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ARTICLE

Synthesis and anticancer activity of benzotriazole derivatives

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Abstract

A series of benzotriazole (BTA) derivatives were synthesized as tyrosine protein kinase inhibitors using fragment-based design strategy. All desired compounds were synthesized with the reaction of benzotriazole, chloroacetonitrile and aromatic aldehyde using Ultrasonic-Microwave method and characterized by IR, ^{1}H and ^{13}C-NMR, mass spectrometry (MS) and elemental analysis. The anticancer activity of these compounds was evaluated by CCK-8 method against carcinoma VX2, lung cancer A549, stomach cancer cell lines MKN45 and MGC *in vitro*. The results showed that all compounds showed good antiproliferative activity. In particular, compound **2.1** showed the most prominent inhibition of VX2 cell lines with IC_{50} of 3.80 ± 0.75 μM. Compound **2.2** exhibited highly potent anticancer activity of stomach MGC cell lines with IC_{50} of 3.72 ± 0.11 μM. A549 and MKN45 cell lines were sensitive to compound **2.5** with IC_{50} of 5.47 ± 1.11 and 3.04 ± 0.02 μM, respectively.

1 | INTRODUCTION

The benzotriazole (BTA) family has emerged as a group of pharmacophoric molecules with a broad spectrum of biological properties, including antifungal,[1,2] antibacterial,[3,4] antiviral,[5] and anticancer activities.[6,7] Protein tyrosine kinases (PTKs) are the crucial class of oncology drugs targets.[8,9] Most of PTKs inhibitors have structures of heterocyclic ring with nitrogen-containing moieties, like BTA as the bioisostere, which competes with ATP for the adenine-binding region of the protein tyrosine kinase to inhibit the growth of cancer cells by blocking the signal transduction of phosphorylation and inhibiting the abnormal expression of PTKs.[10–12] PTKs catalyze phosphorylation of tyrosine in many proteins by the transfer of the γ-phosphoryl group from ATP to regulate the fundamental cellular processes, such as proliferation, differentiation, migration, metabolism, and antiapoptotic signaling.[13] As early as 2001, Sarno et al reported that 4,5,6,7-tetrabromo-*1H*-benzotriazole could dock with the ATP-binding pocket of CK2 in different binding modes, thereby specifically inhibiting its phosphorylation and inducing apoptosis of various cancer cells.[14] Then Makowska et al synthesized more active compounds in 2011 by structural modification of benzene rings and introducing side chains connecting three carbons into 4-methyl-5,6,7-tribromo-*1H*-benzotriazole and 4-ethyl-5,6,7-tribromo-*1H*-benzotriazole.[15] In 2013, a series of 1,3,4-oxadiazole derivatives docking with BTA groups and their anticancer activity were reported. Some of them have strong inhibitory activity against FAK, which is comparable to cisplatin in the treatment of human breast cancer.[6] A series of 4,5,6,7-tetra- bromo-2-(3-chloropropyl)-*2H*-benzotriazole derivatives were synthesized in 2016. The effect of these compounds on human recombinant casein kinase 2α subunit (rhCK2α) and significant cytotoxicity against human T-cell lymphoblast (CCRF-CEM) and breast adenocarcinoma (MCF-7) cell lines had been reported.[16] Regioisomeric BTAs on the different nitrogen atoms are difficult to be synthesized and their anticancer properties remain poorly defined. In this work, we aimed to design novel molecules that can

be used as inhibitors of PTKs with anticancer properties by fragment-based design strategy. We used BTA as starting material to synthesize a series of intermediates with different regioisomeric substituents under ultrasound-microwave conditions before reacted with chloro- or methoxylpyridine aldehydes. The antiproliferative activities of these target BTA compounds were screened in epithelial cancer cell line VX2, human lung cancer cell line A549, human gastric cancer cell lines MGC and MKN45 *in vitro*.

2 | RESULTS AND DISCUSSION

2.1 | Synthesis

Benzotriazole was used as starting material to synthesize 10 target compounds through two steps of substitution and condensation as depicted in Figure 1. BTAs were reacted with chloroacetonitrile to obtain intermediates under ultrasound-microwave conditions, and then 10 BTA derivatives were synthesized with 6-chloronicotinaldehyde and 5-formaldehyde-2-methoxypyridine, respectively. The ultrasonic-microwave reaction was optimized and carried out in this study with a ratio of BTA/chloroacetonitrile or intermediates/aromatic aldehyde of 3:4, microwave 480 W, and temperature of 75°C. Newly synthesized compounds were yellow crystalline powder that were in correspondent with their spectral and elemental analysis data. The structures of compounds are presented in Figure 1. These compounds are insoluble in water, methanol, or ethanol while soluble in toluene, DMF, DMSO, or $CHCl_3$. Physical characterization and yields of the target compounds were summarized in Table 1. According to the infrared spectrum of the intermediate, it was confirmed that the benzotriazole ring was successfully attached to the acetonitrile group with a

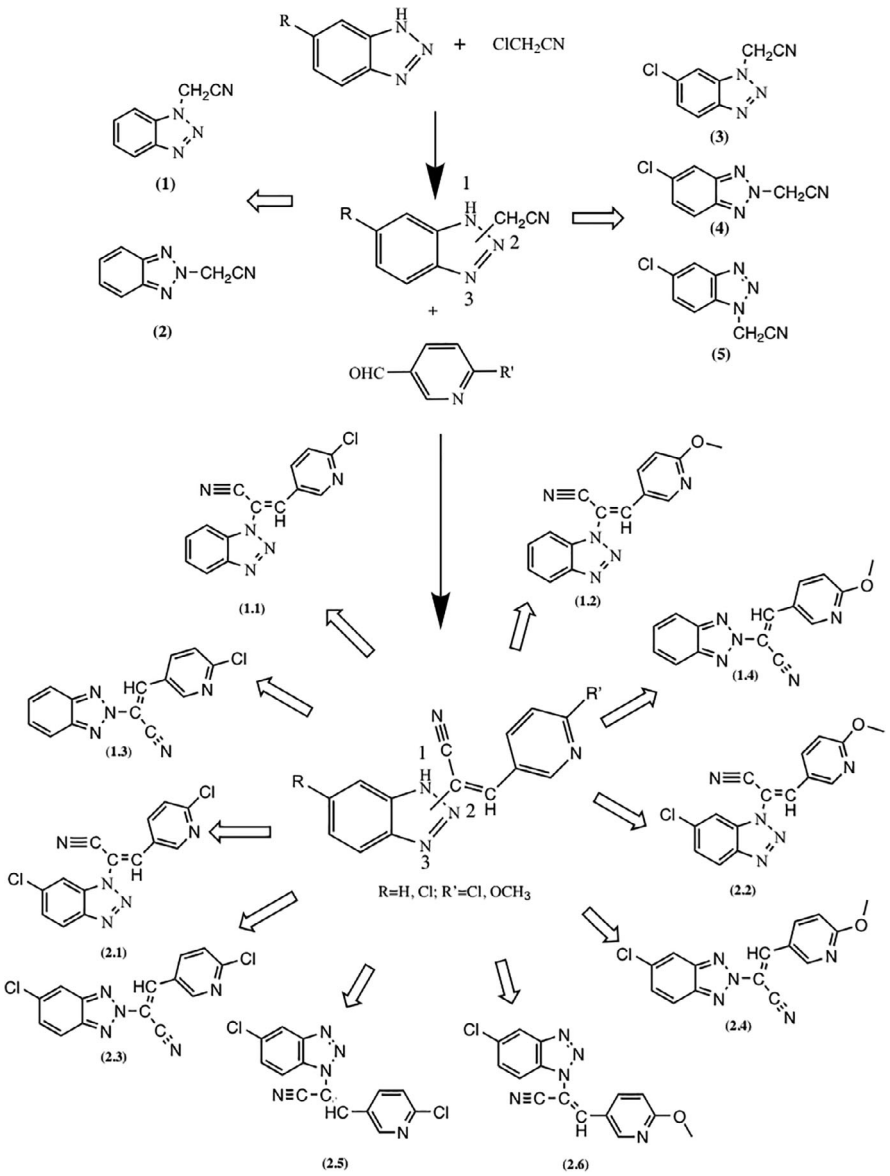

FIGURE 1 Synthesis of target compounds

TABLE 1 Physical characterization and yields of benzotriazole derivatives

Compounds	Molecular formula	Molecular weight	Color	MP (°C)	% Yield
1.1	$C_{14}H_8ClN_5$	281.70	Yellow	144-146	30.5
1.2	$C_{15}H_{11}N_5O$	277.29	Yellow	149-151	18.8
1.3	$C_{14}H_8ClN_5$	281.70	Yellow	233-234	55.7
1.4	$C_{15}H_{11}N_5O$	277.29	Yellow	138-140	23.0
2.1	$C_{14}H_7Cl_2N_5$	316.15	Yellow	166-167	43.7
2.2	$C_{15}H_{10}ClN_5O$	311.73	Yellow	177-179	20.7
2.3	$C_{14}H_7Cl_2N_5$	316.15	Yellow	209-210	60.2
2.4	$C_{15}H_{10}ClN_5O$	311.73	Yellow	133-136	25.6
2.5	$C_{14}H_7Cl_2N_5$	316.15	Yellow	166-167	61.5
2.6	$C_{15}H_{10}ClN_5O$	311.73	Yellow	177-179	25.5

characteristic stretching around 2200 to 2400 cm^{-1}. Furthermore, the regioisomeric BTA intermediates on the different nitrogen atoms were separated and characterized before the condensation reactions. The purification of these isomers were achieved by flash column chromatography based on polarity of these compounds. The region-selectivity of different intermediates was analyzed based on the changes in different proton signals on the benzene ring in ^{1}H-NMR spectra.[17,18] When the aromatic hydrogen proton signals all have signal peaks around 7.50 ppm and the influence of methylene is small, these compounds are predicted to be a 2-substituted intermediate. In 1-substituted intermediates, the chemical shifts of 7-position protons are moved to the high field by the influence of the adjacent methylene group. In 3-substituted intermediates, the chemical shifts of the 6-position proton and the 7-position proton are on the same signal peak, suggesting that the two sites are roughly affected by the influence of chlorine and methylene. Interestingly, region-selective substitution of BTAs at different positions affected the yields of the final products. The yields of 2H-1, 2, 3-benzotriazole derivatives or 3H-1, 2, 3-benzotriazole derivatives were generally higher than the isomers of 1H-1, 2, 3-benzotriazole derivatives presumably due to the less steric effects on 2 or 3-positions comparing to 1 position. Furthermore, yields of BTA derivatives bonded with aromatic aldehydes were impacted by the different substituents on aromatic rings: chlorine on pyridine rings has weak electron-withdrawing effect, while methoxyl group on pyridine rings has strong electron-donating effect. Electron-withdrawing effect in aldehyde promotes the condensation reaction, while the electron-donating group slows down the reaction. Thus the compounds a methoxyl substituted pyridine had lower yields than those with a chlorine substituent. For example, compound **2.5** has the highest yield with 61.5% and compound **1.2** has lowest yield with only 18.8%.

2.2 | Antiproliferative activity

Antiproliferative activities of the newly prepared compounds were evaluated against VX2, A549, MGC, and MKN45 compared with a well-studied PTK inhibitor, Gefitinib, *in vitro*. The results were expressed as median growth inhibitory concentration (IC_{50}) values by using CCK-8 assays. As showed in the Table 2, 10 target compounds had good inhibition to the proliferation of VX2, A549, MGC, and MKN45 cell lines, among which MKN45 were more sensitive to target compounds while less sensitive of A549. Compound **2.5** had the better inhibitory effect on the four cell lines than Gefitinib, while IC_{50} values of 5.47 ± 0.41 μM on VX2, 5.47 ± 1.11 μM on A549, 4.59 ± 0.14 μM on MGC, 3.04 ± 0.02 μM on MKN45, respectively. Concerning the activity against VX2, compounds **1.1**, **1.2**, **1.4**, **2.1**, **2.5** all have better activity than Gefitinib. Compound **2.1** (IC_{50} of 3.80 ± 0.75 μM) emerged as the most potent analogue as it was fourfolds more active than Gefitinib (IC_{50} of 12.88 ± 2.74 μM). Compounds **1.1**, **1.2**, **1.3**, **2.1**, **2.2**, **2.5**, **2.6** all have much better activity than Gefitinib against MGC, while compound **2.2** displayed better inhibitory effect on MGC with an IC_{50} value of 3.72 ± 0.11 μM than that of Gefitinib (IC_{50}: 8.14 ± 0.60 μM). The inhibitory activity of 5-chlorbenzotriazole-series was slightly better than that of non-substituted BTAs. The inhibitory activity of chloronicotin-aldehyde series was better than that of 6-methoxynicotinaldehyde-series. Furthermore, the activity of target compounds substituted at 1 and 3 positions on triazole ring is generally better than that of target compounds substituted at 2 position. The inhibitory activity of compounds of **1.1** and **1.3** were stronger than that of target compounds of **1.2** and **1.4**, suggesting that chloro-substituent on pyridine ring is better than methoxyl group.[19] On the other hand, the chlorine atom on the benzene ring appears to exert limited effects on the inhibitory

TABLE 2 Antiproliferation activity of benzotriazole derivatives on VX2, A549, MGC, MKN45 cell lines measured by CCK-8 assay (unit, μM)

Corresponding effect on cell lines				
Compounds	**VX2**	**A549**	**MGC**	**MKN45**
1.1	5.36 ± 1.40	10.52 ± 0.25	4.65 ± 0.02	11.72 ± 0.23
1.2	8.23 ± 0.96	10.94 ± 3.06	5.20 ± 0.15	9.28 ± 0.16
1.3	56.55 ± 0.21	59.41 ± 0.16	5.79 ± 0.18	5.75 ± 0.05
1.4	12.31 ± 1.51	27.15 ± 0.53	21.77 ± 0.92	11.77 ± 0.25
2.1	3.80 ± 0.75	6.99 ± 0.33	6.04 ± 0.21	9.27 ± 0.22
2.2	30.69 ± 0.59	18.20 ± 0.33	3.72 ± 0.11	3.63 ± 0.08
2.3	26.61 ± 2.28	21.58 ± 0.99	9.78 ± 0.49	11.58 ± 0.75
2.4	39.94 ± 7.98	45.91 ± 0.52	18.72 ± 0.85	12.55 ± 0.43
2.5	5.47 ± 0.41	5.47 ± 1.11	4.59 ± 0.14	3.04 ± 0.02
2.6	19.87 ± 1.87	12.10 ± 2.14	7.29 ± 0.34	3.34 ± 0.10
Gefitinib	12.88 ± 2.74	10.15 ± 0.09	8.14 ± 0.60	6.94 ± 0.28

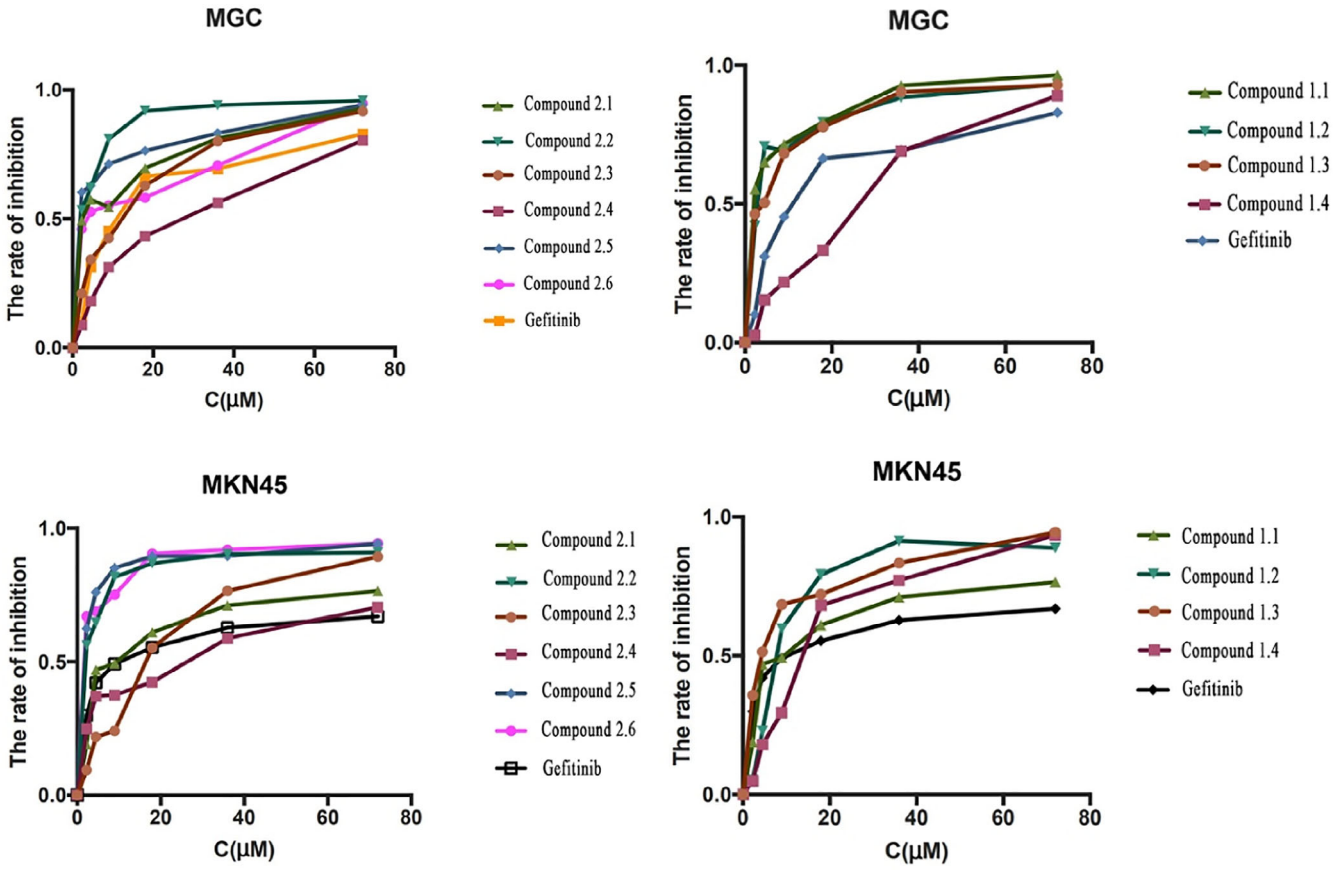

FIGURE 2 Inhibition of cell proliferation of target compounds

activity with a chlrorine on the pyridine (comparing compound **1.1** to **2.1**, **1.3** to **2.3**). In addition, the inhibitory activity of compounds **2.5**, **2.6** were greater than that of compound **2.1**, **2.2** (Figures 2 and 3). Overall, compound **2.5** showed the strongest inhibitory activities across four cell lines, which can be further optimized in future studies.

2.3 | Structure activity discussions

The 10 BTA derivatives have stood out as privileged molecules with good antiproliferative properties that were designed according to the structure of the tyrosine kinase inhibitor, such as Gefitinib. The BTA and pyridine ring in

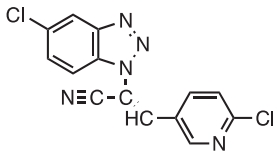

FIGURE 3 Structure of 2-(5-chloro-1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-chloropyridin-3-yl) acrylonitrile, compound 2.5

the target compounds are separated by two carbons. The phenyl ring of BTA and pyridine ring form π-π interaction with tyrosine kinase with increasing the degree of conjugation. The literature survey found that the length of the spacer carbon atoms has an effect on the cancer activity, and two to three units are more favorable in preliminary structure-activity relationship.[20,21] Among them, the target products at 1 or 3 position substitution on BTA ring leave two exposed N atoms easier to interact with hydrogen bond donors on tyrosine protease.[7] The chlorine atoms on BTA and pyridine ring are slightly electron-withdrawing groups, which affect the charge distribution and space morphology of the target compounds. The binding mode of target compounds enhances the affinity between target compound and receptor, which leads to better action. The steric hindrance of substituents is smaller, which is more conducive to the target compound entering the ATP binding region of tyrosine protein kinase and competing with ATP to bind receptor to exhibit a higher inhibition rate. Based on these considerations of structure–activity relationship, the inhibition of compound **2.5** was much higher compared with other compounds. Nonetheless, further structural optimization is needed in the future, in addition to investigate its toxicity toward normal cells.

3 | EXPERIMENTAL

3.1 | Synthesis and characterization

Benzotriazole was used as starting material to synthesize 10 target compounds. The reaction initiated with 27.5 mmol benzotriazole/5-chlorobenzotriazole and 3 mL chloroacetonitrile in an ultrasonic-microwave reactor under 75 mL refluxing ethyl acetate at 480 W to afford intermediates. The BTA intermediates were separated with different ratios of ethyl acetate/petroleum ether as eluent by flash column chromatography. Then 3 mmol intermediates reacted with 4 mmol 6-chloronicotinaldehyde or 6-methoxynicotinaldehyde in 20 mL absolute toluene by catalyzing with 1.2 mL triethylamine to get the desired compounds. The target compounds were separated with different ratios of ethyl acetate/petroleum ether as eluent by flash column chromatography. Analytical thin layer chromatography (TLC) on silica gel G was employed routinely to follow the course of reactions and check the purity of products. All melting points of recrystallized products were measured with a Stuart melting point apparatus. All compounds were characterized by IR, ^{1}H and ^{13}C-NMR, mass spectrometry (MS) and elemental analysis. IR spectra were recorded in KBr disks on Thermo Nicolet model Avatar 330 FT-IR spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded by Avance III, Bruker Biospin at 500/400 MHz in deuterated chloroform or dimethyl sulfoxide ($CDCl_3$/DMSO). Mass spectra were measured on AB3200 QTRAP mass spectrometer.

3.2 | 2-(1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-chloropyridin-3-yl) acrylonitrile (1.1)

IR (KBr, cm^{-1}): 3046, 2230, 1649, 1608, 1579, 1491, 1454, 831, 694; ^{1}H-NMR(500 MHz, $CDCl_3$, δ): 8.70(1H, d, CH aromatic), 8.57(1H, dd, CH aromatic), 8.38(1H, d, ═CH─), 7.91(2H, m, CH aromatic), 7.62(1H, d, CH aromatic), 7.53(2 H, m, CH aromatic); ^{13}C-NMR (400 MHz, DMSO, δ): 109.7, 111.9, 113.9, 120.6, 125.2, 125.3, 126.2, 127.2, 130.1, 131.7, 125.2, 146.0, 151.6, 152.7 ppm; ESI-MS: 282.05 ($C_{14}H_8ClN_5$, $[M + H]^+$); Anal. Calcd for $C_{14}H_8ClN_5$: C, 59.68; H, 2.86; Cl, 12.58; N, 24.86, found C59.62; H, 2.85; N, 24.82.

3.3 | 2-(1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-methoxypyridin-3-yl) acrylonitrile (1.2)

IR (KBr, cm^{-1}): 2956, 2362, 1610, 1574, 1495, 1452, 1290, 1076, 819; ^{1}H-NMR(500 MHz, $CDCl_3$, δ): 8.52(1H, d, CH aromatic), 8.44 (1H, dd, CH aromatic), 8.15 (1H, d, CH aromatic), 7.91 (1H, d, CH aromatic), 7.88(1 H, s, ═CH─), 7.65 (1H, m, CH aromatic), 7.50 (1H, m, CH aromatic), 6.93 (1H, d, CH aromatic), 4.04 (3H, s, ─CH_3); ^{13}C-NMR (400 MHz, DMSO, δ): 54.4, 105.5, 111.6, 111.9, 114.7, 120.5, 121.1, 125.9, 129.8, 132.0, 138.6 (two overlapping signals), 145.8, 151.1, 165.8 ppm; ESI-MS: 278.10 ($C_{15}H_{11}N_5O$, $[M + H]^+$); Anal. Calcd for $C_{15}H_{12}N_5O$: C, 64.97; H, 4.00; N, 25.26; O, 5.77, found C, 64.92; H, 4.03; N, 25.22.

3.4 | 2-(2*H*-benzo[*d*] [1,2,3] triazol-2-yl)-3-(6-chloropyridin-3-yl) acrylonitrile (1.3)

IR (KBr, cm^{-1}): 3017, 2360, 1607, 1572, 1496, 1446, 807, 670; ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 8.74 (1H, d, CH aromatic), 8.51(1H, dd, CH aromatic), 8.48(1H, d, ═CH─), 7.91(2H, m, CH aromatic), 7.52(1H, d, CH aromatic), 7.49(2H, m, CH aromatic); ^{13}C-NMR (400 MHz,

DMSO, δ): 113.1, 114.3, 118.6 (two overlapping signals), 125.3, 126.8 (two overlapping signals), 129.4, 133.6, 139.5, 145.0 (two overlapping signals), 152.1, 153.0 ppm; ESI-MS: 282.05 ($C_{14}H_8ClN_5$, $[M + H]^+$); Anal. Calcd for $C_{14}H_8ClN_5$: C, 59.69; H, 2.86; Cl, 12.58; N, 24.86, found C, 59.73; H, 2.81; N, 24.82.

3.5 | 2-(2*H*-benzo[*d*] [1,2,3] triazol-2-yl)-3-(6-methoxypyridin-3-yl) acrylonitrile (1.4)

IR (KBr, cm^{-1}): 3009, 2258, 1615, 1574, 1496, 1449, 1255, 1091, 841; ^{1}H-NMR(500 MHz, $CDCl_3$, δ): 8.20(1H, d, CH aromatic), 7.90(2 H, m, CH aromatic), 7.80(1 H, s, ═CH─), 7.48(2 H, m, CH aromatic), 6.80(1H, dd, CHaromatic), 5.80 (1H, d, CH aromatic), 3.93(3H, s, $-CH_3$); ^{13}C-NMR(400 MHz, DMSO, δ): 53.7, 110.8, 115.1, 115.2, 118.6 (two overlapping signals), 127.8, 128.0 (two overlapping signals), 138.0, 138.5, 144.4 (two overlapping signals), 146.5, 164.3 ppm; ESI-MS: 278.10($C_{15}H_{11}N_5O$,[-$M + H]^+$); Anal. Calcd for $C_{15}H_{11}N_5O$: C, 64.97; H, 4.00; N, 25.26; O, 5.77, found C, 64.92; H, 3.95; N, 25.22.

3.6 | 2-(6-Chloro-1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-chloropyridin-3-yl) acrylonitrile (2.1)

IR (KBr, cm^{-1}): 3028, 2226, 1652, 1608, 1578, 1507, 1461, 814, 651; ^{1}H-NMR (500 MHz, DMSO, δ): 8.91(1H, d, CH aromatic), 8.47(1H, dd, CH aromatic), 8.35 (2H, m, CH aromatic), 8.29 (1H, d, ═CH─), 7.83(1H, d, CH aromatic), 7.64(1H, dd, CH aromatic); ^{13}C-NMR (400 MHz, DMSO, δ): 109.3, 113.9, 122.0, 125.3, 126.9, 127.2, 132.6, 135.1, 136.2, 139.5, 139.9, 144.7, 151.7, 152.8 ppm; ESI-MS: 317.00 ($C_{14}H_7Cl_2N_5$, $[M + H]^+$); Anal. Calcd for $C_{14}H_7Cl_2N_5$: C, 53.19; H, 2.23; Cl, 22.43; N, 22.15, found C, 53.22; H, 2.19; N, 22.12.

3.7 | 2-(6-Chloro-1*H*-benzo[d] [1,2,3] triazol-1-yl)-3-(6-methoxypyridin-3-yl) acrylonitrile (2.2)

IR (KBr, cm^{-1}): 3008, 2221, 1683, 1602, 1558, 1507, 1456, 1233, 1048, 796, 668; ^{1}H-NMR (500 MHz, DMSO, δ): 8.73 (1H, d, ═CH─), 8.42(1H, dd, CH aromatic), 8.27(3H, m, CH aromatic), 7.62 (1H, dd, CH aromatic), 7.12 (1H, d, CH aromatic), 3.97 (3H, s, $-CH_3$); ^{13}C-NMR (400 MHz, DMSO, δ): 54.4, 106.5, 112.2, 114.2, 114.5, 121.4, 127.7, 129.8, 130.7, 131.7, 136.8, 138.3, 145.2, 148.8, 164.8 ppm; ESI-MS: 312.06 ($C_{15}H_{10}ClN_5O$, $[M + H]^+$); Anal. Calcd for $C_{15}H_{10}ClN_5O$: C, 57.80; H, 3.23; Cl, 11.37; N, 22.47; O, 5.13, found C, 57.85; H, 3.27; N, 22.51.

3.8 | 2-(5-Chloro-2*H*-benzo[*d*] [1,2,3] triazol-2-yl)-3-(6-chloropyridin-3-yl) acrylonitrile (2.3)

IR (KBr, cm^{-1}): 2341, 1636, 1558, 1508, 1457, 818, 669; ^{1}H-NMR(500 MHz, DMSO, δ): 8.89 (1H, d, CH aromatic), 8.81(1H, d, CH aromatic), 8.51 (1H, dd, CH aromatic), 8.26 (1H, d, ═CH─), 8.13 (1H, dd, CH aromatic), 7.82(1H, d, CH aromatic), 7.61(1H, dd, CH aromatic); ^{13}C-NMR (400 MHz, DMSO, δ): 112.9, 114.1, 117.7, 120.6, 125.4, 126.7, 130.5, 133.9, 134.2, 139.5, 143.5, 145.2, 152.2, 153.2 ppm; ESI-MS: 317.00 ($C_{14}H_7Cl_2N_5$, $[M + H]^+$); Anal. Calcd for $C_{14}H_7N_5Cl_2$: C, 53.19; H, 2.23; Cl, 22.43; N, 22.15; found C, 53.22; H, 2.27; N, 22.13.

3.9 | 2-(5-Chloro-2*H*-benzo[*d*] [1,2,3] triazol-2-yl)-3-(6-methoxypyridin-3-yl) acrylonitrile (2.4)

IR (KBr, cm^{-1}): 3017, 2342, 1607, 1572, 1497, 1446, 1260, 1049, 807, 671; ^{1}H-NMR (500 MHz, DMSO, δ): 8.20(1H, d, CH aromatic), 8.73(1H, s, ═CH─), 8,45(1H, dd, CH aromatic), 8.22 (1H, d, CH aromatic), 8.10 (1H, d, CH aromatic), 7.58 (1H, dd, CH aromatic), 7.09 (1H, d, CH aromatic), 3.96 (3H, s, $-CH_3$); ^{13}C-NMR(400 MHz, DMSO, δ): 54.4, 107.5, 112.0, 113.4, 114.8, 125.4, 125.6, 126.8, 131.0, 134.3, 134.5, 144.9, 145.2, 152.1, 164.9 ppm; ESI-MS: 312.05 ($C_{15}H_{10}ClN_5O$, $[M + H]^+$); Anal. Calcd for $C_{15}H_{10}ClN_5O$: C, 57.80; H, 3.23; Cl, 11.37; N, 22.47; O, 5.13, found C, 57.85; H, 3.21; N, 22.52.

3.10 | 2-(5-Chloro-1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-chloropyridin-3-yl) acrylonitrile (2.5)

IR (KBr, cm^{-1}): 2340, 1614, 1573, 1519, 1455, 806, 668; ^{1}H-NMR (500 MHz, DMSO, δ): 8.91 (1H, d, CH aromatic), 8.49 (1H, dd, CH aromatic), 8.44 (1H, m, CH aromatic), 8.36 (1H, s, CH aromatic), 8.20 (1H, d, ═CH─), 7.83(2H, d, CH aromatic); ^{13}C-NMR (400 MHz, DMSO, δ): 109.4, 113.6, 113.8, 119.9, 125.3, 127.1, 130.4, 130.6, 130.7, 135.9, 139.4, 146.7, 151.7, 152.8 ppm; ESI-MS: 317.00 ($C_{14}H_7Cl_2N_5$, $[M + H]^+$); Anal. Calcd for $C_{14}H_7Cl_2N_5$: C, 53.19; H, 2.23; Cl, 22.43; N, 22.15, found C, 53.15; H, 2.25; N, 22.12.

3.11 | 2-(5-Chloro-1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-methoxypyridin-3-yl) acrylonitrile (2.6)

IR (KBr, cm^{-1}): 2341, 1597, 1558, 1506, 1457, 1288, 1048, 822, 668; ^{1}H-NMR (500 MHz, DMSO, δ): 8.73 (1H, d, ═CH─), 8.43(2H, m, CH aromatic), 8.27 (1 H, s, CH aromatic), 8.12 (1H, d, CH aromatic), 7.80(1H, dd, CH aromatic), 7.13 (1H, d, CH aromatic), 3.97 (3H, s, -CH_3); ^{13}C-NMR (400 MHz, DMSO, δ): 54.4, 105.3, 112.0, 113.4, 114.6, 119.8, 121.0, 130.2, 130.4, 131.0, 138.6, 139.1, 146.5, 151.2, 165.8 ppm; ESI-MS: 312.06 ($C_{15}H_{10}ClN_5O$, $[M + H]^+$); Anal. Calcd for $C_{15}H_{10}ClN_5O$: C, 57.80; H, 3.23; Cl, 11.37; N, 22.47; O, 5.13; found C, 57.78; H, 3.20; N, 22.42.

3.12 | Cell viability assays (CCK-8) and IC_{50} determination

CCK-8 method was used to detect the antiproliferative activity of target compounds on VX2, A549, MGC, and MKN45 obtained from Cell Bank of Shanghai Academy of Chinese Sciences *in vitro*. Briefly, Different concentrations of tested compounds and Gefitinib were added to the cells, then measured in an Enspire reader after reaction with the kit. The relation between percent of inhibition and concentration is plotted to get the curve for each cell line showed in Figure 2. IC_{50} was calculated and the results are given in Table 2. Data are presented as mean ± SD. Statistical analysis were performed by GraphPad Prism software (Version 7.00).

4 | CONCLUSION

In summary, 10 BTA derivatives were synthesized according to a fragment-based design strategy as tyrosine protein kinase inhibitors. These compounds were obtained by reaction of BTA with chloroacetonitrile, 6-chloronicotinaldehyde or 6-methoxynicotinaldehyde by ultrasonic-microwave synthesis and characterized by IR, ^{1}H and ^{13}C-NMR, MS and elemental analysis. Anti-proliferative activities of all compounds against VX2, A549, MKN45, and MGC *in vitro* showed that benzotriazole derivatives are a series of good anticancer agents. The most active 2-(5-chloro-1*H*-benzo[*d*] [1,2,3] triazol-1-yl)-3-(6-chloropyridin-3-yl) acrylonitrile (compound **2.5**, Figure 3) with IC_{50} values in the range of 5.47~3.04 μM may be served as a useful lead compound in the development of new chemotherapeutic agents.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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