal. Calcd. For C18H15ClN4O3 (M+H)+: 371.0911. Found: 371.091. Anal. calcd. for C18H15ClN4O3: C, 58.31; H, 4.08; N, 15.11. Found: C, 58.28; H, 4.12; N, 15.04. Compound (4c): white solid; mp 152-154 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.89 (s, 3H, OCH3), 5.05 (s, 2H, OCH2), 7.02 (d, 1H, olefinic proton), 7.20 (dd, 2H, ArH), 7.52 (d, 1H, ArH), 7.56-7.58 (m, 2H, ArH), 7.62 (d, 1H ArH), 7.67 (dd, 1H, ArH), 7.77 (d, 1H, olefinic proton). 13C NMR (125 MHz, chloroform-d, δC ppm): 55.9, 61.7, 112.7, 113.9, 115.8, 122.7, 123.9, 130.4, 130.5, 131.7, 132.4, 144.2, 150.2, 152.7, 156.3, 162.5, 164.5, 190.0. HRMS Anal. Calcd. For C18H15FN4O3 (M+H)+: 355.1206. Found: 355.1202. Anal. calcd. for C18H15FN4O3: C, 61.01; H, 4. 27; N, 15.81. Found: C, 60.92; H, 4.36; N, 15.98. Compound (4d): white solid; mp 176-178 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.84 (s, 3H, OCH3), 3.85 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 6.88 (d, 1H, ArH), 7.13 (d, 1H, olefinic proton), 7.21 (d, 1H, olefinic proton), 7.24 (dd, 1H, ArH), 7.52–7.59 (m, 2H, ArH), 7.68 (dd, 1H, ArH), 7.77–7.81(m, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 55.9, 55.9, 56.0, 61.5, 111.1, 112.5, 112.6, 114.0, 122.4, 123.1, 123.7, 129.8, 132.4, 144.0, 150.2, 150.5, 151.0, 152.8, 155.7, 189.9. HRMS Anal. Calcd. For C20H20N4O5 (M+H)+: 397.1512. Found: 397.1501. Anal. calcd. for C20H20N4O5: C, 60.60; H, 5.09; N, 14.13. Found: C, 60.43; H, 5.31; N, 14.18. Compound (4e): white solid; mp 190-192 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 6.84 (d, 1H, ArH), 7.09 (d, 1H, ArH), 7.13 (d, 1H, olefinic proton), 7.20-7.26 (m, 1H, ArH), 7.30 (d, 1H, ArH), 7.52-7.59 (m, 2H, ArH), 7.67 (d, 1H, olefinic proton), 7.72–7.79 (m, 1H, ArH), 8.40 (s, 1H, OH). 13C NMR (125 MHz, chloroform-d, δC ppm): 56.0, 61.5, 112.6, 114.0, 116.0, 117.8, 122.3, 122.8, 123.7, 130.3, 132.4, 137.5, 143.2, 150.2, 152.8, 155.7, 158.5, 189.9. ESI-MS Anal. calcd. for C18H16N4O4 (M+H)+: 353.125. Found: 353. 12Anal. calcd. for C18H16N4O4: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.31; H, 4. 62; N, 15.88. Compound (4f): white solid; mp 158-160 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.82 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 6.92–6.94 (m, 2H, ArH), 7.12 (s, 1H, ArH), 7.56–7.59 (m, 4H, ArH), 7.68 (d, 1H, olefinic proton), 7.78 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 55. 3, 56.0, 61.5, 112.6, 114.0, 114.0 114.3, 120.6, 123.3, 123.7, 128.9, 130.4, 132.4, 144.1, 150.2, 152.8, 155.7, 161.5, 190.0. HRMS Anal. calcd. for C19H18N4O4 (M+H)+: 367.1406. Found: 367.1407. Anal. calcd. for C19H18N4O4: C, 62.29; H, 4. 95; N, 15.29. Found: C, 62.14; H, 5.24; N, 15.22. Compound (4g): white solid; mp 124-126 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 2.38 (s, 3H, CH3), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 7.13 (d, 1H, olefinic proton), 7.24-7.29 (m, 2H, ArH), 7.45-7.51 (m, 2H, ArH), 7.53-7.59 (m, 2H, ArH), 7.67 (d, 1H, olefinic proton), 7.78 (dd, 1H, ArH); 13C NMR (125 MHz, chloroform-d, δC ppm): 21.4, 56.0, 61.5, 112.6, 114.0, 120.3, 123.3, 123.7, 129.6, 129.9, 132.4, 133.1, 138.3, 139.8, 144.2, 150.2, 152.8, 155.7, 190.0. HRMS Anal. Calcd. For C19H18N4O3 (M+H)+: 351. 1457. Found: 351.1451 Anal. calcd. for C19H18N4O3: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.08; H, 5.22; N, 16.08. Compound (4h): white solid; mp 156-158 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 6. 08 (s, 1H, OH), 6.79–6.81 (m, 2H, ArH), 7.13 (d, 1H, ArH), 7.51–7.57 (m, 4H, ArH), 7.67 (d, 1H, olefinic proton), 7.78 (d, 1H, olefinic proton). 13C NMR (125 MHz, DMSO-d6, δC ppm): 56.0, 61.5, 112.6, 114.0, 116.8, 120.1, 123.3, 123.7, 127.5, 131. 1, 132.4, 135.6, 144.3, 150.2, 152.8, 155.7, 160.0, 190.0. HRMS Anal. calcd. for C18H16N4O4 (M+H)+: 353.125. Found: 353.1244. Anal. calcd. for C18H16N4O4: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.17; H, 4.83; N, 15.84. General procedure for the synthesis of substituted 5-((4-(4,5-dihydro-5-phenyl-1H-pyrazol-3-yl)-2-methoxyphenoxy)methyl)-1H-tetrazole 5a-h: Conventional method: To a solution of substituted (E)-1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one (0.01 mol) 4a-h in 10 mL of ethanol, (0.05 mol) of hydrazine hydrate (99%) was added dropwise. The reaction mixture was heated under reflux for 4-5 h and progress of reaction monitored by TLC. After completion of the reaction, resulting solution was cooled and poured into crushed ice. Solid pyrazolines 5a-h were filtered and recrystallized from ethanol. Sonochemical method: To a solution substituted (E)-1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one (0.01 mol) 4a-h in 10 mL of ethanol, (0.05 mol) of hydrazine hydrate (99%) was added dropwise. Reaction mixture was suspended at centre of the ultrasonic bath and sonicated for 10-15 min. After completion of reaction, separated solid was collected by filtration, washed with water and recrystallized from ethanol. Compound (5a): yellow solid; mp 131-133 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.21 (dd, 1H, pyrazoline CH2), 3.36 (dd, 1H, pyrazoline CH2), 3.88 (s, 3H, OCH3), 4.84 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 7.07 (d, 1H, ArH), 7.25–7.36 (m, 7H, ArH), 7.86 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.7, 56.0, 58.8, 61.5, 107.3, 113.2, 118.7, 122.7, 125.7, 126.7, 127.6, 128.5, 132.2, 141.4, 148.7, 150.5, 151.6, 155.7. HRMS Anal. calcd. for C18H18N6O2 (M+H)+: 351.1569. Found: 351. 1564. Anal. calcd. for C18H18N6O2: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.58; H, 5.46; N, 23.94. Compound (5b): yellow solid; mp 116-118 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.21 (dd, 1H, pyrazoline CH2), 3.36 (dd, 1H, pyrazoline CH2), 3.88 (s, 3H, OCH3), 4.83 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 7.08 (d, 1H, ArH), 7.25– .27 (m, 3H, ArH), 7.30 (d, 1H, ArH), 7.33–7.35 (m, 2H, ArH), 7.85 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm):41.7, 56.0, 58.3, 61.5, 107.3, 113.2, 118.7, 125.4, 126.7, 128.0, 128.6, 132.5, 133.4, 140.1, 148.7, 150.5, 151.6, 155.7. HRMS Anal. calcd. for C18H17ClN6O2 (M+H)+: 385.118. Found: 385.1174. Anal. calcd. for C18H17ClN6O2: C, 56.17; H, 4.45; N, 21.88. Found: C, 56. 03; H, 4.70; N, 21.78. Compound (5c): yellow solid; mp 188-190 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.21 (dd, 1H, pyrazoline CH2), 3.36 (dd, 1H, pyrazoline CH2), 3.88 (s, 3H, OCH3), 4.83 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 7. 00-7.03 (m, 2H, ArH), 7.07 (d, 1H, ArH), 7.25–7.28 (m, 1H, ArH), 7.30-7.32 (m, 2H, ArH), 7.86 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.6, 56.0, 58. 6, 61.5, 107.3, 113.2, 115.5, 115.7, 118.7, 126.7, 127.9, 127.9, 138.3, 138.3, 148.7, 150.5, 151.6, 155.7, 161.3, 163.3. HRMS Anal. calcd. for C18H17FN6O2 (M+H)+: 369.1475. Found: 369.1476. Anal. calcd. for C18H17FN6O2: C, 58.69; H, 4.65; N, 22.81. Found: C, 59.15; H, 4.74; N, 24.34. Compound (5d): yellow solid; mp 160-162 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.23 (dd, 1H, pyrazoline CH2), 3.34 (dd, 1H, pyrazoline CH2), 3.82 (s, 3H, OCH3), 3.83 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 4.87 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 6.88 (d, 1H, ArH), 6.91–6. 94 (m, 1H, ArH), 7.07 (d, 1H, ArH), 7.25–7.30 (m, 2H, ArH), 7.90 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.6, 55.9, 56.0, 58.3, 61.5, 107.3, 110.3, 112.4, 113.2, 118.7, 120.3, 122.7, 126.7, 131.4, 148.3, 148.7, 149.4, 150.5, 151.6, 155.7. HRMS Anal. calcd. for C20H22N6O4 (M+H)+: 411.1781. Found: 411.1775. Anal. calcd. for C20H22N6O4: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.32; H, 5.62; N, 20.45. Compound (5e): yellow solid; mp 210-212 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.23 (dd, 1H, pyrazoline CH2), 3.33 (dd, 1H, pyrazoline CH2), 3.88 (s, 3H, OCH3), 4.86 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 6.70 (ddd, 1H, ArH), 6.77 (td, 1H, ArH), 6.99 (ddt, 1H, ArH), 7.07 (d, 1H, ArH), 7.12 (t, 1H, ArH), 7.26 (dd, 1H, ArH), 7.30 (d, 1H, ArH), 7.91 (d, 1H, ArH), 8.25 (s, 1H, OH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.6, 56.0, 58.5, 61.5, 107.3, 113.2, 113.4, 116.0, 118.7, 119.1, 126.7, 130.1, 144.4, 148.7, 150.5, 151.6, 155.7, 158.0. HRMS Anal. calcd. for C18H18N6O3 (M+H)+: 367.1519. Found: 367.1517. Anal. calcd. for C18H18N6O3: C, 59.01; H, 4.95; N, 22.94. Found: C, 59.12; H, 4.87; N, 23. 07. Compound (5f): yellow solid; mp 170-172 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.21 (dd, 1H, pyrazoline CH2), 3.36 (dd, 1H, pyrazoline CH2), 3.78 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 4.84 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 6.77-6. 83 (m, 2H, ArH), 7.07 (d, 1H, ArH), 7.24-7.34 (m, 4H, ArH), 7.86 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.6, 55.3, 56.0, 58.6, 61.5, 107.3, 110.3, 113.2, 113.8, 118.7, 126.7, 126.9, 129.3, 136.5, 148.7, 150.5, 151.6, 155.7, 159.1. HRMS Anal. calcd. for C19H20N6O3 (M+H)+: 381.1675. Found: 381.1670. Anal. calcd. for C19H20N6O3: C, 59.99; H, 5.30; N, 22.09. Found: C, 60.12; H, 5.39; N, 21. 87. Compound (5g): yellow solid; mp 148-150 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 2.34 (s, 3H, CH3), 3.21 (dd, 1H, pyrazoline CH2), 3.36 (dd, 1H, pyrazoline CH2), 3.88 (s, 3H, OCH3), 4.83 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 7.06-7.08 (m, 2H, ArH), 7.20–7.21 (m, 2H, ArH), 7.23–7.26 (m, 1H, ArH), 7.86 (d, 2H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 21.1, 41.7, 56.0, 58.8, 61.5, 107.3, 13. 2, 118.7, 122.7, 126.1, 126.7, 129.4, 133.0, 138.1, 139.7, 148.7, 150.5, 151.6, 155. 7. HRMS Anal. calcd. for C19H20N6O2 (M+H)+: 365.1726. Found: 365.1721. Anal. calcd. for C19H20N6O2: C, 62.62; H, 5.53; N, 23.06. Found: C, 62.43; H, 5.78; N, 23.05. Compound (5h): yellow solid; mp 128-130 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.21 (dd, 1H, pyrazoline CH2), 3.36 (dd, 1H, pyrazoline CH2), 3.88 (s, 3H, OCH3), 4.84 (t, 1H, pyrazoline CH), 5.30 (s, 2H, OCH2), 6.68- 6. 72 (m, 2H, ArH), 6.96 (s, 1H, ArH), 7.07 (d, 1H, ArH), 7.20 – 7.21 (m, 2H, ArH), 7. 25-7.30 (m, 1H, ArH), 7.86 (d, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.6, 56.0, 58.7, 61.5, 107.3, 113.2, 115.6, 118.7, 122.2, 126.7, 127.7, 133.6, 135.6, 148.7, 150.5, 151.6, 155.7, 157.7. HRMS Anal. calcd. for C18H18N6O3 (M+H)+: 367.1519. Found: 367.1513. Anal. calcd. for C18H18N6O3: C, 59.01; H, 4. 95; N, 22.94. Found: C, 58.83; H, 5.26; N, 22.81. General procedure for the synthesis of substituted 5-((4-(4,5-dihydro-5-phenylisoxazol-3-yl)-2-methoxyphenoxy)methyl)-1H-tetrazole 6a-h: Conventional method: To a solution of substituted (E)-1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one (0.01 mol) 4a-h in 10 mL of ethanol, (0.05 mol) of hydroxylamine hydrochloride was added dropwise. Reaction mixture was heated under reflux for 4-5 h and progress of the reaction monitored by TLC. After completion of reaction, resulting solution was cooled and poured into crushed ice. Solid pyrazolines 6a-h were filtered and recrystallized from ethanol. Sonochemical method: To a solution substituted (E)-1-(4-((1H-tetrazol-5-yl)methoxy)-3-methoxyphenyl)-3-phenylprop-2-en-1-one (0.01 mol) 4a-h in 10 mL of ethanol, (0.05 mol) of hydroxylamine hydrochloride was added dropwise. Reaction mixture was suspended at centre of the ultrasonic bath and

sonicated for 10-15 min. After completion of reaction, separated solid was collected by filtration, washed with water and recrystallized from ethanol. Compound (6a): yellow solid; mp 140-142 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.67–3.74 (m, 2H, isoxazoline CH2), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 5.79-5.81 (t, 1H, isoxazoline CH), 7.01 (d, 1H, ArH), 7.26-7.35 (m, 5H, ArH), 7.40–7.41 (m, 2H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 56.0, 61.5, 79.8, 110.1, 113.4, 121.9, 123.0, 125.6, 126.5, 128.4, 128.5, 132.8, 141.0, 149.1, 150.7, 155.1, 155.7. HRMS Anal. calcd. for C18H17N5O3 (M + H) + : 352.141. Found: 352.1402. Anal. calcd. for C18H17N5O3: C, 61.53; H, 4.88; N, 19.93. Found: C, 61.34; H, 5.12; N, 19.86. Compound (6b): yellow solid; mp 126-128 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.68–3.73 (m, 2H, isoxazoline CH2), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 5.78 (t, 1H, isoxazoline CH), 7.01 (d, 1H, ArH), 7.26 (d, 1H, ArH), 7.32–7.27 (m, 1H, ArH), 7.35–7.29 (m, 1H, ArH), 7.32 (s, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 56.0, 61.5, 76.3, 110.1, 113.4, 121.9, 124.3, 125.6, 127.9, 128.8, 131.4, 134.1, 138.2, 149.1, 150.7, 155.1, 155.7. HRMS Anal. calcd. for C18H16ClN5O3 (M + H) + : 386.102. Found: 386.1014. Anal. calcd. for C18H16ClN5O3: C, 56.04; H, 4.18; N, 18.15. Found: C, 55.89; H, 4.43; N, 18.11. Compound (6c): yellow solid; mp 138-140 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.75–3.65 (m, 2H, isoxazoline CH2), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 5.79 (dd, 1H, isoxazoline CH), 7.01 (d, 1H, ArH), 7.07–7.10 (m, 1H, ArH), 7.26 (d, 1H, ArH), 7.29 (dd, 1H, ArH), 7.38–7.41 (m, 2H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 56.0, 61.5, 110.1, 113.4, 115.8, 115.9, 121.9, 125.6, 127.7, 127.8, 135.7, 135.7, 149.1, 150.7, 155.1, 155.7, 162.1, 164.2. HRMS Anal. calcd. for C18H16FN5O3 (M + H) + : 370.1315. Found: 370.1313. Anal. calcd. for C18H16FN5O3: C, 58.53; H, 4.37; N, 18.96. Found: C, 58.36; H, 4.63; N, 18.92. Compound (6d): yellow solid; mp 120-122 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.71 (dd, 2H, isoxazoline CH2), 3.81 (s, 3H, OCH3), 3.82 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 5.30 (s, 1H, OCH2), 5.79 (t, 1H, isoxazoline CH), 7.00 (d, 1H, ArH), 7.06–7.10 (m, 3H, ArH), 7.26 (d, 1H, ArH), 7.29 (dd, 1H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 55.9, 56.0, 61.5, 81.5, 110.1, 110.6, 112.6, 113.4, 120.3, 121.9, 125.6, 128.5, 132.7, 148.9, 149.1, 149.1, 150.7, 155.1, 155.7. HRMS Anal. calcd. for C20H21N5O5 (M + H) + : 412.1621. Found: 412.1612. Anal. calcd. for C20H21N5O5: C, 58.39; H, 5.14; N, 17.02. Found: C, 58.26; H, 5.30; N, 16.86. Compound (6e): yellow solid; mp 168-170 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.70 (dd, 2H, isoxazoline CH2), 3.88 (s, 3H, OCH3), 5.30 (s, 1H, OCH2), 5.70 (t, 1H, isoxazoline CH), 6.69–6.71 (m, 1H, ArH), 6.83 (dd, 1H, ArH), 7.01 (d, 1H, ArH), 7.16–7.21 (m, 2H, ArH), 7.26 (d, 1H, ArH), 7.29 (dd, 1H, ArH), 8.46 (s, 1H, OH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 56.0, 61.5, 81.8, 110.1, 113.4, 114.4, 115.8, 118.3, 121.9, 125.6, 130.1, 139.1, 149.1, 150.7, 155.1, 155.7, 157.5. HRMS Anal. calcd. for C18H17N5O4 (M + H) + : 368.1359. Found: 368.1358. Anal. calcd. for C18H17N5O4: C, 58.85; H, 4.66; N, 19.06. Found: C, 58.69; H, 4.93; N, 19.01. Compound (6f): yellow solid; mp 130-132 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.67–3.74 (m, 2H, isoxazoline CH2), 3.78 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 5.79 (dd, 1H, isoxazoline CH), 6.83–6.90 (m, 2H, ArH), 7.01 (d, 1H, ArH), 7.26–7.30 (m, 4H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 55.3, 56.0, 61.5, 76.4, 110.1, 113.4, 113.9, 118.7, 121.9, 125.6, 127.3, 130.3, 133.6, 149.1, 150.7, 155.1, 155.7, 159.8. HRMS Anal. calcd. for C19H19N5O4 (M + H) + : 382.1515. Found: 382.1512. Anal. calcd. for C19H19N5O4: C, 59.84; H, 5.02; N, 18.36. Found: C, 59.55; H, 5.40; N, 18.35. Compound (6g): yellow solid; mp 152-154 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 2.34 (s, 2H, CH3), 3.68–3.74 (m, 2H, isoxazoline CH2), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 5.79 (dd, 1H, isoxazoline CH), 7.01 (d, 1H, ArH), 7.15 (dq, 2H, ArH), 7.26–7.30 (m, 5H, ArH). 13C NMR (125 MHz, chloroform-d, δC ppm): 21.1, 41.1, 56.0, 61.5, 76.5, 110.1, 113.4, 121.1, 121.9, 125.6, 126.6, 129.6, 137.6, 137.6, 137.9, 149.1, 150.7, 155.1, 155.7. HRMS Anal. calcd. for C19H19N5O3 (M + H) + : 366.1566. Found: 366.1561. Anal. calcd. for C19H19N5O3: C, 62.46; H, 5.24; N, 19.17. Found: C, 62.28; H, 5.56; N, 19.08. Compound (6h): yellow solid; mp 102-104 °C; 1H NMR (500 MHz, chloroform-d, δH ppm): 3.67–3.74 (m, 2H, isoxazoline CH2), 3.88 (s, 3H, OCH3), 5.30 (s, 2H, OCH2), 5.79 (dd, 1H, isoxazoline CH), 6.78–6.80 (m, 2H, ArH), 7.01 (d, 1H, ArH), 7.26–7.28 (m, 3H, ArH), 7.29 (dd, 1H, ArH), 8.17 (s, 1H, OH). 13C NMR (125 MHz, chloroform-d, δC ppm): 41.1, 56.0, 61.5, 76.5, 110.1, 113.4, 115.7, 119.5, 121.9, 125.6, 127.3, 129.0, 132.5, 149.1, 150.7, 155.1, 155.7, 158.2. HRMS Anal. calcd. for C18H17N5O4 (M + H) + : 368.1359. Found: 368.1351. Anal. calcd. for C18H17N5O4: C, 58.85; H, 4.66; N, 19.06. Found: C, 58.72; H, 4.90; N, 18.89.

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