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Synthesis and biological evaluation of 4-aryl-5-cyano-2*H*-1,2,3-triazoles as inhibitor of HER2 tyrosine kinase

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Abstract—4-Aryl-5-cyano-2*H*-1,2,3-triazoles bearing a variety of substituting groups on 4-phenyl were synthesized. The chemicals, designed as HER2 tyrosine kinase inhibitors, were screened for bioactivity of inhibiting growth of breast cancer MDA-MB-453 cells. The lowest IC_{50} value of inhibiting HER2 tyrosine kinase phosphorylation in breast cancer cells is 6.6 μM and the IC_{50} value of cell growth inhibition is correspondingly 30.9 μM. The lipophilicity of substituting groups on triazoles is the main factor to influence their bioactivities.

1. Introduction

Epidermal growth factor receptor (EGFR) family has four members: HER2 (human epidermal growth factor receptor-2; also known as erbB2) and its relatives HER1 (epidermal growth factor receptor; EGFR), HER3, and HER4. HER2, like other EGFR members, is a transmembranous glycoprotein (p185neu) with intrinsic tyrosine kinase activity encoded by the HER2 protooncogene located on the long arm of chromosome 17 (17q21).[1] Being on the upstream of the signal pathway, it acts as a mayor switch in different signal transduction processes which mediate and sustain the organ physiologic activity.[2] In normal cells, activation of this receptor tyrosine kinase family triggers a rich network of signaling pathways, which control normal cell growth, differentiation, motility, and adhesion in several cell lineages.[3] HER2 overexpression is identified on many tumor cells and the relationship between HER2 status and clinicopathological characteristics in breast cancer has been investigated.[4] Statistically overexpression of HER2 occurs in a number of human cancers, including 25–30% of breast cancer,[5] 28% in pulmonary adenocarcinoma, and 17% of colorectal adenocarcinoma.[6] Its upregulated expression is also related to rapid disease progression, chemoresistance, accelerated relapse as well as poor prognosis and mortality.[7] Signal-transduction pathways depending on HER2 initiate with HER2 forming homodimer with itself or heterodimers with other relatives. The dimerization activates the receptor tyrosine kinases, which phosphorylate special tyrosine residues on proteins.[8] These phosphorylated tyrosine residues initiate downstream multiple signaling pathways associated with cell growth (or differentiation) mainly including the Ras/MAP kinase pathway and PKB/Akt pathway.[9]

The overexpression of HER2 promotes receptor dimerization[8] and results in cell transformation. Clearly, HER2 triggers a diverse signaling network rather than a single pathway. Therefore, HER2 is proving to be an excellent target for antitumor therapy,[10] specific to HER2-overexpressing cancers such as breast cancer.[6] We previously reported that 1,2,3-triazole was discovered as an inhibitor of HER2 tyrosine kinase through computer-aided drug design approach and searched from molecule libraries (see Fig. 1 illustrating the interaction of **3k** with HER2 tyrosine kinase).[11] Recently, some triazoles were synthesized and showed that the triazole derivatives can inhibit HER2 tyrosine kinase phosphorylation in the breast cancer cell, and it has also been testified that the growth of human breast cancer cell MDA-MB-453 can be inhibited by the chemicals.

2. Chemistry

Synthesis of the object chemicals is via a two-step procedure as outlined in Scheme 1. First, arylacetylenes (**1**)

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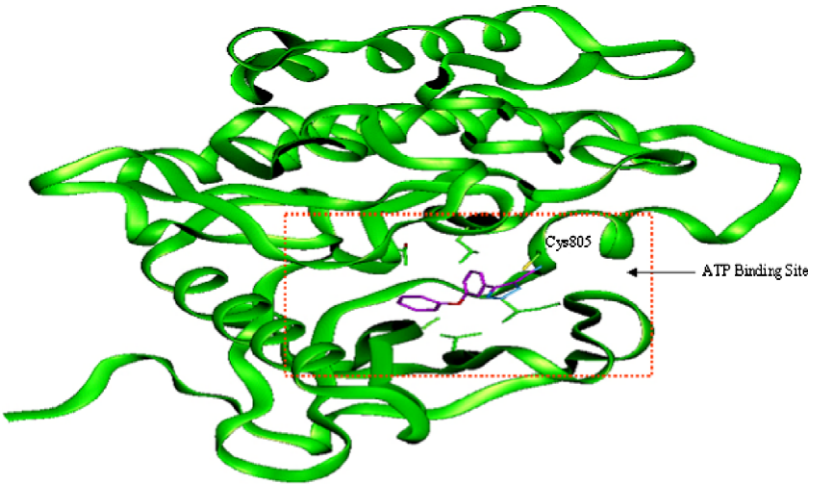
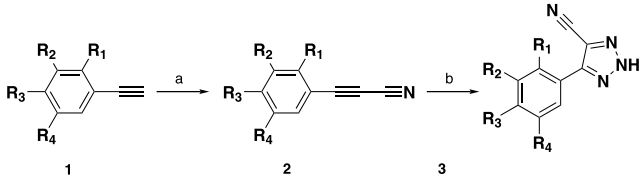


Figure 1. Proposed binding mode of **3k** with the kinase domain of HER2 obtained after docking and molecular dynamics simulation.



Scheme 1. Reagents and conditions: (a) CuCN, $(CH_3)_3SiCl$, NaI (cat.); DMSO/CH_3CN/H_2O, 50 °C, 24–72 h; (b) NaN_3, 90–120 °C, 1.5 h.

reacted with cuprous cyanide in the presence of chlorotrimethylsilane and sodium iodide to generate 3-arylpropynenitriles **2a**–**2l** after more than one day's stirring in the mixture solvent of sulfoxide and acetonitrile.[12] Chemicals **2a**–**2l** on further reaction with NaN_3 afford corresponding 2*H*-1,2,3-triazoles **3a**–**3l** in excellent yields.[13]

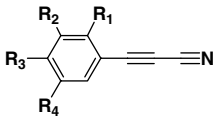
The structure of newly synthesized compounds was confirmed by recording the IR, ^{1}H NMR, and mass spectra. Characterization data of triazoles are given in the text. IR spectrum of compounds showed absorption bands at 3200–2500, 2240, and 2210 cm^{-1} due to N–H, C≡N, and C≡C groups, respectively. The absence of the absorption band corresponding to C≡C stretching frequency of the reactants clearly confirmed the formation of triazoles **3a**–**3l**. The ^{1}H NMR spectrum of target compounds showed a broad singlet at δ 12.5 ppm corresponding to active proton on the triazole ring. Mass spectral data of the newly synthesized compounds were recorded and are also given in the text. All the compounds showed negative molecular ion peaks as the major picks revealing the presence of the active proton. The major peaks appearing in the spectra were due to the loss of the proton on the triazole ring.

3. Results and discussion

3.1. Chemistry

Arylpropynenitriles (**2**) were prepared by the method reported previously.[11] We found that arylpropynenitriles which bear a methoxy group were easily formed comparatively, because after 24 h the reaction was almost finished and the yield of the product was relatively high and did not increase by prolonging the reaction time (see Table 1). It is suggested that the reaction can be accelerated by the electron-donating substituting groups.

Table 1. The reaction time and yield of synthesis of 3-arylpropynenitriles



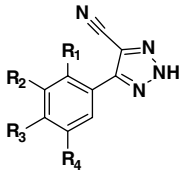
Compound	R_1	R_2	R_3	R_4	Time (h)	Yield[a] (%)
2a	H	H	H	H	48	58
2b	H	H	CH_3	H	48	76
2c	H	H	$(CH_3)_2CH$	H	72	80
2d	H	H	CH_3O	H	24	85
2e	CH_3O	CH_3O	H	H	24	80
2f	H	CH_3O	CH_3O	H	24	70
2g	H	CH_3O	CH_3O	CH_3O	24	75
2h	H	H	F	H	72	75
2i	H	H	Cl	H	48	50
2j	H	H	Br	H	48	50
2k	H	H	PhO	H	72	80
2l	H	F	PhO	H	72	80

[a] Purified yield.

Cycloaddition reactions were performed at different temperatures (90 or 120 °C) for the individual substituting group on arylpropynenitrile. Corresponding higher temperature could enhance the reaction but result in more side products and lower yields. Electron-donating groups had a negative effect on the reaction results. For multi-methoxy group trazoles were obtained at lower yields even though at higher temperature.

1,3-Dipolar cycloaddition between unsymmetrical acetylenes and sodium azide results in tautomers. The crystal

Table 2. In vitro bioactivity of compounds **3a**–**3l**



Compound	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l
IC_{50}[a] (μM)	153.3	211.7	130.7	169.8	241.9	223.7	329.1	35.0	56.7	55.0	30.9	51.7
IC_{50}[b] (μM)	53.5	36.5	13.5	>50	>50	>50	>50	31.6	11.0	7.9	6.6	16.6

[a] Inhibitory growth of MDA-MB-453 cells with MTT assay.
[b] Inhibitory on HER2 tyrosine kinase phosphorylation in MDA-MB-453 cells.

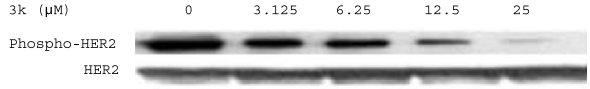


Figure 2. Inhibition of HER2 tyrosine kinase phosphorylation and HER2 receptor protein expression by chemical **3k**. Overexpressing HER2 receptor MDA-MB-453 cells were treated with different concentrations of **3k** for 30 min, then collected and lysed. Western blot analysts was conducted and probed with anti-phospho-HER2 or anti-HER2 antibody.

structures of 4-phenyl-1,2,3-triazole have been determined.[14] 2*H*-Isomer is predominant in solid and gas phases, however 1,2-di*H* equilibrium exists in neutral solution and 1,3-diH^+ equilibrium exists in cation solution, respectively, within these kinds of compounds.

3.2. Bioactivity

In vitro bioactivity of compounds **3a**–**3l** was evaluated, growth inhibition of the human breast cancer MDA-MB-453 cells tested by MTT assay, and phosphorylation inhibition of HER2 tyrosine kinase measured by Western blot assay. The results are summarized in Table 2.

The most potent chemical is compound **3k**. Its IC_{50} value of inhibiting the breast cancer cell growth was 30.9 μM and its IC_{50} value of phosphorylation inhibition was 6.6 μM. However, the compounds with their IC_{50} value of phosphorylation inhibition more than 50 μM showed relatively lower activity of cell growth inhibition. Western blot assay indicated that the phospho-HER2 protein in breast cancer cell MDA-MB-453 was markedly reduced by compound **3k**, while the total number of HER2 protein was not altered (see Fig. 1). In the parallel analysis, the phospho-HER1 protein in the HER1-overexpressing breast cancer cell MDA-MB-468 treated with **3k** was not observed to be reduced through Western blot assay (see Fig. 2). It is suggested that the cell growth inhibition was ascribed mainly to the inhibition of HER2 tyrosine kinase, for decreases of HER phosphorylation could inhibit proliferation of the cell through HER signal-transduction pathway (Fig. 3).

Figure 3. Inhibition of HER1 tyrosine kinase phosphorylation and HER1 receptor protein expression by chemical **3k**. Overexpressing HER1 receptor MDA-MB-468 cells were treated with different concentrations of **3k** for 1 h, then collected and lysed. Western blot analysts was conducted and probed with anti-phospho-HER1 or anti-HER1 antibody.

As potential inhibitors, the structure–activity relationships covering the chemicals and their HER2 phosphorylation inhibition capability can be summarily deduced from the data presented in Table 2. Lipophilic substitute on 4-phenyl of the triazole is favorable for the inhibitory activity and an electron-drawing group does not appear to enhance the inhibitory activity.

4. Conclusion

In summary, we have described the synthesis of 4-aryl-5-cyano-2*H*-1,2,3-triazole derivatives and investigated their bioactivity as HER2 tyrosine kinase inhibitor. The simple method for synthesis of this series of triazole opens wide possibility for easy modification of their chemical structures and properties in the desired direction.

5-Cyano-2*H*-1,2,3-triazole derivatives were testified to inhibit the growth of the human breast cancer cell MDA-MB-453 through inhibiting its phosphorylation of HER2 in the cell. The lipophilicity of substituting groups on derivatives is the main factor to influence their bioactivities, which is rational by the value of IC_{50}.

5. Experimental protocols

5.1. General procedure for chemistry

NMR spectra were recorded on a Varian INOVA500NB 500 MHz spectrometer with Me_4Si as the internal standard. IR spectra were carried out on a FT-IR Tensor 37 spectrophotometer in KBr or as films on KBr pellet. MS spectra were performed on a ZAB-HS instrument or a TSQ QUANTUM. Melting points (mp) were obtained on a WRR Melting point apparatus and the values are

uncorrected. TLC was carried out on glass plates coated with silica gel GF_{254}. Compounds were purified by chromatography on silica gel.

5.2. General procedure for the synthesis of 3-arylpropynenitriles (2a–2l)

A 100 ml flask is charged with dimethylsulfoxide (30 ml) and acetonitrile (10 ml). To this rapidly stirring solution cuprous cyanide(10.7 g, 120 mmol), sodium iodide (0.6 g, 6.0 mmol), and arylacetylene (40 mmol) were added successively. Chlorotrimethylsilane (15.2 ml, 120 mmol) was dropped to the reaction mixture over a 5 min period, via the addition funnel, and the reaction mixture then was heated at 50 °C for 24–72 h. The reaction was terminated by cooling the mixture to room temperature, then 50 ml water was added to the reaction mixture and was extracted with ether (5× 20 ml). The combined organic layer was washed with saturated sodium bicarbonate (50 ml), brine (50 ml) in turn, and then dried with magnesium sulfate overnight. The residue obtained after filtering and evaporation was chromatographed on silica gel using hexane–ethyl acetate (100:5, v/v) as eluent to yield 3-arylpropynenitriles.

5.2.1. 3-Phenylpropynenitrile (2a). IR(Nujol): 2269 cm^{-1} (C≡N), 2145 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 7.4 (3H), 7.6 (2H); MS-FAB (*m*/*z*): 127 (M).

5.2.2. 3-(4-Methylphenyl)propynenitrile (2b). IR(Nujol): 2262 cm^{-1} (C≡N), 2137 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 2.4 (s, 6H), 7.3 (d, 2H), 7.6 (d, 2H) ppm; MS-FAB (*m*/*z*): 141 (M).

5.2.3. 3-(4-Isopropylphenyl)propynenitrile (2c). IR(Nujol): 2266 cm^{-1} (C≡N), 2244 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 1.3 (d, 6H), 3.2 (m, 1H), 7.3 (d, 2H), 7.5 (d, 2H) ppm; MS-FAB (*m*/*z*): 169 (M).

5.2.4. 3-(4-Methoxyphenyl)propynenitrile (2d). IR(Nujol): 2262 cm^{-1} (C≡N), 2137 cm^{-1} (C≡C); ^1H NMR($CDCl_3$, δ): 3.8 (s, 3H), 7.0 (d, 2H), 7.5 (d, 2H) ppm; MS-FAB (*m*/*z*): 157 (M).

5.2.5. 3-(2,3-Dimethoxyphenyl)propynenitrile (2e). IR-(Nujol): 2262 cm^{-1} (C≡N), 2145 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 3.8 (s, 6H), 6.9 (m, 2H), 7.2 (d, 1H) ppm; MS-FAB (*m*/*z*): 187 (M).

5.2.6. 3-(3,4-Dimethoxyphenyl)propynenitrile (2f). IR-(Nujol): 2255 cm^{-1} (C≡N), 2140 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 3.9 (s, 6H), 6.9 (d, 1H), 7.1 (m, 2H) ppm; MS-FAB (*m*/*z*): 187 (M).

5.2.7. 3-(3,4,5-Trimethoxyphenyl)propynenitrile (2g). IR(Nujol): 2262 cm^{-1} (C≡N), 2146 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 3.8 (9H), 6.7 (s, 2H) ppm; MS-FAB (*m*/*z*): 217 (M).

5.2.8. 3-(4-Fluorophenyl)propynenitrile (2h). IR(Nujol): 2268 cm^{-1} (C≡N), 2145 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 7.1 (d, 2H), 7.6 (d, 2H) ppm; MS-FAB (*m*/*z*): 145 (M).

5.2.9. 3-(4-Chlorophenyl)propynenitrile (2i). IR(Nujol): 2260 cm^{-1} (C≡N), 2142 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 7.3 (d, 2H), 7.7 (d, 2H) ppm; MS-FAB (*m*/*z*): 161 (M).

5.2.10. 3-(4-Bromophenyl)propynenitrile (2j). IR(Nujol): 2276 cm^{-1} (C≡N), 2146 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 7.5 (d, 2H), 7.6 (d, 2H) ppm; MS-FAB (*m*/*z*): 205 (M).

5.2.11. 3-(3-Phenoxyphenyl)propynenitrile (2k). IR(Nujol): 2265 cm^{-1} (C≡N), 2146 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 7.3 (m) ppm; MS-FAB (*m*/*z*): 219 (M).

5.2.12. 3-(4-Fluoro-3-phenoxylphenyl)propynenitrile (2l). IR(Nujol): 2260 cm^{-1} (C≡N), 2147 cm^{-1} (C≡C); ^1H NMR ($CDCl_3$, δ): 7.2 (m) ppm; MS (FAB) *m*/*z*: 237 (M).

5.3. General procedure for the synthesis of 4-aryl-5-cyano-2*H*-1,2,3-triazoles (3a–3l)

NaN_3 (0.97 g, 15 mmol) suspended in DMF (10 ml) was strongly stirred at 90 or 120 °C (change according to the variety of arylpropynenitriles). To this flask a solution of arylpropynenitrile (10 mmol) in DMF (4 ml) was added dropwise through an addition funnel and kept the addition lasting 30 min. After the solution was added, the mixture was maintained stirring for another hour at the reaction temperature. The residue obtained after removal of the solvent was treated with water (10 ml). Then the water layer was extracted three times with CH_2Cl_2 to remove the oil and give a transparent light color solution. Finally, the aqueous solution was acidified with 10% HCl resulting in light yellow precipitate which was washed with iced water to yield crude 4-aryl-5-cyano-2*H*-1,2,3-triazoles. The products were purified by column chromatography on silica gel eluted with petrol ether and ethyl acetate (100:10, v/v).

5.3.1. 4-Phenyl-5-cyano-1,2,3-triazoles (3a). Reaction temperature: 90°C; yield: 80%; mp: 165–166 °C (lit.[13] 166–167 °C); IR(KBr): 3250–2500 cm^{-1} (N–H), 2240 cm^{-1} (C≡N) cm^{-1}; ^1H NMR (CD_3COCD_3, δ): 7.6 (m, 3H), 8.0 (m, 2H), 15.2 (br s, 1H) ppm; ESI-MS (*m*/*z*): 169.145 $(M-H)^-$. Anal. Calcd for $C_9H_6N_4$ (170.17): C, 63.52; H, 3.55; N, 32.92. Found: C, 63.75; H, 4.13; N, 32.74.

5.3.2. 4-(4-Methylphenyl)-5-cyano-2*H*-1,2,3-triazoles (3b). Reaction temperature: 90 °C; yield: 80%; mp: 173–174 °C; IR(KBr): 3250–2500 cm^{-1} (N–H), 2240 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 2.4 (3H, s), 7.8 (d, 2H, J = 8.1 Hz), 7.3 (d, 2H, J = 7.9 Hz), 12.6 (br s, 1H) ppm; ESI-MS (*m*/*z*): 184.156 $(M-H)^-$. Anal. Calcd for $C_{10}H_8N_4$ (184.20): C, 65.21; H, 4.38; N, 30.42. Found: C, 65.45; H, 4.59; N, 30.17.

5.3.3. 4-(4-Isopropylphenyl)-5-cyano-2*H*-1,2,3-triazoles (3c). Reaction temperature: 90 °C; yield: 80%; mp: 147–148 °C; IR(KBr): 3250–2500 cm^{-1} (N–H), 2240 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 1.3 (d, 6H, J = 7.0 Hz), 3.0 (m, 1H), 7.4 (d, 2H, J = 8.0 Hz), 7.9 (d, 2H, J = 8.5 Hz), 12.9 (br s, 1H) ppm; ESI-MS

(*m*/*z*): 211.226 $(M-H)^-$. Anal. Calcd for $C_{12}H_{12}N_4$ (212.25): C, 67.90; H, 5.70; N, 26.40. Found: C, 68.18; H, 6.15; N, 26.04.

5.3.4. 4-(4-Methoxyphenyl)-5-cyano-2*H*-1,2,3-triazoles (3d). Reaction temperature: 90 °C; yield: 75%; mp: 202–203 °C; IR(KBr): 3252–2500 cm^{-1} (N–H), 2237 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 4.0 (s, 3H), 7.0 (d, 1H, $J = 8.9$ Hz), 7.9 (d, 1H, $J = 8.8$ Hz), 12.20 (br s, 1H) ppm; ESI-MS (*m*/*z*): 198.105 $(M-H)^-$. Anal. Calcd for $C_{10}H_8N_4O$ (200.20): C, 59.99; H, 4.03; N, 27.99. Found: C, 60.20; H, 4.61; N, 27.62.

5.3.5. 4-(2,3-Dimethoxyphenyl)-5-cyano-2*H*-1,2,3-triazoles (3e). Reaction temperature: 120 °C; yield: 60%; mp: 179–180 °C, IR(KBr): 3255–2968 cm^{-1} (N–H), 2242 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 3.9 (s, 3H), 4.0 (s, 3H), 7.3 (m, 3H), 15.1 (br s, 1H) ppm; ESI-MS (*m*/*z*): 229.211 $(M-H)^-$. Anal. Calcd for $C_{11}H_{10}N_4O_2$ (230.22): C, 57.39; H, 4.38; N, 24.34. Found: C, 57.70; H, 4.81; N, 24.01.

5.3.6. 4-(3,4-Dimethoxyphenyl)-5-cyano-2*H*-1,2,3-triazoles (3f). Reaction temperature: 120 °C; yield: 60%; mp: 247–248 °C; IR(KBr): 3249–2939 cm^{-1} (N–H), 2237 cm^{-1} (C≡N); ^1H NMR (CD3COCD3, δ): 3.912 (3H, s), 3.910 (3H, s), 7.56 (2H, m), 7.16 (dd, 1H, $J = 8.3$ Hz, $J = 17.6$ Hz), 15.3 (1H, br s) ppm; ESI-MS (*m*/*z*): 230.202 $(M-H)^-$. Anal. Calcd for $C_{11}H_{10}N_4O_2$ (230.22): C, 57.39; H, 4.38; N, 24.34. Found: C, 57.68; H, 4.76; N, 24.13.

5.3.7. 4-(3,4,5-Trimethoxyphenyl)-5-cyano-2*H*-1,2,3-triazoles (3g). Reaction temperature: 120 °C; yield 54%; mp: 222–223 °C; IR(KBr): 3197–2940 cm^{-1} (N–H), 2241 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 3.8 (s, 3H), 3.9 (s, 6H), 7.3 (s, 2H), 15.2 (br s, 1H) ppm; ESI-MS (*m*/*z*): 259.191 $(M-H)^-$. Anal. Calcd for $C_{12}H_{12}N_4O_3$ (260.25): C, 55.38; H, 4.65; N, 21.53. Found: C, 55.29; H, 5.21; N, 21.01.

5.3.8. 4-(4-Fluorophenyl)-5-cyano-2*H*-1,2,3-triazoles (3h). Reaction temperature: 120 °C; yield: 80%; mp: 193–195 °C; IR(KBr): 3250–2500 cm^{-1} (N–H), 2240 cm^{-1} (C≡N); ^1H NMR(CD_3COCD_3, δ): 7.3 (d, 2H, $J = 7.9$ Hz), 7.8 (d, 2H, $J = 8.1$ Hz), 12.3 (br s, 1H) ppm; ESI-MS (*m*/*z*): 186.136 $(M-H)^-$. Anal. Calcd for $C_9H_5FN_4$(188.16): C, 57.45; H, 2.68; N, 29.78. Found: C, 57.41; H, 2.94; N, 29.49.

5.3.9. 4-(4-Chlorophenyl)-5-cyano-2*H*-1,2,3-triazoles (3i). Reaction temperature: 120 °C; yield: 80%; mp: 189–190 °C; IR(KBr): 3250–2560 cm^{-1} (N–H), 2243 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 7.5 (d, 2H, $J = 8.6$ Hz), 7.9 (d, 2H, $J = 8.6$ Hz), 15.1 (br s, 1H) ppm; ESI-MS (*m*/*z*): 202.143 $(M-H)^-$. Anal. Calcd for $C_9H_5ClN_4$ (204.62): C, 52.83; H, 2.46; N, 27.38. Found: C, 52.46; H, 2.96; N, 27.17.

5.3.10. 4-(4-Bromophenyl)-5-cyano-2*H*-1,2,3-triazoles (3j). Reaction temperature: 120 °C; yield: 80%; mp: 182–183 °C; IR(KBr): 3224–2534 cm^{-1} (N–H), 2256 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 7.8 (d, 2H, $J = 8.9$ Hz), 7.9 (d, 2H, $J = 8.8$ Hz), 15.3 (br s, 1H); ESI-MS (*m*/*z*): 245.925 $(M-H)^-$. Anal. Calcd for $C_9H_5BrN_4$ (249.07): C, 43.40; H, 2.02; N, 22.49. Found: C, 43.14; H, 2.71; N, 22.12.

5.3.11. 4-(3-Phenoxylphenyl)-5-cyano-2*H*-1,2,3-triazoles (3k). Reaction temperature: 120 °C; yield: 80%; mp: 159–160 °C; IR(KBr): 3250–2500 cm^{-1} (N–H), 2242 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 7.07 (dd, 2H, $J = 1.0$ Hz, $J = 8.6$ Hz), 7.1 (m, 2H), 7.4 (dd, 1H, $J = 7.5$, 8.5 Hz), 7.5 (t, 1H, $J = 8.0$ Hz), 7.6 (s, 1H), 7.7 (d, 1H, $J = 7.8$ Hz), 12.4 (br s, 1H) ppm; ESI-MS (*m*/*z*): 260.125 $(M-H)^-$. Anal. Calcd for $C_{15}H_{10}N_4O$ (262.27): C, 68.69; H, 3.84; N, 21.36. Found: C, 68.41; H, 4.11; N 21.45.

5.3.12. 4-(4-Fluoro-3-phenoxylphenyl)-5-cyano-2*H*-1,2,3-triazoles (3l). Reaction temperature: 120 °C; yield: 80%; mp: 127–129 °C; IR(KBr): 3250–2500 cm^{-1} (N–H), 2245 cm^{-1} (C≡N); ^1H NMR (CD_3COCD_3, δ): 7.05 (dd, 2H, $J = 1.0$ Hz, $J = 8.7$ Hz), 7.15 (t, 1H, $J = 7.4$ Hz), 7.35 (m, 3H), 7.63 (dd, 1H, $J = 1.9$ Hz, $J = 7.5$ Hz), 7.74 (dd, 1H, $J = 2.2$, 4.1, and 8.5 Hz), 12.34 (1H, br s) ppm; ESI-MS (*m*/*z*): 279.161 $(M-H)^-$. Anal. Calcd for $C_{15}H_9FN_4O$ (280.26): C, 64.28; H, 3.24; N, 19.99. Found: C, 63.87; H, 3.53; N, 19.78.

5.4. Biology

5.4.1. Cell and cell culture. Human breast cancer cell line MDA-MB-453 was obtained from ATCC (USA). MDA-MB-453 cells (overexpressing HER2 receptor) were cultured in RPMI-1640 containing 10% of fetal bovine serum (FBS). The cultures were maintained at 37 °C in 5% CO_2. All experiments were conducted on cells in logarithmic growth phase.

5.4.2. MTT assay. A total of 2000 cells in 100 μl culture media with 10% fetal bovine serum were seeded to each well of a 96-well plate. After the addition of each drug in triplicate the cells were incubated at 37 °C. Ten microliters of 5 mg/ml MTT (Sigma) was add to each well. After the addition of MTT, further incubation was carried out for 4 h. At the end of the incubation the culture media were removed and 0.2 ml DMSO was added. The optical density (OD) was measured at 570 nm when the formazan crystals were dissolved in DMSO. The concentrations required to inhibit growth by 50% (IC_{50} values) were calculated from the cytotoxicity curves (Bliss's software).

5.4.3. Determining phosphorylation inhibition of HER2 tyrosine kinase (Western blot method). One milliliter of diluted cells 1×10^6/ml was seeded into 6-well plate per well. Cells treated with different concentration of drug at the same time were washed twice with PBS, lysed in 100 μl lysis buffer, and then centrifuged at 14,000*g* for 10 min. Protein concentrations were measured by the method of BCA (piece) and the IC_{50} values of phosphorylation inhibition were calculated, respectively.

To explore the change of HER, 40 mg of the protein prepared from aforesaid approach was used per lane, for SDS–PAGE. Sample treatment buffer was added and

incubated at 95 °C for 10 min. Following the seperation by 10% SDS–polyacrylamide gel electrophoresis proteins were transferred onto an equilibrated polyvinylidene difluoride membrane (Amersham) by electro-blotting. Afterwards membranes were blocked by 5% non-fat milk and consequently treated with primary antibodies and secondary antibodies. When all these were done the membrane was incubated at room temperature for 2 h. Before illuminant chemical was added, the blot was washed with TBST buffer (10 mmol/l Tris–HCl, pH 7.4, 150 mmol/l NaCl, and 0.1% Tween 20) for 10 min once. And then it was flaked, put into dark box to develop.

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