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# Synthesis and biological evaluation of pyrazole derivatives containing thiourea skeleton as anticancer agents

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ABSTRACT

Two series of pyrazole derivatives designing for potential EGFR kinase inhibitors have been discovered. Some of them exhibited significant EGFR inhibitory activity. Compound *3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide* (**C5**) displayed the most potent EGFR inhibitory activity with $IC_{50}$ of 0.07 μM, which was comparable to the positive control erlotinib. Docking simulation was performed to position compound **C5** into the EGFR active site to determine the probable binding model. Antiproliferative assay results indicating that some of the pyrazole derivatives own high antiproliferative activity against MCF-7. Compound **C5** showed significant antiproliferative activity against MCF-7 with $IC_{50}$ of 0.08 μM. Therefore, compound **C5** with potent inhibitory activity in tumor growth inhibition would be a potential anticancer agent.



## 1. Introduction

Cancer chemotherapy has entered a new era of molecularly targeted therapeutics, which is highly selective and not associated with the serious toxicities of conventional cytotoxic drugs.[1] Receptor protein tyrosine kinases play a key role in signal transduction pathways that regulate cell division and differentiation. Among the growth factor receptor kinases that have been identified as being important in cancer is epidermal growth factor receptor (EGFR) kinase. Activation of EGFR may be because of overexpression, mutations resulting in constitutive activation, or autocrine expression of ligand.[2,3] The role of EGFR has been most thoroughly studied in breast cancer, where it is overexpressed in 25–30% of cases and is correlated with a poor prognosis. EGFR overexpression is also seen in ovarian cancer,[4] lung cancer (especially lung adenocarcinomas)[5–7] and in hormone-refractory prostate cancer.[8]

Compounds that inhibit the kinase activity of EGFR after binding of its cognate ligand are of potential interest as new therapeutic antitumor agents.[9,10]

The thiourea and urea derivatives play important role in anticancer agents because of their good inhibitory activity against receptor tyrosine kinases (RTKs), protein tyrosine kinases (PTKs), and NADH oxidase, which play critical roles in many aspects of tumorigenesis.[11–13] For example, pyrazolopyrimidine ureas bound to the ATP binding site of KDR kinase, the amino thienopyrimidine core mimics the adenine of ATP in its interaction with the hinge region of KDR. Hydrogen bonds are formed between the exocyclic amino group of pyrazolopyrimidine ureas and the backbone carbonyl of Glu 917 and the proximal ring nitrogen and the backbone N–H of Cys 919.[14]

Many pyrazole derivatives are acknowledged to possess a wide range of bioactivities. The pyrazole motif makes up the core structure of numerous biologically active compounds. Thus, some representatives of this heterocycle exhibit anti-viral/anti-tumor,[15–17] antibacterial,[18–21] antiinflamatory,[22] analgesic,[23] fungistatic,[24] and anti-hyperglycemic activity.[25,26] Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazole C-glycoside, pyrazofurin which demonstrated a broad spectrum of antimicrobial activity.[27] However, to our knowledge, few reports have been dedicated to the synthesis and EGFR inhibitory activity of pyrazole derivatives containing thiourea skeleton. Herein, in continuation to extend our research on anticancer compounds with EGFR inhibitory activity,[28,29] we report in the present work the synthesis and structure–activity relationships of a series of pyrazole derivatives containing thiourea skeleton as anticancer agents. Biological evaluation indicated that some of the synthesized compounds are potent inhibitors of EGFR. Compound **C5** displayed the most potent EGFR inhibitory activity with $IC_{50}$ of 0.07 μM, which was comparable to the positive control erlotinib ($IC_{50}$ = 0.03 μM). Docking simulations were performed using the X-ray crystallographic structure of the EGFR in complex with an inhibitor to explore the binding modes of these compounds at the active site.

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## 2. Results and discussion

### 2.1. Chemistry

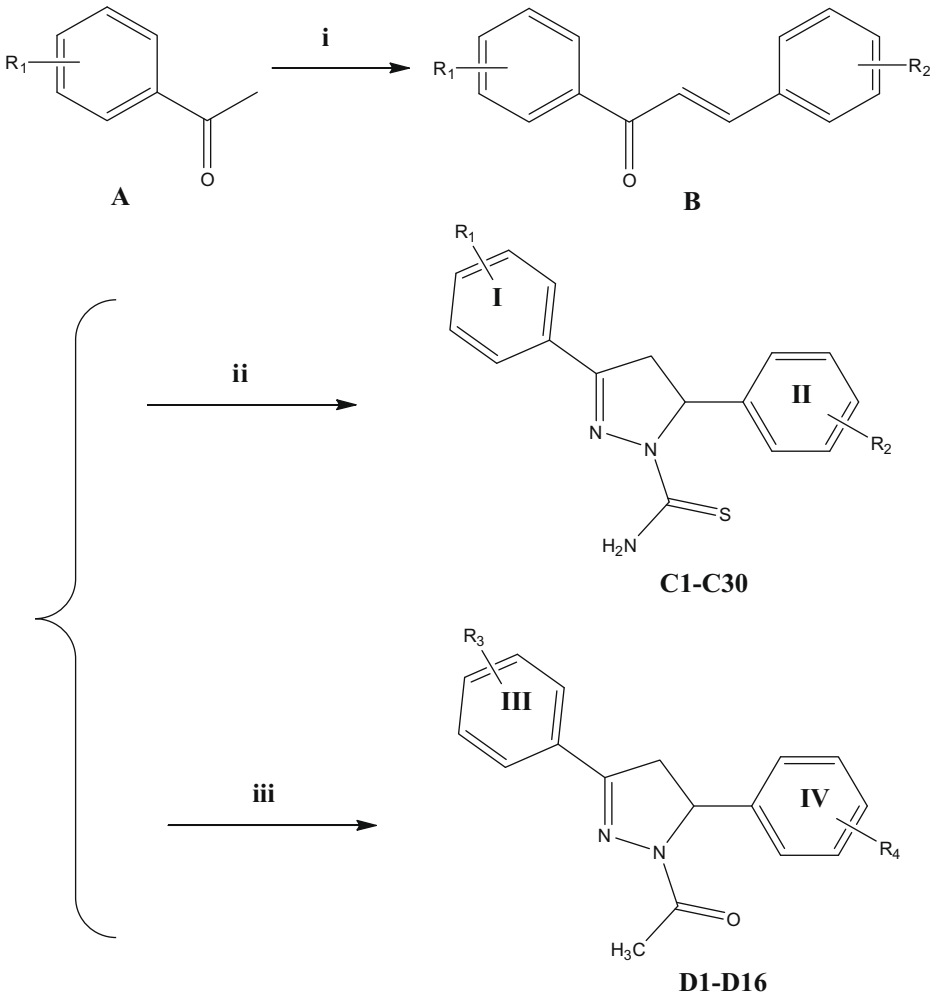
The synthesis of compounds **C1–C30** and **D1–D16** followed the general pathway outlined in Scheme 1. They are prepared in two steps. Firstly, the chalcones were obtained by direct condensation between the aromatic aldehydes and the substituted acetophenone, using 20% potassium hydroxide as catalyst in ethanol. Secondly, for compounds **C1–C30**, cyclization of different chalcones with thiosemicarbazide under basic condition in refluxing ethanol leads to the formation of pyrazole derivatives containing thiourea skeleton. As for compounds **D1–D16**, a solution of chalcones (5 mmol) in 30 mL of acetic acid was added dropwise to 0.6 mL of hydrazine hydrate (12.5 mmol) and kept under stirring at 120 °C for 24 h. The mixture was then poured into ice-water, obtaining the 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives **D1–D16**. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

### 2.2. EGFR inhibitory activity and structure–activity relationship of the pyrazole derivatives C1–C30 and D1–D16

The two series of pyrazole derivatives (**C1–C30** and **D1–D16**) were evaluated for their ability to inhibit the autophosphorylation of EGFR kinases using a solid-phase ELISA assay. The inhibition constant ($IC_{50}$) of the compounds were summarized in Tables 1 and 2. As shown in Table 1, it was observed that pyrazole derivatives containing thiourea skeleton (compounds **C1–C30**) have been found to show fairly good inhibitory activity displaying $IC_{50}$ values between 0.07 and 13.37 μM. In order to help understand the structure–activity relationship, a series of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives (compounds **D1–D16**) have been synthesized and also tested for their EGFR kinase inhibitory activity.

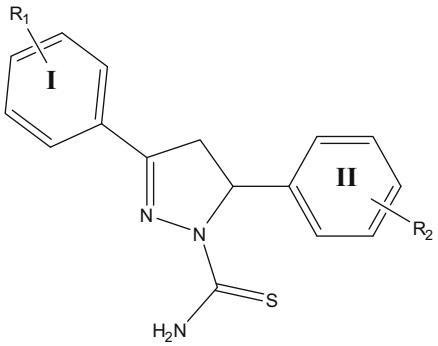
As illustrated in Table 2, compounds **D1**–**D16** displayed moderate EGFR inhibitory activity with $IC_{50}$ ranging from 15.24 to 28.69 μM. These results indicated that the thiourea skeleton in the pyrazole derivatives **C1**–**C30** play an important role in the EGFR inhibitory activity. For example, there is only one difference between compounds **C1**–**C5** and compounds **D9**–**D13**, that is, the former five compounds have the thiourea skeleton. Compounds **C1**–**C5** exhibited potent EGFR inhibitory activity with $IC_{50}$ ranging from 0.07 to 2.17 μM, however, compounds **D9**–**D13** showed moderate EGFR inhibitory activity with $IC_{50}$ more that 15.24 μM. The same rule also applies to compounds **C11**–**C15** and compounds **D1**–**D5** (see Tables 1 and 2).

Subsequently SAR studies were performed by modification of the parent compound to determine how the substituents of the subunits affected the EGFR inhibitory activities. Inspection of the chemical structure of the compounds **C1–C30** (Table 1 and



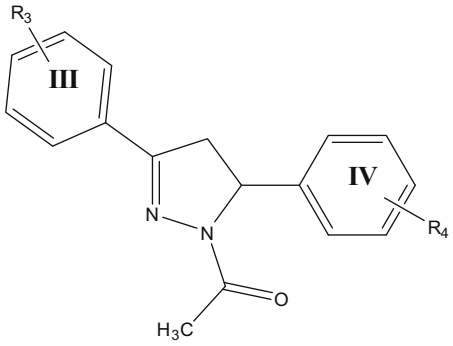

**Scheme 1.** General synthesis of compounds **C1**–**C30** and **D1**–**D16**. Reagents and conditions: (i) substituted aromatic aldehyde, 40% NaOH, $CH_3CH_2OH$; (ii) thiosemicarbazide, $CH_3CH_2OH$, reflux; (iii) $N_2H_4{\cdot}H_2O$, $CH_3COOH$, 120 °C, reflux, 12 h.

**Table 1**
Chemical structures and EGFR inhibitory activity of compounds **C1–C30**




| Compd | $R_1$ | $R_2$ | EGFR ($IC_{50}$, μM) | Compd | $R_1$ | $R_2$ | EGFR ($IC_{50}$, μM) |
|---|---|---|---|---|---|---|---|
| **C1** | 3,4-2$CH_3$ | 4-F | 0.83 | **C16** | 3,4-2Cl | 4-OH | 5.27 |
| **C2** | 3,4-2$CH_3$ | 4-Cl | 1.36 | **C17** | 3,4-2Cl | 4-$NO_2$ | 7.78 |
| **C3** | 3,4-2$CH_3$ | 4-Br | 2.17 | **C18** | 3,4-2Cl | 2-F | 6.56 |
| **C4** | 3,4-2$CH_3$ | 4-$CH_3$ | 0.34 | **C19** | 3,4-2Cl | 2-Cl | 7.28 |
| **C5** | 3,4-2$CH_3$ | 4-$OCH_3$ | 0.07 | **C20** | 3,4-2Cl | 2-Br | 6.43 |
| **C6** | 3,4-2$CH_3$ | 4-OH | 0.13 | **C21** | 3,4-2Br | 4-F | 8.92 |
| **C7** | 3,4-2$CH_3$ | 4-$NO_2$ | 3.06 | **C22** | 3,4-2Br | 4-Cl | 8.15 |
| **C8** | 3,4-2$CH_3$ | 2-F | 5.19 | **C23** | 3,4-2Br | 4-Br | 10.06 |
| **C9** | 3,4-2$CH_3$ | 2-Cl | 3.87 | **C24** | 3,4-2Br | 4-$CH_3$ | 9.83 |
| **C10** | 3,4-2$CH_3$ | 2-Br | 4.21 | **C25** | 3,4-2Br | 4-$OCH_3$ | 8.09 |
| **C11** | 3,4-2Cl | 4-F | 6.27 | **C26** | 3,4-2Br | 4-OH | 11.27 |
| **C12** | 3,4-2Cl | 4-Cl | 7.32 | **C27** | 3,4-2Br | 4-$NO_2$ | 10.64 |
| **C13** | 3,4-2Cl | 4-Br | 6.85 | **C28** | 3,4-2Br | 2-F | 13.37 |
| **C14** | 3,4-2Cl | 4-$CH_3$ | 7.38 | **C29** | 3,4-2Br | 2-Cl | 12.26 |
| **C15** | 3,4-2Cl | 4-$OCH_3$ | 5.74 | **C30** | 3,4-2Br | 2-Br | 10.97 |
| Erlotinib | — | — | 0.03 | — | — | — | — |

**Table 2**
Chemical structures and EGFR inhibitory activity of compounds **D1–D16**




| Compd | $R_3$ | $R_4$ | EGFR ($IC_{50}$, μM) | Compd | $R_3$ | $R_4$ | EGFR ($IC_{50}$, μM) |
|---|---|---|---|---|---|---|---|
| **D1** | 3,4-2Cl | 4-F | 18.35 | **D9** | 3,4-2$CH_3$ | 4-F | 15.56 |
| **D2** | 3,4-2Cl | 4-Cl | 16.14 | **D10** | 3,4-2$CH_3$ | 4-Cl | 19.73 |
| **D3** | 3,4-2Cl | 4-Br | 18.45 | **D11** | 3,4-2$CH_3$ | 4-Br | 20.61 |
| **D4** | 3,4-2Cl | 4-$CH_3$ | 19.32 | **D12** | 3,4-2$CH_3$ | 4-$CH_3$ | 17.49 |
| **D5** | 3,4-2Cl | 4-$OCH_3$ | 17.19 | **D13** | 3,4-2$CH_3$ | 4-$OCH_3$ | 15.24 |
| **D6** | 3,4-2Cl | 2-Cl | 24.15 | **D14** | 3,4-2$CH_3$ | 2-Cl | 21.48 |
| **D7** | 3,4-2Cl | 4-H | 28.69 | **D15** | 3,4-2$CH_3$ | 4-H | 20.04 |
| **D8** | 3,4-2Cl | 3,5-2$OCH_3$ | 25.47 | **D16** | 3,4-2$CH_3$ | 3,5-2$OCH_3$ | 17.31 |
| Erlotinib | — | — | 0.03 | — | — | — | — |

Scheme 1) suggested that it could be divided into two subunits: **I**- and **II**-rings.

As shown in Table 1, structure–activity relationships in compounds **C1–C30** demonstrated that compounds bearing two methyl group at 3-position and 4-position at **I**-ring (compounds **C1–C10**, $IC_{50}$: 0.07–5.19 μM) showed better EGFR inhibitory activity than those with two halogen substituents (compounds **C11–C30**, $IC_{50}$: 5.27–13.37 μM) at the same position. Among the former, com-

pounds **C8–C10** ($IC_{50}$: 3.87–5.19 μM) with substitution on the 2-position of the **II**-ring displayed less inhibitory activity than those with 4-position (compounds **C1–C7**, $IC_{50}$: 0.07–3.06 μM).

As for compounds **C1–C7**, a comparison of the substitution on **II**-ring demonstrated that the electron-donating substituents at 4-position derivatives (compounds **C4–C6**, $IC_{50}$: 0.07–0.34 μM) have more potent EGFR inhibitory activities than the electron-withdrawing substituents ones (compounds **C1–C3** and **C7**, $IC_{50}$: 1.06–3.16 μM). Most significantly, the stronger electron-donating substituents the compounds contained on **II**-ring at 4-position, the more potent it showed, which was illustrated by the potency order $OCH_3 > OH > CH_3$. Among them, compound **C5** displayed the most potent EGFR inhibitory activity with $IC_{50}$ of 0.07 μM, which was comparable to the positive control erlotinib ($IC_{50}$ = 0.03 μM).

### 2.3. The antiproliferative activity and molecular docking study of the top 10 compounds

The binding affinity was evaluated by the hydrogen bonding and binding free energies (ΔGb, kcal/mol). Compound **C5** which displayed the most potent EGFR inhibitory activity was selected for further molecular docking study. Then, molecular docking of the most potent inhibitor **C5** into ATP binding site of EGFR kinase was performed on the binding model based on the EGFR complex structure (1M17.pdb). The binding model of compound **C5** and EGFR is depicted in Figures 1–3. In the binding model, compound **C5** is nicely bound to the EGFR kinase with its N–H group project toward the side chain carbonyl group of D831 (Asp831), forming a more optimal H-bond interaction (distance: 1.94 Å, angle: 127.1°). Based on the significant EGFR inhibitory activity of pyrazole derivatives containing thiourea skeleton, it can be concluded that this H-bond plays an important effect in the EGFR inhibitory. Also, the oxygen atom of the methoxy group of compound **C5** forms hydrogen bond with G697 (Gly697) (distance: 2.20 Å, angle: 156.8°). The predicted binding free energy that includes the intermolecular energy and torsional free energy was used as the criterion for ranking. The selected pose of compound **C5** had an estimated free energy of binding of −10.17 kcal/mol. The estimated free energy of other compounds (**C1–C4, C6–C10**) are ranging from −9.78 to −6.57 kcal/mol. Furthermore, the intermolecular hydrogen bonds of compound **C5** (Fig. 1), whose effect has already been counted in the binding energy, were also investigated in order to find useful information for drug design. This molecular docking result, along with the biological assay data, suggesting that compound **C5** is a potential inhibitor of EGFR.

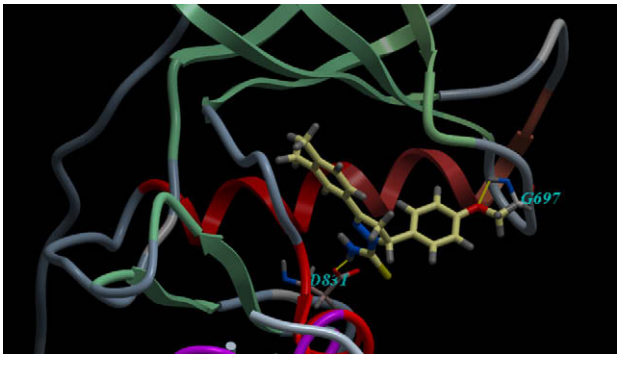



**Figure 1.** Molecular docking modeling of compound **C5** with EGFR kinase: for clarity, only interacting residues are displayed. The H-bond (yellow line) is displayed as line. Compound **C5** is nicely bound to the EGFR kinase with its N–H group project toward the side chain carbonyl group of D831 (Asp831), forming a more optimal H-bond interaction. Also, the oxygen atom of the methoxy group of compound **C5** forms hydrogen bond with G697 (Gly697).

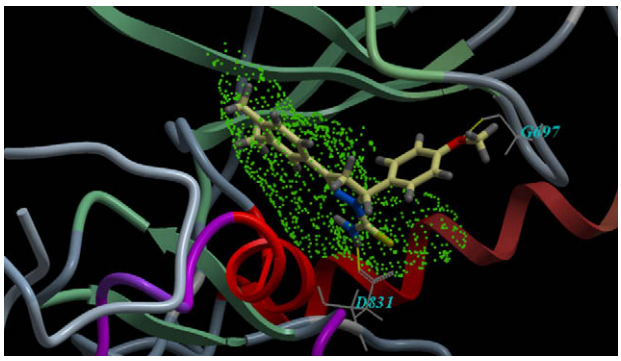



**Figure 2.** Binding model of compound **C5** and EGFR kinase. The H-bond (yellow line) is displayed as line, and the dots show the binding sites of EGFR kinase. For clarity, only interacting residues are displayed.

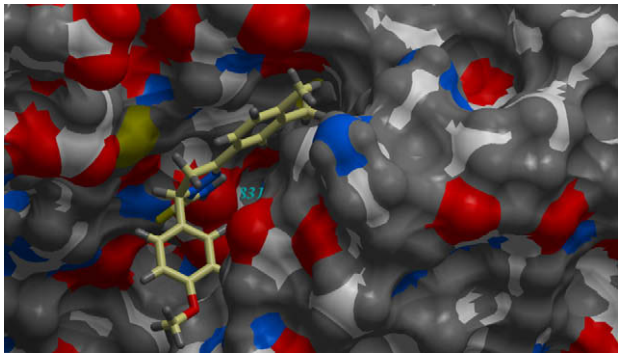


**Figure 3.** 3D model of the interaction between compound **C5** and the EGFR kinase binding site. The EGFR kinase is represented by molecular surface. Compound **C5** is depicted by sticks and balls.

**Table 3**
Antiproliferative activity and docking parameters of the top 10 compounds

| Top compounds | MCF-7 ($IC_{50}$, μM) | Docking parameters ΔGb (kcal/mol) |
|---|---|---|
| **C1** | 0.58 ± 0.03 | −7.76 |
| **C2** | 0.71 ± 0.05 | −8.29 |
| **C3** | 0.64 ± 0.05 | −7.86 |
| **C4** | 0.31 ± 0.07 | −8.62 |
| **C5** | 0.08 ± 0.01 | −10.17 |
| **C6** | 0.17 ± 0.02 | −9.78 |
| **C7** | 0.96 ± 0.06 | −8.07 |
| **C8** | 1.54 ± 0.09 | −7.49 |
| **C9** | 1.83 ± 0.12 | −6.57 |
| **C10** | 1.48 ± 0.08 | −7.14 |
| Erlotinib | 0.02 ± 0.005 | −10.86 |

Besides, the in vitro antiproliferative activity of the top 10 compounds (compounds **C1**–**C7** and **C9**) was studied on a panel of one human tumor cell line (MCF-7), which overexpresses EGFR. As shown in Table 3, out of the top 10 compounds, compounds **C5** and **C6**, which have potent inhibitory activity of EGFR showed high antiproliferative activity against MCF-7 with $IC_{50}$ of 0.08 and 0.17 μM, indicating that these pyrazole derivatives were potent inhibitor of EGFR as antitumor agents. In particular, compound **C5** has demonstrated significant inhibitory activity in tumor growth inhibition and displayed favorable EGFR inhibitory activity.

## 3. Conclusions

Two series of pyrazole derivatives that may function as inhibitors of EGFR and kinases have been prepared, and some of the

synthesized compounds displayed potent EGFR inhibitory. Compound **C5** exhibited the most potent EGFR inhibitory activity with $IC_{50}$ of 0.07 μM, which was comparable to the positive control erlotinib.

Compound **C5** is nicely bound to the EGFR kinase with its N–H group project toward the side chain carbonyl group of Asp831, forming a more optimal H-bond interaction. Also, the oxygen atom of the methoxy group of compound **C5** forms hydrogen bond with Gly697. Antiproliferative assay results indicated that these pyrazole derivatives own high antiproliferative activity against MCF-7. In particular, compound **C5** has demonstrated significant EGFR inhibitory activity and inhibitory activity in tumor growth inhibition as a potential anticancer agent.

## 4. Experimental section

### 4.1. General

Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck). The quantity of silica gel used was 50–100 times the weight charged on the column. Then, the eluates were monitored using TLC. Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and $^1$H NMR spectra were recorded on a Bruker PX500 or DPX300 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm ($\delta$). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

### 4.2. Synthesis

#### 4.2.1. General synthetic procedure of chalcones

Equimolar portions of the appropriately substituted aromatic aldehydes (10 mmol, 1 equiv) and ketones (10 mmol, 1 equiv) were dissolved in approximately 15 mL of ethanol. The mixture was allowed to stir for several minutes at 5–10 °C. A 10 mL aliquot of a 40% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction flask via a self-equalizing addition funnel. The reaction solution was allowed to stir at room temperature for approximately 4 h. Most commonly, a precipitate formed and was then collected by suction filtration.

#### 4.2.2. General synthetic procedure of pyrazole derivatives C1–C30

A mixture of chalcone (0.01 mol), thiosemicarbazide (0.01 mol), and NaOH (0.025 mol) was refluxed in ethanol (25 mL) for 8 h. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol.

**4.2.2.1. 3-(3,4-Dimethylphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C1).** White powder. Mp 223–225 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.29 (s, 6H); 3.12–3.18 (dd, $J_1$ = 3.66, $J_2$ = 17.58 Hz, 1H); 3.74–3.83 (dd, $J_1$ = 11.52, $J_2$ = 17.76 Hz, 1H); 5.92–5.97 (dd, $J_1$ = 3.66, $J_2$ = 11.56 Hz, 1H); 7.09–7.18 (m, 3H); 7.42–7.46 (m, 2H); 7.46–7.48 (m, 1H); 7.50 (s, 1H). MS (ESI): 328.1 ($C_{18}H_{19}FN_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}FN_3S$: C, 66.03; H, 5.54; N, 12.83. Found: C, 66.17; H, 5.72; N, 12.65.

**4.2.2.2. 5-(4-Chlorophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C2).** White powder. Mp 216–217 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 6H); 3.11–3.18 (dd, $J_1$ = 3.66, $J_2$ = 17.73 Hz, 1H); 3.76–3.86 (dd, $J_1$ = 11.52, $J_2$ = 17.73 Hz, 1H); 5.96–6.01 (dd, $J_1$ = 3.66, $J_2$ = 11.34 Hz, 1H); 7.14–7.19 (m, 3H); 7.27–7.30 (m, 2H); 7.41–7.44 (m, 1H); 7.50 (s, 1H). MS (ESI): 344.1 ($C_{18}H_{19}ClN_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}ClN_3S$: C, 62.87; H, 5.28; N, 12.22. Found: C, 62.69; H, 5.42; N, 12.41.

**4.2.2.3. 5-(4-Bromophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C3).** White powder. Mp 231–233 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 6H); 3.11–3.18 (dd, $J_1$ = 3.66, $J_2$ = 17.55 Hz, 1H); 3.76–3.86 (dd, $J_1$ = 11.52, $J_2$ = 17.76 Hz, 1H); 5.94–5.99 (dd, $J_1$ = 3.66, $J_2$ = 11.52 Hz, 1H); 7.08–7.19 (m, 3H); 7.41–7.44 (m, 2H); 7.45–7.47 (m, 1H); 7.51 (s, 1H). MS (ESI): 388.1 ($C_{18}H_{19}BrN_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}BrN_3S$: C, 55.67; H, 4.67; N, 10.82. Found: C, 55.78; H, 4.81; N, 10.64.

**4.2.2.4. 3-(3,4-Dimethylphenyl)-5-p-tolyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C4).** White powder. Mp 198–200 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 9H); 3.14–3.21 (dd, $J_1$ = 3.48, $J_2$ = 17.55 Hz, 1H); 3.74–3.84 (dd, $J_1$ = 11.34, $J_2$ = 17.55 Hz, 1H); 5.96–6.01 (dd, $J_1$ = 3.45, $J_2$ = 11.34 Hz, 1H); 7.08–7.13 (m, 3H); 7.14–7.18 (m, 2H); 7.41–7.44 (m, 1H); 7.51 (s, 1H). MS (ESI): 324.1 ($C_{19}H_{22}N_3S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{21}N_3S$: C, 70.55; H, 6.54; N, 12.99. Found: C, 70.74; H, 6.42; N, 12.76.

**4.2.2.5. 3-(3,4-Dimethylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C5).** White powder. Mp 207–209 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.31 (s, 6H); 3.11–3.19 (dd, $J_1$ = 3.47, $J_2$ = 17.56 Hz, 1H); 3.71 (s, 3H); 3.72–3.81 (dd, $J_1$ = 11.42, $J_2$ = 17.55 Hz, 1H); 5.97–6.03 (dd, $J_1$ = 3.45, $J_2$ = 11.34 Hz, 1H); 7.06–7.11 (m, 3H); 7.15–7.18 (m, 2H); 7.42–7.45 (m, 1H); 7.51 (s, 1H). MS (ESI): 340.1 ($C_{19}H_{22}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{21}N_3OS$: C, 67.23; H, 6.24; N, 12.38. Found: C, 67.48; H, 6.46; N, 12.17.

**4.2.2.6. 3-(3,4-Dimethylphenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C6).** White powder. Mp 218–219 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 6H); 3.12–3.18 (dd, $J_1$ = 3.67, $J_2$ = 17.55 Hz, 1H); 3.74–3.83 (dd, $J_1$ = 11.53, $J_2$ = 17.76 Hz, 1H); 5.93–5.99 (dd, $J_1$ = 3.68, $J_2$ = 11.53 Hz, 1H); 7.09–7.19 (m, 3H); 7.41–7.44 (m, 2H); 7.44–7.46 (m, 1H); 7.50 (s, 1H). MS (ESI): 326.1 ($C_{18}H_{20}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}N_3OS$: C, 66.43; H, 5.88; N, 12.91. Found: C, 66.65; H, 5.69; N, 12.75.

**4.2.2.7. 3-(3,4-Dimethylphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C7).** White powder. Mp 202–203 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.31 (s, 6H); 3.13–3.19 (dd, $J_1$ = 3.66, $J_2$ = 17.55 Hz, 1H); 3.76–3.85 (dd, $J_1$ = 11.51, $J_2$ = 17.73 Hz, 1H); 5.92–5.97 (dd, $J_1$ = 3.65, $J_2$ = 11.51 Hz, 1H); 7.07–7.16 (m, 3H); 7.40–7.43 (m, 2H); 7.45–7.47 (m, 1H); 7.50 (s, 1H). MS (ESI): 355.1 ($C_{18}H_{19}N_4O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81. Found: C, 61.18; H, 5.32; N, 15.64.

**4.2.2.8. 3-(3,4-Dimethylphenyl)-5-(2-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C8).** White powder. Mp 211–213 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 6H); 3.08–3.15 (dd, $J_1$ = 4.04, $J_2$ = 17.89 Hz, 1H); 3.88–3.97 (dd, $J_1$ = 11.53, $J_2$ = 17.96 Hz, 1H); 6.33–6.38 (dd, $J_1$ = 4.09, $J_2$ = 11.43 Hz, 1H); 7.08–7.12 (m, 1H); 7.16–7.26 (m, 3H); 7.39–7.45 (m, 2H); 7.51 (s, 1H). MS (ESI): 328.1 ($C_{18}H_{19}FN_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}FN_3S$: C, 66.03; H, 5.54; N, 12.83. Found: C, 66.19; H, 5.31; N, 12.74.

**4.2.2.9. 5-(2-Chlorophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C9).** White powder. Mp

208–210 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.29 (s, 6H); 3.06–3.14 (dd, $J_1$ = 4.02, $J_2$ = 17.94 Hz, 1H); 3.85–3.95 (dd, $J_1$ = 11.52, $J_2$ = 17.91 Hz, 1H); 6.30–6.35 (dd, $J_1$ = 4.05, $J_2$ = 11.52 Hz, 1H); 7.04–7.07 (m, 1H); 7.15–7.23 (m, 3H); 7.37–7.44 (m, 2H); 7.50 (s, 1H). MS (ESI): 344.1 ($C_{18}H_{19}ClN_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}ClN_3S$: C, 62.87; H, 5.28; N, 12.22. Found: C, 62.63; H, 5.38; N, 12.46.

**4.2.2.10. 5-(2-Bromophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C10).** White powder. Mp 217–219 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.31 (s, 6H); 3.05–3.12 (dd, $J_1$ = 4.06, $J_2$ = 17.96 Hz, 1H); 3.83–3.94 (dd, $J_1$ = 11.51, $J_2$ = 17.93 Hz, 1H); 6.28–6.32 (dd, $J_1$ = 4.03, $J_2$ = 11.52 Hz, 1H); 7.06–7.09 (m, 1H); 7.16–7.23 (m, 3H); 7.38–7.45 (m, 2H); 7.50 (s, 1H). MS (ESI): 388.1 ($C_{18}H_{19}BrN_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{18}BrN_3S$: C, 55.67; H, 4.67; N, 10.82. Found: C, 55.83; H, 4.54; N, 10.74.

**4.2.2.11. 3-(3,4-Dichlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C11).** White powder. Mp 206–208 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.11–3.17 (dd, $J_1$ = 3.65, $J_2$ = 17.51 Hz, 1H); 3.72–3.83 (dd, $J_1$ = 11.32, $J_2$ = 17.73 Hz, 1H); 5.91–5.96 (dd, $J_1$ = 3.67, $J_2$ = 11.51 Hz, 1H); 7.07–7.16 (m, 3H); 7.41–7.45 (m, 2H); 7.47–7.48 (m, 1H); 7.50 (s, 1H). MS (ESI): 368.0 ($C_{16}H_{13}Cl_2FN_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Cl_2FN_3S$: C, 52.18; H, 3.28; N, 11.41. Found: C, 52.35; H, 3.41; N, 11.34.

**4.2.2.12. 5-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C12).** White powder. Mp 211–213 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.11–3.17 (dd, $J_1$ = 3.68, $J_2$ = 17.75 Hz, 1H); 3.75–3.86 (dd, $J_1$ = 11.51, $J_2$ = 17.73 Hz, 1H); 5.94–6.02 (dd, $J_1$ = 3.65, $J_2$ = 11.37 Hz, 1H); 7.13–7.17 (m, 3H); 7.28–7.32 (m, 2H); 7.40–7.44 (m, 1H); 7.51 (s, 1H). MS (ESI): 383.0 ($C_{16}H_{13}Cl_3N_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Cl_3N_3S$: C, 49.95; H, 3.14; N, 10.92. Found: C, 49.82; H, 3.26; N, 10.78.

**4.2.2.13. 5-(4-Bromophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C13).** White powder. Mp 214–216 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.12–3.18 (dd, $J_1$ = 3.68, $J_2$ = 17.53 Hz, 1H); 3.74–3.86 (dd, $J_1$ = 11.53, $J_2$ = 17.76 Hz, 1H); 5.92–5.98 (dd, $J_1$ = 3.66, $J_2$ = 11.53 Hz, 1H); 7.07–7.17 (m, 3H); 7.42–7.45 (m, 2H); 7.47–7.48 (m, 1H); 7.51 (s, 1H). MS (ESI): 427.0 ($C_{16}H_{13}BrCl_2N_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}BrCl_2N_3S$: C, 44.78; H, 2.82; N, 9.79. Found: C, 44.96; H, 2.66; N, 9.98.

**4.2.2.14. 3-(3,4-Dichlorophenyl)-5-p-tolyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C14).** White powder. Mp 223–225 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 3H); 3.12–3.21 (dd, $J_1$ = 3.46, $J_2$ = 17.55 Hz, 1H); 3.76–3.84 (dd, $J_1$ = 11.37, $J_2$ = 17.53 Hz, 1H); 5.94–6.01 (dd, $J_1$ = 3.46, $J_2$ = 11.34 Hz, 1H); 7.06–7.13 (m, 3H); 7.13–7.17 (m, 2H); 7.41–7.43 (m, 1H); 7.50 (s, 1H). MS (ESI): 364.0 ($C_{17}H_{16}Cl_2N_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Cl_2N_3S$: C, 56.05; H, 4.15; N, 11.53. Found: C, 56.18; H, 4.26; N, 11.38.

**4.2.2.15. 3-(3,4-Dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C15).** White powder. Mp 209–210 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.12–3.19 (dd, $J_1$ = 3.46, $J_2$ = 17.53 Hz, 1H); 3.71 (s, 3H); 3.74–3.81 (dd, $J_1$ = 11.43, $J_2$ = 17.53 Hz, 1H); 5.94–6.01 (dd, $J_1$ = 3.46, $J_2$ = 11.34 Hz, 1H); 7.07–7.11 (m, 3H); 7.14–7.18 (m, 2H); 7.42–7.45 (m, 1H); 7.51 (s, 1H). MS (ESI): 380.0 ($C_{17}H_{16}Cl_2N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Cl_2N_3OS$: C, 53.69; H, 3.98; N, 11.05. Found: C, 53.78; H, 3.81; N, 11.24.

**4.2.2.16. 3-(3,4-Dichlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C16).** White powder. Mp 235–237 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.12–3.17 (dd, $J_1$ = 3.67, $J_2$ = 17.53 Hz, 1H); 3.72–3.83 (dd, $J_1$ = 11.56, $J_2$ = 17.76 Hz, 1H); 5.92–5.98 (dd, $J_1$ = 3.68, $J_2$ = 11.51 Hz, 1H); 7.08–7.19 (m, 3H); 7.42–7.44 (m, 2H); 7.44–7.47 (m, 1H); 7.50 (s, 1H). MS (ESI): 366.0 ($C_{16}H_{14}Cl_2N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{13}Cl_2N_3OS$: C, 52.47; H, 3.58; N, 11.47. Found: C, 52.62; H, 3.74; N, 11.59.

**4.2.2.17. 3-(3,4-Dichlorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C17).** White powder. Mp 203–205 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.13–3.18 (dd, $J_1$ = 3.65, $J_2$ = 17.53 Hz, 1H); 3.74–3.83 (dd, $J_1$ = 11.53, $J_2$ = 17.73 Hz, 1H); 5.94–5.97 (dd, $J_1$ = 3.65, $J_2$ = 11.53 Hz, 1H); 7.07–7.14 (m, 3H); 7.40–7.43 (m, 2H); 7.46–7.48 (m, 1H); 7.50 (s, 1H). MS (ESI): 395.0 ($C_{16}H_{13}Cl_2N_4O_2S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Cl_2N_4O_2S$: C, 48.62; H, 3.06; N, 14.17. Found: C, 48.76; H, 3.24; N, 14.34.

**4.2.2.18. 3-(3,4-Dichlorophenyl)-5-(2-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C18).** White powder. Mp 235–236 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.09–3.15 (dd, $J_1$ = 4.16, $J_2$ = 17.86 Hz, 1H); 3.85–3.96 (dd, $J_1$ = 11.53, $J_2$ = 17.96 Hz, 1H); 6.31–6.38 (dd, $J_1$ = 4.21, $J_2$ = 11.43 Hz, 1H); 7.08–7.14 (m, 1H); 7.18–7.26 (m, 3H); 7.36–7.43 (m, 2H); 7.51 (s, 1H). MS (ESI): 368.0 ($C_{16}H_{13}Cl_2FN_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Cl_2FN_3S$: C, 52.18; H, 3.28; N, 11.41. Found: C, 52.32; H, 3.39; N, 11.58.

**4.2.2.19. 5-(2-Chlorophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C19).** White powder. Mp 241–243 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.06–3.12 (dd, $J_1$ = 4.14, $J_2$ = 17.92 Hz, 1H); 3.87–3.95 (dd, $J_1$ = 11.53, $J_2$ = 17.91 Hz, 1H); 6.31–6.35 (dd, $J_1$ = 4.26, $J_2$ = 11.52 Hz, 1H); 7.03–7.07 (m, 1H); 7.17–7.23 (m, 3H); 7.36–7.44 (m, 2H); 7.50 (s, 1H). MS (ESI): 383.0 ($C_{16}H_{13}Cl_3N_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Cl_3N_3S$: C, 49.95; H, 3.14; N, 10.92. Found: C, 49.76; H, 3.27; N, 10.81.

**4.2.2.20. 5-(2-Bromophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C20).** White powder. Mp 229–230 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.07–3.12 (dd, $J_1$ = 4.16, $J_2$ = 17.93 Hz, 1H); 3.86–3.94 (dd, $J_1$ = 11.53, $J_2$ = 17.91 Hz, 1H); 6.26–6.32 (dd, $J_1$ = 4.13, $J_2$ = 11.51 Hz, 1H); 7.04–7.09 (m, 1H); 7.17–7.23 (m, 3H); 7.36–7.45 (m, 2H); 7.50 (s, 1H). MS (ESI): 427.0 ($C_{16}H_{13}BrCl_2N_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}BrCl_2N_3S$: C, 44.78; H, 2.82; N, 9.79. Found: C, 44.92; H, 2.69; N, 9.93.

**4.2.2.21. 3-(3,4-Dibromophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C21).** White powder. Mp 246–247 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.14–3.17 (dd, $J_1$ = 3.66, $J_2$ = 17.51 Hz, 1H); 3.76–3.83 (dd, $J_1$ = 11.31, $J_2$ = 17.73 Hz, 1H); 5.92–5.96 (dd, $J_1$ = 3.66, $J_2$ = 11.56 Hz, 1H); 7.05–7.16 (m, 3H); 7.42–7.45 (m, 2H); 7.46–7.48 (m, 1H); 7.51 (s, 1H). MS (ESI): 455.0 ($C_{16}H_{13}Br_2FN_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_2FN_3S$: C, 42.04; H, 2.65; N, 9.19. Found: C, 42.17; H, 2.74; N, 9.35.

**4.2.2.22. 5-(4-Chlorophenyl)-3-(3,4-dibromophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C22).** White powder. Mp 216–218 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.12–3.17 (dd, $J_1$ = 3.66, $J_2$ = 17.75 Hz, 1H); 3.73–3.86 (dd, $J_1$ = 11.53, $J_2$ = 17.71 Hz, 1H); 5.96–6.04 (dd, $J_1$ = 3.65, $J_2$ = 11.45 Hz, 1H); 7.15–7.17 (m, 3H); 7.26–7.32 (m, 2H); 7.41–7.44 (m, 1H); 7.51 (s, 1H). MS (ESI): 471.0 ($C_{16}H_{13}Br_2ClN_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_2ClN_3S$: C, 40.58; H, 2.55; N, 8.87. Found: C, 40.46; H, 2.68; N, 8.69.

**4.2.2.23. 5-(4-Bromophenyl)-3-(3,4-dibromophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C23).** White powder. Mp 239–241 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.14–3.18 (dd, $J_1$ = 3.69, $J_2$ = 17.53 Hz, 1H); 3.77–3.86 (dd, $J_1$ = 11.51, $J_2$ = 17.76 Hz, 1H); 5.91–5.98 (dd, $J_1$ = 3.66, $J_2$ = 11.57 Hz, 1H); 7.09–7.17 (m, 3H); 7.42–7.46 (m, 2H); 7.47–7.48 (m, 1H); 7.50 (s, 1H). MS (ESI): 515.0

($C_{16}H_{13}Br_3N_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_3N_3S$: C, 37.09; H, 2.33; N, 8.11. Found: C, 37.18; H, 2.45; N, 8.32.

**4.2.2.24. 3-(3,4-Dibromophenyl)-5-p-tolyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C24).** White powder. Mp 211–213 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 3H); 3.13–3.21 (dd, $J_1$ = 3.46, $J_2$ = 17.55 Hz, 1H); 3.74–3.84 (dd, $J_1$ = 11.31, $J_2$ = 17.57 Hz, 1H); 5.96–6.01 (dd, $J_1$ = 3.46, $J_2$ = 11.37 Hz, 1H); 7.08–7.13 (m, 3H); 7.13–7.17 (m, 2H); 7.41–7.43 (m, 1H); 7.50 (s, 1H). MS (ESI): 451.0 ($C_{17}H_{16}Br_2N_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Br_2N_3S$: C, 45.05; H, 3.34; N, 9.27. Found: C, 45.21; H, 3.48; N, 9.36.

**4.2.2.25. 3-(3,4-Dibromophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C25).** White powder. Mp 236–238 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.11–3.17 (dd, $J_1$ = 3.43, $J_2$ = 17.53 Hz, 1H); 3.71 (s, 3H); 3.77–3.81 (dd, $J_1$ = 11.46, $J_2$ = 17.53 Hz, 1H); 5.97–6.01 (dd, $J_1$ = 3.43, $J_2$ = 11.34 Hz, 1H); 7.08–7.11 (m, 3H); 7.16–7.18 (m, 2H); 7.42–7.45 (m, 1H); 7.51 (s, 1H). MS (ESI): 467.0 ($C_{17}H_{16}Br_2N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{15}Br_2N_3OS$: C, 43.52; H, 3.22; N, 8.96. Found: C, 43.68; H, 3.37; N, 8.84.

**4.2.2.26. 3-(3,4-Dibromophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C26).** White powder. Mp 221–223 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.11–3.16 (dd, $J_1$ = 3.63, $J_2$ = 17.51 Hz, 1H); 3.75–3.83 (dd, $J_1$ = 11.53, $J_2$ = 17.76 Hz, 1H); 5.93–5.98 (dd, $J_1$ = 3.68, $J_2$ = 11.53 Hz, 1H); 7.09–7.19 (m, 3H); 7.42–7.45 (m, 2H); 7.46–7.48 (m, 1H); 7.50 (s, 1H). MS (ESI): 453.0 ($C_{16}H_{14}Br_2N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{13}Br_2N_3OS$: C, 42.22; H, 2.88; N, 9.23. Found: C, 42.45; H, 2.69; N, 9.38.

**4.2.2.27. 3-(3,4-Dibromophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C27).** White powder. Mp 246–248 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.12–3.18 (dd, $J_1$ = 3.69, $J_2$ = 17.53 Hz, 1H); 3.76–3.83 (dd, $J_1$ = 11.51, $J_2$ = 17.73 Hz, 1H); 5.92–5.97 (dd, $J_1$ = 3.63, $J_2$ = 11.53 Hz, 1H); 7.09–7.14 (m, 3H); 7.41–7.43 (m, 2H); 7.46–7.48 (m, 1H); 7.50 (s, 1H). MS (ESI): 482.0 ($C_{16}H_{13}Br_2N_4O_2S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_2N_4O_2S$: C, 39.69; H, 2.50; N, 11.57. Found: C, 39.78; H, 2.41; N, 11.69.

**4.2.2.28. 3-(3,4-Dibromophenyl)-5-(2-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C28).** White powder. Mp 236–237 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.07–3.13 (dd, $J_1$ = 4.18, $J_2$ = 17.86 Hz, 1H); 3.83–3.92 (dd, $J_1$ = 11.51, $J_2$ = 17.96 Hz, 1H); 6.35–6.38 (dd, $J_1$ = 4.36, $J_2$ = 11.43 Hz, 1H); 7.12–7.14 (m, 1H); 7.19–7.26 (m, 3H); 7.36–7.43 (m, 2H); 7.51 (s, 1H). MS (ESI): 455.0 ($C_{16}H_{13}Br_2FN_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_2FN_3S$: C, 42.04; H, 2.65; N, 9.19. Found: C, 42.16; H, 2.43; N, 9.31.

**4.2.2.29. 5-(2-Chlorophenyl)-3-(3,4-dibromophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C29).** White powder. Mp 219–221 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.08–3.12 (dd, $J_1$ = 4.26, $J_2$ = 17.92 Hz, 1H); 3.89–3.95 (dd, $J_1$ = 11.53, $J_2$ = 17.91 Hz, 1H); 6.33–6.35 (dd, $J_1$ = 4.26, $J_2$ = 11.52 Hz, 1H); 7.04–7.07 (m, 1H); 7.17–7.26 (m, 3H); 7.36–7.44 (m, 2H); 7.50 (s, 1H). MS (ESI): 471.0 ($C_{16}H_{13}Br_2ClN_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_2ClN_3S$: C, 40.58; H, 2.55; N, 8.81. Found: C, 40.69; H, 2.38; N, 8.72.

**4.2.2.30. 5-(2-Bromophenyl)-3-(3,4-dibromophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (C30).** White powder. Mp 214–216 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): 3.09–3.15 (dd, $J_1$ = 4.21, $J_2$ = 17.91 Hz, 1H); 3.84–3.91 (dd, $J_1$ = 11.52, $J_2$ = 17.93 Hz, 1H); 6.29–6.32 (dd, $J_1$ = 4.26, $J_2$ = 11.51 Hz, 1H); 7.06–7.09 (m, 1H); 7.19–7.23 (m, 3H); 7.34–7.45 (m, 2H); 7.50 (s, 1H). MS (ESI): 515.0 ($C_{16}H_{13}Br_3N_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{12}Br_3N_3S$: C, 37.09; H, 2.33; N, 8.11. Found: C, 37.19; H, 2.42; N, 8.32.

**4.2.3. General synthetic procedure of pyrazole derivatives D1–D16**

A solution of chalcone (5 mmol) in 30 mL of acetic acid was added dropwise to 0.6 mL of hydrazine hydrate (12.5 mmol) and kept under stirring at 120 °C for 24 h. The mixture was then poured in ice-water, obtaining the crude pyrazole derivatives **D1–D16**, which were crystallized from ethanol.

**4.2.3.1. 1-(3-(3,4-Dichlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D1).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 3H); 3.17–3.22 (dd, $J_1$ = 2.91, $J_2$ = 10.98 Hz, 1H); 3.80–3.86 (dd, $J_1$ = 7.32, $J_2$ = 10.98 Hz, 1H); 5.55–5.58 (dd, $J_1$ = 2.94, $J_2$ = 7.14 Hz, 1H); 7.12–7.16 (t, $J$ = 5.31 Hz, 2H); 7.22–7.25 (m, 2H); 7.72–7.78 (m, 2H); 7.97 (d, $J$ = 11.10 Hz, 1H). MS (ESI): 351.0 ($C_{17}H_{14}Cl_2FN_2O$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}Cl_2FN_2O$: C, 58.14; H, 3.73; N, 7.98. Found: C, 58.16; H, 3.71; N, 7.84.

**4.2.3.2. 1-(5-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D2).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 3H); 3.17–3.22 (dd, $J_1$ = 2.90, $J_2$ = 10.96 Hz, 1H); 3.81–3.87 (dd, $J_1$ = 7.30, $J_2$ = 10.91 Hz, 1H); 5.54–5.57 (dd, $J_1$ = 2.96, $J_2$ = 7.14 Hz, 1H); 7.21–7.23 (d, $J$ = 5.13 Hz, 2H); 7.37–7.39 (d, $J$ = 4.95 Hz, 2H); 7.72–7.78 (m, 2H); 7.96–7.97 (d, $J$ = 11.10 Hz, 1H). MS (ESI): 367.0 ($C_{17}H_{14}Cl_3N_2O$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}Cl_3N_2O$: C, 55.54; H, 3.56; N, 7.62. Found: C, 55.52; H, 3.58; N, 7.53.

**4.2.3.3. 1-(5-(4-Bromophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D3).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 3H); 3.17–3.22 (dd, $J_1$ = 2.91, $J_2$ = 10.96 Hz, 1H); 3.81–3.87 (dd, $J_1$ = 7.34, $J_2$ = 10.98 Hz, 1H); 5.52–5.56 (dd, $J_1$ = 2.96, $J_2$ = 7.17 Hz, 1H); 7.21–7.23 (d, $J$ = 5.13 Hz, 2H); 7.37–7.39 (d, $J$ = 4.95 Hz, 2H); 7.72–7.78 (m, 2H); 7.96–7.97 (d, $J$ = 11.10 Hz, 1H). MS (ESI): 410.9 ($C_{17}H_{14}BrCl_2N_2O$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}BrCl_2N_2O$: C, 49.55; H, 3.18; N, 6.80. Found: C, 49.57; H, 3.17; N, 6.68.

**4.2.3.4. 1-(3-(3,4-Dichlorophenyl)-5-p-tolyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D4).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.26 (s, 3H); 2.29 (s, 3H); 3.13–3.17 (dd, $J_1$ = 2.76, $J_2$ = 10.80 Hz, 1H); 3.79–3.85 (dd, $J_1$ = 7.32, $J_2$ = 10.98 Hz, 1H); 5.49–5.52 (dd, $J_1$ = 2.76, $J_2$ = 7.14 Hz, 1H); 7.05–7.13 (dd, $J_1$ = 4.74, $J_2$ = 17.37 Hz, 4H); 7.71–7.77 (m, 2H); 7.96–7.97 (d, $J$ = 11.1 Hz, 1H). MS (ESI): 347.1 ($C_{18}H_{17}Cl_2N_2O$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}Cl_2N_2O$: C, 62.26; H, 4.64; N, 8.07. Found: C, 62.27; H, 4.62; N, 8.21.

**4.2.3.5. 1-(3-(3,4-Dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D5).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.25 (s, 3H); 3.11–3.16 (dd, $J_1$ = 2.73, $J_2$ = 10.80 Hz, 1H); 3.68 (s, 3H); 3.74–3.80 (dd, $J_1$ = 7.14, $J_2$ = 10.80 Hz, 1H); 5.45–5.49 (dd, $J_1$ = 2.73, $J_2$ = 6.93 Hz, 1H); 6.83–6.85 (d, $J$ = 5.31 Hz, 2H); 7.07–7.08 (d, $J$ = 5.31 Hz, 2H); 7.69–7.70 (d, $J$ = 5.13 Hz, 1H); 7.73–7.75 (dd, $J_1$ = 1.29, $J_2$ = 5.13 Hz, 1H); 7.94 (d, $J$ = 11.1 Hz, 1H). MS (ESI): 363.1 ($C_{18}H_{17}Cl_2N_2O_2$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_2$: C, 59.52; H, 4.44; N, 7.71. Found: C, 59.53; H, 4.42; N, 7.85.

**4.2.3.6. 1-(5-(2-Chlorophenyl)-3-(3,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D6).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.30 (s, 3H); 3.15–3.24 (dd, $J_1$ = 2.91, $J_2$ = 10.96 Hz, 1H); 3.82–3.89 (dd, $J_1$ = 7.34, $J_2$ = 10.98 Hz, 1H); 5.51–5.58 (dd, $J_1$ = 2.96, $J_2$ = 7.17 Hz, 1H); 7.21–7.25 (d, $J$ = 5.13 Hz, 2H); 7.35–7.39 (d, $J$ = 4.95 Hz, 2H); 7.72–7.78 (m, 2H); 7.94–7.97 (d, $J$ = 11.10 Hz, 1H). MS (ESI): 367.0 ($C_{17}H_{14}Cl_3N_2O$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}Cl_3N_2O$: C, 55.54; H, 3.56; N, 7.62. Found: C, 55.56; H, 3.53; N, 7.78.

**4.2.3.7. 1-(3-(3,4-Dichlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D7).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.31 (s, 3H); 3.14–3.22 (dd, $J_1$ = 4.74, $J_2$ = 18.27 Hz, 1H); 3.79–3.89 (dd, $J_1$ = 11.88, $J_2$ = 18.30 Hz, 1H); 5.53–5.58 (dd, $J_1$ = 4.77, $J_2$ = 11.91 Hz, 1H); 7.17–7.35 (m, 5H); 7.70–7.79 (m, 2H); 7.70–7.72 (s, 1H). MS (ESI): 333.1 ($C_{17}H_{15}Cl_2N_2O$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}Cl_2N_2O$: C, 61.28; H, 4.23; N, 8.41. Found: C, 61.26; H, 4.25; N, 8.56.

**4.2.3.8. 1-(3-(3,4-Dichlorophenyl)-5-(3,5-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D8).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.26 (s, 3H); 3.12–3.16 (dd, $J_1$ = 2.73, $J_2$ = 10.80 Hz, 1H); 3.68 (s, 6H); 3.73–3.83 (dd, $J_1$ = 7.14, $J_2$ = 10.80 Hz, 1H); 5.45–5.49 (dd, $J_1$ = 2.73, $J_2$ = 6.93 Hz, 1H); 6.81–6.85 (d, $J$ = 5.31 Hz, 2H); 7.06–7.08 (d, $J$ = 5.31 Hz, 2H); 7.69–7.70 (d, $J$ = 5.13 Hz, 1H); 7.72–7.75 (dd, $J_1$ = 1.29, $J_2$ = 5.13 Hz, 1H); 7.95 (d, $J$ = 11.1 Hz, 1H). MS (ESI): 393.1 ($C_{19}H_{19}Cl_2N_2O_3$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{18}Cl_2N_2O_3$: C, 58.03; H, 4.61; N, 7.12. Found: C, 58.05; H, 4.59; N, 7.26.

**4.2.3.9. 1-(3-(3,4-Dimethylphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D9).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.25 (s, 6H); 2.29 (s, 3H); 3.07–3.14 (dd, $J_1$ = 2.76, $J_2$ = 10.86 Hz, 1H); 3.76–3.86 (dd, $J_1$ = 7.32, $J_2$ = 10.80 Hz, 1H); 5.50–5.54 (dd, $J_1$ = 2.76, $J_2$ = 6.96 Hz, 1H); 7.09–7.23 (m, 5H); 7.48–7.50 (d, $J$ = 7.86, 1H); 7.56 (s, 1H); MS (ESI): 311.2 ($C_{19}H_{20}FN_2O$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}FN_2O$: C, 73.53; H, 6.17; N, 9.03. Found: C, 73.55; H, 6.16; N, 9.16.

**4.2.3.10. 1-(5-(4-Chlorophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D10).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.25 (s, 6H); 2.29 (s, 3H); 3.06–3.14 (dd, $J_1$ = 2.76, $J_2$ = 10.86 Hz, 1H); 3.77–3.87 (dd, $J_1$ = 7.32, $J_2$ = 10.80 Hz, 1H); 5.49–5.55 (dd, $J_1$ = 2.76, $J_2$ = 6.96 Hz, 1H); 7.18–7.23 (m, 3H); 7.36–7.39 (d, $J$ = 8.22, 2H); 7.47–7.50 (d, $J$ = 8.04, 2H); 7.56 (s, 1H); MS (ESI): 327.1 ($C_{19}H_{20}ClN_2O$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}ClN_2O$: C, 69.83; H, 5.86; N, 8.57. Found: C, 69.84; H, 5.84; N, 8.64.

**4.2.3.11. 1-(5-(4-Bromophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D11).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.25 (s, 6H); 2.29 (s, 3H); 3.07–3.14 (dd, $J_1$ = 4.56, $J_2$ = 18.09 Hz, 1H); 3.76–3.86 (dd, $J_1$ = 11.88, $J_2$ = 18.09 Hz, 1H); 5.47–5.53 (dd, $J_1$ = 4.38, $J_2$ = 11.70 Hz, 1H); 7.12–7.15 (d, $J$ = 8.43, 2H); 7.20–7.23 (d, $J$ = 7.86, 1H); 7.47–7.55 (m, 4H). MS (ESI): 371.1 ($C_{19}H_{20}BrN_2O$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}BrN_2O$: C, 61.47; H, 5.16; N, 7.55. Found: C, 61.49; H, 5.13; N, 7.69.

**4.2.3.12. 1-(3-(3,4-Dimethylphenyl)-5-p-tolyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D12).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.22 (s, 6H); 2.25 (s, 6H); 3.01–3.06 (dd, $J_1$ = 2.73, $J_2$ = 10.80 Hz, 1H); 3.73–3.79 (dd, $J_1$ = 6.96, $J_2$ = 10.80 Hz, 1H); 5.42–5.46 (dd, $J_1$ = 2.55, $J_2$ = 8.14 Hz, 1H); 7.01–7.02 (d, $J$ = 4.77, 2H); 7.07–7.09 (d, $J$ = 4.74, 2H); 7.17–7.19 (d, $J$ = 4.59, 1H);7.45–7.46 (d, $J$ = 4.74, 1H); 7.53 (s, 1H). MS (ESI): 307.2 ($C_{20}H_{23}N_2O$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{22}N_2O$: C, 78.40; H, 7.24; N, 9.14. Found: C, 78.43; H, 7.22; N, 9.25.

**4.2.3.13. 1-(3-(3,4-Dimethylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D13).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.25 (s, 6H); 2.27 (s, 3H); 3.04–3.11 (dd, $J_1$ = 3.84, $J_2$ = 12.09 Hz, 1H); 3.71 (s, 3H); 3.76–3.82 (dd, $J_1$ = 11.68, $J_2$ = 18.06 Hz, 1H); 5.44–5.47 (dd, $J_1$ = 4.26, $J_2$ = 10.91 Hz, 1H); 6.85–6.87 (d, $J$ = 7.24, 2H); 7.07–7.10 (d, $J$ = 7.22, 2H); 7.20–7.22 (d, $J$ = 4.86, 2H); 7.47–7.56 (m, 2H). MS (ESI): 323.2 ($C_{20}H_{23}N_2O_2$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.53; H, 6.85; N, 8.76.

**4.2.3.14. 1-(5-(2-Chlorophenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D14).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.24 (s, 6H); 2.34 (s, 3H); 2.98–3.06 (dd, $J_1$ = 4.74, $J_2$ = 17.91 Hz, 1H); 3.86–3.96 (dd, $J_1$ = 11.88, $J_2$ = 17.91 Hz, 1H); 5.70–5.76 (dd, $J_1$ = 4.59, $J_2$ = 11.73 Hz, 1H); 7.02–7.05 (m, 1H); 7.19–7.22 (d, $J$ = 7.86 Hz, 1H); 7.27–7.30 (m, 2H); 7.47–7.55 (m, 3H). MS (ESI): 327.1 ($C_{19}H_{20}ClN_2O$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{19}ClN_2O$: C, 69.83; H, 5.86; N, 8.57. Found: C, 69.85; H, 5.84; N, 8.71.

**4.2.3.15. 1-(3-(3,4-Dimethylphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D15).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.25 (s, 6H); 2.30 (s, 3H); 3.05–3.12 (dd, $J_1$ = 4.38, $J_2$ = 18.12 Hz, 1H); 3.77–3.87 (dd, $J_1$ = 11.91, $J_2$ = 18.12 Hz, 1H); 5.49–5.54 (dd, $J_1$ = 4.23, $J_2$ = 11.73 Hz, 1H); 7.15–7.34 (m, 6H); 7.48–7.56 (m, 2H). MS (ESI): 293.2 ($C_{19}H_{21}N_2O$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 78.07; H, 6.87; N, 9.67.

**4.2.3.16. 1-(5-(3,5-Dimethoxyphenyl)-3-(3,4-dimethylphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (D16).** White powder. $^1$H NMR (300 MHz, DMSO-$d_6$): 2.26 (s, 6H); 2.27 (s, 3H); 3.02–3.10 (dd, $J_1$ = 3.84, $J_2$ = 12.09 Hz, 1H); 3.71 (s, 6H); 3.74–3.81 (dd, $J_1$ = 11.68, $J_2$ = 18.06 Hz, 1H); 5.42–5.47 (dd, $J_1$ = 4.26, $J_2$ = 10.91 Hz, 1H); 6.84–6.87 (d, $J$ = 7.24, 2H); 7.06–7.10 (d, $J$ = 7.22, 2H); 7.20–7.22 (d, $J$ = 4.86, 2H); 7.45–7.56 (m, 2H). MS (ESI): 353.2 ($C_{21}H_{25}N_2O_3$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.55; H, 6.87; N, 7.84.

### 4.3. EGFR inhibitory assay

A 1.6 kb cDNA encoded for the EGFR cytoplasmic domain (EGFR-CD, amino acids 645–1186) were cloned into baculoviral expression vector pFASTBacHTc. A sequence that encodes $(His)_6$ was located at the 5′ upstream to the EGFR sequence. Sf-9 cells were infected for 3 days for protein expression. Sf-9 cell pellets were solubilized at 0 °C in a buffer at pH 7.4 containing 50 mM HEPES, 10 mM NaCl, 1% Triton, 10 μM ammonium molybdate, 100 μM sodium vanadate, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin, and 16 μg/mL benzamidine HCl for 20 min followed by 20 min centrifugation. Crude extract supernatant was passed through an equilibrated Ni-NTA superflow packed column and washed with 10 mM and then 100 mM imidazole to remove nonspecifically bound material. Histidine tagged proteins were eluted with 250 and 500 mM imidazole and dialyzed against 50 mM NaCl, 20 mM HEPES, 10% glycerol, and 1 μg/mL each of aprotinin, leupeptin, and pepstatin for 2 h. The entire purification procedure was performed at 4 °C or on ice.

The EGFR kinase assay was set up to assess the level of autophosphorylation based on DELFIA/Time-Resolved Fluorometry. Compounds **C1–C30** and **D1–D16** were dissolved in 100% DMSO and diluted to the appropriate concentrations with 25 mM HEPES at pH 7.4. In each well, 10 μL of compound was incubated with 10 μL (12.5 ng for HER-2 or 5 ng for EGFR) of recombinant enzyme (1:80 dilution in 100 mM HEPES) for 10 min at room temperature. Then, 10 μL of 5 mM buffer (containing 20 mM HEPES, 2 mM $MnCl_2$, 100 μM $Na_3VO_4$, and 1 mM DTT) and 20 μL of 0.1 mM ATP-50 mM $MgCl_2$ was added for 1 h. Positive and negative controls were included in each plate by incubation of enzyme with or without ATP-$MgCl_2$. At the end of incubation, liquid was aspirated, and plates were washed three times with wash buffer. A 75 μL (400 ng) sample of europiumlabeled anti-phosphotyrosine antibody was added to each well for another 1 h of incubation. After washing, enhancement solution was added and the signal was detected by Victor (Wallac Inc.) with excitation at 340 nm and emission at 615 nm. The percentage of autophosphorylation

inhibition by the compounds was calculated using the following equation: 100% − [(negative control)/(positive control − negative control)]. The $IC_{50}$ was obtained from curves of percentage inhibition with eight concentrations of compound. As the contaminants in the enzyme preparation are fairly low, the majority of the signal detected by the anti-phosphotyrosine antibody is from EGFR.

### 4.4. Antiproliferative activities assay

The antiproliferative activities of compounds **C1–C10** were determined using a standard (MTT)-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of $7 \times 10^3$ cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 100 μg/mL. After 48 h, cell survival was determined by the addition of an MTT solution (10 μL of 5 mg/mL MTT in PBS). After 4 h, 100 μL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 18 h; optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. $IC_{50}$ values were determined from replicates of six wells from at least two independent experiments.

### 4.5. Molecular docking study

The automated docking studies were carried out using AutoDock version 4.0. First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 60 Å size ($x$, $y$, $z$) with a spacing of 0.375 Å and grid maps were created representing 17 the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules.

The three-dimensional structures of the aforementioned compounds were constructed using Chem 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger-Hückel charges of ligands were assigned. The crystal structures of EGFR (PDB code: 1M17) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins.

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