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Synthesis and biological evaluation of novel 6-alkoxy-3-aryl-[1,2,4]triazolo[4,3-*b*][1,2,4,5] tetrazines

Run-Jie Shi[1], Zhen-Zhen Yang[1], Ye-Tao Gao[1], Wen-Jin Cai[1], Can Ye[1], Feng Xu[1] and John Wang[2]

Abstract

A series of 6-alkoxy-3-aryl-[1,2,4]triazolo[4,3-*b*][1,2,4,5] tetrazine derivatives is synthesized and evaluated for their antitumor activities. These compounds exhibit potent antiproliferative activities against A549, Bewo, and MCF-7 cells. Molecular docking is performed to study the inhibitor–c-Met kinase interactions, and the results show that 6-ethoxyl-3-phenylethyl-[1,2,4]triazolo[4,3-*b*][1,2,4,5] tetrazine is potently bound to c-Met kinase with two hydrogen bonds and one π–π interaction. Based on these preliminary results, it is thought that compound 6-ethoxyl-3-phenylethyl-[1,2,4] triazolo[4,3-*b*][1,2,4,5] tetrazine with potent inhibitory activity may be a potential anticancer agent.

Keywords

anticancer, c-Met, molecular docking, synthesis, tetrazines, triazoles

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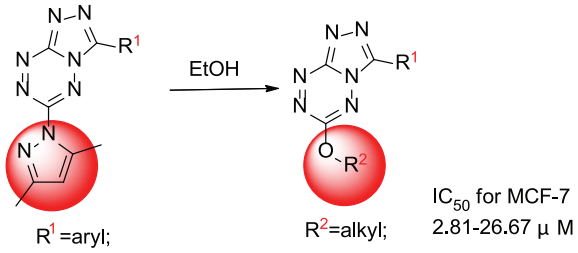


A series of novel 6-alkoxy-3-aryl-[1,2,4]triazolo[4,3-*b*][1,2,4,5]tetrazines is synthesized and their anticancer activities against A549, Bewo, and MCF-7 are tested. The results suggest that these compounds display potent antiproliferative activities.

Introduction

Cancer is one of the most important problems in public health and is the second leading cause of death in the world.[1] Currently, cancer chemotherapy is one of the most effective methods to treat cancer, which represents a constant, global, and interdisciplinary research effort and can heavily promote and extend the quality of life. New antitumor therapies point to novel compounds with potently selective anticancer effects and must therefore exhibit a cytotoxic effect on malignant cells, without damaging normal cells.[2–4]

Most of the known synthetic anticancer drugs are heterocyclic compounds, and several of them correspond to nitrogen heterocycles.[5–7] In the last decades, a large number of 1,2,4,5-tetrazine derivatives have been synthesized and their antitumor activity has been reported, which has led to them emerging as a promising and attractive scaffolds.[8–11] Among them, a 1,4-dihydro-1,2,4,5-tetrazine derivative, N,*N*′-di(*m*-methylpheny)-3,6-dimethyl-1,4-dihydro-1,2,4,5-tetrazine-1,4-dicarboamide (**ZGDHu-1**), has been found with strong inhibition on the proliferation of PANC-1 pancreatic cancer cells via apoptosis and G2/M cell cycle arrest;[12] 3-alkylamino-1,2,4,5-tetrazine derivative **I** exhibits potent antiproliferative activity against H1975 cells;[13] and 3-(4-methylimidazole-1-yl)imidazo[1,2-*b*][1,2,4,5]tetrazine (**II**), an imidazotetrazine derivative, is being used for therapy of malignant tumors in human and animals (Figure 1).[14]

[1]Pharmaceutical Research Institute, Taizhou Vocational & Technical College, Taizhou, P.R. China
[2]ImmuOn Therapeutics, Nantong, P.R. China

Corresponding author:
Feng Xu, Pharmaceutical Research Institute, Taizhou Vocational & Technical College, Taizhou 318000, P.R. China.
Email: xufeng901@126.com

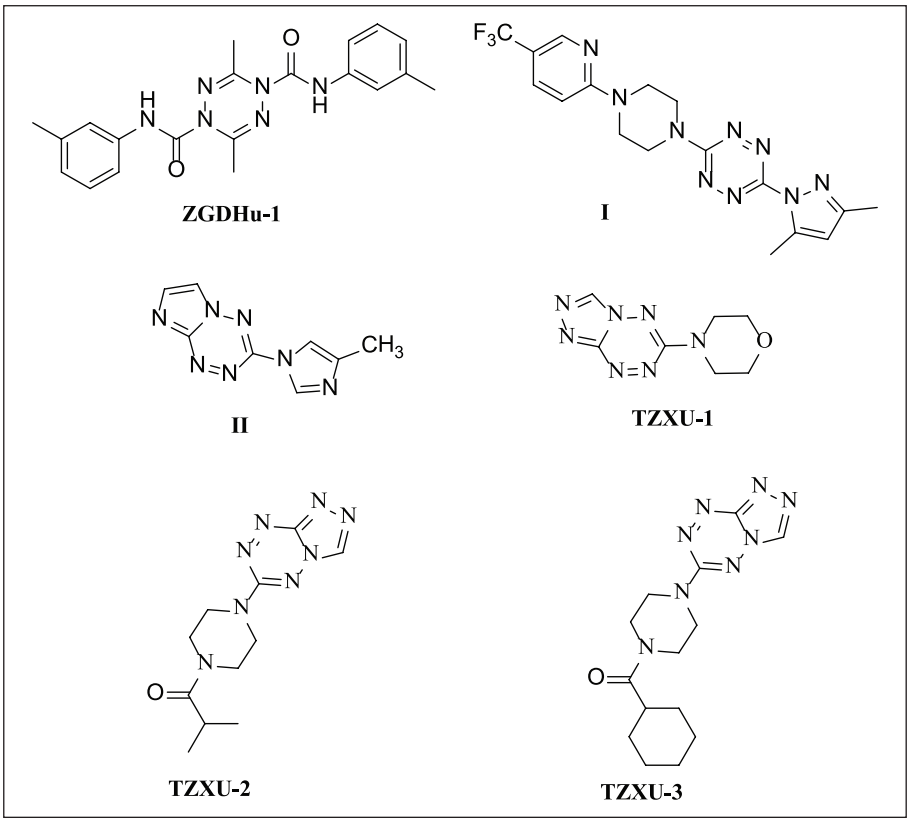


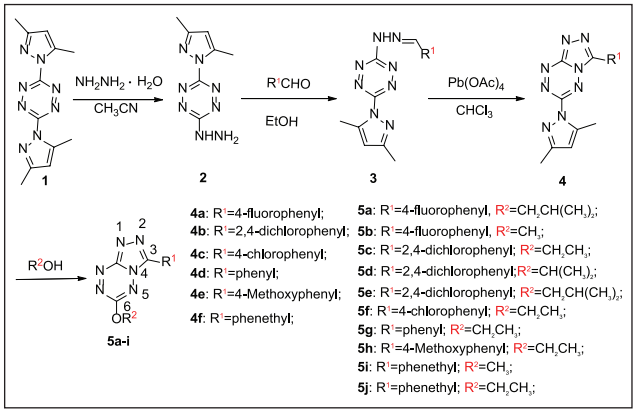
Figure 1. Structures of 1,2,4,5-tetrazine derivatives with anticancer activities.

Our research group has synthesized numerous [1,2,4] triazolo[4,3-*b*][1,2,4,5]tetrazine derivatives, and some of them (**TZXU-1-3**) showed potent antiproliferative activities against MCF-7, Bewo, and HL-60 cells and c-Met kinase inhibitory activities (Figure 1).[15–17] All these synthetic compounds belong to 6-alkylamino-[1,2,4] triazolo[4,3-*b*][1,2,4,5]tetrazine derivatives; however, literature focused on 6-alkoxy-[1,2,4]triazolo[4,3-*b*] [1,2,4,5] tetrazine derivatives are rare. On the basis of the above research, we have prepared some new [1,2,4] triazolo[4,3-*b*][1,2,4,5]tetrazines and have attempted to investigate how the alkoxy groups located at the 6-position of the triazolotetrazine ring influence antitumor activity. This letter reports the synthesis of 10 [1,2,4] triazolo[4,3-*b*][1,2,4,5]tetrazines. Subsequently, in vitro bioassays were performed to evaluate the anticancer activities against human lung adenocarcinoma cells (A549), human placenta choriocarcinoma cells (Bewo), and human breast cancer cells (MCF-7). Furthermore, molecular docking was performed to study the inhibitor c-Met kinase interactions.

Results and discussion

Chemical synthesis

Scheme 1 details the synthesis and structures of the title compounds. The starting material **1** was prepared using the published method.[18] Compound **1** was then reacted with 80% hydrazine hydrate in acetonitrile at room temperature to yield compound **2**,[19] which was then heated



Scheme 1. Synthesis of the target compounds **5**.

with different aromatic aldehydes in ethanol to give compounds to **3** via the Schiff reaction.[20] Compounds **3** were then subjected to intramolecular cyclization with lead tetraacetate as the oxidizing agent.[21] Finally, the title compounds **5** were prepared from the compound **4** by treatment with alkyl alcohols.

The structures of the synthesized compounds were confirmed from their ^{1}H NMR, ^{13}C NMR, infrared (IR), and elemental analysis data. All of these compounds provided satisfactory spectroscopic data, which were consistent with the assigned structures. It should be noted in this context that to the best of our knowledge, all of the title products are reported for the first time. The 1H NMR and ^{13}C NMR spectra of the title compounds are given in the supplementary data.

Anticancer activity in vitro

To test the antitumor activities of the synthesized compounds, we evaluated the antiproliferative activities of compounds **5** against A549, Bewo, and MCF-7 cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results are summarized in Table 1. The active analogs were found to display cytotoxic activity. In terms of the bioactivities against A549, all the compounds were no more effective than the positive control cisplatin (IC_{50} = 33.23 μM), except for compounds **5b** (IC_{50} = 8.08 μM) and **5j** (IC_{50} = 21.68 μM). Particularly, all of the tested compounds presented higher anticancer activities against MCF-7 than the positive control cisplatin (IC_{50} = 50.09 μM), and compound **5j** (IC_{50} = 2.81 μM) showed the most potent biological activity against MCF-7, and its inhibitory effect was close to that of **TZXU-1** (IC_{50} = 2.24 μM). Notably, when the R_2 group was the same (e.g. ethyl), the anticancer activity results against MCF-7 followed the order: **5h** > **5f** > **5c** > **5g**. This revealed that the substituted group on the phenyl ring of the R1 group was critical for enhancing the anticancer potency of the target compounds, and compounds with an electron-donating group (e.g. Me) on the phenyl ring were more effective than those with an electron-withdrawing group (e.g. Cl). This trend is the reverse of our previously reported work[16] regarding the substituted group on the phenyl ring (connected to the piperazine ring) located at the 6-position of the triazolotetrazine ring. That is electron-withdrawing groups (e.g. NO_2, Cl) on the phenyl ring were more effective than an electron-donating group (e.g. Me). This result is of interest when taking into consideration structure optimization.

Table 1. Antitumor activities against MCF-7, Bewo, and HL-60 cell lines in vitro (IC_{50} in μM)[a].

Compound	IC_{50} (μM)		
	A549	Bewo	MCF-7
5a	49.50	86.72	14.40
5b	8.08	106.96	12.39
5c	88.42	90.89	23.50
5d	45.89	70.03	6.89
5e	54.78	69.22	23.47
5f	43.37	83.13	9.76
5g	85.66	147.91	26.67
5h	64.00	56.97	4.72
5i	79.49	87.76	9.68
5j	**21.68**	**20.72**	**2.81**
Cisplatin	33.23	34.86	50.09
TZXU-1[b]	–	1.30	2.24

[a]Values presented are the mean of three experiments.
[b]Data taken from the literature.[15]

Binding mode analysis

To gain a better understanding of the potency of the target compounds and to guide further structure–activity relationship (SAR) studies, we continued to investigate the interaction effect between compound **5j** and the c-Met crystal structure (3EFJ.pdb). The molecular docking was performed by inserting compound **5j** into c-Met kinase at the active site. All docking runs were applied in Surflex-Dock of Sybyl-X 2.0.

The binding modes of compound **5j** and c-Met kinase are depicted in Figure 2(a). As illustrated in Figure 2, compound **5j** is potently bound to the active site of c-Met kinase via hydrophobic interactions and binding is stabilized by two hydrogen bonds and one π–π interaction.

The two nitrogen atoms of the triazole ring formed two hydrogen bonds with the same amido hydrogen of LYS1110 (bond lengths: 1.94 and 2.79 Å, respectively), and the π–π interaction was predicted to form between the two phenyl rings in **5j** (Cg1, Cg1 is centroid of the phenyl ring in **5j**) and PHE1223 (Cg2, Cg2 is centroid of the phenyl ring in PHE1223) (Cg1–Cg2 = 3.83 Å; Figure 2(a)). The enzyme surface model is shown in Figure 2(b), which revealed that the target molecule **5j** was well-embedded in the active pocket of c-Met kinase. This molecular docking result, along with the biological assay data, suggested that compound **5j** is a potential inhibitor of c-Met kinase.

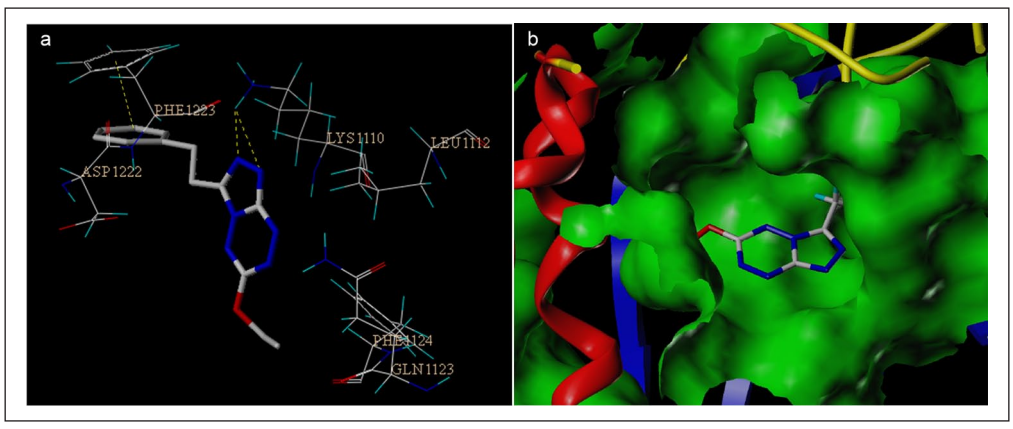


Figure 2. (a) Compound **5j** (colored by atom: carbons: gray; nitrogen: blue; oxygen: red) is bound into c-Met (entry 3EFJ in the Protein Data Bank). The dotted lines show the hydrogen bonds and π–π interaction. (b) The surface model structure of binding compound **5j** with the c-Met complex.

Experimental

Materials and methods

Melting points (m.p.s) were obtained on an XRC-1 apparatus and are uncorrected (Beijing Technical Instrument Co.). IR spectra were recorded as KBr disks of solid materials on a Nicolex FI-IR-170 instrument. The 1H NMR spectra were run on a Bruker AC400 (400 MHz) spectrometer. Compounds were dissolved in DMSO-d_6 or $CDCl_3$, and chemical shifts were referenced to tetramethylsilane (TMS). Mass spectra were obtained on an Agilent 1260 Ion Trap LC-MS 500 analysis system. Elemental analyses were performed on a Thermo-Finnigan Flash EA 1112 instrument. Thin-layer chromatography (TLC) was carried out on silica gel UV-254 plates.

General procedure for the synthesis of compounds **4a–f**

Lead tetraacetate (5.0 g, 11.4 mmol) in chloroform (10 mL) was added to a refluxing mixture of compound **3** (10 mmol) and chloroform (20 mL). The color of the solution changed from wine-red to orange. After substrate **3** was completely consumed (the reaction course was monitored by TLC (AcOEt)), evaporation of the chloroform gave crude yellow compound **4**, which was recrystallized from absolute ethanol.

Compounds **4a**, **4b**, **4d**, and **4e** have been described in our previous report.[17]

*3-(4-Chlorophenyl)-6-(3,5-dimethyl-1H-pyrazol-1-yl)-[1,2,4] triazolo[4,3-b][1,2,4,5]tetrazine (**4c**).* Yellow solid, m.p. 198–200 °C; IR (KBr, cm^{-1}): 2976, 1529, 1396, 1452, 1036, and 830. MS (ES): *m/z* 327 $(M + H)^+$; 1H NMR (400 MHz, DMSO-d_6) δ: 8.43 (d, 2H), 7.75 (d, 2H), 6.25 (s, 1H), 2.80 (s, 3H), 2.41 (s, 3H); Anal. calcd (%) for $C_{14}H_{11}ClN_8$: C, 51.46; H, 3.39; N, 34.29; found: C, 51.52; H, 3.37; N, 34.22.

*6-(3,5-Dimethyl-1H-pyrazol-1-yl)-3-phenethyl-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (**4f**).* Red solid, m.p. 143–145 °C; IR (KBr, cm^{-1}): 3260, 3071, 1589, 1488, and 1418; MS (ES): *m/z* 321 $(M + H)^+$; 1H NMR (400 MHz, DMSO-d_6) δ: 7.16–7.27 (m, 5H, ArH), 6.35 (s, 1H), 3.45(t, J=7.4Hz, 2H), 3.17(d, J=7.4Hz, 2H), 2.58 (s, 3H), 2.27 (s, 3H); Anal. calcd (%) for $C_{16}H_{16}N_8$: C, 59.99; H, 5.03; N, 34.98; found: C, 59.90; H, 5.02; N, 35.03.

General procedure for the synthesis of compounds **5a–j**

Compound **4** (3 mmol) and the alkyl alcohol (20 mL) were mixed and heated to reflux for 5–10 h. After **4** was completely consumed (the reaction course was monitored by TLC (PE/AcOEt = 1:1)), the mixture was cooled to room temperature and the excess alcohol evaporated. The residue was added cooled ethanol (3 mL), resulting in a significant amount of a yellow solid, which was purified by preparative thin-layer chromatography over silica gel PF 254 (2 mm, PE/AcOEt = 2:1).

*3-(4-Fluorophenyl)-6-isobutoxy-[1,2,4]triazolo[4,3-b][1,2,4, 5] tetrazine (**5a**).* Yellow solid, m.p. 128–130 °C; IR (KBr, cm^{-1}): 3074, 2974, 1606, 1547, 1057, and 861. 1H NMR (400 MHz, DMSO-d_6) δ: 8.45 (t, J=8.8 Hz, 2H), 7.52 (t, J=8.8 Hz, 2H), 4.29 (d, J=6.3 Hz, 2H), 2.23 (m, 1H), 1.06 (d, J=6.3 Hz, 2H); 13C NMR (100 MHz, DMSO-d_6) δ: 165.2, 159.4, 152.1, 144.3, 130.2, 130.1, 121.6, 117.2, 117.0, 75.9, 27.6, 19.3 (2C); MS (ES): *m/z* (%) 289.2 ($(M + H)^+$, 100). Anal. calcd for $C_{13}H_{13}FN_6O$: C, 54.16; H, 4.55; N, 29.15; O, 5.55; found: C, 54.25; H, 4.54; N, 29.12; O, 5.57.

*3-(4-Fluorophenyl)-6-methoxy-[1,2,4]triazolo[4,3-b][1,2,4, 5]tetrazine (**5b**).* Yellow solid, m.p. 178–180 °C; IR (KBr, cm^{-1}): 3099, 2977, 1605, 1559, 1052, and 852; 1H NMR (400 MHz, DMSO-d_6) δ: 8.47–8.50 (m, 2H), 7.51 (dd, J_1=10.0, J_2=8.0 Hz, 2H), 4.19 (s, 3H); 13C NMR (100 MHz, DMSO-d_6) δ: 165.3, 159.9, 152.1, 144.3, 130.2, 130.3, 121.5, 117.2, 117.0, 57.2. MS (ES): *m/z* (%) 247.2 ($(M + H)^+$,100). Anal. calcd for $C_{10}H_7FN_6O$: C, 48.78; H, 2.87; N, 34.13; O, 6.50; found: C, 48.69; H, 2.86; N, 34.20; O, 6.48.

*3-(2,4-Dichlorophenyl)-6-ethoxy-[1,2,4]triazolo[4,3-b][1, 2,4,5]tetrazine (**5c**).* Yellow solid, m.p. 146–148 °C; IR (KBr, cm^{-1}): 3098, 2979, 1596, 1549, 1052, and 826; 1H NMR (400 MHz, DMSO-d_6) δ: 7.97 (s, 1H), 7.80 (d, J=8.8 Hz, 1H), 7.71 (d, J=8.8 Hz, 1H), 4.40 (q, J=7.0 Hz, 2H), 1.06 (t, J=7.0 Hz, 3H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.5, 151.5, 143.7, 137.6, 134.8, 134.1, 130.7, 128.6, 122.7, 66.5, 14.2; MS (ES): *m/z* (%) 311.1 ($(M + H)^+$, 100), 333.1 ($(M + Na)^+$, 50); Anal. calcd for $C_{11}H_8Cl_2N_6O$: C, 42.46; H, 2.59; N, 27.01; O, 5.14; found: C, 42.57; H, 2.59; N, 26.93; O, 5.13.

*3-(2,4-Dichlorophenyl)-6-isopropoxy-[1,2,4]triazolo[4,3-b][1, 2,4,5]tetrazine (**5d**).* Yellow solid, m.p. 128–130 °C; IR (KBr, cm^{-1}): 3094, 2988, 1547, 1399, 1053, and 824; 1H NMR (400 MHz, DMSO-d_6) δ: 7.98 (s, 1H), 7.79 (d, J=8.4 Hz, 1H), 7.72 (d, J=8.4 Hz, 1H), 5.06 (sext, J=6.1 Hz, 1H), 1.42(d, J=4.1 Hz, 6H); 13C NMR (100 MHz, DMSO-d_6) δ: 158.9, 151.4, 143.6, 137.5, 134.8, 134.1, 130.7, 128.5, 122.7, 74.8, 21.5 (2C); MS (ES): *m/z* (%) 325.1 ($(M + H)^+$, 100), 347.1 ($(M + Na)^+$, 23); Anal. calcd for $C_{12}H_{10}Cl_2N_6O$: C, 44.33; H, 3.10; N, 25.85; O, 4.92; found: C, 44.43; H, 3.11; N, 25.82; O, 4.91.

*3-(2,4-Dichlorophenyl)-6-isobutoxy-[1,2,4]triazolo[4,3-b][1,2, 4,5]tetrazine (**5e**).* Yellow solid, m.p. 168–170 °C; IR (KBr, cm^{-1}): 3058, 2977, 1583, 1538, 1038, and 821; 1H NMR (400 MHz, DMSO-d_6) δ: 7.97 (s, 1 Hz), 7.80 (d, J=8.4 Hz, 1H), 7.22 (d, J=8.4 Hz, 1H), 4.13(d, J=6.4 Hz, 2H), 2.14 (m, 1H), 1.00 (d, J=6.7 Hz, 6H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.7, 151.5, 143.6, 137.6, 134.8, 134.0, 130.7, 128.5, 122.7, 75.9, 27.6, 19.2 (2C); MS (ES): *m/z* (%) 340.2 ($(M + 2)^+$, 100), 361.2 ($(M + Na)^+$, 25); Anal. calcd for $C_{13}H_{12}Cl_2N_6O$: C, 46.03; H, 3.57; N, 24.78; O, 4.72; found: C, 46.12; H, 3.57; N, 24.70; O, 4.70.

*3-(4-Chlorophenyl)-6-ethoxy-[1,2,4]triazolo[4,3-b][1,2,4,5] tetrazine (**5f**).* Yellow solid, m.p. 220–222 °C; IR (KBr, cm^{-1}): 3090, 2994, 1539, 1054, and 851; 1H NMR (400 MHz,

DMSO-d_6) δ: 8.41 (d, J=8.6 Hz, 2H), 7.73 (d, J=8.6 Hz, 2H), 4.56 (q, J=7.0 Hz, 2H), 1.50 (t, J=7.0 Hz, 3H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.2, 152.2, 144.1, 136.5, 130.0 (2C), 129.3 (2C), 123.9, 66.5, 14.3; MS (ES): *m/z* (%) 277.0 ($(M+H)^+$, 100). Anal. calcd for $C_{11}H_9ClN_6O$: C, 47.75; H,3.28; N, 30.37; O, 5.78; found: C, 47.90; H, 3.27; N, 30.27; O, 5.77.

*6-Ethoxy-3-phenyl-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (**5g**).* Yellow solid, m.p. 120–122 °C; IR (KBr, cm^{-1}): 3068, 2986, 1573, 1541, 1054, 747, and 705; 1H NMR (400 MHz, DMSO-d_6) δ: 8.43–8.45 (m, 2H), 7.65–7.71 (m, 3H, ArH), 4.58 (q, J=7.0 Hz, 2H), 1.53(t, J=7.0 Hz, 3H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.2, 152.2, 144.1, 131.8, 130.7 (2C), 128.8 (2C), 125.9, 66.4, 14.3; MS (ES): *m/z* (%) 243.1 ($(M+H)^+$, 100); Anal. calcd for $C_{11}H_{10}N_6O$: C, 54.54; H, 4.16; N, 34.69; O, 6.60; found: C, 54.61; H, 4.14; N,34.75; O, 6.58.

*3-(4-Methoxyphenyl)-6-ethoxy-[1,2,4]triazolo[4,3-b][1,2, 4,5] tetrazine (**5h**).* Yellow solid, m.p. 143–145 °C; IR (KBr, cm^{-1}): 3086, 2991, 1529, 1044, and 850; 1H NMR (400 MHz, DMSO-d_6) δ: 8.38 (d, J=8.6 Hz, 2H), 7.19 (d, J=8.6 Hz, 2H), 4.56 (q, J=7.0 Hz, 2H), 3.83 (s, 3H), 1.50 (t, J=7.0 Hz, 3H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.2, 152.2, 144.3, 136.2, 129.5 (2C), 125.7 (2C), 123.6, 66.4, 55.3, 14.2; MS (ES): *m/z* (%) 273.1 ($(M+H)^+$, 100); Anal. calcd for $C_{12}H_{12}N_6O$: C, 52.94; H, 4.44; N, 30.87; O, 11.75; found: C, 52.82; H, 4.46; N, 30.95; O, 11.78.

*3-Phenylethyl-6-methoxy-[1,2,4]triazolo[4,3-b][1,2,4,5] tetrazine (**5i**).* Yellow solid, m.p. 98–100 °C; IR (KBr, cm^{-1}): 3098, 2945, 1579, 1551, 1041, 745, and 707; 1H NMR (400 MHz, DMSO-d_6) δ: 7.16–7.27 (m, 5H, ArH), 4.09 (s, 3H), 3.37 (t, J=7.7 Hz, 2H), 3.15 (t, J=7.7 Hz, 2H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.8, 151.5, 147.8, 140.5, 128.9 (2C), 128.8 (2C), 126.8, 57.0, 31.6, 25.4; MS (ES): *m/z* (%) 257.3 ($(M+H)^+$, 100); Anal. calcd for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.72; N, 32.79; O, 6.24; found: C, 56.35; H, 4.71; N, 32.75; O, 6.22.

*3-Phenylethyl-6-ethoxyl-[1,2,4]triazolo[4,3-b][1,2,4,5] tetrazine (**5j**).* Yellow solid, m.p. 83–85 °C; IR (KBr, cm^{-1}): 3024, 2999, 1601, 1541, 1037, 748, and 698; 1H NMR (400 MHz, DMSO-d_6) δ: 7.14–7.26 (m, 5H, ArH), 4.47 (q, J=7.0 Hz, 2H), 3.36 (t, J=7.7 Hz, 2H), 3.13 (t, J=7.7 Hz, 2H), 1.46 (t, J=7.0 Hz, 3H); 13C NMR (100 MHz, DMSO-d_6) δ: 159.1, 151.5, 147.7, 140.5, 128.9 (2C), 128.8 (2C), 126.8, 66.2, 31.6, 25.4, 14.2; MS (ES): *m/z* (%) 271.5 ($(M+H)^+$, 100), 293.3 ($(M+Na)^+$, 83); Anal. calcd for $C_{13}H_{14}N_6O$: C, 57.77; H, 5.22; N, 31.09; O,5.92; found: C, 57.70; H, 5.20; N, 31.15; O, 5.93.

Biological evaluation

In vitro cancer cell growth inhibition assay

The antiproliferative activities of the compounds **5** against several human cancer cell lines were assayed by standard MTT assay procedures. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium at 37 °C with 5% CO_2 and 95% air, supplemented with 10% (v/v) bovine calf serum. Cells were plated in 96-well plates at a density of 10,000 cells per well. After 24 h, the cells were treated with various concentrations of compounds from 0.5 to 50 μg mL^{-1}. Wells containing culture medium without cells were used as blanks and cisplatin was assayed over the same time as a positive control. The cells were further incubated for 72 h. The cytotoxicity was measured by adding 5 mg mL^{-1} of MTT to each well with incubation for another 4 h. The formazan crystals were dissolved by adding 150 μL of DMSO to each well. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from plots of % viability against the dose of each compound added. Each assay was performed in triplicate.

Molecular docking

Molecular docking was performed with the Surflex-Dock program interfaced with Sybyl-X 2.0. The programs adapted an empirical scoring function and a patented searching engine.[22] The ligand was docked into the corresponding protein binding site guided by protomol, which is an idealized representation of a ligand that makes every potential interaction with a binding site. In this work, the crystal structure of c-Met complexed with 2-benzyl-5-{4-[(6,7-dimethoxyquinolin-4-yl)oxy]-3-fluorophenyl}-3-methylpyrimidin-4(3H)-one (PDB entry: 3EFJ) was extracted from the Brookhaven Protein Database (PDB; http://www.rcsb.org/pdb). At the beginning of docking, all the water and ligands were removed and random hydrogen atoms were added. Next, the receptor structure was minimized over 10,000 cycles using the Powell method in Sybyl-X 2.0. All the compounds were constructed using a sketch molecular module. Hydrogen and Gasteiger-Hückel charges were added to every molecule. Then, their geometries were optimized by the conjugate gradient method in the TRIPOS force field. The energy convergence criterion is 0.001 kcal mol^{-1}. Finally, ligand-based mode was adopted to generate the "protomol," leaving the threshold at their default value of 1.

Conclusion

In summary, several of 6-alkoxy-3-aryl-[1,2,4]triazolo[4,3-*b*][1,2,4,5]tetrazine derivatives have been prepared and evaluated for their anticancer activities against three cancer cell lines (MCF-7, Bewo, and HL-60). Compound **5b** showed substantially strong anticancer activity against A549 in vitro with an IC_{50} value of 8.08 μM. Compound **5j** showed the most potent biological activity against MCF-7 and Bewo with IC_{50} values of 2.81 and 20.72 μM, respectively, and the molecular docking result between **5j** and c-Met kinase (3EFJ) suggested that compound **5j** was a potential inhibitor of c-Met kinase.

Declaration of conflicting interests

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ORCID iD


Feng Xu https://orcid.org/0000-0002-1696-2242

Supplemental material

Supplemental material for this article is available online.

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