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Synthesis, molecular docking and evaluation of thiazolyl-pyrazoline derivatives as EGFR TK inhibitors and potential anticancer agents

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ABSTRACT

Fourty-two thiazolyl-pyrazoline derivatives were synthesized to screen for their EGFR kinase inhibitory activity. Compound 4-(4-chlorophenyl)-2-(3-(3,4-dimethylphenyl)-5-p-tolyl-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (**11**) displayed the most potent EGFR TK inhibitory activity with IC_{50} of 0.06 μM, which was comparable to the positive control. Molecular docking results indicated that compound **11** was nicely bound to the EGFR kinase. Compound **11** also showed significant antiproliferative activity against MCF-7 with IC_{50} of 0.07 μM, which would be a potential anticancer agent.

Protein kinases catalyze the phosphorylation of tyrosine and serine/threonine residues in various proteins involved in the regulation of all functions.[1] They can be broadly classified as receptor kinases such as EGFR, and non-receptor kinases. Inappropriate or uncontrolled activation of many of these kinases has been shown to result in uncontrolled cell growth.[2] The EGFR PTKs have been identified as interesting targets for medicinal chemistry programs especially in cancer therapy.[3–6] Compounds that inhibit the kinase activity of EGFR after binding its cognate ligand are of potential interest as new therapeutic antitumor agents.[7,8]

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities,[9,10] recently found applied in drug development for the treatment of allergies,[11] inflammation,[12] schizophrenia,[13] bacterial,[14] HIV infections,[15] and more recently for the treatment of cancer.[16]

Lin et al. reported that 2,7-diamino-thiazolo[4,5-*d*]pyrimidines analogues showed modest to potent EGFR TK inhibitory activity, with IC_{50} values ranging from micromolar to single digit nanomolar.[17] 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,[18–20] antibacterial,[21,22] antiinflamatory,[23] analgesic,[24] fungistatic[25] and anti-hyperglycemic activity.[26] Robert D. Hubbard et al. have discovered a series of EGFR TK inhibitors with pyrazolopyrimidine scaffold.[27]

Herein, in continuation to extend our research on anticancer compounds with EGFR TK inhibitory activity,[28] we report in the present work the synthesis and structure–activity relationships of a series of thiazolyl-pyrazoline derivatives as anticancer agents. Biological evaluation indicated that some of the synthesized compounds are potent inhibitors of EGFR TK. Compound **11** displayed the most potent EGFR TK inhibitory activity with IC_{50} of 0.06 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 TK in complex with an inhibitor to explore the binding modes of these compounds at the active site.

The synthesis of thiazolyl-pyrazoline derivatives **4–45** followed the general pathway outlined in Scheme 1. Firstly, the chalcones (compound **2**) were obtained by direct condensation between the aromatic aldehydes and the substituted acetophenone, using 20% potassium hydroxide as catalyst. Secondly, cyclization of different chalcones with thiosemicarbazide under basic condition leads to the formation of pyrazole derivatives containing thiourea skeleton (compound **3**). Finally, thiazolyl-pyrazoline derivatives **4–45** were obtained by reacting compound **3** with substituted 2-bromoacetophenone. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. The general synthetic procedure and spectroscopic data of compounds **4–45** can be found in the Supplementary data. Most of the synthetic compounds are soluble in DMSO and $CHCl_3$.

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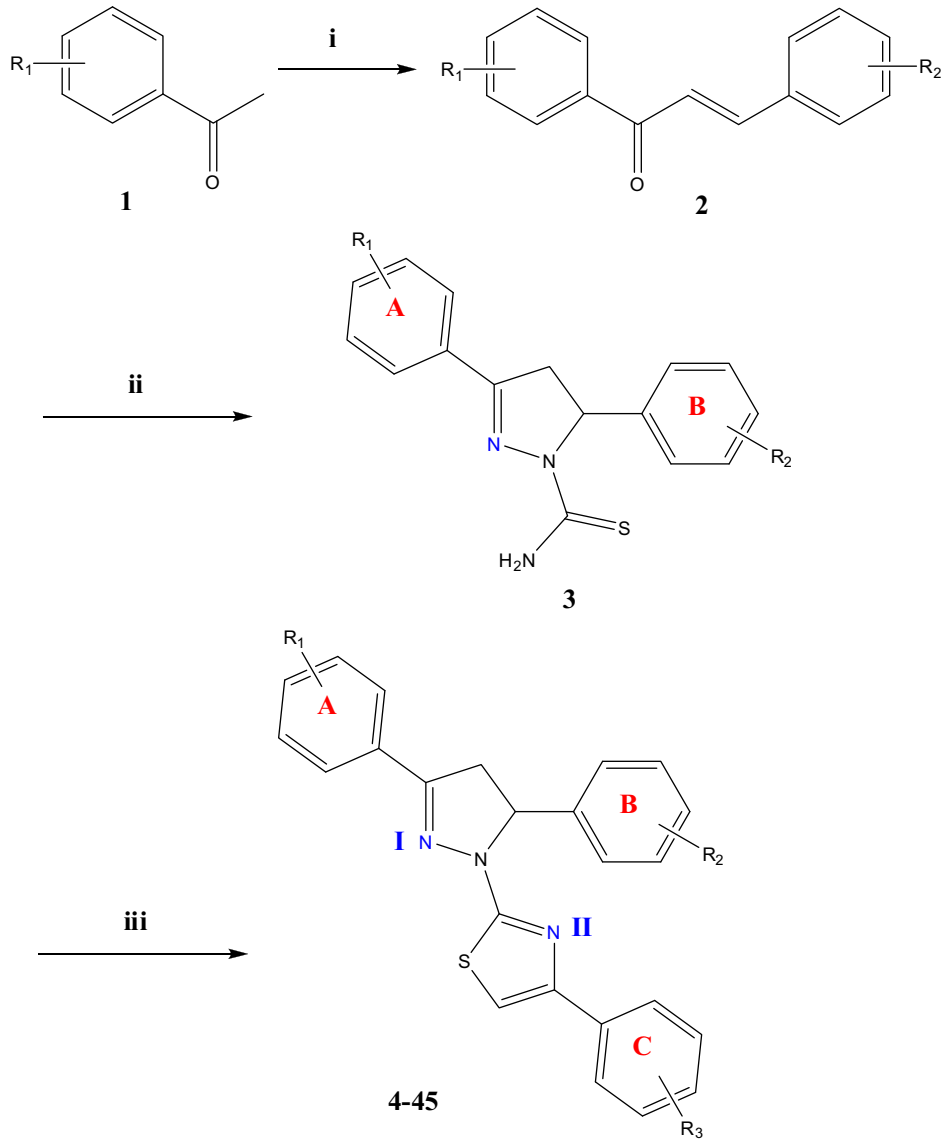


Scheme 1. General synthesis of compounds **4**–**45**. Reagents and conditions: (i) Substituted aromatic aldehyde, 40% NaOH, CH_3CH_2OH; (ii) thiosemicarbazide, NaOH, CH_3CH_2OH, reflux; (iii) substituted 2-bromoacetophenone, reflux, 4 h. **I** and **II** represent the atom number binding to the amino acid active site.

The synthesized compounds **4–45** were evaluated for their activity to inhibit the autophosphorylation of EGFR kinases. The inhibition constants (IC_{50}) of the compounds are summarized in Table 1. As shown in Table 1, the synthesized compounds showed fairly good inhibitory activity displaying IC_{50} values between 0.06 and 28.17 μM. Subsequently SAR studies were performed by modification of the parent compound to determine how the substituents of the subunits affect the EGFR inhibitory activities. Inspection of the chemical structures of the compounds **4–45** (Table 1 and Scheme 1) suggested that they could be divided into three subunits: **A**-, **B**- and **C**-rings.

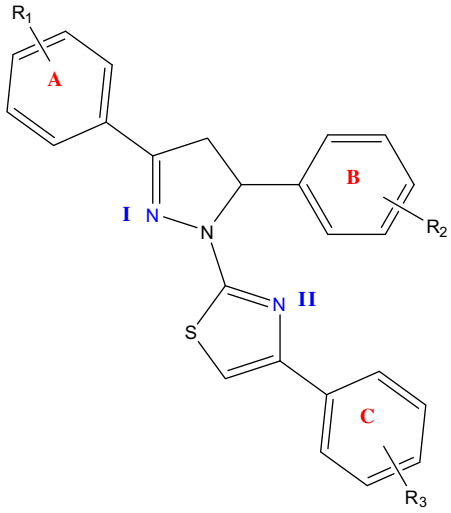
As shown in Table 1, structure–activity relationships in compounds **4–45** demonstrated that compounds bearing one methoxy group at 4-position at **A**-ring (compounds **32–45**, IC_{50}: 5.07–8.63 μM) showed better EGFR inhibitory activity than those with one bromine atom substituent (compounds **18–31**, IC_{50}: 8.95–28.17 μM) at the same position. After we modified the substituents on **A**-ring with two methyl group at 3-position and 4-position (compounds **4–17**, IC_{50}: 0.06–3.08 μM), the EGFR inhibitory activity of compounds **4–17** was remarkably increased compared to compounds **32–45** (IC_{50}: 5.07–8.63 μM).

Among compounds **4–17**, compounds **11–17** (IC_{50}: 0.06–1.04 μM) with a chlorine atom substitution on the 4-position of the **C**-ring displayed more inhibitory activity than those without 4-position substitution derivatives (compounds **4–10**, IC_{50}: 1.73–3.85 μM). Furthermore, as for compounds **11–17**, a comparison of the substitution on **B**-ring demonstrated that the electron-withdrawing substituents at 4-position derivatives (compounds **11–13** and **17**, IC_{50}: 0.06–0.63 μM) had more potent EGFR inhibitory activities than the electron-donating substituents ones (compounds **14–16**, IC_{50}: 0.82–1.04 μM). Among the former, compound **11** displayed the most potent EGFR inhibitory activity with IC_{50} of 0.06 μM, which was comparable to the positive control erlotinib (IC_{50} = 0.03 μM).

To help understand the SARs observed at the EGFR and guide further SAR studies, molecular docking of the most potent inhibitor **11** 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 **11** and EGFR is depicted in Figure 1.

As shown in Figure 1, compound **11** was nicely bound to the EGFR kinase with three hydrogen bonds: $N^{I}\cdots O$/Leu768 (distance:

Table 1
Chemical structures and EGFR TK inhibitory activity of compounds **4–45**



Compd	R_1	R_2	R_3	EGFR (IC_{50}, μM)	Compd	R_1	R_2	R_3	EGFR (IC_{50}, μM)
4	3,4-2CH_3	4-F	4-H	1.73 ± 0.12	**25**	4-Br	4-F	4-Cl	24.16 ± 2.74
5	3,4-2CH_3	4-Cl	4-H	2.36 ± 0.28	**26**	4-Br	4-Cl	4-Cl	17.84 ± 1.89
6	3,4-2CH_3	4-Br	4-H	1.84 ± 0.16	**27**	4-Br	4-Br	4-Cl	25.74 ± 2.83
7	3,4-2CH_3	4-CH_3	4-H	2.67 ± 0.31	**28**	4-Br	4-CH_3	4-Cl	28.17 ± 2.91
8	3,4-2CH_3	4-OCH_3	4-H	3.08 ± 0.42	**29**	4-Br	4-OCH_3	4-Cl	19.83 ± 1.94
9	3,4-2CH_3	4-OH	4-H	1.92 ± 0.19	**30**	4-Br	4-OH	4-Cl	21.52 ± 2.46
10	3,4-2CH_3	4-NO_2	4-H	3.85 ± 0.56	**31**	4-Br	4-NO_2	4-Cl	24.37 ± 2.72
11	3,4-2CH_3	4-F	4-Cl	0.06 ± 0.009	**32**	4-OCH_3	4-F	4-H	5.71 ± 0.61
12	3,4-2CH_3	4-Cl	4-Cl	0.19 ± 0.012	**33**	4-OCH_3	4-Cl	4-H	4.94 ± 0.43
13	3,4-2CH_3	4-Br	4-Cl	0.37 ± 0.026	**34**	4-OCH_3	4-Br	4-H	5.56 ± 0.56
14	3,4-2CH_3	4-CH_3	4-Cl	1.04 ± 0.11	**35**	4-OCH_3	4-CH_3	4-H	7.48 ± 0.70
15	3,4-2CH_3	4-OCH_3	4-Cl	0.82 ± 0.057	**36**	4-OCH_3	4-OCH_3	4-H	6.92 ± 0.69
16	3,4-2CH_3	4-OH	4-Cl	0.97 ± 0.064	**37**	4-OCH_3	4-OH	4-H	5.35 ± 0.48
17	3,4-2CH_3	4-NO_2	4-Cl	0.63 ± 0.049	**38**	4-OCH_3	4-NO_2	4-H	8.63 ± 0.73
18	4-Br	4-F	4-H	11.24 ± 1.68	**39**	4-OCH_3	4-F	4-Cl	5.07 ± 0.52
19	4-Br	4-Cl	4-H	9.37 ± 0.83	**40**	4-OCH_3	4-Cl	4-Cl	7.78 ± 0.68
20	4-Br	4-Br	4-H	8.95 ± 0.78	**41**	4-OCH_3	4-Br	4-Cl	6.31 ± 0.57
21	4-Br	4-CH_3	4-H	19.64 ± 2.15	**42**	4-OCH_3	4-CH_3	4-Cl	4.68 ± 0.38
22	4-Br	4-OCH_3	4-H	10.17 ± 1.46	**43**	4-OCH_3	4-OCH_3	4-Cl	6.27 ± 0.49
23	4-Br	4-OH	4-H	22.36 ± 2.34	**44**	4-OCH_3	4-OH	4-Cl	7.66 ± 0.81
24	4-Br	4-NO_2	4-H	15.72 ± 1.82	**45**	4-OCH_3	4-NO_2	4-Cl	8.43 ± 0.94
Erlotinib	—	—	—	0.03 ± 0.007	—	—	—	—	—

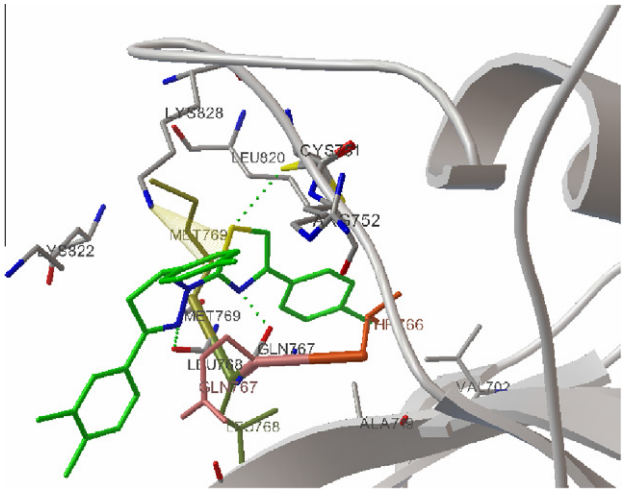


Figure 1. Molecular docking modeling of compound **11** with EGFR kinase. The H-bonds are displayed as green dot lines. Compound **11** was nicely bound to the EGFR kinase with three hydrogen bonds: $N^{I}\cdots$O/Leu768 (distance: 2.35 Å, angle: 127.5°), $N^{II}\cdots$O/Gln767 (distance: 1.82 Å, angle: 121.8°) and S···N/Cys751 (distance: 2.87 Å, angle: 97.3°). The modeling also suggested that there was a π-cation interaction between the thiazole ring of compound **11** and Lys828.

2.35 Å, angle: 127.5°), $N^{II}\cdots$O/Gln767 (distance: 1.82 Å, angle: 121.8°) and S···N/Cys751 (distance: 2.87 Å, angle: 97.3°). The modeling also suggested that there was a π-cation interaction between the thiazole ring of compound **11** and Lys828. π-Cation interaction energies are of the same order of magnitude as hydrogen bonds or salt bridges and play an important role in stabilizing the three dimensional structure of a protein.[29] Also, the binding model of erlotinib and EGFR was introduced to make a comparison, which is shown in Figure 2. As illustrated in Figure 2, one nitrogen atom of the quinazoline skeleton forms a hydrogen bond with Met769, and the other quinazoline nitrogen atom is not within H-bonding distance of the Thr766 side chain, but a water molecule bridges this gap.[30]

In addition, we also selected the top seven compounds **11–17** which had better EGFR inhibitory activity to test their in vitro antiproliferative activity against human tumor cell line (MCF-7). The results are showed in Table 2. We can see from Table 2 that compound **11** exhibited significant antiproliferative activity against MCF-7 with IC_{50} of 0.07 μM, which was comparable to the positive control erlotinib (IC_{50} = 0.02 μM). Compounds **12–17** also displayed good antiproliferative activity against MCF-7 with IC_{50} ranging from 0.16 to 1.47 μM. In particular, compound **11** demonstrated significant EGFR inhibitory activity and potent antiprolifer-

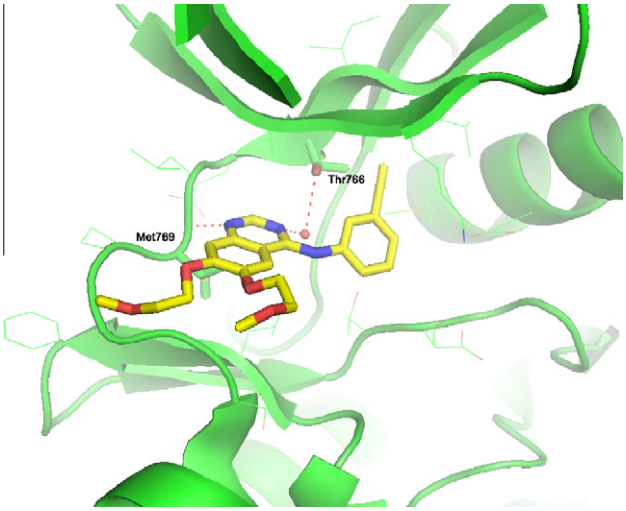


Figure 2. Molecular docking modeling of erlotinib with EGFR kinase. The H-bonds are displayed as green dot lines.

Table 2
Antiproliferative activity of the top seven compounds

Top compounds	MCF-7 (IC_{50}, μM)
11	0.07 ± 0.008
12	0.16 ± 0.02
13	0.26 ± 0.03
14	0.68 ± 0.07
15	0.92 ± 0.09
16	1.47 ± 0.12
17	0.43 ± 0.05
Erlotinib	0.02 ± 0.005

ative activity, and could act as a potential anticancer agent deserving further study.

In conclusion, fourty-two thiazolyl-pyrazoline derivatives that may function as inhibitors of EGFR kinases were prepared, and some of them exhibited potent EGFR inhibitory. Compound **11** displayed the most potent EGFR inhibitory activity with IC_{50} of 0.06 μM, which was comparable to the positive control erlotinib. Molecular docking study indicated that compound **11** was nicely bound to the EGFR kinase with three hydrogen bonds. Compounds **11** also exhibited significant antiproliferative activity against MCF-7 with IC_{50} of 0.07 μM, which was comparable to the positive control. Therefore, compound **11** would be a potent anticancer agent with significant EGFR TK inhibitory activity.

Acknowledgments

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