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Short communication

Design and synthesis of novel 2′-hydroxy group substituted 2-pyridone derivatives as anticancer agents

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ABSTRACT

We have synthesized a series of novel 2-pyridone derivatives with 1,2,3-triazole and evaluated their anti-tumor activities *in vitro*. The bioassays showed that the majority of the resultant compounds exerted inhibitory effects on six human cancer cell lines to various extents. In particular, compound **10k** showed the best anti-tumor activities (IC_{50} values of A549, HeLa and SW480 cancer cell lines were 0.86 ± 0.17 μM, 0.54 ± 0.23 μM and 0.21 ± 0.13 μM, respectively).

1. Introduction

Cancer is one of the leading causes of death and only second to heart diseases [1]. The current treatment options for cancers are unsatisfactory due to the poor efficacy and relatively significant side effects [2]. Moreover, the growing incidence of drug resistance also results in serious medical problems [3]. During decades of research, several classes of small molecule drug have been studied deeply as antiproliferative agents (Fig. 1). Colchicine, first isolated in 1820 from *Colchicum autumnale* [4], was limited in the use of anti-gout due to its severe side effects. Paclitaxel, which was isolated in 1966 [5],is used in the treatment of non-small-cell lung cancer (NSCLC) and other epithelial malignancies [6]. Furthermore, CA-4 was a natural cis-stilbene product which was first isolated by Pieet, *etc.* from the bark of African willow tree *Combretum caffrum* [7]. This natural product strongly inhibits tubulin polymerization by binding to the colchicines binding site which had drawn great interests to the scientists round the world. A lot of CA-4 analogs had been reported [1,8]. CA-4P, the most promising compound in the form of a water-soluble phosphate prodrug, is currently in phase II and III clinical trials for the treatment of solid tumors [7,9,10]

However, many of the currently available anticancer agents are unable to differentiate between the normal cells and the tumor cells. Thereby, it is hard to overcome primary or secondary resistance mechanisms evolved in the cancer cells [11]. For these reasons mentioned above, there is an urgent need to develop a new kind of chemotherapeutic agent working on multiple targets or novel single chemical entity that could modulate several targets of a multi-factorial disease.

In our previously study, we have designed and synthesized a series of 2-pyridone derivatives possessing good anti-HBV activity [12]. In continuation of pursuing more potent anti-HBV agents, we found that though the modification of 2′-hydroxyl group would reduce the anti-HBV activity of 2-pyridone derivatives, they showed good to excellent antiproliferative against the human cancer cell lines. Consequently, we reported herein the design and synthesis of 2′-modified derivatives of novel 2-pyridones and their evaluation as anticancer agents against six human cancer cell lines, *viz.* A549 (human lung adenocarcinoma), HeLa (Henrietta Lacks strain of cancer cell line), QGY (human hepatocellular carcinoma cell line), SGC7901 (human gastric cancer cells), MDA (human breast cancer cell line) and SW480 (colorectal cancer cell line).

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2. Chemistry

The synthetic route of compound **7** was depicted in Scheme 1. Compound **5** was synthesized by a reported procedure [13]. Coupling of compound **5** with methyl acrylate by a Heck reaction afforded the intermediate **6**. In the presence of triethylamine, the key intermediate (KI) **7** was obtained by reacting compound **6** with *m*-methylanline [12]. The KI **7** was reacted with substituted chlorides or chloroformic esters to give the target compounds **8a**–**8l** (Scheme 2). Compound **9** was obtained by reacted KI **7** with 3-bromoprop-1-yne and the target compounds **10a**–**10t** were synthesized through click reaction with substituted benzyl bromides or benzyl chlorides (Scheme 3).

3. Pharmacology

The potential anticancer activity of all the synthesized target compounds was tested in A549, HeLa, QGY7701, SGC7901, MDA-MB-231 and SW480 using MTT assay, and 5-fluorouracil (5-Fu) was used as a reference antiproliferative drug. The antiproliferative activity of each compound was expressed as the concentration of compound that achieved 50% inhibition (IC_{50}).

4. Results and discussion

As showing in Table 1, all the synthesized compounds possessed good to excellent antiproliferative activities against the six cell lines and most of them were more active than the positive control (5-Fu) and different substitutions were important for the antiproliferative activity. Compounds **8a**–**8l**, **8k** (IC_{50} against A549, HeLa, and SW480 were 0.59 ± 0.13 μM, 0.46 ± 0.06 μM and 0.52 ± 0.23 μM, respectively) possessed the best inhibitory effects against six human cancer cell lines, which were 9.5, 35.9 and 30.2 folds more potent than 5-Fu against A549, HeLa, and SW480 cancer cells, respectively. When the benzyloxy (compound **8k**) was replaced by *para*- or *ortho*-chloro benzyl (compounds **8g** and **8h**), the anticancer activity could be maintained except for the SGC7901 and MDA-MB-231 cell lines. Moreover, *para*-chloro substituted on phenyl was preferable. Methyl derivative (compound **8e**) showed no anticancer activity against five cancer cell lines of all. Furthermore, substituted phenyl derivatives (compounds **8a**, **8c**, **8d** and **8f**) showed better anti-cancer potency than the alkyl group ones (compounds **8b** and **8e**), which maybe the poor flexibility affected the anti-cancer activity severely. Meanwhile, the resultant compounds with propoxy or ethoxy group (compounds **8j** or **8l**), did not show good anti-cancer performance. A simple conclusion could be drawn that the flexibility and aromatic substituent were important for the anti-cancer activity.

Based on the conclusion above, compounds **10a**–**10t** were designed and synthesized via click chemistry approach (Fig. 2). Apparently, the issues about flexibility and aromatic substituent could be realized easily. However, the drawback was that when the 1,2,3-triazole ring was between aromatic group and 2′-oxygen atom, it could affect the anti-cancer activity by forming excess hydrogen bonds with receptor protein. Anyway, the anti-cancer activity (Table 1) indicated that most of them showed good activities.

Compound **10a**, which had no substituted on the phenyl group, was more potent than the positive control 5-Fu against A549, HeLa and SW480 cancer cell lines. When a chloro-group was introduced on the 4 position of the phenyl group, compound **10k** showed 6.5, 30.6, and 74.8 folds more potent than 5-Fu against A549, HeLa and SW480 cancer cells. When the chloro-group was replaced by fluoro- and methyl group (compound **10o** and **10r**), the anti-cancer activity also maintained. However, the antiproliferative activity of the analogs was lost or decreased severely while the chloro-, fluoro-and methyl groups were moved to 3 or 2 position (compounds **10i**, **10j**, **10m**, **10n**, **10p** and **10q**). The same regularity could also be seen from compounds **10b** to **10g**. More interestingly, a *tert*-butyl substituent on the 4 position of the phenyl group (compound **10t**) showed best antiproliferative against HeLa and SW480 cancer cell lines (0.34 ± 0.23 and 0.11 ± 0.08 μM, respectively) among all the target compounds, which were 48.5 and 142.7 folds more potent than 5-Fu. Thereby, a simple conclusion could be made that the substituent on the 4 position was preferable for compounds **10a**–**10t**.

5. Conclusion

In the present study, a library of novel 2-pyridone derivatives were designed and synthesized and their anticancer activities

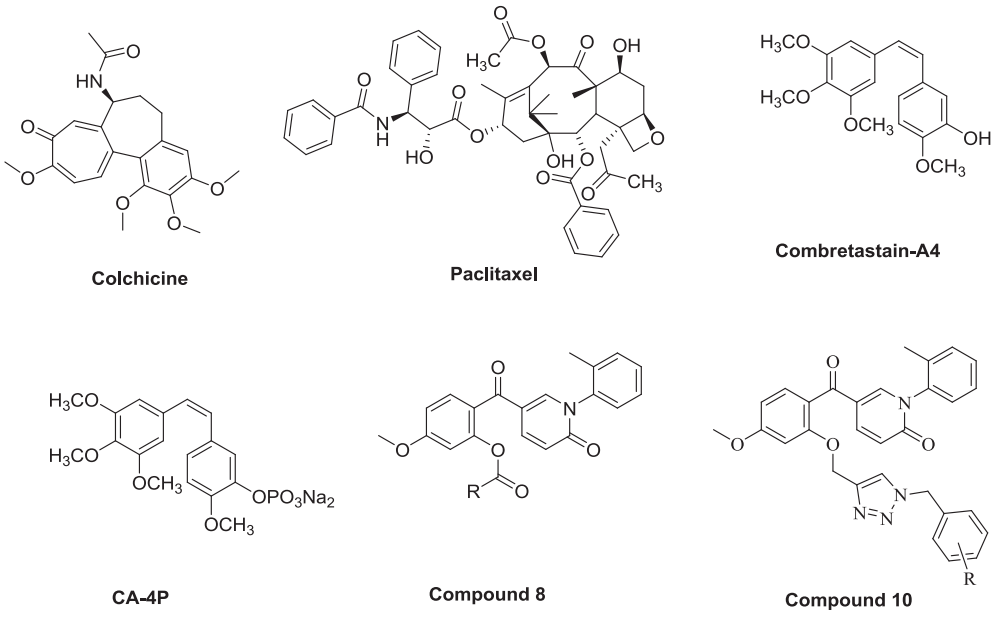
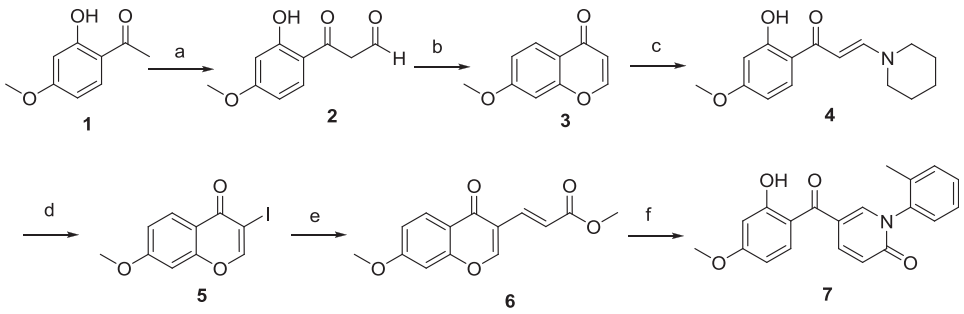


Fig. 1. The structures of anticancer agents and compounds **8** and **10**.



Scheme 1. Synthesis of the key intermediate compound **7**. Reagents and conditions: a. Na, ether, ethyl formate, 0 °C, yield 95%; b. CH_3COOH, conc HCl, 100 °C, 30 min, yield 98%; c. piperidine, methanol, reflux, yield 99%; d. dichloromethane, I_2, over night, yield 97%; e. $PdCl_2(Ph_3P)_2$, CuI, K_2CO_3, THF/H_2O, 100 °C, yield 80%; f. 2-methylaniline, methanol, reflux, yield 83%.

in vitro against six human cancer cell lines were evaluated. From the structure–activity relationship (SAR) of the substituents on the 2′-hydroxy group of the target compounds, the aromatic group, which was linked through a flexible linker to the 2′-hydroxy group, was needed and substituents at the *para*-position of the aromatic group were better for the anticancer activity (compounds **8g**, **10k**, **10o**, **10r** and **10t**). The further chemical and biological studies are under way. Moreover, due to the solubility of the compounds was unsatisfactory, some hydroxyl groups could be introduced to the molecule. Our finding would provide very useful clues for the design and development of more potent anticancer agents based on the novel 2-pyridone scaffold.

6. Experimental procedures

6.1. Chemistry

Melting points were measured on an uncorrected X-5 Digital melting point apparatus (Gongyi City Yuhua Instrument Co., Ltd; China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ as solvents. Chemical shift are given in ppm (δ). The mass spectra were recorded on an Esquire 3000 LC-MS mass spectrometer. Silica gel thin-layer chromatography was performed on precoated plates GF-254 (Qingdao Haiyang Chemical, China). All solvents and reagents were analytical pure and no further purification is needed. All starting materials were commercially available.

6.1.1. Synthesis of the target compounds

*6.1.1.1. General procedure for the synthesis of compounds **8a**–**8l**.* Substituted acyl chloride (4.5 mmol) was added dropwise into a solution of triethylamine (0.3 g, 3.3 mmol) and compound **7** (1 g, 3 mmol) in dry CH_2Cl_2 (15 mL). The reaction mixture was stirred at 20 °C for 1 h. 10% HCl solution (20 mL) was added to quench the reaction. The CH_2Cl_2 layer was washed by saturated $NaHCO_3$ solution (20 mL), water and dried with $MgSO_4$. CH_2Cl_2 was removed and the crude product was recrystallized in ethyl acetate to give the target compound.

*6.1.1.1.1. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 4-methylbenzoate (**8a**).* White solid, m.p.: 209–211 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.547 (s, 3H), 3.765 (s, 3H), 3.878 (s, 3H), 6.608 (d, 1H, J = 9.6 Hz), 6.830 (d, 1H, J = 2.4 Hz), 6.888 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 7.019 (d, 1H, J = 7.5 Hz), 7.321 (m, 3H), 7.487 (m, 3H), 7.509 (m, 1H), 7.813 (d, 1H, J = 1.8 Hz), 7.824 (dd, 1H, J_1 = 9.9 Hz, J_2 = 4.2 Hz), 8.044 (m, 2H). ESI-MS (m/z): 470.3 [M + 1].

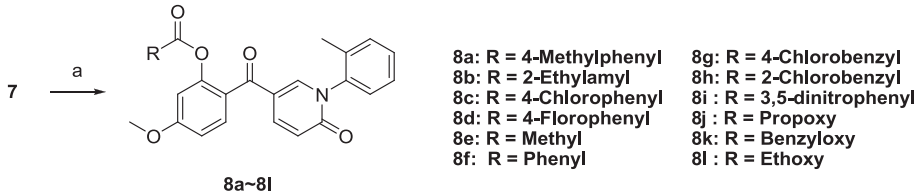
*6.1.1.1.2. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 2-ethylhexanoate (**8b**).* White powder, m.p.: 210–211 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.966 (m, 6H), 1.257 (m, 4H), 1.577 (m, 2H), 1.678 (m, 2H), 2.180 (s, 3H), 2.237 (m, 1H), 3.854 (s, 3H), 6.597 (d, 1H, J = 1.8 Hz), 6.633 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.4 Hz), 6.656 (d, 1H, J = 2.6 Hz), 7.196 (d, 1H, J = 7.5 Hz), 7.342 (m, 3H), 7.427 (d, 1H, J = 8.4 Hz), 7.794 (d, 1H, J = 1.6 Hz), 7.949 (dd, 1H, J_1 = 6.6 Hz, J_2 = 1.8 Hz). ESI-MS (m/z): 462.6 [M + 1].

*6.1.1.1.3. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 4-chlorobenzoate (**8c**).* White powder, m.p.: 185–186 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.047 (s, 3H), 3.865 (s, 3H), 6.607 (d, 1H, J = 9.6 Hz), 6.831 (d, 1H, J = 2.4 Hz), 6.888 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 7.019 (d, 1H, J = 7.5 Hz), 7.321 (m, 3H), 7.487 (m, 2H), 7.509 (m, 1H), 7.813 (d, 1H, J = 1.8 Hz), 7.824 (dd, 1H, J_1 = 9.9 Hz, J_2 = 4.2 Hz), 8.044 (m, 2H). ESI-MS (m/z): 474.2 [M + 1].

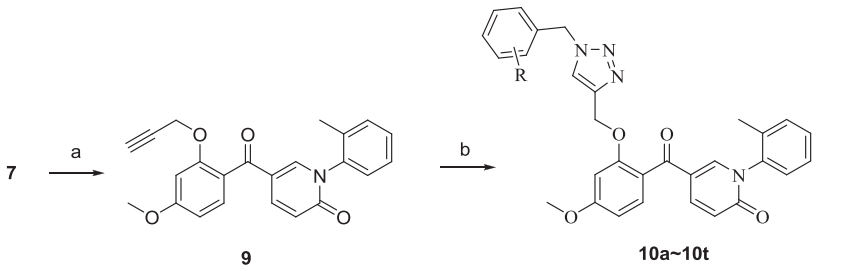
*6.1.1.1.4. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 4-fluorobenzoate (**8d**).* White crystal, m.p.: 95–96 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.046 (s, 3H), 3.866 (s, 3H), 6.611 (d, 1H, J = 9.6 Hz), 6.821 (d, 1H, J = 2.4 Hz), 6.874 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.4 Hz), 7.021 (d, 1H, J = 7.5 Hz), 7.318 (m, 3H), 7.447 (m, 2H), 7.521 (m, 1H), 7.812 (d, 1H, J = 1.8 Hz), 7.821 (dd, 1H, J_1 = 9.9 Hz, J_2 = 4.2 Hz), 8.007 (m, 2H). ESI-MS (m/z): 458.5 [M + 1].

*6.1.1.1.5. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl acetate (**8e**).* White powder, m.p.: 99–100 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.780 (s, 3H), 2.237 (s, 3H), 3.855 (s, 3H), 6.590 (d, 1H, J = 1.8 Hz), 6.626 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.4 Hz), 6.652 (d, 1H, J = 2.6 Hz), 7.196 (d, 1H, J = 7.5 Hz), 7.442 (m, 3H), 7.428 (d, 1H, J = 8.4 Hz), 7.797 (d, 1H, J = 1.6 Hz), 7.951 (dd, 1H, J_1 = 6.6 Hz, J_2 = 1.8 Hz). ESI-MS (m/z): 378.4 [M + 1].

*6.1.1.1.6. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl benzoate (**8f**).* White crystal, m.p.: 76–78 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.023 (s, 3H), 3.878 (s, 3H), 6.608 (d, 1H,



Scheme 2. Synthesis of the target compounds **8a**–**8l**. Reagents and conditions: a. substituted acyl chlorides, CH_2Cl_2, Et_3N, 20 °C, 1 h.



Scheme 3. Synthesis of the target compounds **10a**–**10t**. Reagents and conditions: a. 3-bromoprop-1-yne, K_2CO_3, CH_2Cl_2, 20 °C, 6 h; b. substituted benzyl chlorides, NaN_3, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, acetone, r.t., 2 h.

J = 9.6 Hz), 6.830 (d, 1H, J = 2.4 Hz), 6.888 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 7.019 (d, 1H, 7.5 Hz), 7.321 (m, 3H), 7.487 (m, 3H), 7.509 (m, 1H), 7.813 (d, 1H, J = 1.8 Hz), 7.824 (dd, 1H, J_1 = 9.9 Hz, J_2 = 4.2 Hz), 8.044 (m, 2H). ESI-MS (*m*/*z*): 440.4 [M + 1].

*6.1.1.1.7. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 2-(4-chlorophenyl)acetate (**8g**).* White crystal, m.p.: 79–80 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.120 (s, 3H), 3.578 (s, 2H), 3.898 (s, 3H), 6.618 (d, 1H, J = 9.3 Hz), 6.748 (d, 1H, J = 2.4 Hz),

Table 1
IC_{50} determination of cytotoxicity of target compounds against human cancer cell lines.

Compd.	R	Cytotoxicity IC_{50} ± SEM (μM)[b]					
		A549[a]	HeLa[a]	QGY7701[a]	SGC7901[a]	MDA-MB-231[a]	SW480[a]
5-Fu[c]	–	5.62 ± 0.72	16.52 ± 0.93	14.30 ± 0.055	6.04 ± 0.83	13.65 ± 0.82	15.71 ± 0.86
8a	*p*-Methylphenyl	3.24 ± 0.59	3.27 ± 0.72	>30	3.96 ± 0.77	0.54 ± 0.12	2.31 ± 0.32
8b	2-Ethylamyl	2.95 ± 0.21	2.28 ± 0.33	>30	1.48 ± 0.70	0.37 ± 0.11	3.35 ± 0.24
8c	*p*-Chlorophenyl	5.23 ± 0.62	1.56 ± 0.53	>30	5.79 ± 0.43	1.84 ± 0.15	1.33 ± 0.32
8d	*p*-Fluorophenyl	3.68 ± 0.66	6.86 ± 0.80	9.64 ± 0.82	3.62 ± 0.02	2.35 ± 0.37	2.78 ± 0.07
8e	Methyl	>30	>30	>30	6.21 ± 0.83	>30	>30
8f	Phenyl	4.47 ± 0.72	7.29 ± 0.59	>30	1.42 ± 0.43	1.18 ± 0.41	3.12 ± 0.11
8g	*p*-Chlorobenzyl	0.49 ± 0.13	0.58 ± 0.43	5.68 ± 0.52	3.48 ± 0.33	2.44 ± 0.49	0.58 ± 0.33
8h	o-Chlorobenzyl	1.94 ± 0.83	0.87 ± 0.08	6.05 ± 0.59	1.45 ± 0.49	2.97 ± 0.83	1.17 ± 0.43
8i	3,5-Dinitrophenyl	3.89 ± 0.40	5.33 ± 0.93	>30	2.44 ± 0.43	3.28 ± 0.67	2.75 ± 0.74
8j	Propoxy	6.13 ± 0.23	7.03 ± 0.47	>30	4.18 ± 0.72	2.11 ± 0.99	5.97 ± 0.09
8k	Benzyloxy	0.59 ± 0.13	0.46 ± 0.06	4.33 ± 0.59	0.69 ± 0.43	0.75 ± 0.58	0.52 ± 0.23
8l	Ethoxy	5.83 ± 0.41	7.44 ± 0.77	>30	3.35 ± 0.46	>30	4.56 ± 0.83
9	–	7.59 ± 0.93	7.82 ± 0.59	9.08 ± 0.73	6.94 ± 0.49	>30	5.33 ± 0.36
10a	H	3.64 ± 0.73	4.23 ± 0.59	>30	1.25 ± 0.44	>30	2.51 ± 0.43
10b	*o*-Bromo	5.35 ± 0.09	5.22 ± 0.31	>30	1.23 ± 0.13	>30	3.62 ± 0.41
10c	m-Bromo	4.46 ± 0.33	4.52 ± 0.13	>30	5.68 ± 0.63	>30	2.32 ± 0.46
10d	*p*-Bromo	5.45 ± 0.72	4.25 ± 0.43	4.99 ± 0.83	2.29 ± 0.03	2.47 ± 0.08	>30
10e	*o*-Cyano	8.32 ± 0.83	8.05 ± 0.89	>30	>30	>30	>30
10f	*m*-Cyano	7.28 ± 0.77	9.62 ± 0.52	>30	>30	>30	>30
10g	*p*-Cyano	4.31 ± 0.43	0.89 ± 0.03	2.87 ± 0.72	>30	0.43 ± 0.33	>30
10h	*m*-Nitro	3.61 ± 0.40	3.12 ± 0.48	3.01 ± 0.43	0.55 ± 0.06	1.39 ± 0.93	4.42 ± 0.72
10i	*o*-Chloro	0.69 ± 0.09	1.70 ± 0.33	4.72 ± 0.11	>30	0.38 ± 0.33	0.39 ± 0.05
10j	*m*-Chloro	1.53 ± 0.07	3.71 ± 0.59	>30	>30	0.38 ± 0.02	1.37 ± 0.19
10k	*p*-Chloro	0.86 ± 0.17	0.54 ± 0.23	>30	1.10 ± 0.59	0.32 ± 0.72	0.21 ± 0.13
10l	2,4-Dichloro	2.56 ± 0.73	3.51 ± 0.13	6.43 ± 0.72	0.41 ± 0.55	1.19 ± 0.43	2.22 ± 0.40
10m	*o*-Fluoro	3.96 ± 0.63	4.83 ± 0.81	>30	>30	1.14 ± 0.33	3.28 ± 0.83
10n	*m*-Fluoro	3.06 ± 0.43	5.75 ± 0.82	>30	>30	1.24 ± 0.43	2.23 ± 0.13
10o	*p*-Fluoro	0.48 ± 0.44	8.48 ± 0.59	5.44 ± 0.39	0.19 ± 0.43	1.46 ± 0.62	2.37 ± 0.93
10p	*o*-Methyl	3.21 ± 0.83	4.86 ± 0.53	4.63 ± 0.22	>30	1.34 ± 0.05	3.07 ± 0.43
10q	*m*-Methyl	3.25 ± 0.11	3.27 ± 0.18	6.38 ± 0.23	>30	0.21 ± 0.45	2.45 ± 0.09
10r	*p*-Methyl	0.61 ± 0.33	0.38 ± 0.03	>30	5.48 ± 0.59	0.49 ± 0.08	0.48 ± 0.13
10s	*p*-Methoxyl	1.54 ± 0.08	0.99 ± 0.43	>30	1.28 ± 0.63	0.59 ± 0.06	>30
10t	*p*-*tert*-Butyl	1.73 ± 0.49	0.34 ± 0.23	3.39 ± 0.07	3.52 ± 0.44	3.35 ± 0.45	0.11 ± 0.08

[a] Abbreviations: A549: human lung adenocarcinoma cell line; HeLa: human cervical carcinoma cell line; QGY7701: human hepatoma cell line; SGC7901: human gastric adenocarcinoma cell line; MDA-MB-231: human breast carcinoma cell line; SW480: human colon carcinoma cell line.
[b] The IC_{50} value was defined as the concentration at which 50% survival of cells was observed.
[c] Used as a positive control.

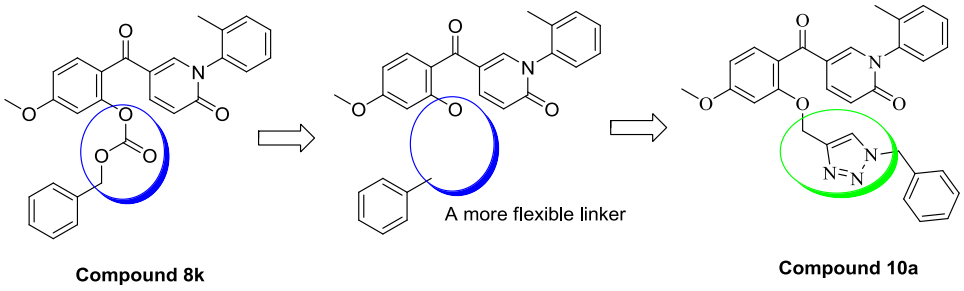


Fig. 2. The design of the target compounds **10a**–**10t**.

6.842 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz), 7.121 (d, 1H, $J = 8.6$ Hz), 7.351 (m, 7H), 7.496 (d, 1H, $J = 8.7$ Hz), 7.740 (d, 1H, $J = 2.4$ Hz), 7.875 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz). ESI-MS (*m*/*z*): 488.6 [M + 1].

*6.1.1.1.8. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 2-(2-chlorophenyl)acetate (**8h**).* White crystal, m.p.: 84–85 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.121 (s, 3H), 3.573 (s, 2H), 3.899 (s, 3H), 6.618 (d, 1H, $J = 9.3$ Hz), 6.750 (d, 1H, $J = 2.4$ Hz), 6.838 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz), 7.109 (d, 1H, $J = 8.6$ Hz), 7.551 (m, 7H), 7.516 (d, 1H, $J = 8.7$ Hz), 7.738 (d, 1H, $J = 2.4$ Hz), 7.785 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz). ESI-MS (*m*/*z*): 488.3 [M + 1].

*6.1.1.1.9. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl 2-(3,5-dinitrophenyl)acetate (**8i**).* Yellow crystal, m.p. 68–70 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.178 (s, 3H), 3.917 (s, 3H), 6.669 (d, 1H, $J = 9.6$ Hz), 6.882 (d, 1H, $J = 2.4$ Hz), 6.921 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz), 7.186 (m, 1H), 7.367 (m, 3H), 7.557 (d, 1H, $J = 8.4$ Hz), 7.83 (m, 2H), 9.277 (m, 3H). ESI-MS (*m*/*z*): 530.6 [M + 1].

*6.1.1.1.10. 5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl propyl carbonate (**8j**).* Yellow powder, m.p.: 97–98 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.180 (s, 3H), 2.383 (t, 1H, $J = 2.4$ Hz), 3.854 (s, 3H), 4.658 (d, 2H, $J = 2.4$ Hz), 6.597 (d, 1H, $J = 1.8$ Hz), 6.633 (d, 1H, $J = 2.4$ Hz), 6.656 (d, 1H, $J = 9.6$ Hz), 7.196 (d, 1H, $J = 7.5$ Hz), 7.342 (m, 3H), 7.427 (d, 1H, $J = 7.4$ Hz), 7.794 (d, 1H, $J = 1.6$ Hz), 7.949 (dd, 1H, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz). ESI-MS (*m*/*z*): 422.2 [M + 1].

*6.1.1.1.11. Benzyl 5-methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl carbonate (**8k**).* Yellow powder, m.p.: 108–109 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.131 (s, 3H), 3.839 (s, 3H), 5.198 (s, 2H), 6.618 (d, 1H, $J = 9.3$ Hz), 6.748 (d, 1H, $J = 2.4$ Hz), 6.842 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz), 7.121 (d, 1H, $J = 8.6$ Hz), 7.351 (m, 8H), 7.496 (d, 1H, $J = 8.7$ Hz), 7.740 (d, 1H, $J = 2.4$ Hz), 7.875 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz). ESI-MS (*m*/*z*): 470.3 [M + 1].

*6.1.1.1.12. Ethyl 5-methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenyl carbonate (**8l**).* Light yellow powder, m.p.: 116–118 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.331 (t, 3H, $J = 7.2$ Hz), 2.192 (s, 3H), 3.856 (s, 3H), 4.219 (q, 2H, $J = 7.2$ Hz), 6.678 (d, 1H, $J = 9.6$ Hz), 6.763 (d, 1H, $J = 2.4$ Hz), 6.847 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.4$ Hz), 7.218 (m, 1H), 7.367 (m, 3H), 7.506 (d, 1H, $J = 8.7$), 7.784 (m, 1H), 7.938 (dd, 1H, $J_1 = 9.9$ Hz, $J_2 = 2.7$ Hz). ESI-MS (*m*/*z*): 408.9 [M + 1].

*6.1.2. Synthesis of 5-(4-methoxy-2-(prop-2-ynyloxy)benzoyl)-1-o-tolylpyridin-2(1H)-one (**9**)*

A solution of compound **7** (1 g, 3mmol), K_2CO_3 (0.4 g, 3 mmol) and 3-bromoprop-1-yne (0.4 g, 3.3 mmol) in CH_2Cl_2 (15 mL) was stirred at 20 °C for 6 h. 10% HCl solution (20 mL) was added to quench the reaction after which completed when monitored by TLC. Consequently, the CH_2Cl_2 layer was washed by $NaHCO_3$ solution (20 mL), water and dried with $MgSO_4$. CH_2Cl_2 was removed and the crude product was re-crystallized in ethyl acetate. White crystal was obtained, 0.98 g, yield 87.6%, m.p.: 148–149 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.180 (s, 3H), 2.383 (t, 1H, $J = 2.4$ Hz), 3.854 (s, 3H), 4.658 (d, 2H, $J = 2.4$ Hz), 6.597 (d, 1H, $J = 1.8$ Hz), 6.633 (d, 1H, $J = 2.4$ Hz), 6.656 (d, 1H, $J = 9.6$ Hz), 7.196 (d, 1H, $J = 7.5$ Hz), 7.342 (m, 3H), 7.427 (d, 1H, $J = 7.4$ Hz), 7.794 (d, 1H, $J = 1.6$ Hz), 7.949 (dd, 1H, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz). ESI-MS(*m*/*z*): 374.1 [M + 1].

*6.1.3. General procedure for the synthesis of compounds **10a**–**10t***

To a solution of substituted benzyl chloride (3.3 mmol) in acetone (9 mL) with stirring at r.t. for 30 min. Then NaN_3 (0.24 g, 3.6 mmol), compound **9** (1.12 g, 3 mmol), saturated $CuSO_4$ solution (10 mL) and catalytic amount of sodium ascorbate were added. The mixture was stirred at room temperature for 2 h. The mixture was poured into ice-water (200 mL) with severe stirring. The solution was extracted with EtOAc and the EtOAc layer was washed with brine, dried with $MgSO_4$ and concentrated to afford target compound.

*6.1.3.1. 5-(2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10a**).* White crystal, m.p.: 85–86 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.045 (s, 3H), 3.843 (s, 3H), 5.164 (s, 2H), 5.484 (s, 2H), 6.563 (m, 2H), 6.657 (s, 1H), 6.993 (d, 1H, $J = 9.3$ Hz), 7.254 (m, 2H), 7.387 (m, 7H), 7.736 (d, 1H, $J = 2.4$ Hz), 7.845 (d, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.7$ Hz). ESI-MS (*m*/*z*): 507.3 [M + 1].

*6.1.3.2. 5-(2-((1-(2-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10b**).* White crystal, m.p.: 97–98 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.879 (s, 3H), 3.789 (s, 3H), 5.178 (s, 2H), 5.775 (s, 2H), 6.494 (d, 1H, $J = 9.6$ Hz), 6.609 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz), 6.853 (d, 1H, $J = 1.8$ Hz), 7.251 (m, 3H), 7.455 (m, 3H), 7.558 (m, 1H), 7.635 (m, 2H), 7.805 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.882 (d, 1H, $J = 1.2$ Hz), 7.908 (s, 1H). ESI-MS (*m*/*z*): 585.1 [M + 1].

*6.1.3.3. 5-(2-((1-(3-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one **10c**.* White crystal, m.p.: 85–86 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.138 (s, 3H), 3.849 (s, 3H), 5.166 (s, 2H), 5.732 (s, 2H), 6.195 (d, 1H, $J = 9.4$ Hz), 6.664 (dd, 1H, $J_1 = 9.4$ Hz, $J_2 = 2.4$ Hz), 6.884 (d, 1H, $J = 1.8$ Hz), 7.685 (m, 2H), 7.395 (m, 4H), 7.448 (m, 1H), 7.586 (m, 2H), 7.811 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.888 (d, 1H, $J = 1.2$ Hz), 7.877 (s, 1H). ESI-MS (*m*/*z*): 585.1 [M + 1].

*6.1.3.4. 5-(2-((1-(4-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one **10d**.* White crystal, m.p.: 148–150 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.889 (s, 3H), 3.788 (s, 3H), 5.155 (s, 2H), 5.547 (s, 2H), 6.499 (d, 1H, $J = 9.6$ Hz), 6.531 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.849 (d, 1H, $J = 2.1$ Hz), 7.210 (m, 2H), 7319 (m, 5H), 7.533 (m, 2H), 7.696 (d, 1H, $J = 1.8$ Hz), 7.803 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 8.091 (s, 1H). ESI-MS (*m*/*z*): 585.2 [M + 1].

*6.1.3.5. 2-((4-((5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (**10e**).* White crystal, m.p.: 192–193 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.881 (s, 3H), 3.789 (s, 3H), 5.178 (s, 2H), 5.775 (s, 2H), 6.494 (d, 1H, $J = 9.6$ Hz), 6.609 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz), 6.853 (d, 1H, $J = 1.8$ Hz), 7.251 (m, 3H), 7.355 (m, 3H), 7.538 (m, 1H), 7.682 (m, 2H), 7.805 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.882 (d, 1H, $J = 1.2$ Hz), 7.908 (s, 1H). ESI-MS (m/z): 532.3 [M + 1].

*6.1.3.6. 3-((4-((5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (**10f**).* White crystal, m.p.: 167–168 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.138 (s, 3H), 3.849 (s, 3H), 5.166 (s, 2H), 5.732 (s, 2H), 6.195 (d, 1H, $J = 9.4$ Hz), 6.664 (dd, 1H, $J_1 = 9.4$ Hz, $J_2 = 2.4$ Hz), 6.884 (d, 1H, $J = 1.8$ Hz), 7.685 (m, 2H), 7.395 (m, 4H), 7.448 (m, 1H), 7.586 (m, 2H), 7.811 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.888 (d, 1H, $J = 1.2$ Hz), 7.877 (s, 1H). ESI-MS (m/z): 532.6 [M + 1].

*6.1.3.7. 4-((4-((5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (**10g**).* White crystal, m.p.: 194–196 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.889 (s, 3H), 3.788 (s, 3H), 5.155 (s, 2H), 5.547 (s, 2H), 6.499 (d, 1H, $J = 9.6$ Hz), 6.531 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.849 (d, 1H, $J = 2.1$ Hz), 7.210 (m, 2H), 7319 (m, 5H), 7.533 (m, 2H), 7.696 (d, 1H, $J = 1.8$ Hz), 7.803 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 8.091 (s, 1H). ESI-MS (m/z): 532.1 [M + 1].

*6.1.3.8. 3-((4-((5-Methoxy-2-(6-oxo-1-o-tolyl-1,6-dihydropyridine-3-carbonyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)phenyl nitrate (**10h**).* Yellow crystal, m.p.: 123–124 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.869 (s, 3H), 3.784 (s, 3H), 5.172 (s, 2H), 5.743 (s, 2H), 6.469 (d, 1H, $J = 9.6$ Hz), 6.607 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 1.4$ Hz), 6.848 (d, 1H, 1.4 Hz), 7.187 (d, 1H, $J = 0.9$ Hz), 7.313 (m, 4H), 7.671 (m, 3H), 7.798 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.7$ Hz), 8.173 (m, 3H). ESI-MS (m/z): 552.5 [M + 1].

*6.1.3.9. 5-(2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10i**).* White crystal, m.p.: 95–96 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.882 (s, 3H), 3.787 (s, 3H), 5.177 (s, 2H), 5.772 (s, 2H), 6.491 (d, 1H, $J = 9.6$ Hz), 6.610 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz), 6.853 (d, 1H, $J = 1.8$ Hz), 7.251 (m, 3H), 7.364 (m, 3H), 7.538 (m, 1H), 7.677 (m, 2H), 7.805 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.882 (d, 1H, $J = 1.2$ Hz), 7.910 (s, 1H). ESI-MS (m/z): 541.2 [M + 1].

*6.1.3.10. 5-(2-((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10j**).* White crystal, m.p.: 83–84 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.135 (s, 3H), 3.889 (s, 3H), 5.168 (s, 2H), 5.734 (s, 2H), 6.191 (d, 1H, $J = 9.6$ Hz), 6.654 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.856 (d, 1H, $J = 1.8$ Hz), 7.674 (m, 2H), 7.368 (m, 4H), 7.436 (m, 1H), 7.586 (m, 2H), 7.811 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.863 (d, 1H, $J = 1.2$ Hz), 7.874 (s, 1H). ESI-MS (m/z): 541.5 [M + 1].

*6.1.3.11. 5-(2-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10k**).* White crystal, m.p.: 132–133 °C ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.887 (s, 3H), 3.786 (s, 3H), 5.157 (s, 2H), 5.547 (s, 2H), 6.501 (d, 1H, $J = 9.6$ Hz), 6.531 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.849 (d, 1H, $J = 2.1$ Hz), 7.233 (m, 2H), 7332 (m, 5H), 7.533 (m, 2H), 7.697 (d, 1H, $J = 1.8$ Hz), 7.805 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 8.091 (s, 1H). ESI-MS (m/z): 541.8 [M + 1].

*6.1.3.12. 5-(2-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10l**).* White crystal, m.p.: 139–140 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.899 (s, 3H), 3.790 (s, 3H), 5.168 (s, 2H), 5.649 (s, 2H), 6.495 (d, 1H, $J = 9.6$ Hz), 6.611 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.850 (d, 1H, $J = 1.4$ Hz), 7.221 (m, 2H), 7.331 (m, 4H), 7.423 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 2.1$ Hz), 7.674 (m, 2H), 7.807 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.7$ Hz), 8.042 (s, 1H). ESI-MS (m/z): 575.4 [M + 1].

*6.1.3.13. 5-(2-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10m**).* White crystal, m.p.: 123–124 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.887 (s, 3H), 3.789 (s, 3H), 5.159 (s, 2H), 5.620 (s, 2H), 6.502 (d, 1H, $J = 9.6$ Hz), 6.609 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 1.4$ Hz), 6.850 (d, 1H, $J = 2.4$ Hz), 7.228 (m, 5H), 7.339 (m, 4H), 7.697 (d, 1H, $J = 2.4$ Hz), 7.806 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.7$ Hz), 8.038 (s, 1H). ESI-MS (m/z): 524.3[M + 1].

*6.1.3.14. 5-(2-((1-(3-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10n**).* White crystal, m.p.: 81–83 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.138 (s, 3H), 3.849 (s, 3H), 5.166 (s, 2H), 5.732 (s, 2H), 6.195 (d, 1H, $J = 9.4$ Hz), 6.664 (dd, 1H, $J_1 = 9.4$ Hz, $J_2 = 2.4$ Hz), 6.884 (d, 1H, $J = 1.8$ Hz), 7.685 (m, 2H), 7.395 (m, 4H), 7.448 (m, 1H), 7.586 (m, 2H), 7.811 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.888 (d, 1H, $J = 1.2$ Hz), 7.877 (s, 1H). ESI-MS (m/z): 524.1 [M + 1].

*6.1.3.15. 5-(2-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10o**).* White crystal, m.p.: 86–87 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.878 (s, 3H), 3.789 (s, 3H), 5.150 (s, 2H), 5.548 (s, 2H), 6.503 (d, 1H, $J = 9.6$ Hz), 6.611 (dd, 1H, $J_1 = 11.4$ Hz, $J_2 = 1.8$ Hz), 6.849 (d, 1H, $J = 2.1$ Hz), 7.208 (m, 3H), 7.332 (m, 6H), 7.696 (d, 1H, $J = 2.4$ Hz), 7.808 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 8.072 (s, 1H). ESI-MS (m/z): 524.6 [M + 1].

*6.1.3.16. 5-(2-((1-(2-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10p**).* White crystal, m.p.: 146–147 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.281 (s, 3H), 2.321 (s, 3H), 3.789 (s, 3H), 5.174 (s, 2H), 5.765 (s, 2H), 6.492 (d, 1H, $J = 9.6$ Hz), 6.612 (dd, 1H, $J_1 = 9.4$ Hz, $J_2 = 2.1$ Hz), 6.851 (d, 1H, $J = 1.8$ Hz), 7.256 (m, 3H), 7.555 (m, 3H), 7.568 (m, 1H), 7.684 (m, 2H), 7.815 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.862 (d, 1H, $J = 1.2$ Hz), 7.918 (s, 1H). ESI-MS (m/z): 521.5 [M + 1].

*6.1.3.17. 5-(2-((1-(3-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10q**).* White crystal, m.p.: 83–85 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.253 (s, 3H), 2.332 (s, 3H), 3.889 (s, 3H), 5.178 (s, 2H), 5.764 (s, 2H), 6.493 (d, 1H, $J = 9.6$ Hz), 6.622 (dd, 1H, $J_1 = 9.4$ Hz, $J_2 = 2.1$ Hz), 6.855 (d, 1H, $J = 1.8$ Hz), 7.266 (m, 3H), 7.532 (m, 3H), 7.568 (m, 1H), 7.684 (m, 2H), 7.812 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 1.8$ Hz), 7.867 (d, 1H, $J = 1.2$ Hz), 7.915 (s, 1H). ESI-MS (m/z): 521.1 [M + 1].

*6.1.3.18. 5-(2-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10r**).* White crystal, m.p.: 85–86 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.889 (s, 3H), 2.543 (s, 3H), 3.788 (s, 3H), 5.155 (s, 2H), 5.547 (s, 2H), 6.499 (d, 1H, $J = 9.6$ Hz), 6.531 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.849 (d, 1H, $J = 2.1$ Hz), 7.210 (m, 2H), 7319 (m, 5H), 7.533 (m, 2H), 7.696 (d, 1H, $J = 1.8$ Hz), 7.803 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 8.091 (s, 1H). ESI-MS (m/z): 521.4 [M + 1].

*6.1.3.19. 5-(2-((1-(4-Methoxylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10s**).* White crystal, m.p.: 98–99 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.889 (s, 3H), 3.743 (s, 3H), 3.788 (s, 3H), 5.155 (s, 2H), 5.547 (s, 2H), 6.499 (d, 1H, $J = 9.6$ Hz), 6.531 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 6.849 (d, 1H, $J = 2.1$ Hz), 7.210 (m, 2H), 7319 (m, 5H), 7.533 (m, 2H), 7.696 (d, 1H,

$J = 1.8$ Hz), 7.803 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.4$ Hz), 8.091 (s, 1H). ESI-MS (m/z): 537.2 [M + 1].

*6.1.3.20. 5-(2-((1-(4-tert-Butylbenzyl)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxybenzoyl)-1-o-tolylpyridin-2(1H)-one (**10t**).* White crystal, m.p.: 83–84 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.232 (s, 9H), 1.896 (s, 3H), 3.785 (s, 3H), 5.144 (s, 2H), 5.501 (s, 2H), 6.491 (d, 1H, $J = 9.6$ Hz), 6.607 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.1$ Hz), 6.844 (d, 1H, $J = 2.1$ Hz), 7.207 (m, 2H), 7.337 (m, 7H), 7.700 (d, 1H, $J = 2.4$ Hz), 7.803 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 2.7$ Hz), 8.040 (s, 1H). ESI-MS (m/z): 563.7 [M + 1].

6.2. Biological assay

6.2.1. Screening of the in vitro anti-cancer activities

Cytotoxic effects were examined in the A549, HeLa, QGY, SGC7901, MDA and SW480 cells. *In vitro* anti-cancer activity was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and manifested by IC_{50} of the compounds [14–16]. Various cancer cell lines were obtained from Shanghai Institute of Biological Sciences (SIBS), CAS. IC_{50} calculation was done according to the National Committee for Clinical Laboratory Standards (NCCLS). All compounds were dissolved in DMSO with the stock concentration of 10 g/L (stored at 4 °C), and diluted with fresh medium promptly before drug administration. Cells were seeded in 96-well plates at the density of 8000 cells/well. 24 h later, compounds at various concentrations were applied in duplicate to cells, which were incubated at 37 °C in a humidified incubator with 5% CO_2. After 48 h, 20 μL of MTT (Sigma) at 5 mg/mL in PBS (filter sterilized, light protected, and stored at 4 °C) was added to each well. After 4 h of incubation at 37 °C, MTT was converted to a blue formazan product by mitochondrial succinate dehydrogenase. The yielding product was eluted from cells by adding 150 mL of DMSO. The absorbance at 570 nm wavelength was determined with an ELX800 microplate spectrophotometer. The IC_{50} value was defined as the concentration under which 50% of the cells could survive.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.06.046.

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