Bioorganic & Medicinal Chemistry Letters 20 (2010) 6282–6285

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel thienopyridine derivatives as specific anti-hepatocellular carcinoma (HCC) agents: Synthesis, preliminary structure–activity relationships, and in vitro biological evaluation

Xiu-Xiu Zeng [a,b,†], Ren-Lin Zheng [a,†], Tian Zhou [a,†], Hai-Yun He [a], Ji-Yan Liu [c], Yu Zheng [a], Ai-Ping Tong [a], Ming-Li Xiang [a], Xiang-Rong Song [a], Sheng-Yong Yang [a], Luo-Ting Yu [a], Yu-Quan Wei [a], Ying-Lan Zhao [a,*], Li Yang [a,*]

[a] State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medicinal School, Sichuan University, Chengdu 610041, Sichuan, China
[b] Department of Pharmaceutical and Bioengineering, School of Chemical Engineering, Sichuan University, Chengdu 610065, Sichuan, China
[c] Department of Medical Oncology, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medicinal School, Sichuan University, Chengdu 610041, Sichuan, China

ARTICLE INFO

Article history:
Received 25 March 2010
Revised 27 July 2010
Accepted 18 August 2010
Available online 21 August 2010

Keywords:
Hepatocellular carcinoma (HCC)
HCC-specific anticancer activity
Thienopyridine
Structure–activity relationships (SARs)
G_0/G_1 cell cycle arrest

ABSTRACT

Novel thienopyridine derivatives **1b**–**1r** were synthesized, based on a hit compound **1a** that was found in a previous cell-based screening of anticancer drugs. Compounds **1a**–**1r** have the following features: (1) their anticancer activity in vitro was first reported by our group. (2) The most potent analog **1g** possesses hepatocellular carcinoma (HCC)-specific anticancer activity. It can specifically inhibit the proliferation of the human hepatoma HepG2 cells with an IC_{50} value of 0.016 μM (compared with doxorubicin as a positive control, whose IC_{50} was 0.37 μM). It is inactive toward a panel of five different types of human cancer cell lines. (3) Compound **1g** remarkably induces G_0/G_1 arrest and apoptosis in HepG2 cells in vitro at low micromolar concentrations. These results, especially the HCC-specific anticancer activity of **1g**, suggest their potential in targeted chemotherapy for HCC.

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer death and its incidence is increasing worldwide.[1] Liver transplantation is considered to be the only curative therapy at present but a majority (>80%) of patients with advanced and unresectable HCC are not suitable for transplantation or surgical resection.[2] Chemotherapy is one of the commonly used treatment options, especially for patients with unresectable tumors. However, the use of conventional cytotoxic drugs, including doxorubicin, cisplatin, fluorouracil, etc., has not shown any improvement in survival, and severe adverse effects were observed frequently in patients with these treatments.[3] Thus, there is an urgent need to develop targeted chemotherapeutic agents for HCC. The success of sorafenib, which was found to prolong significantly the survival of patients with advanced HCC,[4] suggested that small-molecule targeted chemotherapy is a promising strategy to combat this cancer.[5]

Our research group has been interested in the design, screening, synthesis, and biological evaluation of novel tumor growth inhibitors and apoptosis inducers as potential new anticancer agents.[6] In a previous cell-based screening of anticancer drugs,[7] we found a 3-amino-thieno[2.3-*b*]pyridine derivative, **1a** (Fig. 1) that can inhibit efficiently the growth of human hepatocellular carcinoma HepG2 cells, and induce their apoptosis in vitro.

Although 3-amino-thieno[2.3-*b*]pyridine derivatives demonstrate various biological activities, such as inhibition of IκB kinase-β (IKK-β),[8] modulation of muscarinic acetylcholine receptors (mAChRs),[9] agonist activity towards luteinizing hormone (LH) receptor,[10] promotion of bone formation,[11] etc., their antitumor activity was seldom reported.[12] To the best of our knowledge, the antitumor activity of 6-aryl-3-amino-thieno[2,3-*b*]pyridine derivatives **1a** is being first reported by our group. Here we report

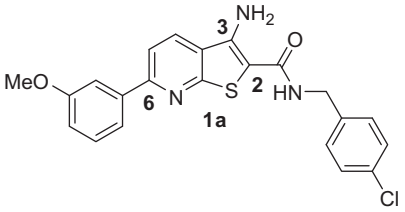
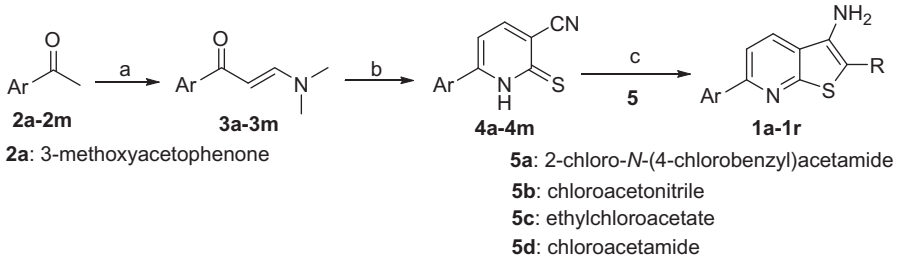


Figure 1. The structure of hit compound **1a**.

* Corresponding authors. Tel.: +86 28 85164063; fax: +86 28 85164060 (L.Y.).
E-mail addresses: alancenxb@sina.com (Y.-L. Zhao), yangli@scu.edu.cn (L. Yang).
† These authors contributed equally to this paper.

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.08.088



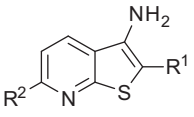
Scheme 1. Synthesis of analogs **1a**–**1r**.[15] Reagents and conditions: (a) DMF-DMA (2.0 equiv), reflux, 4–24 h, 75–95%; (b) DABCO (0.5 equiv), EtOH, reflux, 2–4 h, 40–97%; (c) compound **5**, 10% aq KOH/DMF, rt, 10–15 min and then 85 °C, 6 h, 50–80%.

the synthesis, the structure–activity relationship (SAR) study, and preliminary biological evaluation of this novel class of anticancer agents.

As illustrated in Scheme 1, compound **1a**[13] was prepared readily through the condensation of 6-aryl-3-cyanopyridine-2-(1*H*)-thione **4a** with compound 2-chloro-*N*-(4-chlorobenzyl)acetamide **5a**, via the Thorpe-Ziegler cyclization.[14] The key intermediate **4a** was prepared as follows: readily available acetophenone **2a** was reacted with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) to give enaminone **3a**. Subsequent condensation with cyanothioacetamide, catalyzed by DABCO, yielded **4a** in 80% overall yield. Using the same synthetic strategy shown in Scheme 1, analogs **1b**–**1e** were prepared through the condensation of **4b** with **5a**, chloroacetonitrile (**5b**), ethyl chloroacetate (**5c**), and chloroacetamide (**5d**), respectively. Compounds **1g–1r** were synthesized in parallel from the corresponding aryl ketones **2** with the same synthetic sequence of **1a**–**1e**. The only exception was the analog **1f**, which was prepared via the hydrolysis of the parent compound **1d** under basic conditions. The structures of **1a–1r** were fully characterized by ^{1}H NMR, ^{13}C NMR, and ESI-MS analysis.

As shown in Table 1, 17 analogs of **1a** were synthesized to survey the SAR of the 2- and 6-positions on the thieno[2,3-*b*]pyridine scaffold by evaluating the cell growth inhibitory activity in human hepatocellular carcinoma HepG2 cells, using the MTT assay.

Table 1
Cytotoxicity data for **1a**–**1r**



Compound	R^1	R^2	HepG2 IC_{50}[a] (μM)
1a	*N*-(4-Cl–Bn)NHCO–	3-MeO–Ph–	9.4
1b	*N*-(4-Cl–Bn)NHCO–	4-MeO–Ph–	>100
1c	–CN	4-MeO–Ph–	68
1d	$-CO_2Et$	4-MeO–Ph–	>100
1e	$-CONH_2$	4-MeO–Ph–	0.019
1f	–COOH	4-MeO–Ph–	>100
1g	$-CONH_2$	3-MeO–Ph–	0.016
1h	$-CONH_2$	2-MeO–Ph–	>100
1i	$-CONH_2$	3,4-Di MeO–Ph–	0.72
1j	$-CONH_2$	3,4-Di Cl–Ph–	0.29
1k	$-CONH_2$	2-F–Ph–	0.70
1l	$-CONH_2$	3-F–Ph–	0.63
1m	$-CONH_2$	4-F–Ph–	1.89
1n	$-CONH_2$	3,5-Di CF_3–Ph–	6.32
1o	$-CONH_2$	Biphenyl	44.7
1p	$-CONH_2$	3-Pyridyl	4.76
1q	$-CONH_2$	2-Thiazolyl	1.30
1r	$-CONH_2$	2-Furanyl	1.97
Doxorubicin	—	—	0.37

[a] The cytotoxicity effects of various compounds on HepG2 cells were determined by the MTT assay,[16] and the results were expressed as the mean IC_{50} calculated from three independent experiments.

Among them, the most potent analog **1g** significantly inhibited the proliferation of the human hepatoma HepG2 cells at low nanomolar concentrations (IC_{50} = 16 nM). With compounds **1a**–**1f** in hand, we started our SAR studies at the 2-position (R^1). The importance of the unsubstituted formamide group was revealed by the dramatic loss of activity upon replacement with substituted formamide (**1a** and **1b**), a cyano group (**1c**), formate ester (**1d**), or a carboxylic group (**1f**). The activity of the analog with unsubstituted amide (**1e**, IC_{50} = 0.019 μM) at 2-position was about 500–5000-fold greater than that of the substituted amide analogs (**1a, 1c, 1d**).

After identifying the most potent 2-position substituent, we next turned to examining the SAR at the 6-position (R^2). As shown in Table 1, introduction of a *meta*-methoxy substituent on the phenyl ring (**1g**) in the R^2 group was well tolerated, whereas the *ortho*-substituted analog (**1h**) led to almost total loss of activity. The slightly reduced potency of 3,4-disubstituted analogs (**1i** and **1j**) suggested that the modification at the 3,4-position of the phenyl ring does not compromise activity. Introduction of the electron-withdrawing fluoro group to the phenyl ring at the *ortho*-, *meta*-, and *para*-positions (**1k–1m**) and the strongly electron-withdrawing trifluoromethyl group (**1n**) were also not deleterious to the activity of the parent compound. However, the introduction of a bulky phenyl ring (**1o**) in the R^2 had a detrimental effect on the potency: that is, a fivefold decrease in potency, compared with that of the parent compound (**1a**). Replacement of the phenyl ring in R^2 with heteroaryl rings, such as pyridine (**1p**), thiazole (**1q**), and furan (**1r**) groups yielded compounds with IC_{50} values in the low micromolar range, but showed no clear SAR trends, and a moderate decrease in potency was observed compared with **1g**. It is noteworthy that the most potent analog **1g** (IC_{50} = 0.016 μM) exhibited a nearly 20-fold improvement in inhibitory activity against HepG2 cells over that of doxorubicin as a positive control (IC_{50} = 0.37 μM).

To further study the cytotoxic profile, the hit compound **1a** and the most potent analog **1g** were selected for further evaluation for inhibitory activity against a panel of five different types of human cancer cell lines: breast cancer cell line MCF-7, colorectal cancer cell line HCT-116, lung cancer cell line A549, prostate cancer cell line PC-3, and epithelial cancer cell line A431. Interestingly, compound **1a** showed broad-spectrum antiproliferative activities whereas compound **1g** had a specific cytotoxic effect against HepG2 cells over the other five common types of human cancer cell lines in vitro (Table 2). In addition, compound **1g** also had significant cytotoxicity against human hepatoma Bel-7402 and SMMC-7721 cells, with IC_{50} values <10 μM.

These results suggested that the lead compound **1g** might have different mechanisms of action compared with the parent compound **1a** and its potential in targeted chemotherapy for HCC. To the best of our knowledge, there has been no reported instance of small molecules with HCC-specific anticancer activity. The novel HCC-specificity suggests that **1g** might target one or several HCC-specific targets. Therefore, further mechanism studies of

Table 2
The anti-proliferation activities of compounds **1a**, **1g**, and doxorubicin against various cancer cell lines

Compound	IC_{50}[a] (μM)							
	Bel-7402	SMMC-7721	HepG2	MCF-7	A549	A431	HCT-116	PC-3
1a	13.1	7.6	6.0	9.9	13.4	38.3	35.4	7.4
1g	7.4	0.90	0.016	>100	>100	>100	>100	>100
Doxorubicin	0.049	1.06	0.37	0.75	9.2	0.37	0.07	0.60

[a] The cytotoxicity effects of compounds on various cancer cells were determined by the MTT assay, and the results were expressed as the IC_{50}, that were means calculated from three independent experiments.

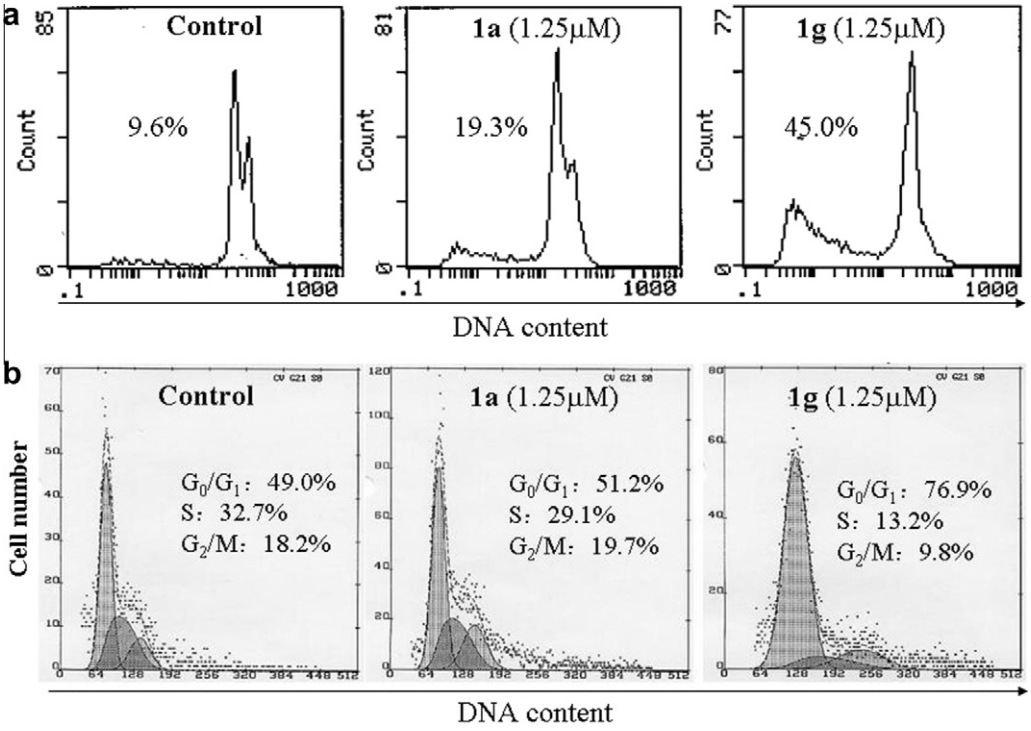


Figure 2. Effects of **1a**, **1g**, and control on the induction of apoptosis and cell cycle distribution. (a) DNA fluorescence histograms of PI-stained HepG2 cells. Cells were treated with 1.25 μM of **1a** and **1g** for 36 h, and the cells in the sub-G_1 phase were considered as apoptotic cells ($n = 5$). (b) Effects of control and 1.25 μM of **1a** and **1g** on cell cycle distribution for 36 h.

1g may provide insight into novel targets for targeted chemotherapy for HCC.

Flow cytometric analysis was used to identify and measure the apoptotic cells (sub-G_1 cells) and the cell cycle after propidium iodide (PI) staining, as described previously[17] to further evaluate the mechanism of the hit compound **1a** and the lead compound **1g**.

As can be seen in Fig. 2a, a 36 h exposure of HepG2 cells to compounds **1a** and **1g** (1.25 μM) resulted in a distinct sub-G_1 peak that represents the population of apoptotic cells.[18] Moreover, a significant accumulation of cells in the G_0/G_1 phase, accompanied by a decrease in G_2/M and S phase was observed after treatment with compound **1g**, whereas no G_0/G_1 arrest was observed after treatment with compound **1a** (Fig. 2b). The percentage of cells in the G_0/G_1 phase was 49.0% for the control, and 51.2% and 76.9% for compounds **1a** and **1g**, respectively. These results suggest that compound **1g** could remarkably induce G_0/G_1 arrest and apoptosis in the human hepatoma cell line HepG2 in vitro at low micromolar concentrations.

In conclusion, novel 3-aminothieno [2,3-*b*]pyridine-2-carboxamide derivatives have been synthesized to survey the SAR of substituents on the thieno[2,3-*b*]pyridine scaffold. The SAR analysis indicated that the substituents of the 2- and 6-positions play a crucial role in the antiproliferative activities. The most potent analog **1g** exhibited an IC_{50} value of 0.016 μM (compared with doxorubicin as a positive control, with an IC_{50} of 0.37 μM) against HepG2 cells. It was inactive toward a panel of five common types of human cancer cell lines. These results, especially the HCC-specific anticancer activity of **1g**, suggest their potential in targeted chemotherapy for HCC. Further lead optimization and mechanism studies are worth pursuing.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC-81000982), National 863 Project (2006AA03Z356) and the Youth Foundation of Sichuan University (No. 2008110). We thank the Analytical and Testing Center of Sichuan University for NMR measurements.

Supplementary data

The list of starting materials **2a**–**2m**, experimental details, and spectroscopic characterization of compounds **3a**, **4a**, **1a**–**1f** and **1h**–**1r**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.088.

References and notes

1. Pisani, P.; Parkin, D. M.; Bray, F.; Ferlay, J. *Int. J. Cancer* **1999**, *83*, 18.
2. Thomas, M. B.; Zhu, A. X. *J. Clin. Oncol.* **2005**, *23*, 2892.
3. Simonetti, R. G.; Liberati, A.; Angiolini, C.; Pagliaro, L. *Ann. Oncol.* **1997**, *8*, 117.
4. Keating, G. M.; Santoro, A. *Drugs* **2009**, *69*, 223.
5. Spangenberg, H. C.; Thimme, R.; Blum, H. E. *Nat. Rev. Gastroenterol. Hepatol.* **2009**, *6*, 423.
6. For representative papers of our groups, see: (a) Zheng, Y. Z.; Zhao, Y. L.; Deng, X. Q.; Yang, S. Y.; Mao, Y. Q.; Li, Z. G.; Jiang, P. D.; Zhao, X.; Wei, Y. Q. *Cancer Invest.* **2009**, *27*, 286; (b) Jiang, P. D.; Zhao, Y. L.; Shi, W.; Deng, X. Q.; Xie, G.; Mao, Y. Q.; Li, Z. G.; Zheng, Y. Z.; Yang, S. Y.; Wei, Y. Q. *Cell. Physiol. Biochem.* **2008**, *22*, 431; (c) Xie, H. Z.; Li, L. L.; Ren, J. X.; Zou, J.; Yang, L.; Wei, Y. Q.; Yang, S. Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1944; (d) Wang, H. Y.; Cao, Z. X.; Li, L. L.; Jiang, P. D.; Zhao, Y. L.; Luo, S. D.; Yang, L.; Wei, Y. Q.; Yang, S. Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4972; (e) Luo, Y. F.; Xu, Y. B.; Chen, L. J.; Hu, J.; Peng, C.; Xie, D. C.; Shi, J. Y.; Huang, W. C.; Xu, G. B.; Peng, M.; Han, J.; Li, R.; Yang, S. Y.; Wei, Y. Q. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4702; (f) Wei, Y. Q.; Wang, Q. R.; Zhao, X.; Yang, L.; Tian, L.; Lu, Y.; Kang, B.; Lu, C. J.; Huang, M. J.; Lou, Y. Y.; Xiao, F.; He, Q. M.; Shu, J. M.; Xie, X. J.; Mao, Y. Q.; Lei, S.; Luo, F.; Zhou, L. Q.; Liu, C. E.; Zhou, H.; Jiang, Y.; Peng, F.; Yuan, L. P.; Li, Q.; Wu, Y.; Liu, J. Y. *Nat. Med.* **2000**, *6*, 1160.
7. Deng, X. Q.; Wang, H. Y.; Zhao, Y. L.; Xiang, M. L.; Jiang, P. D.; Cao, Z. X.; Zheng, Y. Z.; Luo, S. D.; Yu, L. T.; Wei, Y. Q.; Yang, S. Y. *Chem. Biol. Drug. Des.* **2008**, *71*, 533.
8. (a) Wu, J. P.; Fleck, R.; Brickwood, J.; Capolino, A.; Catron, K.; Chen, Z. D.; Cywin, C.; Emeigh, J.; Foerst, M.; Ginn, J.; Hrapchak, M.; Hickey, E.; Hao, M. H.; Kashem, M.; Li, J.; Liu, W. M.; Morwick, T.; Nelson, R.; Marshall, D.; Martin, L.; Nemoto, P.; Potocki, I.; Liuzzi, M.; Peet, G. W.; Scouten, E.; Stefany, D.; Turner, M.; Weldon, S.; Zimmitti, C.; Spero, D.; Kelly, T. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5547; (b) Morwick, T.; Berry, A.; Brickwood, J.; Cardozo, M.; Catron, K.; Turi, M. D.; Emeigh, J.; Homon, C.; Hrapchak, M.; Jacober, S.; Jakes, S.; Kaplita, P.; Kelly, T. A.; Ksiazek, J.; Liuzzi, M.; Magolda, R.; Mao, C.; Marshall, D.; McNeil, D.; Prokopowicz, A.; Sarko, C.; Scouten, E.; Sledziona, C.; Sun, S. X.; Watrous, J.; Wu, J. P.; Cywin, C. L. *J. Med. Chem.* **2006**, *49*, 2898.
9. Shirey, J. K.; Xiang, Z.; Orton, D.; Brady, A. E.; Johnson, K. A.; Williams, R.; Ayala, J. E.; Rodriguez, A. L.; Wess, J.; Weaver, D.; Niswender, C. M.; Conn, P. J. *Nat. Chem. Biol.* **2008**, *4*, 42.
10. Straten, N. C. R. V.; Schoonus-Gerritsma, G. G.; Someren, R. G. V.; Draaijer, J.; Adang, A. E. P.; Timmers, C. M.; Hanssen, R. G. J. M.; Boeckel, C. A. A. V. *ChemBioChem* **2002**, *10*, 1023.
11. Satoru, N.; Akira, N.; Tsuyoshi, S.; Keiji, S., Kiyoshi, O. WO2007043581, 2007.
12. (a) Hayakawa, I.; Shioya, R.; Agatsuma, T.; Furukawa, H.; Sugano, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3411; (b) Claudia, R.; Alexander, L.; Alexander, S.; Mohammed, D.; Siegfried, L.; Andreas, K.; Jochen, H. WO2006074919, 2006.
13. The sample of **1a** for screening was purchased from Specs Company. Because there is no synthetic literature of compound **1a**, we had to synthesize compound **1a** and its analogs.
14. (a) Litvinov, V. P.; Dotsenko, V. V.; Krivokolysko, S. G. *Russ. Chem. Bull.* **2005**, *54*, 864; (b) Yakovlev, M. Y.; Kadushkin, A. V.; Granik, V. G. *Khim. Farm. Zh.* **1996**, *30*, 36.
15. Typical procedure for the synthesis of **1a**–**1e** and **1g**–**1r**.
    Compound **1g** was prepared as follows: To a suspension of **4a** (1.0 g, 4.2 mmol) in DMF (6 ml) was added aq KOH solution (1.8 M, 3.0 ml) followed by the addition of chloroacetamide (0.5 g, 5 mmol) at room temperature. The resulting mixture was stirred for 10–15 min at room temperature and then heated to 85 °C for 6 h after the addition of additional aqueous KOH solution (1.8 M, 3.0 ml). After the reaction mixture was allowed to cool to room temperature, the precipitate was collected by filtration, and washed with cold ethanol, then recrystallized from ethanol to give a yellow solid (0.95 g, 75% yield), Mp: 234–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.51 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.72 (s, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.06 (dd, J = 8.0, 2.0 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.95, 159.72, 158.67, 156.10, 145.51, 139.39, 131.61, 129.92, 125.31, 119.29, 116.36, 115.34, 112.02, 97.47, 55.18; ESI-MS: m/z 334.16 $[M+Cl]^-$.
16. Briefly, cells (2000/well) were seeded in 96-well plates and cultured for 24 h, followed by treatment with the compounds for 48 h. Ten microliters of 10 mg/ml MTT was added per well and incubated for another 2.5 h at 37 °C. Then the supernatant fluid was removed and 150 μL/well DMSO was added for 15–20 min. The absorbance (OD) of each well was measured at 570 nm, using a SpectraMAX M5 microplate spectrophotometer (Molecular Devices).
17. Peng, F.; Wei, Y. Q.; Tian, L.; Yang, L.; Zhao, X.; Lu, Y.; Mao, Y. Q.; Kan, B.; Lei, S.; Wang, G. S.; Jiang, Y.; Wang, Q. R.; Luo, F.; Zou, L. Q.; Liu, J. Y. *J. Cancer Res. Clin. Oncol.* **2002**, *128*, 223.
18. Wei, Y. Q.; Zhao, X.; Kariya, Y.; Fukata, H.; Teshigawara, K. *Uchida. Cancer Res.* **1994**, *54*, 4952.