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Design, Synthesis, Molecular Docking Studies, and Biological Evaluation of Pyrazoline Incorporated Isoxazole Derivatives

T. Radhika[a, b, 1], A. Vijay[a], B. V. Harinadha[a], and B. Madhavareddy[a]

[a] *Department of Pharmaceutical Chemistry, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, Telangana, 500028 India*

[b] *Department of pharmacy, University College of Technology, Osmania University, Hyderabad, Telangana, India*

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Abstract—A novel series of pyrazoline incorporated isoxazole derivatives were designed and synthesized. The synthesized compounds were characterized by ^{1}H NMR, IR and ESI-MS spectra. In addition, all the synthesized compounds were docked with the target human DHFR (PDB ID: 1KMS). Among all the compounds, compound 5-(4-methoxyphenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone proved to be the most potent exhibiting the highest binding affinity with a docking score of 153.763. All the synthesized compounds were screened for anticancer activity against human breast cancer cell lines MCF-7 and MDA-MB-231 through MTT assay. Out of all the synthesized compounds (5-(4-methoxyphenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone posses good activity with IC_{50} values ranging from 3–4 μg/mL. Further all the compounds were screened for antitubercular assay against the strain H_{37}Rv and multidrug resistant strain DKU 156, among all four compounds exhibited significant activity at 6.25 μg/mL concentrations. Thus the MIC value may be in between the range of 3.12 and 6.25 μg/mL.

Keywords: anticancer, binding affinity, docking, isoxazole, pyrazoles, antitubercular

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INTRODUCTION

Cancer is the second leading cause of mortality worldwide [1], and currently available anticancer drugs lead to several side effects with drug resistance [2]. Therefore, there is an extreme necessity to discover and develop novel anticancer agents with enhanced tumor selectivity, safety, and competency. In recent years, isoxazoles and their derivatives have been found greater consideration because of their varied biological activities like anticancer [3–8], antibacterial [9], anti-inflammatory [10, 11], and antiviral [12]. On the other hand pyrazolines were found to be lead structure in many of the biological active compounds [13–15] 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 [16–20].

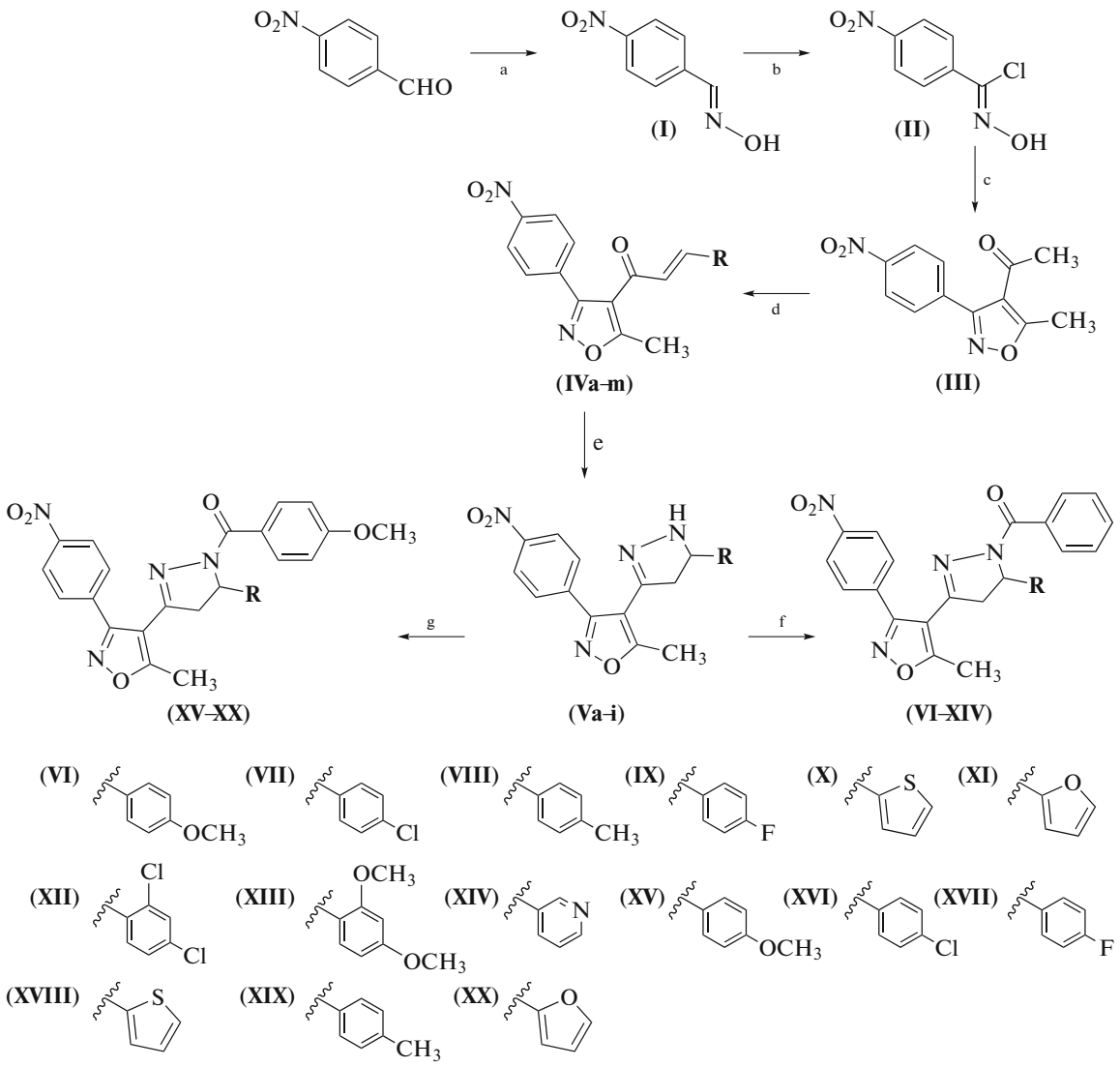
In view of the various biological opportunities and anticancer activity and antitubercular activities [21–23] of pyrazoles and isoxazole pharmacophores promoted us to synthesize the title compounds presuming that their incorporation into a single structural entity could produce new compounds with good anticancer and antitubercular activities, in the present study, we planned to design target compounds by incorporating pyrazole ring on isoxazole ring. Furthermore, on the molecular design level, modifications were done at 5th position of pyrazoline ring by using different substituted aldehydes, in order to explain the structural activity relationship, exploring the influence of substituents on the anticancer activity by regulating the electronic and steric effects. Various substitutes were introduced in the terminal phenyl ring with the purpose of increase in anticancer activity. The synthesized compounds were screened for their in vitro cytotoxic activity against two cancer cell lines, MCF-7 and MDA-MB-231 human breast cancer cell lines by MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay method. Molecular docking study was performed for synthesized compounds against human DHFR protein to explore their binding interactions at the active site. Consequently, in recent years, this receptor has gained much scientific focus as a target for the design of novel anticancer agents. In addition the synthesized compounds were screened for anti-tubercular activity against the strain H_{37}Rv and multidrug resistant strain DKU 156.

[1] Corresponding author: e-mail: radhikavanam25@gmail.com.

RESULTS AND DISCUSSION

Chemistry

A series of (5-(4-substitutedphenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)(subphenyl)methanone (**VI**–**XX**) were synthesized in following six steps (Scheme 1). In the first step, 4-nitrobenzaldehydeoxime (**I**) was prepared by the reaction of 4-nitrobenzaldehyde with hydroxylamine hydrochloride. In the second step, 1-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)ethanone (**III**) was prepared from in situ synthesized *N*-hydroxy-4-nitrobenzimidoyl chloride (**II**) and acetylacetone in methanol. In the next step, the compound (**III**) was condensed with various substituted aromatic aldehydes in the presence of NaOH under reflux for 30 to 50 min to obtain intermediate 1-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-3-(substituted-phenyl)prop-2-en-1-one (**IV**). In the next step, intermediate (**IV**) was refluxed with hydrazine hydrate in ethanol for 3–4 h to result in 4-(5-(4-substitutedphenyl) -4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (**V**). In the final step, (5-(4-substituted-phenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)(subphenyl)methanone were synthesized in quantitative yields from the reaction of the intermediate (**V**) with substituted benzoyl chlorides. All the derivatives were characterized by ^{1}H NMR, IR and ESI-MS spectra.In H^{1}NMR of compound aromatic protons appeared as a multiplet in the region δ 6.8–8.2, three protons of CH_3 appeared as a singlet at δ 2.5, pyrazoline CH_2 protons appeared as double doublet at 3.0 and 3.8. Pyrazoline CH proton appear as triple doublet at δ 5.6.



Scheme 1. Synthesis of the titled compounds. Reagents and conditions: (a) NH_2OH (2.0 equiv.), sodium acetate, ethanol, 80–90°C, 3 h (b) *N*-chlorosuccinamide (1.0 equiv.), DMF solution, rt, 16 h; (c) acetylacetone (1.0 equiv.), KOH (10% soln), ethanol, rt, 3 h (d) substituted aldehydes (1.0 equiv.), NaOH solution (10%), 25°C, 2 h. (e) $NH_2NH_2 \cdot H_2O$ (1.0 equiv.), ethanol, 80°C. (f) Substitued benzoylchloride (1.0 equiv.), THF : TEA (1 : 3), Str, 2 h (g) methoxy benzoyl chloride THF : TEA (1 : 3).

Table 1. Cytotoxic activity of synthesized compounds expressed (expressed as IC_{50} values, μg/mL)

Compound	IC_{50}, μg/mL		Compound	IC_{50}, μg/mL	
	MCF-7	MDA-MB-231		MCF-7	MDA-MB-231
(VI)	3.31 ± 0.19	3.85 ± 0.07	**(XIV)**	11.98 ± 0.19	14.00 ± 0.09
(VII)	5.54 ± 0.10	7.00 ± 0.16	**(XV)**	9.04 ± 0.06	10.65 ± 0.09
(VIII)	ND	ND	**(XVI)**	123.46 ± 0.13	130.23 ± 0.07
(IX)	124.21 ± 0.07	94.83 ± 0.09	**(XVII)**	47.44 ± 0.08	26.25 ± 0.03
(X)	5.89 ± 0.21	5.98 ± 0.04	**(XVIII)**	8.92 ± 0.02	8.34 ± 0.06
(XI)	28.43 ± 0.36	11.25 ± 0.06	**(XIX)**	27.11 ± 0.011	26.08 ± 0.03
(XII)	5.99 ± 0.06	8.72 ± 0.04	**(XX)**	ND	ND
(XIII)	8.34 ± 0.07	11.16 ± 0.03	Std (Taxol)	0.015	0.015

Cytotoxic Activity

New pyrazoline builtin isoxazole compounds were evaluated for antiproliferative activity against MDA-MB-231 and MCF-7 human breast cancer cell lines by MTT assay ([3-(4,5-dimethylthiazol-2-yl)-2,5-*d* iphenyltetrazolium bromide]) with Taxol as a positive control. Table 1 depicts IC_{50} values obtained with the titled compounds. All the titled compounds have shown moderate to significant anticancer activity. Among all the tested compounds **(VI)**, **(VII)**, **(X)**, **(XII)** and **(XVIII)** exhibited effective anticancer activity against MCF-7 and MDA-MB-231 cell line with IC_{50} value of 3–9 μg/mL and compound 6a shown significant cytotoxic activity with IC_{50} values of 3.31 and 3.85 μg/mL Apoptotic features of MDA-MB-231 and MCF-7 was studied by fluorescence microscopy analysis.

Anti-Tubercular Activity

Screening of all the synthesized compounds for anti-tubercular activity is done against strain $H_{37}Rv$ and multidrug resistant strain DKU 156. by using Microplate Alamar Blue asay (MABA) method. The MIC (μg/mL) values of test compounds are compared with that the standard drugs. Results of the standard and test compounds reported in Table 2. Among all the compounds, compounds **(VI)**, **(X)**, **(XVI)** and **(XVII)** exhibited significant activity at 6.25 μg/mL concentrations. Thus the MIC value may be in between the range of 3.12 and 6.25 μg/mL and the other compounds showed moderate activity results were compared to the standard drugs Pyrazinamide, Ciprofloxacin and Streptomycin etc.

Molecular Docking Studies

Docking studies. Molecular docking was performed using DHFR protein by using Lib Dock protocol in order identify the binding interactions with the targeted protein. Different poses were generated for each ligand and scored using a Lib Dock scoring function which estimates their corresponding Lib Dock scores with different orientations. Based on the docked score all the compounds were ranked. Table 3 shows the calculated binding scores of the compounds in the active site of the human DHFR enzyme. Compound 6a had the highest docking score of 153.763 kcal/mol indicating the better binding affinity against the target protein human DHFR (1KMS) shown in Fig. 1. By the docking of compound **(VI)** with human DHFR, it was observed that there was a formation of three hydrogen bonds between the ligand and two interaction residues of the binding site. Nitrogen (N(8)) of compound **(VI)** formed the hydrogen bond with ALA9 involving hydrogen atom of the amine group (A: ALA 9:HN – 6a:N8 1.698000) with a hydrogen bond distance of 1.698 Å and oxygen (O(9)) atom of compound 6a formed the hydrogen bond involving hydrogen atom of an amine group of ALA9 (A: ALA 9:HN–6a:O9) with a hydrogen bond distance of 2.369 Å. There was a single hydrogen bond formation between oxygen (O(36)) atom of compound 6a and a hydrogen atom (HG1) of THR56 (A: THR 56:HG1–6a:O36) with a hydrogen bond distance of 1.965 Å. It was observed that some close interactions are formed by the amino acid residues ILE16 (4), VAL8 (1) and THR56 (3) along with H bond. Apart from **(VI)** other compounds **(VIII)**, **(IX)**, **(X)**, **(XIV)** and **(XVIII)** also shown good libdock score with H-bonding interactions.

EXPERIMENTAL

All the chemicals and solvents used were of synthetic grade from SD fine chemicals Ltd (Mumbai,India) and Avra chemicals pvt Ltd Hyderabad. Reaction completion was monitored by analytical thin layer chromatography (TLC) using E.Merck 0.25 mm silica gel plates by observing under UV light (256 nm) and iodine chamber.Synthesized compounds were purified by recrystallization and the purity of compounds checked by single spot in TLC. Mobile phase for TLC was determined based on trial and error method.Melting points determined in open capillary tubes using ANALAB melting point apparatus and

Table 2. Anti-tubercular activity of standard and test compounds

S.NO	Compound	100 μg/mL	50 μg/mL	25 μg/mL	12.5 μg/mL	6.25 μg/mL	3.12 μg/mL	1.6 μg/mL	0.8 μg/mL	0.4 μg/mL
1	Pyrazinamide	S	S	S	S	S	S	R	R	R
2	Ciprofloxacin	S	S	S	S	S	S	R	R	R
3	Streptomycin	S	S	S	S	S	R	R	R	R
4	**(VI)**	S	S	S	S	S	R	R	R	R
5	**(VII)**	S	S	S	S	R	R	R	R	R
6	**(VIII)**	S	S	S	R	R	R	R	R	R
7	**(IX)**	S	S	R	R	R	R	R	R	R
8	**(X)**	S	S	S	S	S	R	R	R	R
9	**(XI)**	S	S	S	S	R	R	R	R	R
10	**(XII)**	S	S	S	R	R	R	R	R	R
11	**(XIII)**	S	S	S	R	R	R	R	R	R
12	**(XIV)**	S	S	S	R	R	R	R	R	R
13	**(XV)**	S	S	S	R	R	R	R	R	R
14	**(XVI)**	S	S	S	S	S	R	R	R	R
15	**(XVII)**	S	S	R	R	R	R	R	R	R
16	**(XVIII)**	S	S	S	S	S	R	R	R	R
17	**(XIX)**	S	S	S	R	R	R	R	R	R
18	**(XX)**	S	S	R	R	R	R	R	R	R

R—Resistant.
S—Sensitive.

were uncorrected. All the H^1 NMR spectra were recorded on Variant 400 MHz spectrometer using DMSO and $CDCl_3$ as solvent and Tetra Methyl Silane (TMS) as an internal standard; chemical shift values are listed on δ scale. FTIR spectra recorded on Schimadzu FT-IR spectrophotometer by using 1% potassium bromide discs.Mass spectra of the compounds were recorded on Electronic ionization mass spectra on Aglient 1100 series.

Table 3. Calculated docking scores, H-bond count and binding energy of the targeted compounds inside the DHFR Protein

Compound	Libdock score	H-Bond count	Binding energy
(VI)	153.7	3	201.25
(VII)	114.634	1	196.38
(VIII)	135.823	3	121.26
(IX)	135.9	2	167.89
(X)	144.739	1	245.41
(XI)	139.932	1	327.23
(XII)	124.159	3	171.98
(XIII)	90.786	2	467.23
(XIV)	136.819	2	703.67
(XV)	124.78	2	553.21
(XVI)	131.258	1	78.34
(XVII)	128.127	2	156.26
(XVIII)	135.159	2	123.22
(XIX)	130.249	2	66.21
(XX)	132.342	1	301.23

Synthesis of 1-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-3-(substitutedphenyl)prop-2-en-1-one [24] **(IVa–m).** Equimolar quantity of isoxazole ketone (0.01 mmol) and substituted aryl aldehyde (0.001 mmol) were dissolved in 15–20 mL of alcohol to it NaOH (10%) added and stirred on magnetic stirrer over a period of 30 minutes to 1 hour. Reaction was monitored by TLC.

Synthesis of 4-(5-(4-substitutedphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)-isoxazole (Va–i). Intermediate (**IVa–m**) and hydrazine hydrate (0.001 mmol) were taken in ethanol refluxed for 2 h and the reaction was monitored by TLC. The reaction mixture was poured into crushed ice to obtain the solid product and it was filtered under suction, and recrystallised from aq. methanol.

Synthesis of (5-(4-substitutedphenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)(subphenyl)methanone (VI–XX). Intermediate **(Va–i)** (0.001 mmol) was dissolved in a mixture of THF : TEA (1 : 3) to 0.001 mmol of benzoyl chloride or substituted methoxy benzoyl chloride was added drop wise. The above reaction mixture was stirred on a magnetic stirrer for 1 h. After completion of the reaction, the reaction mixture was poured into crushed ice and wash thoroughly with $NaHCO_3$.The obtained solid was filtered and. recrystallized from ethanol.

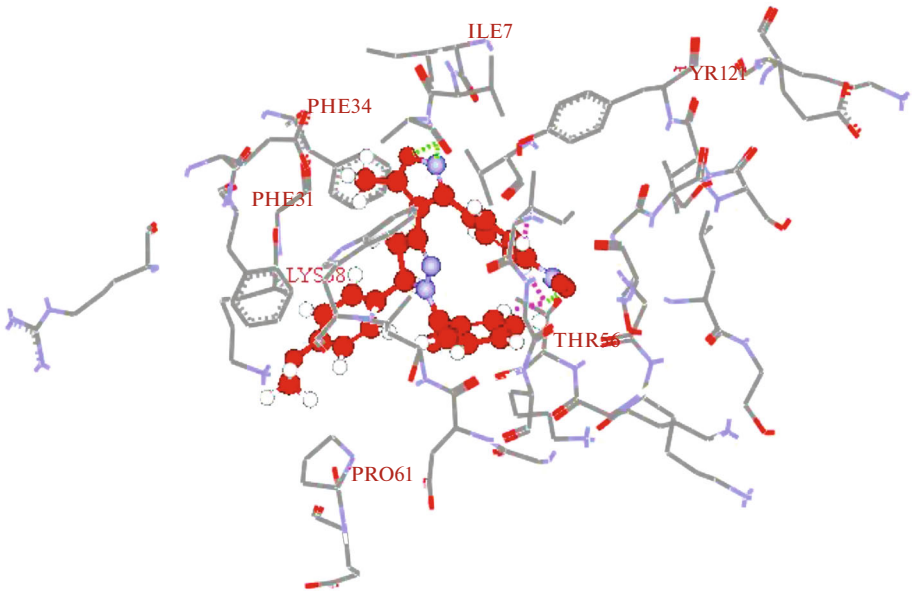


Fig. 1. The docking conformation of compounds (**VI**) in the binding site of protein human DHFR (1KMS).

4-(5-(4-Methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (Va). Yield 71%, mp 124–130°C. IR spectrum, ν, cm^{-1}: 2925, 1602, 1523, 1348, 1241, 1032. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 3.8 (s, 3H, OCH_3), 3.9 (dd, 1H, CH_2), 4.9 (td, 1H, CH), 6.8–7.4 (m, 4H, ArH), 7.6 (s, H, NH), 7.6–8.1 (m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.5, 162.4, 159.2, 153.5, 136.7, 131.3, 129.9, 128.2, 127.5, 125.7, 118.7, 109.7, 62.1, 55.4, 45.3, 13.1.ESI-MS: *m/z* 379 (*M* + 1) observed for $C_{20}H_{18}N_4O_4$. Anal. calcd.: C, 63.48; H, 4.79; N, 14.81; O, 16.91. Found: C, 64.54; H, 5.08; N, 13.78; O, 16.60.

4-(5-(4-Chlorophenyl)-4, 5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (Vb). Yield 70%, mp 128–131°C. IR spectrum, ν, cm^{-1}: 2925, 1602, 1523, 1348, 753. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.0 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 5.1 (td, 1H, CH), 7.0–7.4 (m, 4H, ArH), 7.6 (s, H, NH), 7.8–8.2 (m, 4H, ArH).^{13}C NMR(100 MHz, DMSO-d_6) δ 171.3, 165.6, 152.2, 148.9, 142.6, 136.3, 133.1, 129.5, 127.1, 126.5, 125.9, 108.8, 64.4, 45.9, 13.5. ESI-MS: *m/z* 379 (*M* + 1) observed for $C_{19}H_{15}ClN_4O_3$. Anal. calcd.: C, 59.61; H, 3.95; Cl, 9.261; N, 14.64; O, 12.54. Found: C, 58.74; H, 4.38; Cl, 8.56; N, 14.72; O, 13.60.

4-(5-(4-Tolyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (Vc). Yield 68%, mp 126–129°C. IR spectrum, ν, cm^{-1}: 2924, 1601, 1520, 1347, 1001. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.5 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.0 (dd, 1H, CH_2), 3.9 (dd, 1H,CH_2), 4.9 (td, 1H, CH), 7.1–7.4 (m, 4H, Ar–H), 7.6 (S, 1H, NH), 7.7–8.1 (m, 4H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 161.6, 152.8, 148.6, 141.0, 136.2, 135.8, 129.1, 127.6, 126.4, 124.5, 110.5, 55.8, 42.4, 21.8, 13.1.ESI-MS: *m/z* 363 (*M* + 1) observed for $C_{20}H_{18}N_4O_3$, Anal. calcd.: C, 66.29, H, 5.01, N, 15.46, O, 13.25. Found: C, 65.34; H, 5.12, N, 15.85; O,13.69.

4-(5-(4-Fluorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (Vd). Yield 67%, mp 121–127°C. IR spectrum, ν, cm^{-1}: 2923, 1603, 1522, 1348, 1001. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 4.9 (td, 1H, CH), 7.1–7.5 (m, 4H, ArH), 7.6 (s, H, NH), 7.7–8.1 (m, 4H, ArH).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9, 161.6, 160.5, 152.1, 148.9, 140.2, 135.7, 128.8, 126.5, 124.4, 109.8, 62.4, 42.9, 13.5. ESI-MS: *m/z* 379 (*M* + 1) observed for $C_{19}H_{15}FN_4O_3$. Anal. calcd.: C, 62.29; H, 4.13; F, 5.19; N, 15.29; O, 13.10. Found: C, 61.45; H, 4.28; F, 5.85; N, 15.02; O, 13.4.

5-Methyl-3-(4-nitrophenyl)-4-(5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)isoxazole (Ve). Yield 74%, mp 109–115°C. IR spectrum, ν, cm^{-1}: 2930, 1603, 1521, 1347. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.0 (dd, 1H, CH_2), 4.0 (dd, 1H, CH_2), 5.1 (td, 1H, CH), 6.8–7.3 (m, 3H, Ar–H), 7.6 (S, 1H, NH), 7.7–8.2 (m, 4H, Ar–H).^{13}C NMR (100 MHz, DMSO-d_6) δ 171.8, 161.9, 152.2, 148.4, 136.7, 129.1,128.2, 127.6, 126.4, 124.5, 109.8, 58.8, 43.4, 13.1. ESI-MS: *m/z* 355 (*M* + 1) observed for $C_{17}H_{14}N_4O_3S$. Anal. calcd.: C, 57.62; H, 3.98; N, 15.81; O, 13.54; S, 9.05. Found: C, 58.34; H, 4.12, N, 15.96; O, 13.29; S, 8.29.

4-(5-(Furan-2-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-(4-nitrophenyl)isoxazole (Vf). Yield 72%,

mp 114–121°C. IR spectrum, ν, cm^{-1}: 2928, 1602, 1519, 1346. ^1H NMR (400 MHz, DMSO-d_6) δ 2.5 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 4.0 (dd, 1H, CH_2), 5.0 (td, 1H, CH), 6.9–7.4 (m, 3H, Ar–H), 7.6 (S, 1H, NH), 7.8–8.2 (m, 4H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 161.6, 152.7, 148.8, 142.5, 136.2, 127.5, 126.4, 114.5, 109.1, 52.8, 43.4, 13.3. ESI-MS: m/z 339 (M + 1) observed for $C_{17}H_{14}N_4O_4$. Anal. calcd.: C, 60.35; H, 4.17; N, 16.56; O, 18.92. Found: C, 61.04; H, 4.07, N, 15.79; O, 19.10.

4-(5-(2,4-Dichlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (Vg). Yield 69%, mp 126–131°C. IR spectrum, ν, cm^{-1}: 2930, 1600, 1520, 1348, 752. ^1H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.0 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 5.1 (td, 1H, CH), 7.0–7.4 (m, 4H, ArH), 7.6 (s, H, NH), 7.8–8.2 (m, 3H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 165.6, 152.04, 151.1, 148.9, 142.5, 136.3, 127.1, 126.5, 124.5, 120.2, 109.8, 51.4, 41.9, 13.2. ESI-MS: m/z 418 (M + 1) observed for $C_{19}H_{15}Cl_2N_4O_3$. Anal. calcd.: C, 54.69; H, 3.38; Cl, 16.99; N, 13.43; O, 11.50. Found: C, 54.24; H, 3.69; Cl, 17.20; N, 13.72; O, 11.15.

4-(5-(2, 4-Methoxy phenyl)-4, 5-dihydro-1*H*-pyrazol-3-yl)-5-methyl-3-(4-nitrophenyl)isoxazole (Vh). Yield 71%, mp 121–125°C. IR spectrum, ν, cm^{-1}: 2929, 1600, 1520, 1348, 1240, 1030. ^1H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 3.0 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 5.0 (td, 1H, CH), 6.8–7.4 (m, 4H, ArH), 7.6 (s, H, NH), 7.8–8.4, 8.2–8.4 (m, 3H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 161.4, 158.6, 157.1, 155.7, 148.3, 135.9, 133.6, 128.9, 127.2, 125.4, 121.6, 115.5, 109.6, 62.3, 51.4, 43.6, 13.4. ESI-MS: m/z 409 (M + 1) observed for $C_{21}H_{20}N_4O_5$. Anal. calcd.: C, 61.76; H, 4.94; N, 13.72; O, 19.59. Found: C, 60.92; H, 5.04; N, 13.76; O, 20.28.

5-Methyl-3-(4-nitrophenyl)-4-(5-(pyridine-3-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)isoxazole (Vi). Yield 71%, mp 118–122°C. IR spectrum, ν, cm^{-1}: 2930, 1602, 1524, 1348. ^1H NMR (400 MHz, DMSO-d_6) δ 2.5 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 4.0 (dd, 1H, CH_2), 5.0 (td, 1H, CH), 7.2–7.4 (m, 2H, ArH), 7.6 (s, H, NH), 7.8–8.4 (m, 6H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.5, 160.9, 152.1, 150.1, 148.5, 145.2, 135.1, 132.4, 127.5, 124.9, 122.2, 109.8, 59.4, 42.9, 13.5. ESI-MS: m/z 350 (M + 1) observed for $C_{18}H_{15}N_5O_3$. Anal. calcd.: C, 61.89; H, 4.33; N, 20.05; O, 13.74. Found: C, 61.56; H, 4.12; N, 21.02; O, 13.3.

(5-(4-Methoxyphenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)(phenyl)methanone (VI): Yield 77%, mp 120–135°C. IR spectrum, ν, cm^{-1}: 2929, 1629, 1520, 1348, 1240, 1030. ^1H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 3.8, 3.0 (dd, 2H, pyrazoline), 5.6 (td, 1H, pyrazoline), 6.8–7.1 (m, 4H, ArH), 7.2–7.5 (m, 5H, Ar–H), 7.8–8.2 (m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 165.3, 160.4, 158.2, 148.5, 136.1, 134.2, 132.3, 128.9, 128.2, 127.5, 127.0, 126.7, 125.7, 114.7, 109.7, 62.8, 56.7, 42.3, 13.1. ESI-MS: m/z 483 (M + 1) observed for $C_{27}H_{22}N_4O_5$. Anal. calcd.: C, 67.21; H, 4.60; N, 11.61; O, 16.58. Found: C, 65.26; H, 5.78; N, 11.51; O, 17.68.

(5-(4-Chlorophenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-1*H*-pyrazol-1-yl)(phenyl)methanone (VII): Yield 71%, mp 100–110°C. IR spectrum, ν, cm^{-1}: 2922, 1629, 1520, 1348, 750. ^1H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 5.7 (td, 1H, CH), 6.8–7.0 (m, 4H, ArH), 7.1–7.4 (m, 5H, ArH), 7.6–8.1 (m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 166.1160.4, 158.6, 148.3, 139.3, 137.2, 135.6, 132.5, 132.1, 128.9, 128.0, 127.5, 126.5, 125.9, 109.5, 66.9, 42.7, 13. ESI-MS: m/z 488 (M + 1) observed for $C_{26}H_{19}ClN_4O_4$. Anal. calcd.: C, 64.14; H, 3.93; Cl, 7.28; N, 11.51, O, 13.14. Found: C, 65.14; H, 4.20; Cl, 6.98; N, 10.56; O, 13.02.

(3-(5-Methyl-3-(4-nitrophenyl)isoxazol-4-yl)-5-p-tolyl-1*H*-pyrazol-1-yl)(phenyl)methanone (VIII). Yield 72%, mp 125–137°C. IR spectrum, ν, cm^{-1}: 2923, 1629, 1520, 1348. ^1H NMR (400 MHz, DMSO-d_6) δ 2.4 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 3.8 (dd, 1H,CH_2), 5.7 (td, 1H, CH), 7.1–7.3 (m, 6H, Ar–H), 7.4 (t, 2H, Ar–H), 7.6–8.1 (m, 5H, Ar–H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 169.4, 158.1, 152.8, 148.2, 139.1, 136.6, 135.7, 133.7, 130.7, 128.5, 127.6, 127.0, 126.4, 123.3, 109.5, 62.4, 42.4, 21.5, 13.1. ESI-MS: m/z 467 (M + 1) observed for $C_{27}H_{22}N_4O_4$. Anal. calcd.: C, 69.52, H, 4.75, N, 12.01, O, 13.72. Found: C, 68.01; H, 5.12, N, 11.45; O, 15.42.

(5-(4-Fluorophenyl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-1*H*-pyrazol-1-yl)(phenyl)methanone (IX). Yield 70%, mp 98–100°C. IR spectrum, ν, cm^{-1}: 2993, 1629, 1520, 1348, 1000. ^1H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.1 (dd, 1H, CH_2), 4.1 (dd, 1H, CH_2), 5.8 (td, 1H, CH), 6.8–7.2 (m, 4H, Ar–H), 7.4–7.6 (m, 4H, ArH), 7.8–8.1 (m, 5H, Ar–H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 165.3, 162.5, 158.8, 148.5, 139.7, 137.7, 134.2, 132.3, 128.9, 127.5, 127.0, 126.7, 125.9, 114.7, 109.2, 62.8, 42.3, 13.1. ESI-MS: m/z 471 (M + 1) observed for $C_{26}H_{19}FN_4O_4$. Anal. calcd.: C, 66.38; H, 4.07; F, 4.04; N, 11.91; O, 13.60. Found: C, 67.05; H, 5.12; F, 3.98; N, 12.01; O, 11.84.

(5-(1-Thiophen2-yl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-1*H*-pyrazol-1-yl)(phenyl)methanone (X): Yield 74%, mp 99–112°C. IR spectrum, ν, cm^{-1}: 2993, 1629, 1520, 1348. ^1H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 2.9 (dd, 1H, CH_2), 4.0, (dd, 1H, CH_2), 5.8 (td, 1H, CH), 6.8–7.3 (m, 3H, Ar–H), 7.6–7.9 (m, 5H, ArH), 7.9–8.3 (m, 4H,

ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 160.7, 158.9, 148.5, 137.3, 137.0, 135.5, 132.1, 129.9, 129.3, 127.5, 126.5, 126.0, 123.6, 121.5, 109.3, 62.3, 45.0, 13.1. ESI-MS: *m/z* 459 (*M* + 1) observed for $C_{24}H_{18}N_4O_4S$. Anal. calcd.: C, 62.87; H, 3.96; N, 12.22; O, 13.96; S, 6.99. Found: C, 61.56; H, 4.21; N,1 1.45; O, 14.23; S, 8.55.

(5-(Furfu-2-yl)-3-(5-methyl-3-(4-nitrophenyl)-isoxazol-4-yl)-1*H*-pyrazol-1-yl)(phenyl)methanone (XI). Yield 75%, mp 105–118°C. IR spectrum, ν, cm^{-1}: 2925, 1629, 1520, 1348. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 2.9 (dd, 2H, CH_2), 4.0 (dd, 2H, CH_2), 5.5 (td, 1H, CH), 6.8–7.0 (dd, 2H, ArH), 7.2–7.7 (m, 6H, ArH), 7.9–8.1 (m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 165.4, 162,1, 160.7, 158.9, 148.5, 137.3, 137.0, 135.5, 132.1, 129.9, 129.3, 127.5, 126.5, 126.0, 123.6, 121.5, 109.3, 62.3, 45.0, 13.1. ESI-MS: *m/z* 443 (*M* + 1) observed for $C_{24}H_{18}N_4O_5$. Anal. calcd.: C, 65.15; H, 4.10; N, 12.66; O, 18.08. Found: C, 66.26; H, 4.14; N, 11.98; O, 17.62.

(5-(2,4-Dichlorophen4-yl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-1*H*-pyrazol-1-yl)(phenyl) methanone (XII). Yield 70%, mp 121–123°C. IR spectrum, ν, cm^{-1}: 2925, 1629, 1520, 1348, 750. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.1 (dd, 1H, CH_2), 4.1 (dd, 1H,CH_2), 5.6 (td, 1H, CH), 6.8–7.2 (m, 3H, ArH), 7.6–7.9 (m, 5H, ArH), 8.2–8.4 (m, 4H, ArH).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 160.4, 158.9, 155.7, 148.5, 139.7, 137.1, 136.2, 135.0, 133.6, 132.5, 130.4, 128.9, 128.0, 127.5, 125.8, 123.3, 117.5, 109.7, 62.6, 45.3, 13.0. ESI-MS: *m/z* 522 (*M* + 1) observed for $C_{24}H_{18}Cl_2N_4O_4$. Anal. calcd.: C, 59.90; H, 3.48; Cl, 13.60, N, 10.75; O, 12.28. Found: C, 59.19; H, 3.68; Cl, 13.96, N, 9.78; O, 13.39.

(5-(2,4-Dimethoxyphen4-yl)-3-(5-methyl-3-(4-nitrophenyl)isoxazol-4-yl)-1*H*-pyrazol-1-yl)(phenyl)methanone (XIII). Yield 68%, mp 125–138°C. IR spectrum, ν, cm^{-1}: 2929, 1629, 1520, 1348, 1240, 1030. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 3.0 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 5.7 (td, 1H, CH), 6.6–7.0 (m, 2H, ArH), 7.4–7.9 (m, 4H, ArH), 8.2–8.4 (m, 6H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 168.1, 160.4, 158.6, 157.4, 155.7, 148.3, 139.7, 136.2, 133.6, 128.9, 127.3, 125.8, 123.0, 120.3, 114.5, 109.4, 62.3, 45.6, 13.1. ESI-MS: *m/z* 513 (*M* + 1) observed for $C_{28}H_{24}N_4O_6$. Anal. calcd.: C, 65.62; H, 4.72; N, 10.93; O, 18.73. Found: C, 67.12; H, 4.14; N, 10.56; O, 18.18.

(3-(5-Methyl-3-(4-nitrophenyl)isoxazole-4-yl)-5-(pyridine-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)phenyl)-methanone (XIV). Yield 73%, mp 119–129°C. IR spectrum, ν, cm^{-1}: 2925, 1590, 1629, 1520, 1348, 750. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 3.0 (dd, 1H, CH_2), 3.9 (dd, 1H, CH_2), 5.8 (td, 1H, CH), 7.1–7.5 (m, 6H, ArH), 7.6–7.8 (m, 4H, ArH), 8.0–8.4 (m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 167.4, 160.8, 152.3, 148.1, 147.2, 146.4, 141.2, 137.8, 135.5, 133.3, 132.4, 128.8, 127.4, 126.6, 123.9, 109.4, 62.1, 45.4, 13.1. ESI-MS: *m/z* 454 (*M* + 1) observed for $C_{25}H_{19}N_5O_4$. Anal. calcd.: C, 66.22; H, 4.22; N, 15.44; O, 14.11. Found: C, 65.85; H, 4.54; N, 15.98; O, 13.63.

(4-Methoxyphenyl)-)-5-(4-methoxyphenyl)-3-(5-methyl-3-(4-nitrophenylisoxazole-4-yl)-4,5-dihydro-1*H*-prazol-1yl)methanone (XV). Yield 66%, mp 117–126°C. IR spectrum, ν, cm^{-1}: 2929, 1629, 1520, 1348, 1240, 1030. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.6 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 3.0 (dd, 1H, CH_2), 4.1 (dd, 1H, CH_2), 5.7 (td, 1H, CH), 6.9–7.2 (m, 4H, ArH), 7.5–7.8 (m, 4H, ArH), 8.0–8.3 (m, 4H, ArH).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 165.2, 160.4, 158.9, 155.0, 148.0, 138.4, 132.5, 130.0, 127.9, 127.7, 126.4, 124.4, 116.4, 109.7, 62.2, 55.6, 42.3, 13.2. ESI-MS: *m/z* 513 (*M* + 1) observed for $C_{27}H_{24}N_4O_6$. Anal. calcd.: C, 65.62; H, 4.72; N, 10.93; O, 18.73. Found: C, 64.69; H, 4.34; N, 11.35; O, 19.63.

(5-(4-Chlorophenyl)-)-3-(5-methyl-3-(4-nitrophenyl)isoxazole-4-yl)-4,5-dihydro-1*H*-prazol-1yl)(4-methoxyphenyl)methanone (XVI). Yield 75%, mp 119–127°C. IR spectrum, ν, cm^{-1}: 2922, 1629, 1520, 1348, 1240, 1030, 750. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.8 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 3.0 (dd, 1H, CH_2), 4.0 (dd, 1H, CH_2), 5.8 (td, 1H, CH), 7.1–7.4 (m, 5H, ArH), 7.6–7.7 (m, 3H, ArH) 7.8–8.1 (m, 4H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 167.2, 165.1, 160.7, 158.3, 148.5, 139.3, 136.1, 134.2, 130.0, 128.8, 128.0, 127.0, 125.1, 123.0, 118.7, 109.8, 66.1, 55.6, 45.6, 13.2. ESI-MS: *m/z* 517 (*M* + 1) observed for $C_{27}H_{21}ClN_4O_5$. Anal. calcd.: C, 62.73; H, 4.09; Cl, 6.86, N, 10.84; O, 15.48. Found: C, 61.85; H, 4.86; Cl, 7.02; N, 11.34; O, 14.93.

(5-(4-Fluorophenyl)-)-3-(5-methyl-3-(4-nitrophenyl)isoxazole-4-yl)-4,5-dihydro-1*H*-prazol-1yl)(4-methoxyphenyl)methanone (XVII). Yield 72%, mp 123–129°C. IR spectrum, ν, cm^{-1}: 2993, 1629, 1520, 1348, 1242, 1034, 1005. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.6 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 3.0 (dd, 1H, CH_2), 4.0 (dd, 1H, CH_2), 5.7 (td, 1H, CH) 7.1–7.6 (m, 6H, ArH), 7.7–8.1 (m, 6H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 165.3, 162.5, 160.2, 158.8, 152.2, 148.5, 136.0, 130.3, 128.7, 128.1, 126.7, 124.4, 116.7, 109.2, 62.8, 56.0, 45.2, 13.1. ESI-MS: *m/z* 501 (*M* + 1) observed for $C_{27}H_{21}FN_4O_5$. Anal. calcd.: C, 64.80; H, 4.23; F, 3.80, N, 11.19; O, 15.98. Found: C, 65.15; H, 4.54; F, 4.02; N, 11.34; O, 14.95.

(4-Methoxyphenyl)(3-(5-methyl-3-(4-nitrophenyl) isoxazole-4-yl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)methanone (XVIII). Yield 72%, mp 123–128°C. IR spectrum, ν, cm^{-1}: 2925, 1629, 1520, 1348, 1245, 1032. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.1, 3.1 (dd, 2H, pyra-

zoline), 5.8 (td, 1H, pyrazoline), 6.8–7.5 (m, 4H, ArH), 7.7–7.9 (m, 4H, ArH), 8.0–8.3 (m, 3H, ArH).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 168.1, 165.6, 160.7, 158.9, 151.9, 148.3, 139.7, 135.0, 129.6, 128.7, 128.0, 126.9, 126.2, 124.8, 114.7, 109.5, 58.6, 54.3, 42.3, 13.1. ESI-MS: *m/z* 489 (*M* + 1) observed for $C_{25}H_{20}N_4O_5S$. Anal. calcd.: C, 61.47; H, 4.13, N, 11.47; O, 16.38, S, 6.56. Found: C, 62.15; H, 4.32; N, 10.89; O, 16.95; S, 5.69.

(4-Methoxyphenyl)(3-(5-methyl-3-(4-nitrophenyl) isoxazole-4-yl)-5-(*p*-tolyl)-4,5-dihydro-1-*H*-pyrazol-1-yl)methanone (XIX): Yield 71%, mp 123–133°C. IR spectrum, ν, cm^{-1}: 2923, 1629, 1520, 1348, 1243, 1031. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.4 (s, 3H, CH_3), 2.7 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 2.9 (dd, 1H, CH_2), 4.0 (dd, 1H, CH_2), 5.6 (td, 1H, CH), 7.1–7.6 (m, 6H, ArH), 7.7–8.2 (m, 6H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 167.2, 165.1, 160.3, 157.4, 148.5, 139.0, 136.7, 135.2, 130.0, 128.6, 128.1, 127.2, 125.5, 122.9, 119.1, 109.1, 65.3, 55.3, 45.0, 21.0, 13.3. ESI-MS: *m/z* 497 (*M* + 1) observed for $C_{28}H_{24}N_4O_5$. Anal. calcd.: C, 67.73; H, 4.87, N, 11.28; O, 16.11. Found: C, 67.28; H, 4.98; N, 10.89; O, 16.85.

(5-(Furan-2-yl)-3-(5-methyl-3-(4-nitrophenyl)-isoxazol-4-yl)-1*H*-pyrazol-1-yl)(methoxyphenyl)methanone (XX): Yield 73%, mp 119–129°C. IR spectrum, ν, cm^{-1}: 2925, 1629, 1520, 1348, 1240, 1030. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.7 (s, 3H, CH_3), 3.1 (dd, 1H, pyrazoline), 4.0 (dd, 1H, pyrazoline), 5.8 (td, 1H, pyrazoline), 6.8–7.2 (d, 2H, ArH), 7.2–7.6 (m, 5H, ArH), 7.7–8.2 (m, 5H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 167.2, 165.1, 160.3, 157.4, 148.5, 139.0, 136.7, 135.2, 130.0, 128.6, 128.1, 127.2, 125.5, 122.9, 119.1, 109.1, 65.3, 55.3, 45.0, 21.0, 13.3. ESI-MS: *m/z* 473 (*M* + 1) observed for $C_{25}H_{20}N_4O_6$. Anal. calcd.: C, 63.56; H, 4.27, N, 11.86; O, 20.32. Found: C, 64.82; H, 4.34; N, 11.09; O, 19.75.

CONCLUSION

The results of the present study demonstrated the synthesis of pyrazoline incorporated isoxazole derivatives and insilco evaluation for their efficacy as anticancer compounds through docking against hDHFR. Compound **6a** is recognised as the most hopeful anticancer compound among all the synthesized derivatives based on its highest docking score.All the synthesized compounds were screened for anticancer activity by MTT assay among these compounds (**VI**), (**IX**), (**X**) and (**XII**) has exhibited significant anticancer activity. All the compounds screened for antitubercular activity compounds (**VI**), (**X**), (**XVI**) and (**XVIII**) exhibited significant activity at 6.25 μg/mL concentrations. Thus the MIC value may be in between the range of 3.12 and 6. 25 μg/mL.

Thus, based on the in silico docking studies, cytotoxic activity on cell lines it can be proposed that compound VI have drug like properties and may further probed to expand effective anti-cancer lead candidates and also some of the compounds can be developed as antitubercular drugs.

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COMPLIANCE WITH ETHICAL STANDARDS

This article doesnot contain any studies involving human participants performed by any of the authors and doesnot contain any studies involving animals performed by any of the author.

Conflict of Interests

The authors report no conflicts of interest.

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