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Design, synthesis and biological evaluation of thiazolidinone derivatives as potential EGFR and HER-2 kinase inhibitors

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ABSTRACT

Two series of thiazolidinone derivatives designing for potential EGFR and HER-2 kinase inhibitors have been discovered. Some of them exhibited significant EGFR and HER-2 inhibitory activity. Compound *2-(2-(5-bromo-2-hydroxybenzylidene)hydrazinyl)thiazol-4(5H)-one* (**12**) displayed the most potent inhibitory activity (IC_{50} = 0.09 μM for EGFR and IC_{50} = 0.42 μM for HER-2), comparable to the positive control erlotinib. Docking simulation was performed to position compound **12** into the EGFR active site to determine the probable binding model. Antiproliferative assay results indicating that some of the thiazolidinone derivatives own high antiproliferative activity against MCF-7. Compound **12** 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] The first group of these novel anticancer drugs is those targeting mutant or aberrantly expressed oncogenic growth factor receptor and non-receptor tyrosine kinases involved in mitogenic or proliferative signal transduction pathways in cancer cells.[2] 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 (also known as erb-B1 or HER-1) and the related human epidermal growth factor receptor HER-2 (also known as erbB-2). Deregulation of growth-factor signaling due to hyperactivation of the ErbB receptors (primarily EGFR and HER-2) is seen in several cancer types.[3,4] Activation of EGFR may be because of overexpression, mutations resulting in constitutive activation, or autocrine expression of ligand. In contrast, activation of HER-2 occurs mainly by overexpression, which leads to spontaneous homodimerization and activation of downstream signaling events in a ligand-independent manner.[5,6] The role of EGFR and HER-2 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 and HER-2 overexpression is also seen in ovarian cancer,[7] lung cancer (especially lung adenocarcinomas)[8–10] and in hormone-refractory prostate cancer.[11] Compounds that inhibit the kinase activity of EGFR and/or HER-2 after binding of its cognate ligand are of potential interest as new therapeutic antitumor agents.[12,13]

Thiazolidinone derivatives have been investigated for a range of pharmacologic indications such as anti-inflammatory,[14] antimicrobial,[15] antiproliferative,[16,17] antiviral,[18] anticonvulsant,[19,20] antifungal,[21] and antibacterial[22] activities but their anticancer effects have been less widely documented.[23] Since the tyrosine phosphorylation event catalyzed by EGFR or HER-2 propagates the signal for cell division and since deregulation of these kinases has been associated with disease, an inhibitor of this event may have potential therapeutic value. Moreover, these two receptor kinases have a high sequence homology in their catalytic domains. In view of the facts above mentioned, in this study, we describe the synthesis and the SAR of two series of thiazolidinone derivatives. Biological evaluation indicated that some of the synthesized compounds are potent inhibitors of EGFR and HER-2. 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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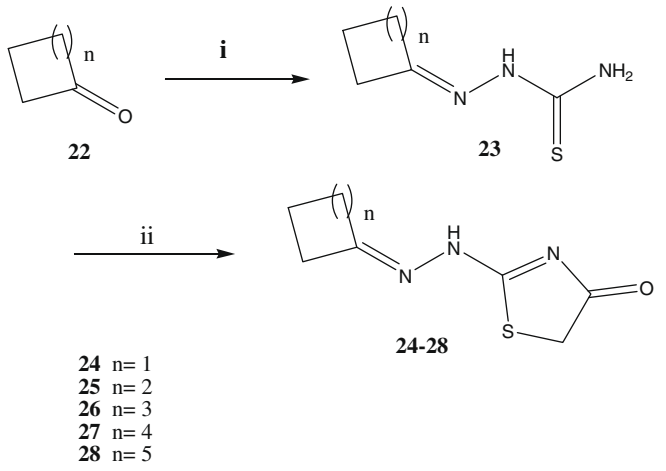
2. Results and discussion

2.1. Chemistry

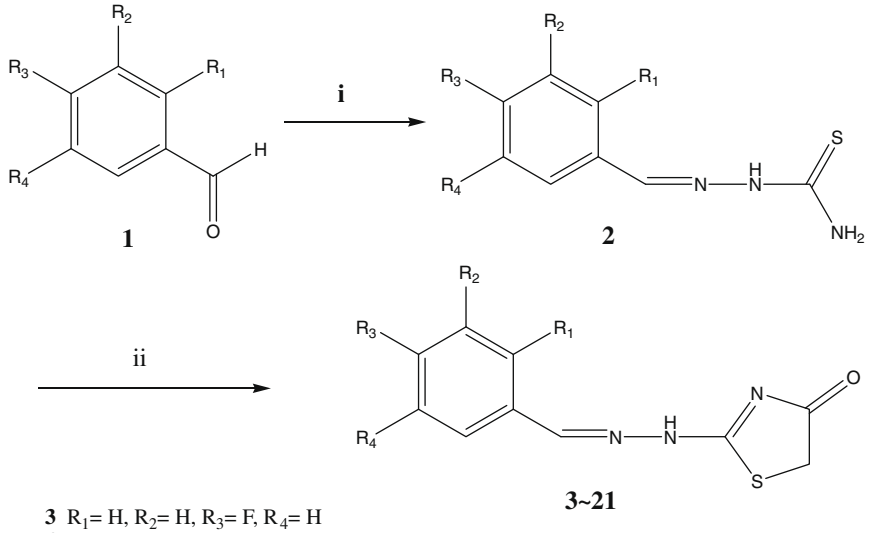
The synthesis of compounds **3–21** followed the general pathway outlined in Scheme 1. Firstly, to a stirred solution of thiosemicarbazide in ethanol were added substituted aldehydes. The mixture was refluxing for 2–6 h. Then, thiosemicarbazones (compound **2**) were obtained with yields of 76–85%. In the next step, treatment of thiosemicarbazones with ethyl bromoacetate in refluxing ethanol containing a catalytic amount of freshly fused sodium acetate afforded the thiazolidinone derivatives **3–21**. Similarly, the synthesis of compounds **24–28** followed the general pathway outlined in Scheme 2. In this series, cyclobutanone and its analogues were introduced to react with thiosemicarbazide instead of substituted aldehydes. The mixture was refluxing for 4–8 h. Then, the thiosemicarbazones (compound **23**) were obtained with yields of 75–87%. Subsequently, compounds **24–28** were obtained by treatment of thiosemicarbazones with ethyl bromoacetate in refluxing ethanol containing a catalytic amount of freshly fused sodium acetate. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.



Scheme 2. General synthesis of compounds **24–28**. Reagents and conditions: (i) thiosemicarbazide, 2-propanol, acetic acid; (ii) $BrCH_2COOEt$, AcONa, EtOH, reflux.

2.2. Biological activity

The synthesized thiazolidinone derivatives were evaluated for their ability to inhibit the autophosphorylation of EGFR and HER-2 kinases using a solid-phase ELISA assay. The results were summarized in Table 1. For the given compounds, we observed that the IC_{50} value for inhibition of HER-2 kinase is, in general, higher than that observed for EGFR kinase but have the same trends. This is possibly due, in part, to the fact that in the enzyme assays we used higher concentration of the purified HER-2 kinase than EGFR kinase. It is evident that there is also a reasonable correlation be-



3 R_1= H, R_2= H, R_3= F, R_4= H
4 R_1= H, R_2= H, R_3= Cl, R_4= H
5 R_1= H, R_2= H, R_3= Br, R_4= H
6 R_1= H, R_2= H, R_3= CH_3, R_4= H
7 R_1= H, R_2= H, R_3= OCH_3, R_4= H
8 R_1= H, R_2= H, R_3= NO_2, R_4= H
9 R_1= H, R_2= H, R_3= OH, R_4= H
10 R_1= OH, R_2= H, R_3= H, R_4= F
11 R_1= OH, R_2= H, R_3= H, R_4= Cl
12 R_1= OH, R_2= H, R_3= H, R_4= Br
13 R_1= OH, R_2= Cl, R_3= H, R_4= Cl
14 R_1= OH, R_2= Br, R_3= H, R_4= Br
15 R_1= H, R_2= F, R_3= H, R_4= H
16 R_1= H, R_2= Cl, R_3= H, R_4= H
17 R_1= H, R_2= Br, R_3= H, R_4= H
18 R_1= H, R_2= CH_3, R_3= H, R_4= H
19 R_1= H, R_2= OCH_3, R_3= H, R_4= H
20 R_1= H, R_2= NO_2, R_3= H, R_4= H
21 R_1= H, R_2= OH, R_3= H, R_4= H

Scheme 1. General synthesis of compounds **3–21**. Reagents and conditions: (i) thiosemicarbazide, 2-propanol, acetic acid; (ii) $BrCH_2COOEt$, AcONa, EtOH, reflux.

Table 1
Inhibition (IC_{50}) of EGFR and HER-2 kinases and inhibition (IC_{50}) of cell proliferation

Compounds	Enzyme assays (IC_{50}, μM)		MCF-7 (IC_{50}, μM)
	EGFR	HER-2	
3	2.18	3.89	1.58 ± 0.24
4	1.94	2.75	1.82 ± 0.45
5	1.72	2.43	0.97 ± 0.08
6	3.26	5.83	2.05 ± 0.17
7	5.78	9.17	3.16 ± 0.84
8	6.82	8.46	2.77 ± 0.56
9	4.71	6.93	1.67 ± 0.34
10	0.97	3.59	0.27 ± 0.09
11	0.56	1.78	0.18 ± 0.05
12	0.09	0.42	0.06 ± 0.02
13	1.46	4.51	1.56 ± 0.18
14	1.24	3.73	0.62 ± 0.12
15	13.53	21.75	7.78 ± 0.43
16	10.78	14.34	4.31 ± 0.59
17	7.46	10.26	2.42 ± 0.12
18	23.49	31.78	8.19 ± 0.68
19	31.25	45.51	9.73 ± 1.12
20	26.61	37.64	12.57 ± 2.41
21	19.32	27.83	11.79 ± 1.97
24	>50	>50	46.3 ± 4.78
25	>50	>50	38.5 ± 3.65
26	41	>50	32.1 ± 3.29
27	36	47	27.6 ± 2.94
28	27	31	18.4 ± 1.78
Erlotinib	0.03	0.16	0.02 ± 0.005

tween the EGFR and HER-2 inhibitory activities; thus, this is not surprising in view of the high sequence homology of the catalytic domains of these two kinases. As shown in Table 1, a number of thiazolidinone derivatives exhibited significant EGFR and HER-2 inhibitory activity. Among them, compound **12** displayed the most potent inhibitory activity (IC_{50} = 0.09 μM for EGFR and IC_{50} = 0.42 μM for HER-2), comparable to the positive control erlotinib (IC_{50} = 0.03 μM for EGFR).

Structure–activity relationships in these thiazolidinone derivatives demonstrated that compounds with aromatic ring (compounds **3–21**) showed better inhibitory activity than those with aliphatic ring (compounds **24–28**). Among the former, compounds with substitution on the 3-position of benzene ring (compounds **15–21**) displayed less inhibitory activity than those with 4-position (compounds **3–9**). Besides, compounds **10–14** exhibited potent EGFR inhibitory activity with IC_{50} ranging from 0.09 to 1.46 μM. All of them have one hydroxyl group on the 2-position of benzene ring. Among them, a comparison of the substitution on benzene ring demonstrated that 4-position-halogen-substituted derivatives (compounds **10**–**12**) have more potent EGFR inhibitory activity than others (compounds **13** and **14**), and the potency order is F < Cl < Br. Compound **12** displayed the most potent EGFR inhibitory activity with IC_{50} of 0.09 μM, which was comparable to the positive control.

In addition, in order to help understand the SARs observed at the EGFR and guide further SAR studies, molecular docking of the most potent inhibitor **12** 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 **12** and EGFR is depicted in Figure 1. In the binding model, compound **12** is nicely bound to the region with the hydroxyl group project toward the mercapto group of Met 769, with the hydroxyl group forming a more optimal H-bond interaction, and nitrogen atom of thiazolidinone ring of compound **12** also forms hydrogen bond with the side chain mercapto group of Cys 751. This molecular docking result, along with the enzyme assay data, suggesting that compound **12** is a potential inhibitor of EGFR.

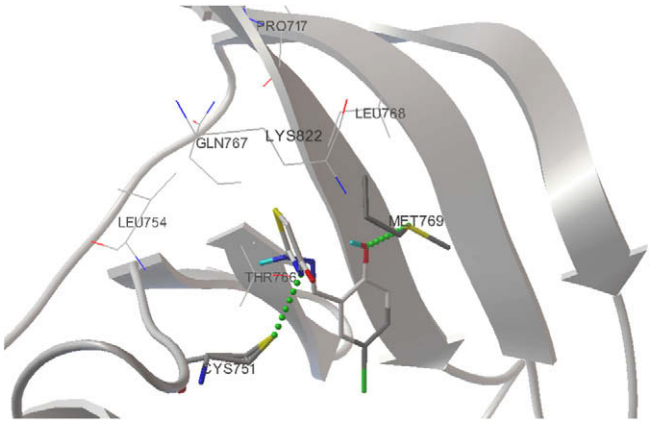


Figure 1. Molecular docking modeling of compound **12** with EGFR kinase: the hydroxyl group of compound **12** forms hydrogen bond with mercapto group of Met 769, and the nitrogen atom of thiazolidinone ring of compound **12** also forms hydrogen bond with the side chain mercapto group of Cys 751.

Besides, the in vitro antiproliferative activity of the synthesized thiazolidinone derivatives was studied on a panel of one human tumor cell line (MCF-7), which overexpresses EGFR and, to a less extent HER-2. Compounds **10–14**, which have potent inhibitory activity of EGFR and HER-2 showed high antiproliferative activity against MCF-7 with IC_{50} ranging from 0.06 to 1.56 μM, indicating that these thiazolidinone derivatives were potent inhibitor of EGFR and HER-2 as antitumor agents. In particular, compound **12** has demonstrated significant inhibitory activity in tumor growth inhibition and displayed favorable EGFR and HER-2 inhibitory activity.

3. Conclusions

Two series of thiazolidinone derivatives that may function as inhibitors of EGFR and HER-2 kinases have been prepared, and some of the synthesized compounds displayed potent EGFR and HER-2 inhibitory. Compound **12** displayed the most potent inhibitory activity (IC_{50} = 0.09 μM for EGFR and IC_{50} = 0.42 μM for HER-2), comparable to the positive control erlotinib. The EGFR molecular docking model suggested that compound **12** is nicely bound to the region with the hydroxyl group project toward the mercapto group of Met 769, with the hydroxyl group forming a more optimal H-bond interaction, and nitrogen atom of thiazolidinone ring of compound **12** also forms hydrogen bond with the side chain mercapto group of Cys 751. Antiproliferative assay results indicated that these thiazolidinone derivatives own high antiproliferative activity against MCF-7; In particular, compound **12** has demonstrated significant EGFR and HER-2 inhibitory activity and inhibitory activity in tumor growth inhibition as a potential anticancer agent.

4. Experimental section

4.1. Chemistry

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 (δ). Elemental

analyses were performed on a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values.

4.2. General method of synthesis of compounds 3–21

To a solution of compound **1** (0.01 mol) in ethanol (20 mL) was added an equimolar amount of thiosemicarbazide (0.91 g, 0.01 mol). The reaction mixture was heated under reflux for 2–6 h, partially concentrated and cooled. The separated solid product was filtered off, dried, and recrystallized from ethanol to give compound **2**. A mixture of compound **2** (0.005 mol), ethyl bromoacetate (0.83 g, 0.005 mol), anhydrous sodium acetate (1.64 g, 0.02 mol), and absolute ethanol (30 mL) was refluxed for 8–12 h. The product obtained upon cooling was collected by filtration, washed with water, dried, and recrystallized from a mixture of EtOH to give compounds **3–21**.

4.2.1. 2-(2-(4-Fluorobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (3)

White powder. Yield: 80%; mp: 134–136 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.49 (s, 2H); 7.26–7.28 (d, J = 7.84 Hz, 2H); 7.65–7.67 (d, J = 8.26 Hz, 2H); 8.38 (s, 1H). MS (ESI): 238.0 ($C_{10}H_9FN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8FN_3OS$: C, 50.62; H, 3.40; N, 17.71. Found: C, 50.87; H, 3.27; N, 17.59.

4.2.2. 2-(2-(4-Chlorobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (4)

White powder. Yield: 83%; mp: 138–139 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.47 (s, 2H); 7.29–7.31 (d, J = 7.86 Hz, 2H); 7.70–7.72 (d, J = 8.29 Hz, 2H); 8.41 (s, 1H). MS (ESI): 254.0 ($C_{10}H_9ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8ClN_3OS$: C, 47.34; H, 3.18; N, 16.56. Found: C, 47.12; H, 3.31; N, 16.73.

4.2.3. 2-(2-(4-Bromobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (5)

White powder. Yield: 78%; mp: 132–134 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.45 (s, 2H); 7.23–7.26 (d, J = 7.81 Hz, 2H); 7.64–7.67 (d, J = 8.24 Hz, 2H); 8.38 (s, 1H). MS (ESI): 297.0 ($C_{10}H_9BrN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8BrN_3OS$: C, 40.28; H, 2.70; N, 14.09. Found: C, 40.39; H, 2.56; N, 14.16.

4.2.4. 2-(2-(4-Methylbenzylidene)hydrazinyl)thiazol-4(5*H*)-one (6)

White powder. Yield: 82%; mp: 125–127 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 2.35 (s, 3H); 4.49 (s, 2H); 7.27–7.29 (d, J = 7.86 Hz, 2H); 7.66–7.68 (d, J = 8.25 Hz, 2H); 8.39 (s, 1H). MS (ESI): 234.0 ($C_{11}H_{12}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.76; H, 4.61; N, 18.19.

4.2.5. 2-(2-(4-Methoxybenzylidene)hydrazinyl)thiazol-4(5*H*)-one (7)

White powder. Yield: 84%; mp: 129–131 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 3.76 (s, 3H); 4.47 (s, 2H); 7.25–7.27 (d, J = 7.81 Hz, 2H); 7.64–7.67 (d, J = 8.26 Hz, 2H); 8.37 (s, 1H). MS (ESI): 250.0 ($C_{11}H_{12}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86. Found: C, 53.21; H, 4.63; N, 16.97.

4.2.6. 2-(2-(4-Nitrobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (8)

White powder. Yield: 77%; mp: 145–146 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.48 (s, 2H); 7.35–7.37 (d, J = 7.83 Hz, 2H); 7.73–7.75(d, J = 8.27 Hz, 2H); 8.46 (s, 1H). MS (ESI): 265.0 ($C_{10}H_9N_4O_3S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8N_4O_3S$: C, 45.45; H, 3.05; N, 21.20. Found: C, 45.67; H, 3.24; N, 21.34.

4.2.7. 2-(2-(4-Hydroxybenzylidene)hydrazinyl)thiazol-4(5*H*)-one (9)

White powder. Yield: 75%; mp: 149–151 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.46 (s, 2H); 7.27–7.29 (d, J = 7.86 Hz, 2H); 7.69–7.71(d, J = 8.23 Hz, 2H); 8.41 (s, 1H); 10.78 (s, 1H). MS (ESI): 236.0 ($C_{10}H_{10}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86, N, 17.86. Found: C, 51.18; H, 3.64; N, 17.64.

4.2.8. 2-(2-(5-Fluoro-2-hydroxybenzylidene)hydrazinyl)thiazol-4(5*H*)-one (10)

White powder. Yield: 78%; mp: 157–159 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.53 (s, 2H); 6.94–6.96 (d, J = 8.51 Hz, 1H); 7.31–7.34(m, 1H); 7.63–7.65 (m, 1H); 8.69 (s, 1H); 10.79 (s, 1H). MS (ESI): 254.0 ($C_{10}H_9FN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8FN_3O_2S$: C, 47.43; H, 3.18; N, 16.59. Found: C, 47.57; H, 3.32; N, 16.73.

4.2.9. 2-(2-(5-Chloro-2-hydroxybenzylidene)hydrazinyl) thiazol-4(5*H*)-one (11)

White powder. Yield: 75%; mp: 163–165 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.51 (s, 2H); 6.97–6.98 (d, J = 8.55 Hz, 1H); 7.36–7.38(m, 1H); 7.68–7.69 (m, 1H); 8.71 (s, 1H); 10.81 (s, 1H). MS (ESI): 270.0 ($C_{10}H_9ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8ClN_3O_2S$: C, 44.53; H, 2.99; N, 15.58. Found: C, 44.71; H, 3.12; N, 15.67.

4.2.10. 2-(2-(5-Bromo-2-hydroxybenzylidene)hydrazinyl) thiazol-4(5*H*)-one (12)

White powder. Yield: 80%; mp: 169–170 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.50 (s, 2H); 6.92–6.93 (d, J = 8.53 Hz, 1H); 7.48–7.49 (m, 1H); 7.80–7.81 (m, 1H); 8.62 (s, 1H); 10.84 (s, 1H). MS (ESI): 313.9 ($C_{10}H_9BrN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8BrN_3O_2S$: C, 38.23; H, 2.57; N, 13.38. Found: C, 38.47; H, 2.78; N, 13.46.

4.2.11. 2-(2-(3,5-Dichloro-2-hydroxybenzylidene)hydrazinyl) thiazol-4(5*H*)-one (13)

White powder. Yield: 77%; mp: 161–163 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.52 (s, 2H); 7.69–7.70 (m, 1H); 7.80 (s, 1H); 8.73 (s, 1H); 11.63 (s, 1H). MS (ESI): 303.9 ($C_{10}H_8Cl_2N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_7Cl_2N_3O_2S$: C, 39.49; H, 2.32; N, 13.82. Found: C, 39.23; H, 2.48; N, 13.74.

4.2.12. 2-(2-(3,5-Dibromo-2-hydroxybenzylidene)hydrazinyl) thiazol-4(5*H*)-one (14)

White powder. Yield: 79%; mp: 173–175 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.51 (s, 2H); 7.64–7.66 (m, 1H); 7.83 (s, 1H); 8.71 (s, 1H); 11.80 (s, 1H). MS (ESI): 391.8 ($C_{10}H_8Br_2N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_7Br_2N_3O_2S$: C, 30.56; H, 1.80; N, 10.69. Found: C, 30.74; H, 1.98; N, 10.83.

4.2.13. 2-(2-(3-Fluorobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (15)

White powder. Yield: 74%; mp: 177–178 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.48 (s, 2H); 7.37 (s, 1H); 7.53–7.56 (m, 1H); 7.63–7.66 (m, 2H); 8.41 (s, 1H). MS (ESI): 238.0 ($C_{10}H_9FN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8FN_3OS$: C, 50.62; H, 3.40; N, 17.71. Found: C, 50.78; H, 3.57; N, 17.63.

4.2.14. 2-(2-(3-Chlorobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (16)

White powder. Yield: 72%; mp: 182–184 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.49 (s, 2H); 7.39 (s, 1H); 7.56–7.59 (m, 1H); 7.67–7.69 (m, 2H); 8.45 (s, 1H). MS (ESI): 254.0 ($C_{10}H_9ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8ClN_3OS$: C, 47.34; H, 3.18; N, 16.56. Found: C, 47.51; H, 3.29; N, 16.79.

4.2.15. 2-(2-(3-Bromobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (17)

White powder. Yield: 76%; mp: 179–181 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.47 (s, 2H); 7.36 (s, 1H); 7.51–7.55 (m, 1H); 7.62–7.67 (m, 2H); 8.43 (s, 1H). MS (ESI): 297.9 ($C_{10}H_9BrN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8BrN_3OS$: C, 40.28; H, 2.70; N, 14.09. Found: C, 40.42; H, 2.97; N, 14.18.

4.2.16. 2-(2-(3-Methylbenzylidene)hydrazinyl)thiazol-4(5*H*)-one (18)

White powder. Yield: 70%; mp: 188–189 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 2.36 (s, 3H); 4.46 (s, 2H); 7.34 (s, 1H); 7.53–7.57 (m, 1H); 7.64–7.69 (m, 2H); 8.47 (s, 1H). MS (ESI): 234.0 ($C_{11}H_{12}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.82; H, 4.56; N, 18.15.

4.2.17. 2-(2-(3-Methoxybenzylidene)hydrazinyl)thiazol-4(5*H*)-one (19)

White powder. Yield: 72%; mp: 185–187 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 3.76 (s, 3H); 4.43 (s, 2H); 7.36 (s, 1H); 7.57–7.61 (m, 1H); 7.72–7.75 (m, 2H); 8.51 (s, 1H). MS (ESI): 250.0 ($C_{11}H_{12}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86. Found: C, 53.23; H, 4.64; N, 16.67.

4.2.18. 2-(2-(3-Nitrobenzylidene)hydrazinyl)thiazol-4(5*H*)-one (20)

White powder. Yield: 78%; mp: 191–193 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.45 (s, 2H); 7.39 (s, 1H); 7.66–7.68 (m, 1H); 7.75–7.78 (m, 2H); 8.53 (s, 1H). MS (ESI): 265.0 ($C_{10}H_9N_4O_3S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_8N_4O_3S$: C, 45.45; H, 3.05; N, 21.20. Found: C, 45.62; H, 3.18; N, 21.37.

4.2.19. 2-(2-(3-Hydroxybenzylidene)hydrazinyl)thiazol-4(5*H*)-one (21)

White powder. Yield: 77%; mp: 196–197 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 4.41 (s, 2H); 7.33 (s, 1H); 7.57–7.61 (m, 1H); 7.67–7.69 (m, 2H); 8.57 (s, 1H); 10.78 (s, 1H). MS (ESI): 236.0 ($C_{10}H_{10}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86; N, 17.86. Found: C, 51.19; H, 3.78; N, 17.64.

4.3. General method of synthesis of compounds 24–28

To a solution of compound **22** (0.01 mol) in ethanol (20 mL) was added an equimolar amount of thiosemicarbazide (0.91 g, 0.01 mol). The reaction mixture was heated under reflux for 2–6 h, partially concentrated and cooled. The separated solid product was filtered off, dried, and recrystallized from ethanol to give compound **23**. A mixture of compound **23** (0.005 mol), ethyl bromoacetate (0.83 g, 0.005 mol), anhydrous sodium acetate (1.64 g, 0.02 mol), and absolute ethanol (30 mL) was refluxed for 8–12 h. The product obtained upon cooling was collected by filtration, washed with water, dried, and recrystallized from a mixture of EtOH to give compounds **24–28**.

4.3.1. 2-(2-Cyclobutylidenehydrazinyl)thiazol-4(5*H*)-one (24)

White powder. Yield: 80%; mp: 80–82 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 1.65–1.69 (m, 4H); 2.24–2.27 (m, 2H); 4.41 (s, 2H). MS (ESI): 184.0 ($C_7H_{10}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_7H_9N_3OS$: C, 45.88; H, 4.95; N, 22.93. Found: C, 45.64; H, 4.82; N, 22.82.

4.3.2. 2-(2-Cyclopentylidenehydrazinyl)thiazol-4(5*H*)-one (25)

White powder. Yield: 84 %; mp: 82–83 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 1.67–1.72 (m, 4H); 2.31–2.39 (m, 4H); 4.40 (s, 2H). MS (ESI): 197.0 ($C_8H_{12}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_8H_{11}N_3OS$: C, 48.71; H, 5.62; N, 21.30. Found: C, 48.58; H, 5.75; N, 21.45.

4.3.3. 2-(2-Cyclohexylidenehydrazinyl)thiazol-4(5*H*)-one (26)

White powder. Yield: 81 %; mp: 86–88 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 1.53–1.56 (m, 4H); 1.63–1.64 (m, 2H); 2.26–2.28 (m, 2H); 2.49–2.52 (m, 2H); 4.41 (s, 2H). MS (ESI): 211.0 ($C_9H_{14}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20. Found: C, 51.31; H, 6.43.

4.3.4. 2-(2-Cycloheptylidenehydrazinyl)thiazol-4(5*H*)-one (27)

White powder. Yield: 83 %; mp: 89–90 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 1.50–1.54 (m, 4H); 1.68–1.69 (m, 2H); 2.17–2.18 (m, 2H); 2.24–2.26 (m, 2H); 2.45–2.48 (m, 2H); 4.43 (s, 2H). MS (ESI): 226.0 ($C_{10}H_{16}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{10}H_{15}N_3OS$: C, 53.31; H, 6.71; N, 19.89. Found: C, 53.46; H, 6.84; N, 19.76.

4.3.5. 2-(2-Cyclooctylidenehydrazinyl)thiazol-4(5*H*)-one (28)

White powder. Yield: 78 %; mp: 92–94 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 1.56–1.59 (m, 4H); 1.64–1.68 (m, 2H); 1.92–1.95 (m, 2H); 2.14–2.16 (m, 2H); 2.28–2.30 (m, 2H); 2.41–2.44 (m, 2H); 4.41 (s, 2H). MS (ESI): 239.1 ($C_{11}H_{18}N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{11}H_{17}N_3OS$: C, 55.20; H, 7.16; N, 17.56. Found: C, 55.42; H, 7.34; N, 17.72.

4.4. Preparation, and purification of HER-2 and EGFR and inhibitory assay

A 1.7 Kb cDNA encoded for human HER-2 cytoplasmic domain (HER-2-CD, amino acids 676–1245) and 1.6 kb cDNA encoded for the EGFR cytoplasmic domain (EGFR-CD, amino acids 645–1186) were cloned into baculoviral expression vectors pBlueBacHis2B and pFASTBacHTc (Huakang Company China), separately. A sequence that encodes $(His)_6$ was located at the 5′ upstream to the HER-2 and EGFR sequences. 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. Histidinetagged 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.[24]

Both EGFR and HER-2 kinase assays were set up to assess the level of autophosphorylation based on DELFIA/Time-Resolved Fluorometry. Compounds **3–21** and **24–28** 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 × 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 or HER-2.

4.5. Cell proliferation assay

The antiproliferative activity was determined using a standard (MTT)-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of 7×10^3 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 6 wells from at least three independent experiments.

4.6. Molecular docking modeling

Molecular docking of compound **12** into the three-dimensional EGFR complex structure (1M17.pdb, downloaded from the PDB) was carried out using the AutoDock software package (version 4.0) as implemented through the graphical user interface AutoDockTools (ADT 1.4.6).

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References and notes

1. Seymore, L. *Cancer Treat. Rev.* **1999**, *25*, 301.
2. Adjei, A. A. *J. Natl. Cancer Inst.* **2001**, *93*, 1062.
3. Yarden, Y.; Sliwkowski, M. X. *Mol. Cell. Biol.* **2001**, *2*, 127.
4. Hynes, N. E.; Stern, D. F. *Biochim. Biophys. Acta* **1994**, *1198*, 165.
5. Slamon, D. J.; Clark, G. M.; Wong, S. G.; Levin, W. J.; Ullrich, A.; McGuire, W. L. *Science* **1987**, *235*, 177.
6. Slamon, D. J.; Godolphin, W.; Jones, L. A. *Science* **1989**, *244*, 707.
7. Scheurle, D.; Jahanzeb, M.; Aronsohn, R. S.; Watzek, L.; Narayanan, R. H. *Anticancer Res.* **2000**, *20*, 2091.
8. Cox, G.; Vyberg, M.; Melgaard, B.; Askaa, J.; Oster, A.; O'Byrne, K. J. *Int. J. Cancer* **2001**, *92*, 480.
9. Gullick, W. J. *Br. Med. Bull.* **1991**, *47*, 87.
10. Moscatello, D. K.; Holgado-Mudruga, M.; Godwin, A. K.; Ramirez, G.; Gunn, G.; Zoltick, P. W.; Biegel, J. A.; Hayes, R. L.; Wong, A. J. *Cancer Res.* **1995**, *55*, 5536.
11. Wikstrand, C. J.; McLendon, R. E.; Friedman, A.; Bigner, D. D. *Cancer Res.* **1997**, *57*, 4130.
12. Bridges, A. J. *Curr. Med. Chem.* **1999**, *6*, 825.
13. Boschelli, D. H. *Drugs Fut.* **1999**, *24*, 515.
14. Ottana, R.; Maccari, R.; Barreca, M. L.; Bruno, G.; Rotondo, A.; Rossi, A.; Chiricosta, G.; Di Paola, R.; Sautebin, L.; Cuzzocrea, S.; Vigorita, M. G. *Bioorg. Med. Chem.* **2005**, *13*, 4243.
15. Vicini, P.; Geronikaki, A.; Anastasia, K.; Incerti, M.; Zani, F. *Bioorg. Med. Chem.* **2006**, *14*, 3859.
16. Gududuru, V.; Hurh, E.; Dalton, J. T.; Miller, D. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5289.
17. Ottana, R.; Carotti, S.; Maccari, R.; Landini, I.; Chiricosta, G.; Caciagli, B.; Vigorita, M. G.; Mini, E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3930.
18. Elbarbary, A. A.; Khodair, A. I.; Pedersen, E. B.; Nielsen, C. *Monatsh. Chem.* **1994**, *125*, 593.
19. Rydzik, E.; Szadowska, A.; Kaminska, A. *Acta Pol. Pharm.* **1984**, *41*, 459.
20. Troutman, H. D.; Long, L. M. *J. Am. Chem. Soc.* **1948**, *70*, 3436.
21. Liu, H. L.; Li, Z. C.; Anthonsen, T. *Molecules* **2000**, *5*, 1055.
22. Kalaiya, S. B.; Parikh, A. R. *J. Indian Chem. Soc.* **1987**, *64*, 172.
23. Dayam, R.; Aiello, F.; Deng, J. X.; Wu, Y.; Garofalo, A.; Chen, X. Y.; Neamati, N. *J. Med. Chem.* **2006**, *49*, 4526.
24. Tsou, H. R.; Mamuya, N.; Johnson, B. D.; Reich, M. F.; Gruber, B. C.; Ye, F.; Nilakantan, R.; Shen, R.; Discafani, C.; DeBlanc, R.; Davis, R.; Koehn, F. E.; Greenberger, L. M.; Wang, Y. F.; Wissner, A. *J. Med. Chem.* **2001**, *44*, 2719.