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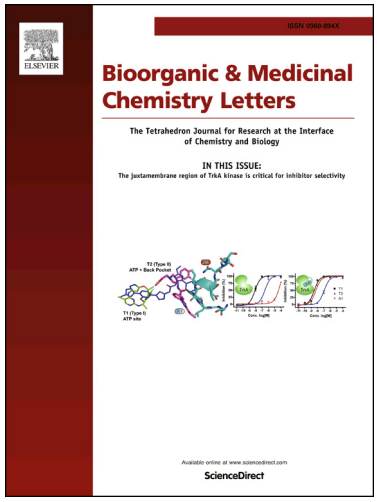

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PII: S0960-894X(17)30245-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.03.010
Reference: BMCL 24762

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 23 November 2016
Revised Date: 19 February 2017
Accepted Date: 6 March 2017

Please cite this article as: He, G-X., Yuan, J-M., Zhu, H-M., Wei, K., Wang, L-Y., Kong, S-L., Mo, D-L., Pan, C-X., Su, G-F., Synthesis and antitumor evaluation of 2,3-diarylbenzofuran derivatives on HeLa cells, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.03.010

Bioorganic & Medicinal Chemistry Letters
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Synthesis and antitumor evaluation of 2,3-diarylbenzofuran derivatives on HeLa cells

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
2,3-diarylbenzofuran
multistep one-pot reaction
antitumor activity against HeLa cells

ABSTRACT

2,2-Dihydroxyarylethanones, readily prepared from the commercially available aromatic ethyl ketones, were reacted with resorcinol, 3-methoxyphenol or 2-methoxyphenol in multi steps one-pot manner promoted by trifluoroacetic acid to furnish the 2,3-diarylbenzofuran derivatives in 22~95% yield. Sixteen targeted compounds were synthesized and characterized by ^{1}H NMR, ^{13}C NMR and HRMS. MTT assay indicated that most compounds possessed effectively inhibitory activities against the proliferation of Hela cell. Among them, **4f** had the highest inhibitory activities, with the IC_{50} being 13.40 ± 2.04 μmol/L. Cell cycle analysis, Annexin V-FITC/propidium iodide dual staining assay and western blotting analysis revealed that **4f** inhibited the proliferation of Hela cell through apoptosis induction in a dose-dependent manner *via* obviously up-regulated the levels of Bak and Bim, while striking down-regulated the level of Bcl-2 and Bcl-xL protein.

Benzofuran scaffold presents as a key structural unit in numerous bioactive natural products and synthetic pharmaceutical compounds.[1] Many benzofuran derivatives exhibit a broad range of biological activities such as anticancer,[2] antimicrobial,[3] antifungal,[4] antioxidative,[5] anti-HIV activities,[6] some of which were served as acetylcholinesterase inhibitors[7] and histone deacetylase inhibitors.[8]

Among the structurally diverse benzofuran derivatives, the 2,3-diarylbenzo[*b*]furan has gained considerable attention.[9] Structurally, its framework was the combination of three pharmacologically important subunits of benzofuran, diarylmethane[10] and 1,2-diphenylethylene (stilbene).[11] Many bioactive natural products also had been found possessing a 2,3-diarylbenzo[*b*]furan scalfold.[9a,9g,9l-9n] In particular, the derivatives present one or more unprotected hydroxyl groups occupying an especially important position, since this functional group was widely found in natural medicines[9a,9e,9g,9l-9n] and some representative compounds were depicted in Fig. 1.

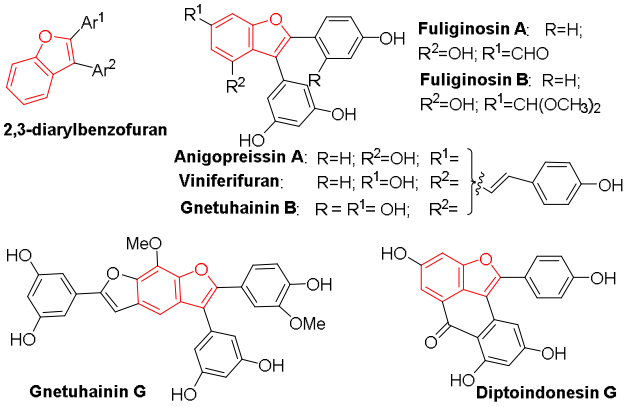

Fig. 1. Selective examples for bioactive natural products containing the 2,3-diarylbenzo[*b*]-furan core

Up to now, many synthetic methodologies developed to access to these important heterocyclic compounds were focused on the transition metal-catalyzed protocol which was usually required for expensive metal catalysts and complex ligands.[9a-9k] And most of the available protocols were difficult to enlarge to provide enough samples for pharmacological studies. What's more, very few of them could provide direct synthesis of 2,3-diarylbenzo[*b*]-furan derivatives with unprotected hydroxyl groups. Therefore, to develop an efficient, material readily available and easy-to-handle procedure for synthesis of 2,3-diaryl benzo[*b*]furans, especially those can directly access the products with unprotected hydroxyl groups, are still very much desired.

Herein, we would like to report a simple and efficient protocol for the synthesis of 2,3-diarylbenzofuran derivatives with at least one unprotected hydroxyl group from readily available aromatic

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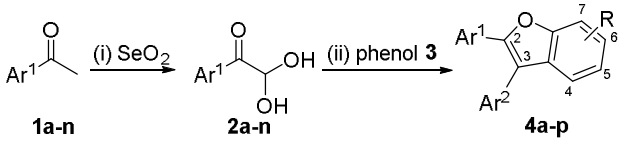
ethyl ketones and phenols, whose inhibitory activities against the proliferation of HeLa cells *in vitro* were investigated.

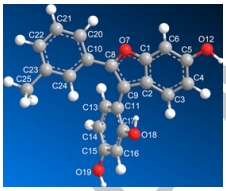
Inspired by the reactions of ninhydrin with phenols reported by Kim and Jung[12] (Scheme 1A, see Supporting Information), we considered that a novel route for the synthesis of benzoins (Scheme 1B, see SI) might be developed based on the indeno[1,2-*b*]benzofuranones synthesis procedure. Prompted by this idea, the 2,2-dihydroxy-1-phenylethanone **2a**, readily prepared from acetophenone followed the literature procedure[13] with slight modification, was mixed with resorcinol (**3a**) and subjected to AcOH, BF_3-Et_2O, H_2SO_4, CF_3CO_2H (TFA) from room temperature to reflux conditions. When H_2SO_4 was used, the reaction was complicated and the reactants easily carbonized. With AcOH or BF_3-Et_2O, the reaction was incomplete and a lot of reactants were recovered. TFA gave the best result and a new product could be isolated in 38% yield, and structural analysis indicated that it was not benzoin derivative but 2,3-diarylbenzofuran derivative.

After further optimizing reaction conditions (Scheme 2), different 2,2-dihydroxy-1-aryl-ethanones **2** reacted with phenols to afford sixteen 2,3-diarylbenzofuran derivatives **4** in 22-95% yield (Table 1). The 3D structure of **4f** indicated that the dihedral angle between Ar^2 and benzofuran ring is about 45º (see SI).



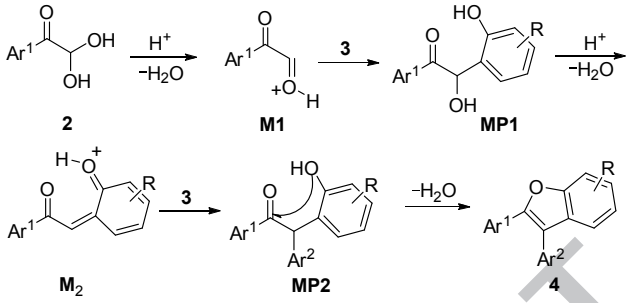
3: resorcinol, *m*-methoxyphenol, *o*-methoxyphenol

4a, **4d-4p**: R= 5-OH, Ar^2=2,4-diOH-C_6H_3;
4b: R= 5-OCH_3, Ar^2=2-OH-4-OCH_3-C_6H_3
4c: R= 6-OCH_3, Ar^2=2-OH-3-OCH_3-C_6H_3


3D structure of **4f**

Scheme 2. General methods to prepare 2,3-diarylbenzofuran derivatives **4**. Reagents and conditions: (i) SeO_2 (2 mmol), 1,4-dioxane (1 mL), H_2O (2 drops), 80 °C, 7 h, 61~82%; (ii) phenols **3** (2.5 mmol, 3 equiv.), CF_3CO_2H (1 mL), THF (1 mL), 80~100 °C, 18~24 h, 22~95%.

The plausible mechanism[14] for the formation of **4**, might start from the protonation of **2** to form the carbocation **M1**, which could undergo a Friedel-Crafts reaction with **3** to give the intermediate **MP1**. Then the **MP1** was further protonated and converted to carbocation **M2**, followed by another Friedel-Crafts reaction with **3** to afford the intermediate **MP2**. **MP2** continued to undergo an intramolecular condensation, with the driving force being aromatized to the benzofuran ring and afforded the target compound **4** (Scheme 3).



Scheme 3. Plausible mechanism for the formation of **4**

Since the 2,3-diarylbenzofuran derivatives **4** possess the structural characteristic of the anti-estrogens agents used to treat the estrogen receptor-dependent cancer, we evaluated their inhibitory activities against the proliferation of HeLa cell lines *in vitro* by using MTT assay and the results were summarized in Table 2.

Table 2. Anticancer activities of **4** against HeLa cell lines

Compd.	IC_{50}/(μM) [a]	Compd.	IC_{50}/(μM) [a]
4a	29.70	**4i**	29.21
4b	28.88	**4j**	28.31
4c	57.78	**4k**	32.74
4d	47.54	**4l**	36.72
4e	>100	**4m**	32.02
4f	13.40	**4n**	13.71
4g	26.87	**4o**	19.89
4h	29.91	**4p**	21.15
HCPT	10.04		

[a]Each IC_{50} value was calculated from three independent experiments performed in triplicate. Errors were within the range of 5~10% of the reported values.

As shown in Table 2, most compounds exhibited cytotoxicity activities against the proliferation of HeLa cell with IC_{50} values of micromole level, among which **4f** had the highest inhibitory activities, with the IC_{50} being 13.40 μM, almost similar potency to the positive control **HCPT**. Among the natural benzofuran derivatives, only the anti-cancer activities of Diptoindonesin G and Anigopreissin A derivatives were reported.[15] Diptoindonesin G[15a] showed anti-proliferation effect against P-388 cells (IC_{50} =13.2±1.1μM) and Permethylated Anigopreissin A exhibited cytotoxicity towards HepG2 and MCF7 cells, with the IC_{50} being 0.24±0.05 and 0.85 ± 0.04 μM, respectively.[15b]

To investigate the molecular mechanism of the targeted compounds underlying the inhibitory effect on HeLa cell, **4f** was chosen to conduct further bioactive investigation.

Table 1. Chemical structure and yield of synthesized compounds **4a-4p**

Compd.	Ar^1	Yield (%) [a]	Compd.	Ar^1	Yield (%) [a]
4a	Ph	60	**4i**	4-CH_3-C_6H_4	57
4b	Ph	36	**4j**	4-F-C_6H_4	68
4c	Ph	22	**4k**	4-Cl-C_6H_4	79
4d	2-CH_3-C_6H_4	74	**4l**	4-Br-C_6H_4	94
4e	2-Br-C_6H_4	95	**4m**	4-HO-C_6H_4	66
4f	3-CH_3-C_6H_4	44	**4n**	4-MeO-C_6H_4	72
4g	3-Br-C_6H_4	94	**4o**	3-pyridinyl	24
4h	3-MeO-C_6H_4	65	**4p**	2-naphthyl	73

[a] Isolated yield

Firstly, HeLa cell lines were treated with different concentrations of **4f** and the cell inhibition was measured by MTT assay. The results in Fig. 2 revealed that the cell inhibition increased remarkably when the concentrations of **4f** changed from 10 μM to 20 μM, the cell inhibition would increase from about 30% to 65%. These data indicated that compound **4f** might be a potential anticancer agent in a dose-dependent manner.

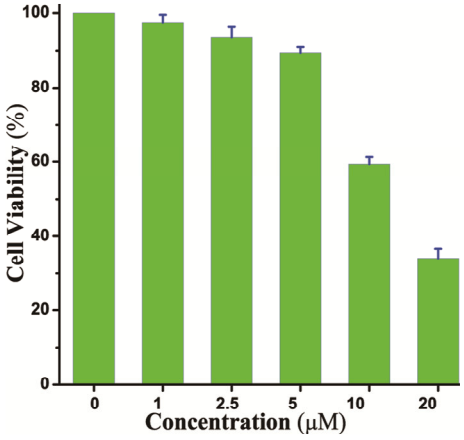


Fig. 2. Dose-dependent manner effect of **4f** on HeLa cells

Then, the effect of **4f** on the cell cycle distribution was investigated. As shown in Fig. 3, the populations of every period had no obvious change with an increasing concentration of **4f** after 48 h, which indicated that **4f** might inhibit the cell proliferation via other signaling pathways rather than by blocking the function of the cell cycle.

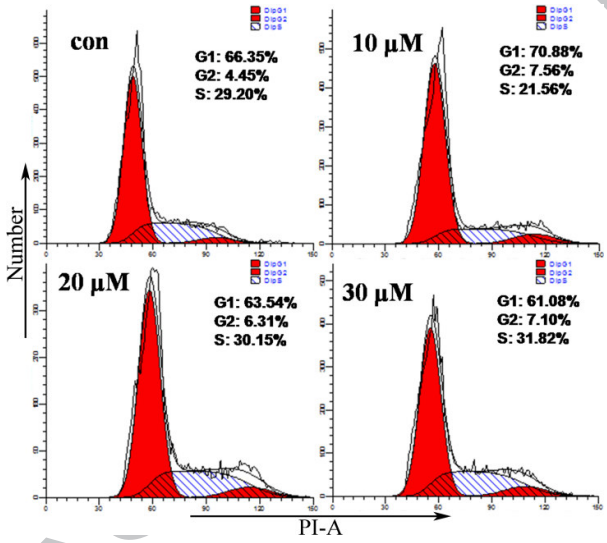


Fig. 3. Results of cell cycle distribution by flow cytometry. Cells was treated with increasing concentrations of **4f** (10 μM, 20 μM, 30 μM, respectively) for 48 h and untreated HeLa as a control.

Thirdly, the Annexin V-FITC/propidium iodide assay to determine the mode of cancer cells death induced by **4f** was conducted. As shown in Fig. 4, the apoptosis ratios of the HeLa cell treated with **4f** in different concentration were found obviously increasing from 15.3% (10 μM of **4f**), 27.5% (20 μM of **4f**) to 46.5% (30 μM of **4f**). These results indicated that the cell death induced by the **4f** was mainly caused by the apoptotic pathway.

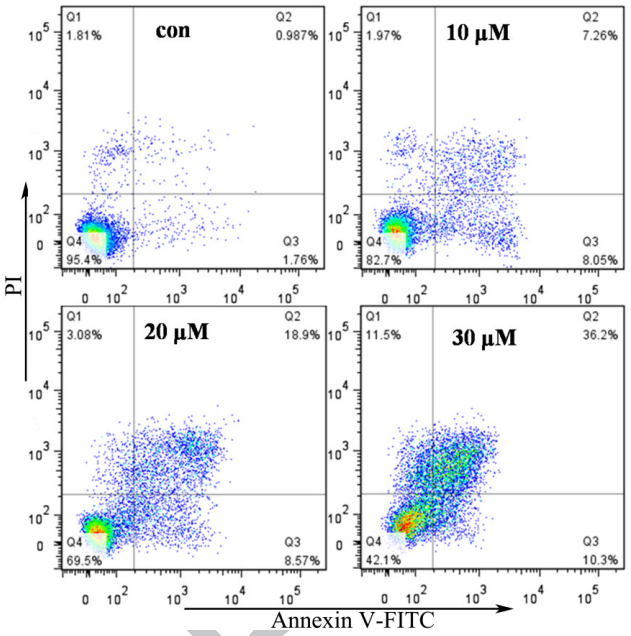


Fig. 4. Induction of apoptosis by **4f** in HeLa cells with the density of 0 μM 10 μM, 20 μM, 30 μM, for 24 h, then stained with fluorescence FITC and PI, analyzed by flow cytometer.

Finally, the western blot analysis results in Fig. 5 revealed that **4f** could obviously up-regulate the levels of Bak and Bim protein, while striking down-regulated the level of Bcl-2 and Bcl-xL protein, which further confirmed the underlying mechanism of the inducing apoptosis by **4f**.

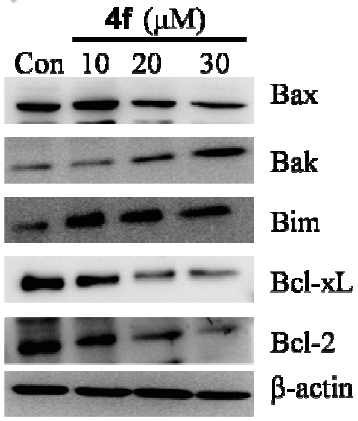


Fig. 5. Western blot analysis of the HeLa cells treated with **4f** (0 μM, 10 μM, 20 μM, 30 μM, respectively) on the cell apoptosis proteins. *β*-actin served as the internal control.

In conclusion, we developed a novel protocol for synthesis of 2,3-diarylbenzofuran derivatives in one-pot two steps starting from readily available aromatic ethyl ketone and resorcinol, 3-methoxyphenol or 2-methoxyphenol. This protocol provides an efficient, material readily available and easy-to-handle procedure to afford the 2,3-diarylbenzo[*b*]furans with bioactive important unprotected hydroxyl group in moderate to high yield. The results of antitumor activity evaluation *in vitro* against HeLa cells indicated that most of the targeted compounds exhibit potent cytotoxicity with IC_{50} values at micromole level. They could inhibit the proliferation of HeLa cell through apoptosis inducing in a dose-dependent manner by up-regulating the levels of Bak and Bim, and down-regulating the level of Bcl-2 and Bcl-xL protein, and **4f** could serve as a potential anticancer reagent for further investigation.

Acknowledgments

Financial support by National Natural Science Foundation of China (21162002, 21462008), Guangxi Natural Science Foundation (2014GXNSFAA118053, 2015GXNSFDA139009), and State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources, Ministry of Science and Technology of China (CMEMR2011-17, CMEMR2012-A014, CMEMR2014-A01) were greatly appreciated.

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Synthesis and antitumor evaluation of 2,3-diarylbenzofuran derivatives on HeLa cells

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Guo-Xue He[†], Jing-Mei Yuan[†], Hai-Miao Zhu, Kai Wei, Ling-Yun Wang, Shi-Lin Kong, Dong-Liang Mo, Cheng-Xue Pan[*], Gui-Fa Su[*]