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Synthesis and Characterization of Some Novel Aryl and Heteroaryl Chalcone Derivatives of 3-(3,4,5-Trimethoxyphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde for Assessing Their Potentials as Anticancer Agents[1]

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Abstract—In the present study, a novel series of nine derivatives of 1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole substituted chalcones **6a–6i** were synthesized with an aim to assess their anticancer activities. The chalcone derivatives were synthesized by the Claisen condensation of 1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole-4-carbaldehyde **4** with various aryl and heteroaryl acetophenones **5a–5i** by treatment with alcoholic sodium hydroxide in methanol at reflux temperature in good to excellent yields. The newly synthesized derivatives were well characterized by IR, NMR, and HRMS spectroscopic techniques. The title compounds were evaluated for *in vitro* antibacterial activity against a panel of gram-positive and gram-negative bacteria and screened for anticancer activity employing EGFR lung protein based on *in silico* molecular docking studies. The study results showed IC_{50} value ranging between 39–94 μg/mL compared to the standard Camptothecin with an IC_{50} value of 47 μg/mL which indicates the anticancer potential of the majority of the synthesized compounds.

Keywords: chalcone, pyrazole, antibacterial, anticancer, molecular docking

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INTRODUCTION

Chalcones have attracted close attention due to their synthetic and biological applications. Chalcones are important starting materials for the synthesis of various heterocyclic derivatives and possess diverse pharmacological activities such as anticancer [1], anti-HIV [2], antituberculosis [3], antiinflammatory [4], and anti-microbial [5] activities. The Claisen–Schmidt condensation between arylmethylketone and aryl aldehyde in the presence of alcoholic alkali was the most convenient method for the synthesis of chalcones [6] and the reactive α,β-unsaturated keto group is responsible for their biological activity. Pyrazole containing molecules are known to possess various biological activities, excellent drug-like properties (Fig. 1) and high oral bioavailability [7]. Some pyrazole derivatives are known to exhibit anticancer activity through inhibition of various targets such as epidermal growth factor (EGF), tumor growth factor (TGF), fibroblast growth factor (FGF), telomerase and different kinases that are significant for the management of cancer [8–10]. Substituted pyrazole derivatives play essential role in biologically active compounds and are known to possess drug like properties (Fig. 1). Important biological activities were reported for some substituted pyrazoles and their derivatives, including antibacterial, antifungal, antitumor, analgesic, antipyretic, anti-inflammatory, antioxidant and anticancer activities [11–14]. Trimethoxy substituted pyrazoles (Fig. 2) also exhibit different pharmacological activities like anticancer [7, 15], antiproliferative, antitubulin [16] and antimicrobial [17].

Keeping in mind various pharmacological activities of pyrazole and chalcone motif, the authors designed and synthesised a series of nine novel 1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole substituted aryl

[1] The text was submitted by the authors in English.

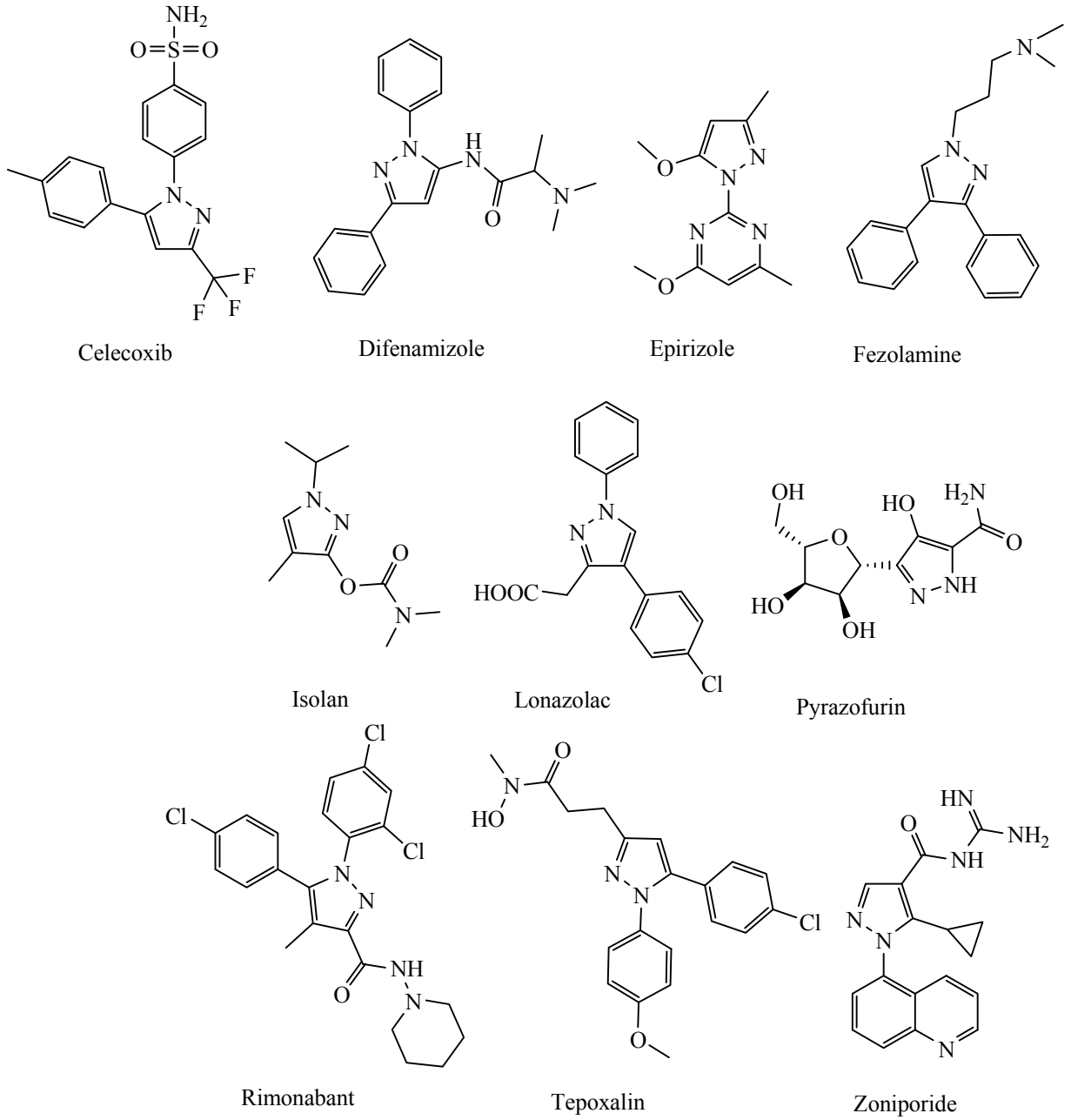


Fig. 1. Drugs containing pyrazole moiety.

and heteroaryl chalcones **6a–6i** for evaluation of their antibacterial and anticancer activities.

RESULTS AND DISCUSSION

The synthetic approach to compounds **6a–6i** (Scheme 1) involved easily accessible 1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole carboxaldehyde **4** and was based on the reported earlier procedure [18]. In the first step of the reaction 3,4,5-trimethoxy acetophenone **1** was treated with phenyl hydrazine **2** in the presence of acetic acid and methanol. Refluxing for 3h led to the acetophenone phenylhydrazone derivative **3** in 96% yield. In the second step the intermediate **3** was treated with Vilsmeier–Haack reagent (DMF–$POCl_3$) at –10°C to room temperature for 1.5 h to afford 1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole carboxaldehyde **4**. The obtained aldehyde **4** underwent the Claisen–Schmidt reaction with various aryl and heteroaryl substituted acetophenones **5a–5i** in the

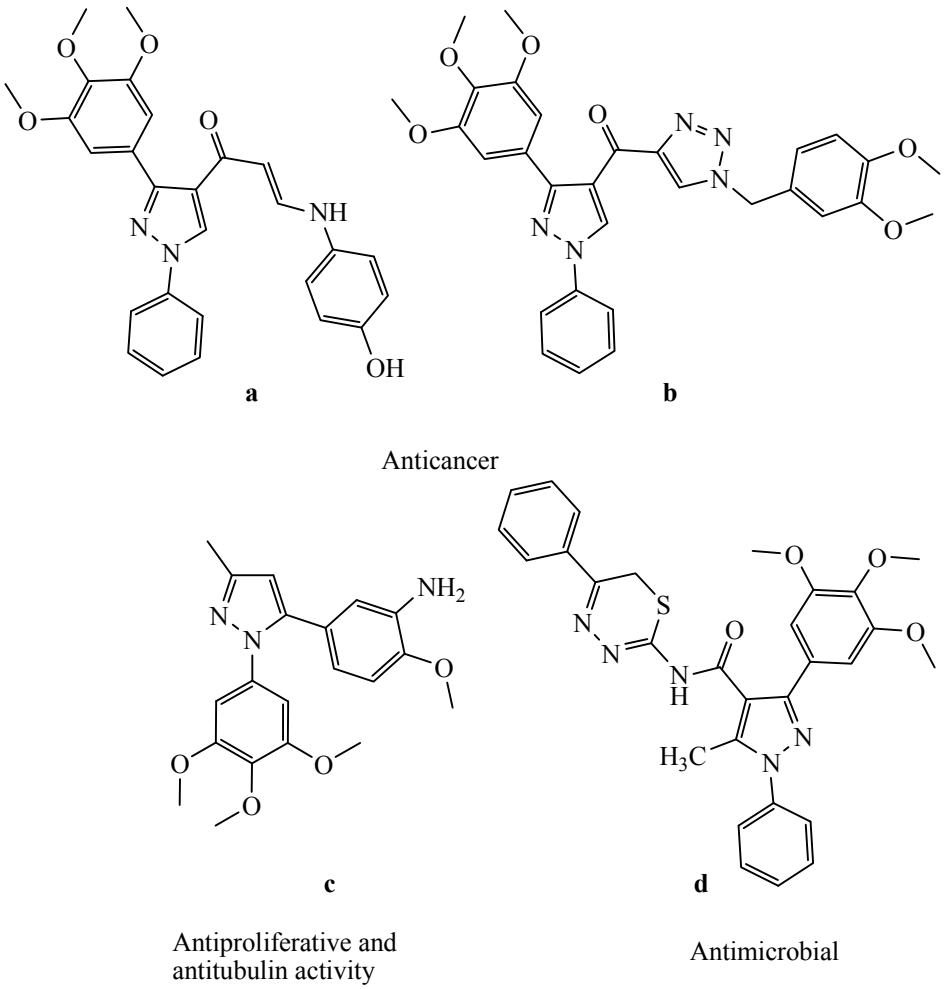


Fig. 2. Trimethoxy substituted pyrazoles possessing various biological activities.

presence of alcoholic alkali to afford the title compounds **6a–6i** in 62–85% yield.

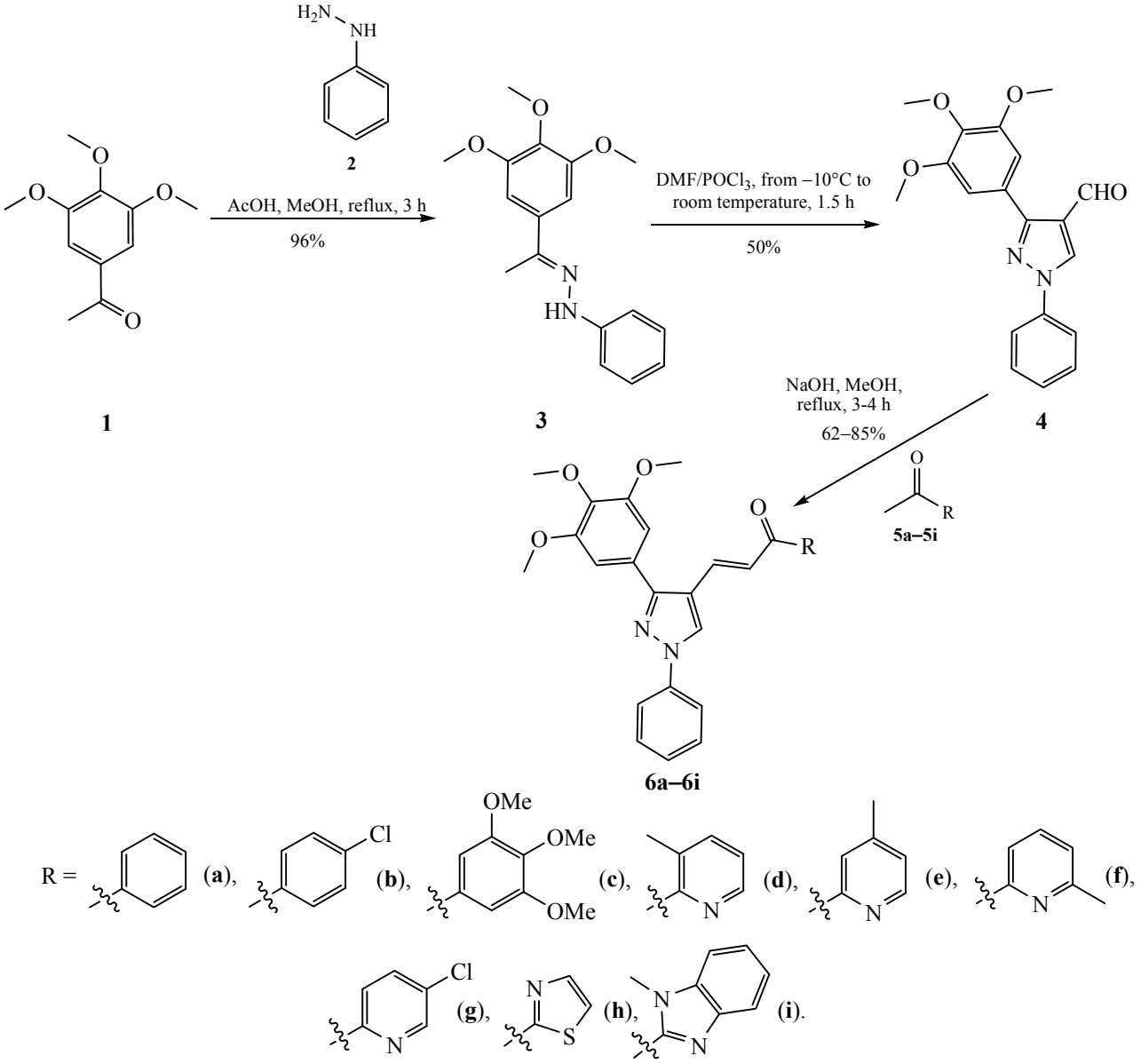
The structures of newly synthesized compounds **6a–6i** were characterized by IR, ^{1}H, and ^{13}C NMR, and HRMS spectral analysis.

Biological evaluation. *Antibacterial activity.* Chalcone derivatives **6a–6i** were screened for their antibacterial activity (zone of inhibition) against *Escherichia coli* (MTCC 2692), *Pseudomonas aeruginosa* (MTCC 2453) as gram negative and *Staphylococcus aureus* (MTCC 902) and *Bacillus subtilis* (MTCC 441) as gram positive bacterial strains using the cup plate method [19, 20]. Antibacterial activity of the synthesised compounds was compared with standard drugs ampicillin and streptomycin at concentrations 10, 20, and 30 μL (Table 1). According to the test data it was evident that the compounds **6b** and **6d** showed moderate activity against bacterial strains *E. coli, S. aureus,* and *bacillus* and all other compounds showed no activity. Compounds **6b**, **6h** demonstrated lower activity against gram negative bacteria *E. coli* and all other compounds were determined to be inactive (Table 1).

Cytotoxic activity. Compounds **6a–6i** were screened for their *in vitro* cytotoxic potential against human lung carcinoma (A549) cell line using the MTT assay (Table 2). The product **6c** demonstrated the highest potential among all synthesised compounds at micromolar concentration. It was evident that the compounds containing 3,4,5-trimethoxy phenyl **6c**, phenyl **6a**, benzimidazole **7,** and 3-pyridyl **6d** substituted chalcone derivatives exhibited pronounced anticancer activity.

Molecular docking. The common H-bonding interactions were estimated between all docked compounds and LYS347, LYS356 and GLU354. The

Scheme 1. Synthetic route for the preparation of pyrazole substituted aryl and heteroaryl chalcones **6a–6i**.



H-bonding interactions with other surrounding residues in the hydrophobic binding pocket were also considered. Strong H-bonding interactions between the methoxyl oxygen (O_{23}) of compound **6c** with the hydrogen atom of LYS356, carbonyl oxygen (O_2) with hydrogen atoms of LYS356 and LYS347 and benzopyrone hydrogen with hydrogen atom of LYS347 were observed (Fig. 3a). Benzopyrone oxygen of compound **6a** was likely to interact with hydrogen atoms of GLU354 and oxygen (O_{23}) with LYS347 (Fig. 3b).

Adsorption, digestion, metabolism, excretion and toxicity (ADMET) studies. The compounds were subjected to ADMET studies using mol inspiration server (Table 3).

The data indicated that the drug likeness score was good. The analytical data of the compounds also confirmed the nonmutagenic and nontoxic nature except the products **6b** and **6i.**

EXPERIMENTAL

All chemicals were purchased from Alfa Aesar. Melting points were determined (uncorrected) in open glass capillaries on a Stuart SMP30 apparatus. IR spectra (KBr pellets) were recorded on a Shimadzu FTIR 8400S spectrophotometer. ^{1}H NMR (400 MHz) and

Table 1. Antibacterial activity of compounds **6a–6i**[a]

Comp. no.	Microorganism	Zone of inhibition, mm						Comp. no.	Microorganism	Zone of inhibition, mm					
		concentration of ampicillin, μL			concentration of streptomycin, μL					concentration of ampicillin, μL			concentration of streptomycin, μL		
		10	20	30	10	20	30			10	20	30	10	20	30
Standard	*E. coli*	3.0	3.2	3.4	3.0	3.2	3.5	**6e**	*E. coli*	–	–	–	–	–	–
	Pseudomonas	1.6	2.1	2.4	3.0	3.1	3.2		*Pseudomonas*	–	–	–	–	–	–
	S. aureus	1.7	1.8	2.2	3.1	3.2	3.4		*S. aureus*	–	–	–	–	–	–
	Bacillus	3.4	3.6	3.7	3.1	3.5	3.7		*Bacillus*	–	–	–	–	–	–
6a	*E. coli*	–	–	–	–	–	–	**6f**	*E. coli*	–	–	–	–	–	–
	Pseudomonas	–	–	–	–	–	–		*Pseudomonas*	–	–	–	–	–	–
	S. aureus	–	–	–	–	–	–		*S. aureus*	–	–	–	–	–	–
	Bacillus	–	–	–	–	–	–		*Bacillus*	–	–	–	–	–	–
6b	*E. coli*	24	25	27	14	16	17	**6g**	*E. coli*	–	–	–	–	–	–
	Pseudomonas	–	–	–	14	15	16		*Pseudomonas*	–	–	–	–	–	–
	S. aureus	–	–	–	15	16	18		*S. aureus*	–	–	–	–	–	–
	Bacillus	–	–	–	15	17	20		*Bacillus*	–	–	–	–	–	–
6c	*E. coli*	–	–	–	–	–	–	**6h**	*E. coli*	26	28	29	–	–	–
	Pseudomonas	–	–	–	–	–	–		*Pseudomonas*	–	–	–	–	–	–
	S. aureus	–	–	–	–	–	–		*S. aureus*	–	–	–	–	–	–
	Bacillus	–	–	–	–	–	–		*Bacillus*	–	–	–	–	–	–
6d	*E. coli*	–	–	–	11	12	13	**6i**	*E. coli*	–	–	–	–	–	–
	Pseudomonas	–	–	–	–	–	–		*Pseudomonas*	–	–	–	–	–	–
	S. aureus	–	–	–	10	12	14		*S. aureus*	–	–	–	–	–	–
	Bacillus	–	–	–	10	11	12		*Bacillus*	–	–	–	–	–	–

[a] (–) No activity.

Table 2. *In vitro* cytotoxic activity of compounds **6a–6i**

Compound	IC_{50}, μg/mL	Compound	IC_{50}, μg/mL
6a	44.04 ± 0.01	**6f**	63.31 ± 0.01
6b	94.33 ± 0.02	**6g**	65.44 ± 0.01
6c	39.94 ± 0.01	**6h**	72.53 ± 0.01
6d	48.35± 0.02	**6i**	44.20 ± 0.01
6e	67.11 ± 0.01	Camptothecin (SD)	47.36 ± 0.02

^{13}C NMR (100 MHz) spectra were measured in $CDCl_3$ and DMSO-d_6 on a Bruker DPX 400 spectrometer using TMS as the internal standard. HRMS spectra were measured on a Xevo QTof mass spectrometer. TLC was performed on silica gel 60 F24 (Merck pre-coated plates) for testing purity of the compounds and monitoring the reactions. The spots were visualized under UV light and in iodine vapour.

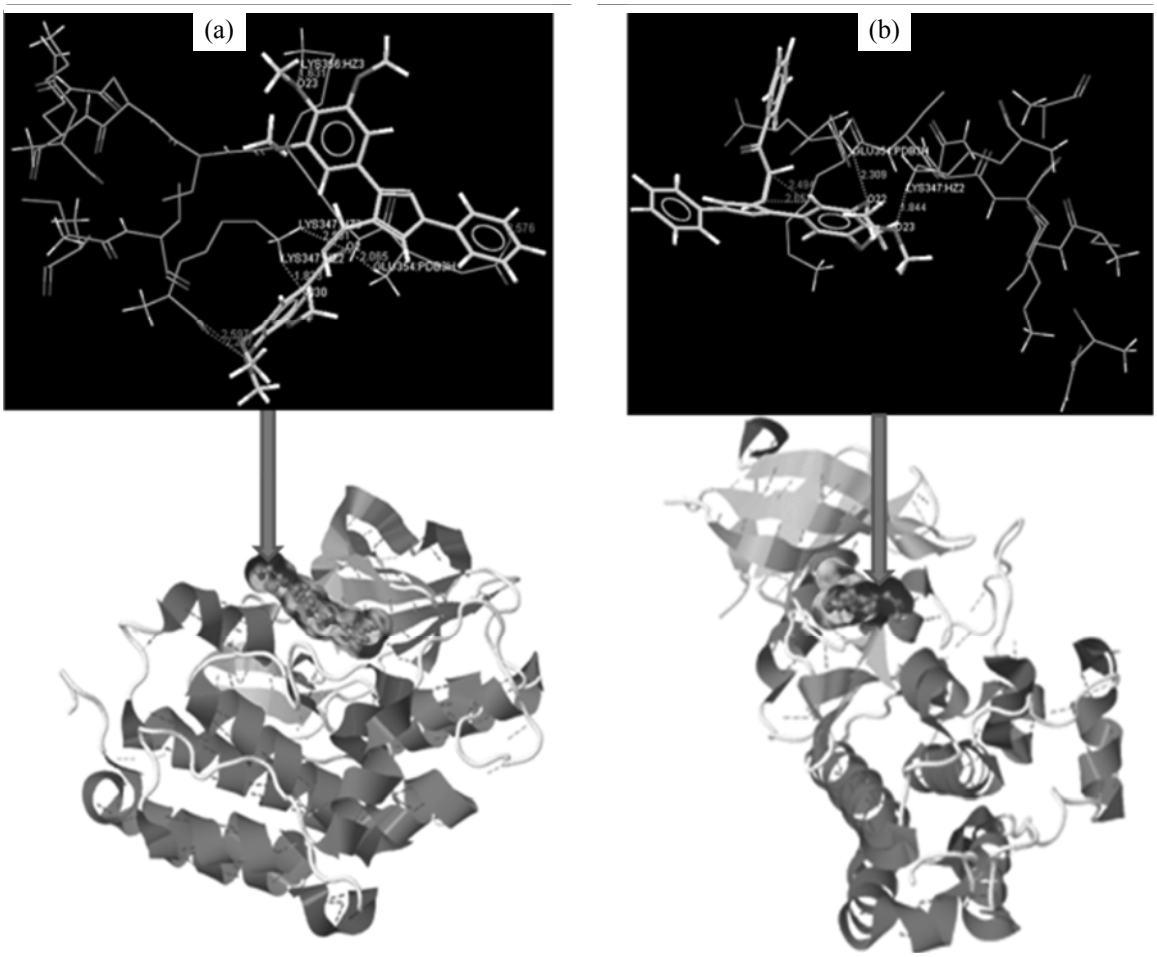


Fig. 3. (a) Interactions of compound **6c** with EGFR protein and (b) interactions of compound **6a** with EGFR.

1-[1-(3,4,5-Trimethoxyphenyl)ethylidene]-2-phenylhydrazine (3) [18]. To a solution of 1-(3,4,5-trimethoxyphenyl) ethanone **1** (5 g, 19 mmol) in methanol (40 mL) was added phenyl hydrazine **2** (2.1 g, 19 mmol) followed by glacial acetic acid (15 mL) at room temperature. The reaction mixture was refluxed for 3 h (TLC monitoring: 20% EtOAc in hexane, R_f 0.5). Upon evaporation of the solvent under reduced pressure, the mixture was diluted with ice water (25 mL), stirred for 15 min at 10°C. The precipitated solid was filtered off, washed with ice water (20 mL) and *n*-pentane (20 mL) and dried under vacuum to

Table 3. ADMET study data for the compounds **6a–6i**

Comp. no.	Mutagenic	Tumorigenic	Irritant	Reproductive effect	*c*log *P*	Solubility	Dry likeness	Dry score
6a	–	–	+	–	2.47	–1.42	1.27	0.27
6b	+	–	–	+	1.26	–1.62	2.57	0.21
6c	–	–	–	+	1.70	–3.86	0.21	0.12
6d	–	–	–	–	1.88	–2.00	2.12	0.41
6e	–	–	–	–	1.88	–2.00	2.05	0.28
6f	–	+	–	–	1.49	–4.76	0.60	0.24
6g	–	–	–	+	2.52	–5.73	2.88	0.18
6h	–	–	–	–	2.85	–1.39	1.02	0.32
6i	+	–	–	+	3.01	–3.84	3.44	0.21

afford the product **3** as pale yellow solid. Yield 6.0 g, 96 %, mp 82–83°C. ^{1}H NMR spectrum, δ, ppm: 2.23 s (3H, CH_3), 3.66 s (3H, OCH_3), 3.82 s (6H, $2OCH_3$), 6.71–6.73 m (1H, Ar-H), 7.03 s (2H, Ar-H), 7.19 d (4H, Ar-H, J = 3.6 Hz), 9.17 s (1H, NH). M 301.15 $[M + H]^+$.

3-(3,4,5-Trimethoxypheyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (4) [21]. Phosphorus oxychloride (6.2 mL, 66 mmol) was added to *N,N*-dimethylformamide (26.5 mL, 339 mmol) at –5 to 0°C drop wise over a period of 10 min and stirred at the same temperature for 30 min. The compound **3** (6.6 g, 22 mmol) dissolved in DMF (10 mL) was added dropwise at –5°C over a period of 15 min. The reaction mixture was warmed up to room temperature and stirred for 1.5 h. After completion, the reaction mixture was slowly added to ice cold water and basified with saturated $NaHCO_3$ solution to adjust pH 7–8, and stirred for 1 h at room temperature. The precipitated solid was filtered off, washed with water and dried under vacuum to get the crude compound which was washed with methanol, filtered and dried under vacuum to afford pure compound **4** as off white solid, yield 50%, mp 105–107°C. ^{1}H NMR spectrum, δ, ppm: 3.72 s (3H, OCH_3), 3.85 s (6H, $2OCH_3$), 7.29 s (2H, Ar-H), 7.43 t (1H, Ar-H, J = 7.44 Hz), 7.57 t (2H, Ar-H, J = 7.84 Hz), 7.99 d (2H, Ar-H, J = 8.28 Hz), 9.30 s (1H, pyrazole-H), 9.99 s (1H, -CHO). M 339.23 $[M + H]^+$.

General procedure for the synthesis of compounds 6a–6i [22]. A suspension of aldehyde intermediate **4** (0.64 mmol) and ketone **5a–5i** (0.64 mmol) in methanol (5 mL) was treated with solid sodium hydroxide (0.83 mmol) and refluxed for 3–4 h. After completion of the process, the precipitated solid was filtered off, washed with water and dried under vacuum to give a pure compounds **6a–6i**.

(*Z*)-1-Phenyl-3-[1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl]prop-2-en-1-one (6a). Off white solid, yield 71%, mp 185–187°C. IR spectrum, ν, cm^{-1}: 1658 (C=O), 1593 (C=C), 1121 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.74 s (3H, OCH_3), 3.83 s (6H, $2OCH_3$), 6.91 s (2H, Ar-H), 7.40 t (1H, Ar-H, J = 7.44 Hz), 7.55–7.61 m (4H, Ar-H), 7.64–7.68 m (1H, Ar-H), 7.76 s (1H, Ar-H), 7.79 s (1H, Ar-H), 7.94 d (2H, Ar-H, J = 7.68 Hz), 8.04–8.06 m (2H, Ar-H), 9.40 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 55.9, 60.1, 105.9, 117.7, 118.7, 121.4, 127.1, 128.1, 128.7, 129.6, 132.9, 134.5, 137.6, 137.9, 138.9, 152.9, 153.1, 188.9. HRMS: found $[M + H]^+$, 441.1765. $C_{27}H_{24}N_2O_4$. Calculated 440.17.

(*Z*)-1-(4-Chlorophenyl)-3-[1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl]prop-2-en-1-one (6b). Off white solid, yield 62%, mp 177–180°C. IR spectrum, ν, cm^{-1}: 1656 (C=O), 1587 (C=C), 1126 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.91 s (9H, OCH_3), 6.90 s (2H, Ar-H), 7.31–7.37 m (2H, Ar-H) 7.44–7.53 m (4H, Ar-H), 7.80 d (2H, Ar-H, J = 7.6 Hz), 7.88–7.96 m (3H, Ar-H), 8.34 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 55.7, 60.3, 105.5, 117.5, 118.8, 119.7, 125.9, 126.9, 129.0, 135.9, 138.7, 144.0, 152.8, 167.9, 168.0, 180.8. HRMS: found $[M + H]^+$ 475.1393. $C_{27}H_{23}ClN_2O_4$. Calculated 474.13.

(*Z*)-3-[1-Phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (6c). Off white solid, yield 70%, mp 179–181°C. IR spectrum, ν, cm^{-1}: 1654 (C=O), 1578 (C=C), 1123 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.74 s (3H, OCH_3), 3.76 s (3H, OCH_3), 3.83 s (6H, $2OCH_3$), 3.87 s (6H, $2OCH_3$), 6.91 s (2H, Ar-H), 7.33 s (2H, Ar-H), 7.41 t (1H, Ar-H, J =7.4 Hz), 7.58 t (2H, Ar-H, J = 7.6 Hz), 7.77 d (2H, Ar-H, J = 0.96 Hz), 7.93 d.d (2H, Ar-H, J = 1.0, 8.52 Hz), 9.32 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 54.4, 54.5, 58.6, 104.3, 104.4, 116.2, 117.1, 119.3, 125.3, 126.1, 126.8, 127.8, 131.5, 132.7, 136.5, 137.5, 140.4, 151.3, 151.5, 185.9. HRMS: found $[M + H]^+$ 531.1935. $C_{30}H_{30}N_2O_7$. Calculated 530.21.

(*Z*)-1-(3-Methylpyridin-2-yl)-3-[1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl]prop-2-en-1-one (6d). Off white solid, yield 75%, mp 150–152°C. IR spectrum, ν, cm^{-1}: 1668 (C=O), 1592 (C=C), 1125 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 2.44 s (3H, CH_3), 3.72 s (3H, OCH_3), 3.81 s (6H, $2OCH_3$), 6.86 s (2H, Ar-H), 7.38 t (1H, Ar-H, J = 7.4 Hz), 7.48–7.59 m (3H, Ar-H), 7.63–7.72 m (2H, Ar-H), 7.79 d (1H, Ar-H, J = 7.36 Hz), 7.97 d (2H, Ar-H, J = 7.68 Hz), 8.51–8.52 m (1H, Ar-H), 9.34 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 18.9, 55.9, 60.1, 105.8, 117.5, 118.7, 123.9, 125.7, 127.3, 128.8, 129.6, 133.3, 135.1, 137.9, 138.9, 139.8, 146.1, 152.8, 153.0, 192.1. HRMS: found $[M + H]^+$ 456.1923. $C_{27}H_{25}N_3O_4$. Calculated 455.18.

(*Z*)-1-(4-Methylpyridin-2-yl)-3-(1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl)prop-2-en-1-one (6e). Off white solid, yield 72%, mp 189–190°C. IR spectrum, ν, cm^{-1}: 1670 (C=O), 1587 (C=C), 1127 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 2.42 s (3H, CH_3), 3.75 s (3H, OCH_3), 3.84 s (6H, $2OCH_3$), 6.92 s (2H, Ar-H), 7.38 t (1H, Ar-H, J = 7.32 Hz), 7.51 d

(1H, Ar-H, J = 4.32 Hz), 7.56 t (2H, Ar-H, J = 7.76 Hz), 7.86–7.91 m (2H, Ar-H), 8.0 d (2H, Ar-H, J = 7.92 Hz), 8.17 d (1H, Ar-H, J = 16.0 Hz), 8.61 d (1H, Ar-H, J = 4.84 Hz), 9.40 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 54.5, 58.8, 104.3, 116.3, 117.3, 119.3, 125.5, 126.0, 127.1, 127.9, 128.4, 133.6, 134.8, 136.6, 137.5, 151.6, 186.4. HRMS: found $[M + H]^+$ 456.1923. $C_{27}H_{25}N_3O_4$. Calculated 455.18.

(*Z*)-1-(6-Methylpyridin-2-yl)-3-[1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl]prop-2-en-1-one (6f). Pale yellow solid, yield 78%, mp 90–92°C. IR spectrum, ν, cm^{-1}: 1664 (C=O), 1584 (C=C), 1123 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 2.58 s (3H, CH_3), 3.75 s (3H, OCH_3), 3.83 s (6H, $2OCH_3$), 6.93 s (2H, Ar-H), 7.39 t (1H, Ar-H, J = 7.36 Hz), 7.51–7.58 m (3H, Ar-H), 7.85–7.92 m (3H, Ar-H), 7.99 d (2H, Ar-H, J = 7.8 Hz), 8.13 d (1H, Ar-H, J = 16.1 Hz), 9.33 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 22.5, 54.3, 58.6, 104.3, 116.3, 117.2, 117.9, 118.8, 124.8, 125.2, 126.0, 126.7, 127.6, 132.8, 135.4, 136.5, 137.4, 151.4, 155.8, 187.1. HRMS: found $[M + H]^+$ 456.1923. $C_{27}H_{25}N_3O_4$. Calculated 455.18.

(*Z*)-1-(5-Chloropyridin-2-yl)-3-(1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl)prop-2-en-1-one (6g). Off white solid, yield 83%, mp 190–191°C. IR spectrum, ν, cm^{-1}: 1667 (C=O), 1590 (C=C), 1128 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.74 s (3H, OCH_3), 3.84 s (6H, $2OCH_3$), 6.92 s (2H, Ar-H), 7.39 t (1H, Ar-H, J = 7.44 Hz), 7.56 t (2H, Ar-H, J = 7.6 Hz), 7.89 d (1H, Ar-H, J = 15.96 Hz), 8.0 d (2H, Ar-H, J = 7.68 Hz), 8.08–8.10 m (2H, Ar-H), 8.14–8.18 m (1H, Ar-H), 8.80 d (1H, Ar-H, J = 2.16 Hz), 9.42 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 75.7, 80.0, 125.8, 137.6, 138.6, 139.5, 143.6, 146.7, 147.3, 148.4, 149.1, 154.9, 155.1, 156.9, 157.9, 158.8, 167.1, 171.7, 172.9. HRMS: found $[M + H]^+$ 476.1342. $C_{26}H_{22}ClN_3O_4$. Calculated 475.13.

(*Z*)-1-(4,5-Dihydrothiazol-2-yl)-3-[1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-4-yl]prop-2-en-1-one (6h). Pale yellow solid, yield 76%, mp 174–178°C. IR spectrum, ν, cm^{-1}: 1621 (C=O), 1601 (C=C), 1129 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.74 s (3H, OCH_3), 3.85 s (6H, $2OCH_3$), 6.93 s (2H, Ar-H), 7.39 t (1H, Ar-H, J = 7.36 Hz), 7.56 t (2H, Ar-H, J = 7.72 Hz), 7.83 d (1H, Ar-H, J = 15.96 Hz), 8.0 d (2H, Ar-H, J = 7.96 Hz), 8.10 d (1H, Ar-H, J = 16 Hz), 8.18 d (1H, Ar-H, J = 2.96 Hz), 8.24 d (1H, Ar-H, J = 2.96 Hz), 9.46 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 54.5, 58.8, 104.3, 116.3, 117.3, 119.3, 125.5, 126.0, 127.2, 127.9, 128.4, 133.6, 134.8, 136.6, 137.5, 151.6, 186.4. HRMS: found $[M + H]^+$ 448.1231. $C_{24}H_{21}N_3O_4S$. Calculated 447.13.

(*Z*)-1-(1-Methyl-1*H*-benzo[*d*]imidazol-2-yl)-3-[1-phenyl-3-(3,4,5-trimethoxyphenyl]-1*H*-pyrazol-4-yl) prop-2-en-1-one (6i). Pale yellow solid, yield 85%, mp 190–192°C. IR spectrum, ν, cm^{-1}: 1657 (C=O), 1587 (C=C), 1126 (C–O–C). ^{1}H NMR spectrum, δ, ppm: 3.75 s (3H, OCH_3), 3.86 s (6H, $2OCH_3$), 4.14 s (3H, NCH_3), 6.94 s (2H, Ar-H), 7.38 q (2H, Ar-H, J = 7.4 Hz), 7.47 t (1H, Ar-H, J = 7.4 Hz), 7.57 t (2H, Ar-H, J = 7.64 Hz), 7.74 d (1H, Ar-H, J = 8.24 Hz), 7.81 d (1H, Ar-H, J = 8.12 Hz), 7.95 d (1H, Ar-H, J = 15.92 Hz), 8.02 d (2H, Ar-H, J = 7.96 Hz), 8.10 d (1H, Ar-H, J = 15.96 Hz), 9.48 s (1H, pyrazole-H). ^{13}C NMR spectrum, δ, ppm: 32.2, 56.0, 60.1, 106.0, 111.5, 117.5, 118.8, 120.8, 122.5, 123.5, 125.5, 127.3, 129.1, 129.6, 134.7, 136.8, 138.0, 138.9, 141.1, 146.9, 153.1, 182.2. HRMS: found, $[M + H]^+$ 495.1906. $C_{29}H_{26}N_4O_4$. Calculated 494.20.

Antibacterial bioassay. In vitro antibacterial activity of the compounds **6a–6i** was studied against four bacterial strains *Escherichia coli* (MTCC 2692), *Pseudomonas aeruginosa* (MTCC 2453) *Staphylococcus aureus* (MTCC 902), and *Bacillus subtilis* (MTCC 441) by the cup plate method using literature protocol [19, 20]. The stock solution of the antibiotic was prepared (1 mg/mL) and concentration of the test antibiotic was determined. It was sterilized in Muller–Hinton agar medium in an autoclave at 121°C at 15 lbs pressure for 15 min. Suspension of 1 mL of standard test organism was added to Muller Hinton medium and mixed thoroughly at 50°C. The above mixture was poured into a petri dish to form a layer of about 3 mm thick and allowed to solidify. The cups with the known dilutions were added to the standard drug solution with known dilutions and stored carefully in the refrigerator for diffusion for 20 min. The condensed water was wiped carefully from the lid of the petri dishes with sterile cotton plugs. The petri dishes were incubated at 37°C for 18–24 h. Size of the zones of inhibition was recorded against each cavity and measured in mm with the help of antibiotic zone reader. The antibacterial activity of the samples was assessed using different concentrations of the sample.

Anticancer activity measurement. Human lung carcinoma (A549) cell lines were obtained from NCCS Pune and kept in RPMI 1640 medium (#AL199A, Himedia) supplemented with 10% Fetal Bovine Serum

(a) (b)

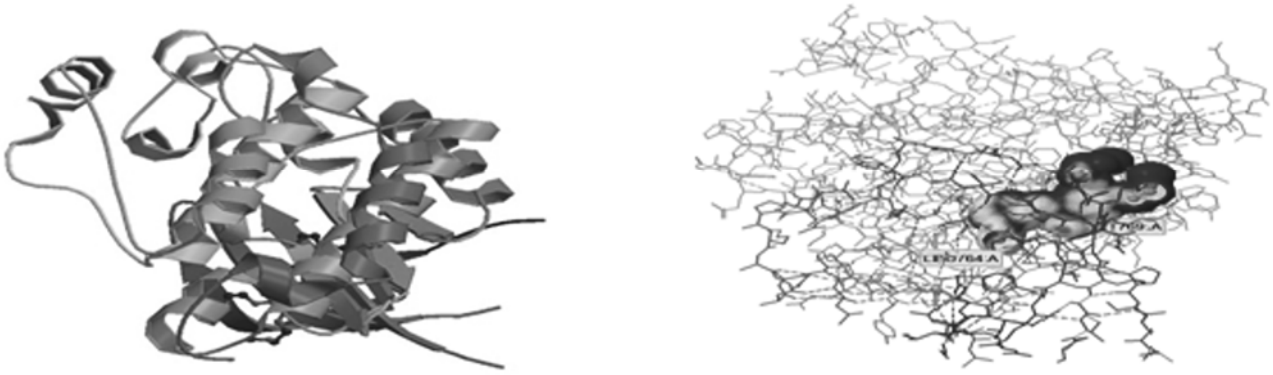


Fig. 4. (a) Structure of EGFR and (b) active site of EGFR.

(#RM10432, Himedia). The cells were stored to confluency at 37°C in an incubator (Healforce, China) with humidified atmosphere and 5% CO_2. There was seeded 200µl cell suspension in a 96-well plate at required cell density (20 000 cells per well) without the test agent and the cells were allows to grow for ca 12 h. Test samples of concentrations of 25, 50, 75, 100, and 125 µg/mL of compounds and 40 µg/mL of drugs were added for the growth of the cells separately and the plates were incubated for 24 h at 37°C in the 5% CO_2 atmosphere. After the incubation period, the plates were taken out of the incubator, the spent media removed and MTT reagent (Camptothecin) was added to the final concentration of 0.5 mg/mL of total volume and the plates were incubated for 3 h. The MTT reagent was removed and 100 µL of solubilizing solution (DMSO) were added. Absorbance was recorded on an ELISA reader at 570 and 630 nm. The IC_{50} value was obtained by using linear regression equation i.e., $y = mx + c$ where, $y = 50$, m and c values were derived from the viability graph.

Molecular docking. The structure of EGFR of *Homo sapiens* was obtained from PDB database. After the unnecessary chains and hetero atoms were removed using SPDBV software, hydrogens were added to the protein and used for active site identification. The active site of EGFR was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities is based on precise computational geometry methods that include alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings as well as cavities. The program specifies the atoms lining pockets, pocket openings and buried cavities, the volume and area of pockets and cavities, and the area and circumference of mouth openings [23]. Docking has been carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows the partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the EGFR. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA include population size (100), selection pressure (1.1), number of operations (10 000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. In the process of docking the default algorithm speed was selected and the ligand binding site in the EGFR was defined within a 10 Å radius with the centroid as CE atom of GLN-111. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5 Å RMSD. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected [24]. Gold Score performs a force field based scoring function and is made up of four components which included Protein-ligand hydrogen bond energy (external H-bond); Protein-ligand Van-der-Waals energy (external vdw); Ligand internal Van-der-Waals energy (internal vdw) and Ligand intramolecular hydrogen bond energy (internal H-bond). The external Van-der-Waals score was multiplied by a factor of 1.375 when the total fitness score was computed. This

was however an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

$$\text{Gold Score} = S\,(\text{hb\_ext}) + S(\text{vdw\_ext}) + S(\text{hb\_int}) + S(\text{vdw\_int}),$$

where S(hb_ext) is the protein-ligand hydrogen bond score, S(vdw_ext) is the protein-ligand van der Waals score, S(hb_int) is the score from intramolecular hydrogen bond in the ligand, and S(vdw_int) is the score from intramolecular strain in the ligand.

The final stable structure of the EGFR (PDB ID: 4HJO) obtained is shown in Fig. 4a. From the CASTp results, it was identified that EGFR protein contained GLN27, GLN111, GLY41, LYS42, GLU154, PRO155, ALA415, GLN408, ILE94 amino acid residues in the active site region (Fig. 4b).

CONCLUSIONS

Nine novel 1-phenyl-3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole substituted aryl and heteroaryl chalcones were synthesized in high yields by the treatment of aldehyde **4** with various aryl and heteroaryl acetophenones **5a–5i**. Antibacterial activity results revealed that compound **6d** exhibited high activity against bacterial strains *E. coli, S. aureus,* and *bacillus*. According to anticancer activity tests data, the compounds **6a**, **6c, 6d,** and **6i** showed potent activities at micro molar concentration being compared to the standard drug Camptothecin. The ADMET studies results confirmed that the synthesized compounds could exhibit inhibitory character in cancer therapy.

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