Identification of novel ROS inducer by merging the fragments of piperlongumine and dicoumarol

Xiaojuan Xu, Xia Fang, Jun Wang, Hong Zhu

PII: S0960-894X(16)30837-X
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.08.016
Reference: BMCL 24142

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 20 May 2016
Revised Date: 14 July 2016
Accepted Date: 6 August 2016

Please cite this article as: Xu, X., Fang, X., Wang, J., Zhu, H., Identification of novel ROS inducer by merging the fragments of piperlongumine and dicoumarol, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.08.016

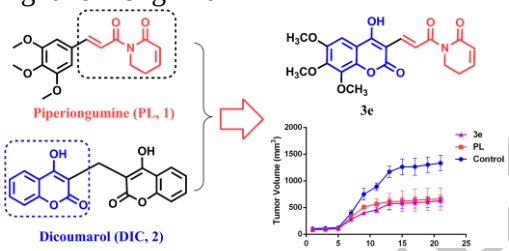
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Identification of novel ROS inducer by merging the fragments of piperlongumine and dicoumarol

Leave this area blank for abstract info.

Xiaojuan Xu[a, *], Xia Fang[a], Jun Wang[a] and Hong Zhu[a]



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Identification of novel ROS inducer by merging the fragments of piperlongumine and dicoumarol

Xiaojuan Xu[a, *], Xia Fang[a], Jun Wang[a] and Hong Zhu[a]

[a] *School of Chemistry and Chemical Engineering, Yancheng Teachers University, Yancheng, China*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Piperlongumine
Dicoumarol
antitumor
Reactive oxygen species
Biological activity

ABSTRACT

A series of novel ROS inducers were designed by merging the fragments of piperlongumine and dicoumarol. Most of these derivatives showed potent in vitro activity against three cancer cell lines and good selectivity towards normal lung cells. The most potent and selective compound **3e** was proven to exhibit obvious ROS elevation and excellent *in vivo* antitumor activity with suppressed tumor growth by 48.46% at the dose of 5 mg/kg. Supported by these investigation, these findings encourage further investigation around this interesting antitumor chemotype.

* Corresponding author. Tel.: +86 0515 8823 3186; fax: +86 0515 8823 3186; e-mail: xxjyctu@163.com.

Cancer remains one of the most threatening diseases in the world. Lots of financial resources have been devoted to discover cancer-related drugs.[1-2] Over the past decades notable progress has been achieved on developing new antitumor active drugs, but there is still urgent to develop new and safe drugs to target tumor cell specific mechanisms. There is a new strategy to target emerging hallmark of cancer cells such as oxidative stress which is a key bioenergetics alterations observed in cancer cells.[3] Cancer cells are usually exposed to elevated levels of reactive oxygen species (ROS) and excessive ROS levels irreversibly damage DNA and lipids which would ultimately lead to apoptosis of cancer cells.[4-5]

Compared with cancer cells, normal cells seem to have much lower ROS levels of endogenous oxidative stress in culture and *in vivo*.[6] Actually, developing compounds that exploit ROS in cancers is a novel and promising therapeutic approach in drug discovery.[6-7] A number of small molecule ROS inducers have been discovered with diverse scaffolds and exert their anticancer activity dependent on the high basal ROS levels uniquely present in cancer cells.[8-10] Among them, piperlongumine (PL), a small molecule ROS generator was found to selectively kill cancer cells in recent years.[11-14] The tumor specific effect of PL on cytotoxicity and ROS shows a new strategy for selective targeting of cancer cells.[10-11] Since the natural product has been reported to be efficient ROS inducers, many derivatives of PL have been synthesized to explore the structure and biological activity relationship.[10-11, 15-16] Several key pharmacophores, such as C2-C3 and C7-C8 double bonds, have been regarded as necessary for PL's toxicity to cancer cells.[11] Meanwhile, another natural product dicoumarol (DIC), chemically termed as 3,3'-methylenebis (4-hydroxycoumarin) shows cytotoxic effects on several malignant cell types as previously reported in various in vitro and in vivo studies and is considered as an anticancer agents.[17]

On careful review at PL and DIC, it was noticed that these two natural products may share same skeleton. In view of this, we initially merged the two natural products into a new structure of **3a** that retained the key pharmacophores for antitumor activity. In order to expand and identify novel PL derivatives, we designed a series of new redox-modulating agents (**3a-3g**) which were based on the merged structure (Fig. 1). Eight compounds have been synthesized and tested for antitumor activity both *in vitro* and *in vivo*.

The synthesis of the target compounds **3a-3h** were shown in Scheme 1. Compound **5** was synthesized via the Vilsmeier Haack reaction by refluxing substituted 4-hydroxy-2-oxo-2H-1-benzopyrans (**4**) with dimethyl formamide and phosphorous oxychloride. Condensation substituted 4-hydroxy-2-oxo-2H-chromene-3-carbaldehyde (**5**) with malonic acid in pyridine yielded the intermediate substituted (*E*)-3-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)acrylic acids (**6**). Treatment of **6** with oxalyl chloride in anhydrous toluene under N_2 gave the acyl chlorides **7** which were reacted with piperidin-2-ones to afford PL derivatives **9** using anhydrous THF as solvent and Et_3N as catalyst. Subsequently, treatment of compound **9** with phenylselenyl chloride gave the α-phenylseleno imide **10** which then subject to oxidation with hydrogen peroxide to obtain the target compounds **3a-3g** in 70~80% yields. In addition, palladium-carbon catalyzed hydrogenation of the double bond of 3a to provide the target compound **3h** in 56% yield. The structures of all target compounds were confirmed by ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS). Before these compounds were used in biological experiments, they were purified by silica gel column chromatography and HPLC was used to determine their purity (all > 95%).

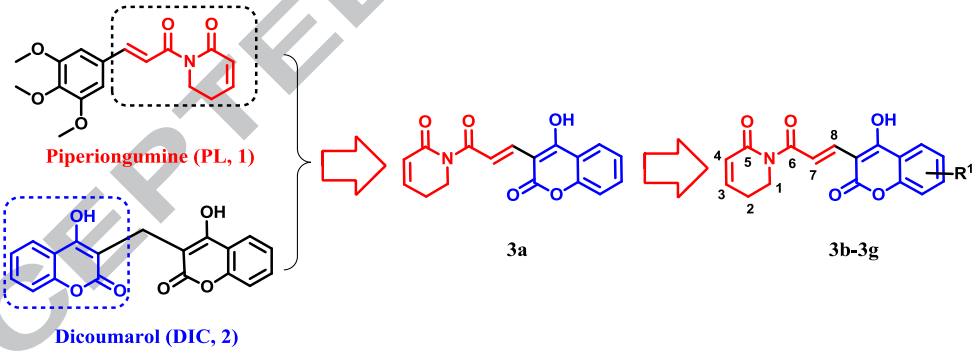


Figure 1. Representative structures of known inhibitors of NQO1.

Cytotoxicity studies were first performed on all of the PL derivatives (**3a-3h**). Cell survival was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay. We utilized four cancer cell lines (non-small cell lung cancinoma A549, human colorectal carcinoma HCT116, hepatocellular carcinoma HepG2) and human lung normal cells MRC-5 to compare the cytotoxicity of these compounds. As illustrated in Table 1, most of these PL derivatives showed potent activities against the three cancer cell lines. In particular, the 6,7,8-methoxy substituted derivative **3e** exhibited IC_{50} values less than 10 μM against all the cancer cell lines. Reduction or replacement of the methoxy group of the most active compound **3e** with mono-methoxy, methyl or halogen groups resulted in less potent derivatives **3a-3d** and **3f-3g** respectively. Saturated derivative **3h** was not potent against all cell lines, indicated the necessity of the presence of C_3-C_4 and C_7-C_8 double bonds in PL derivatives. The target compounds were further evaluated for their toxicity towards the human lung normal cells MRC-5, a superior safety profile of most compounds (**3a-3e**) was confirmed. Due to the selective toxic towards cancer cell lines, **3e** was selected for further evaluation.

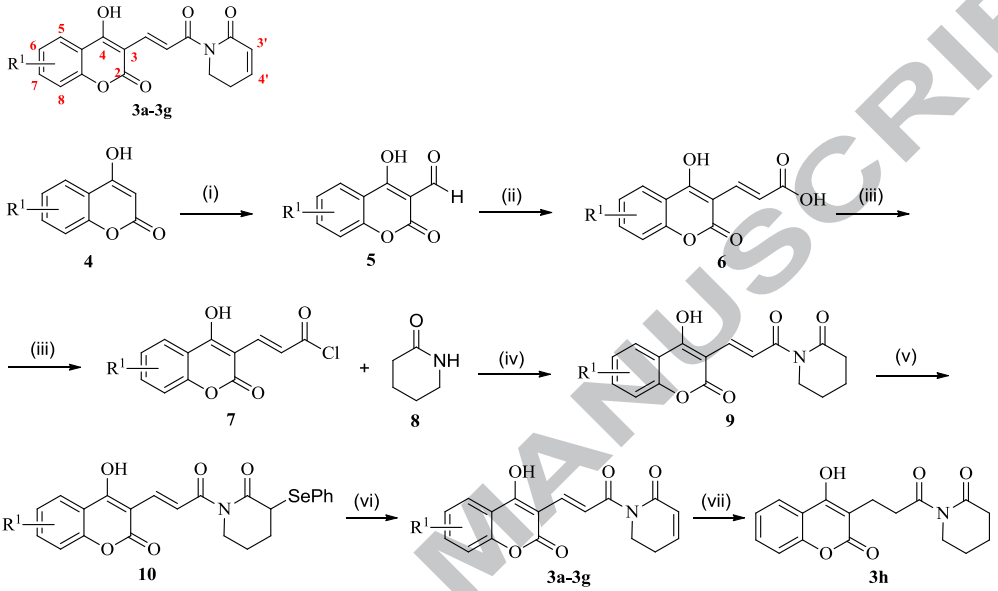
It is supported that these PL derivatives exerted their antitumor activity by accumulating ROS in cancer cells, we next determined the effect the representative compound **3e** on cellular ROS level in A549 cancer cells by fluorescence microscopy. 2',7'-Dichlorfluorescein diacetate (DCFH-DA) is a specific oxidation-sensitive fluorescent probe to detect total intracellular production of ROS. After being uptaken by cells, DCFH-DA is hydrolyzed by cellular esterases to dichlorodihydrofluorescin (DCFH), which is trapped within the cell. The nonfluorescent DCFH is then oxidized to fluorescent dichlorofulorescent by action of cellular ROS. As shown in Fig. 2A, after treating A549 cells with 5 μM or 10 μM **3e** for 1 h, a marked increase in ROS levels was caused. Besides, remarkable elevation of the ROS

production was also observed in long time treatment (24 h and 48 h) with 2 μM **3e** (Fig. 2). The results indicated that **3e** induced ROS production in dose- and time-dependent manners in cancer cells. It was speculated that the induced ROS production of **3e** in cancer cell lines may relate to the interaction with recombinant thioredoxin reductase (TrxR) and alters the antioxidant system. Further investigation is underway by our laboratory.[18-23] In addition, the increased ROS levels might be exerted in A549 cancer cell lines mediated apoptosis.

Table 1

In vitro antitumor activity of derivatives

Cpds	R	IC_{50} (μM)			
		A549	HCT116	Hep G2	MRC-5
3a	H	12 ± 2.2	15 ± 2.1	6.7 ± 0.5	22 ± 2.2
3b	C_6,C_7-Me	15 ± 1.3	5.1 ± 1.1	7.8 ± 0.3	35 ± 1.9
3c	C_6-OCH_3	9.8 ± 1.2	10 ± 0.6	7.1 ± 1.3	53 ± 4.1
3d	C_7- OCH_3	11 ± 2.4	18 ± 0.2	12 ± 1.2	31 ± 5.9
3e	C_6,C_7,C_8-OCH_3	4.2 ± 0.1	3.2 ± 0.1	5.7 ± 0.9	57 ± 1.2
3f	C_7-Cl	6.5 ± 2.0	12 ± 1.0	15 ± 1.3	19 ± 1.1
3g	C_7-F	18 ± 0.3	11 ± 1.6	9.7 ± 0.9	8.9 ± 2.3
3h	-	> 90	> 90	> 90	58 ± 8.1
PL	-	10 ± 2.1	9.2 ± 3.5	8.9 ± 1.8	37 ± 5.4



Scheme 1. Synthesis of the derivatives of PL. Reagents and conditions: (i) DMF/$POCl_3$, 45 °C, 8 h, 72%; (ii) malonic acid, pyridine, benzene, reflux, 6 h, 60%; (iii) $SOCl_2$, toluene, 80 °C, 4 h; (iv) THF, Et_3N, rt, 12 h, 60-70%. (v) PhSeCl, LDA, THF, -50~-78 °C, 5-7 h, 20-30%; (vi) H_2O_2, THF, 0 °C, 30-60 min, 70-80%. (vii) H_2, Pd/C, r.t., 10 h, 56%.

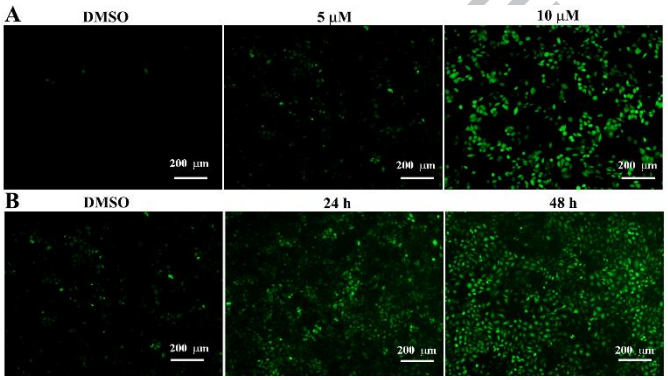


Figure 2. Induction of ROS in A549 cells. (A) The A549 cells were treated with different concentration of **3e** for 1 h followed by the incubation with DCFH-DA (10 μM) for 30 min. (B) The A549 cells were treated with a fixed concentration of **3e** (2 μM) for 24 h and 48 h followed by the incubation with DCFH-DA (10 μM) for 30 min. The fluorescence images were obtained by inverted fluorescence microscopy.

We next examined apoptosis in A549 cells following treatment with **3e**. When A549 cells were incubated with different concentration of **3e** for 24 h followed by DAPI staining, an increasing number of cells displayed condensed nuclei, a characteristic morphology of cells undergoing apoptosis (Fig. 3). A specific analysis revealed nuclei with dose-dependent chromatin condensation and characteristic morphological changes of apoptosis, in cell cultures with **3e**.

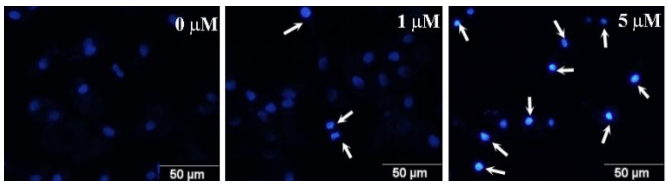


Figure 3. Compound **3e** induced apoptosis in A549 cells in a dose-dependent manner.

To verify the potential drug-like property of compound **3e**, then the compound was further selected to determine its physicochemical property before *in vivo* evaluation. As shown in Table 2, compound **3e** exhibited acceptable Log $D_{7.4}$ value and much improved solubility than PL. In addition, **3e** also displayed acceptable membrane permeability, with *Pe* value of 20.21×10^{-6} cm/s. Permeability is an important property reflecting the ability of molecules to diffuse through the cell membrane. Taking together, compound **3e** possessed balanced properties between hydrophilicity and hydrophobicity. This compound could be selected for further evaluation *in vivo* due to its potent *in vitro* activity and proper physicochemical property.

Table 2

Physicochemical properties of compound **3e**.

Cpds	Log D, pH = 7.4	instrinsic solubility (μg/mL)	*Pe*, pH = 7.4 (10^{-6} cm/s)[a]
3e	0.42	315.8	20.21
PL	2.84	0.75	24.56

[a]Ketoprofen (1.98 × 10^{-6} cm/s) and propranolol (117.21 × 10^{-6} cm/s) are internal standards in permeability determinations.

Having obtained the favorable *in vitro* cellular activity, selectivity for normal cells and proper physicochemical property, compound **3e** was selected for further *in vivo* antitumor activity studies in A549 tumor xenografts mouse model (Fig. 4). Nude mice bearing established A549 tumor xenografts were injection with compound **3e** (5 mg/kg daily over a 21-day period). PL was employed as a positive control drug. As shown in Fig. 4, the results demonstrated that significant antitumor effects were observed in mice treated with **3e** which was administered intraperitoneally (ip) every other day for three weeks. Compound **3e** (tumor growth inhibition = 48.46%) showed the same level of *in vivo* antitumor potency with the control PL (tumor growth inhibition = 41.23%). Furthermore, in the mice treated by **3e**, no significant body weight loss was observed compared with the vehicle control group. Therefore, the results indicated a low toxic compound **3e** which would be promising anticancer therapeutic for the treatment of cancer.

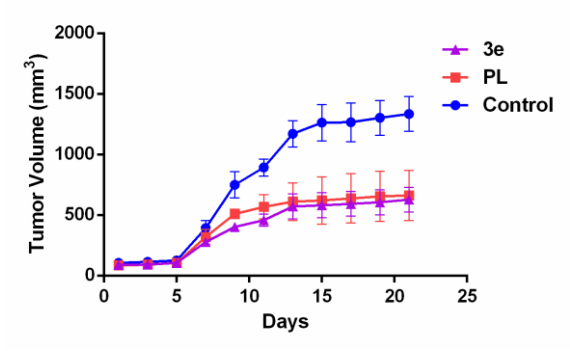


Figure 4. Compound **3e** retards the tumor growth *in vivo* in A549 tumor xenografted nude. The tumor volume measurement. PL (5 mg/kg), **3e** (5 mg/kg) ($p < 0.05$).

In this present work, a key scaffold of 3-(4-hydroxy-2-oxo-2H-chromen-3-yl)acryloyl)-5,6-dihydropyridin-2(1*H*)-one was merged from two natural products, PL and DIC. Based on the scaffold, a series of novel PL derivatives were designed, synthesized and evaluated. These derivatives were characterized and evaluated as efficient antitumor agents *in vitro* and showed modest selectivity for human lung normal cells MRC-5. Among them, the most potent compound **3e** also showed superior safety profile than the control PL *in vitro*. Furthermore, this highlighting compound **3e** was proven to exhibit obvious ROS elevation and excellent *in vivo* antitumor potency. Supported by these investigation, these findings encourage further investigation around this interesting antitumor chemotype.

Acknowledgments

We are thankful for the financial support of the National Natural Science Foundation of China (no. 21102125).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at

References and notes

1 Available online: www.who/int/cancer/en (accessed 02.09.2015).
2 Wiemann, J.; Heller, L.; Csuk, R. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 907−909.
3 Ai, Y.; Zhu, B.; Ren, C.; Kang, F.; Li, J.; Huang, Z.; Lai, Y.; Peng, S.; Ding, K.; Tian, J.; Zhang, Y. *J. Med. Chem.* **2016**, *59*, 1747−1760.
4 Dickinson, B. C.; Chang, C. J. *Nat. Chem. Biol.* **2011**, *7*, 504−511.
5 Szatrowski, T. P.; Nathan, C. F. *Cancer Res.* **1991**, *51*, 794−798.
6 Kawanishi, S.; Hiraku, Y.; Pinlaor, S.; Ma, N. *Biol. Chem.* **2006**, *387*, 365−372.
7 Wondrak, G. T.; Antioxid. R*edox Signal.* **2009**, *11*, 3013−3069.
8 Adams, D. J.; Boskovic, Z. V.; Theriault, J. R.; Wang, A. J.; Stern, A. M.; Wagner, B. K.; Shamji, A. F.; Schreiber, S. L. *ACS Chem. Biol.* **2013**, *8*, 923−929.
9 Trachootham, D.; Alexandre, J.; Huang, P. *Nat. Rev. Drug Discov.* **2009**, *8*, 579−591.
10 Adams, D. J.; Dai, M.; Pellegrino, G.; Wagner, B. K.; Stern, A. M.; Shamji, A. F.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 15115−15120.
11 Wu, Y.; Min, X.; Zhuang, C.; Li, J.; Yu, Z.; Dong, G.; Yao, J.; Wang, S.; Liu, Y.; Wu, S.; Zhu, S.; Sheng, C.; Wei, Y.; Zhang, H.; Zhang, W.; Miao, Z. *Eur. J. Med. Chem.* **2014**, *82*, 545−551.
12 Bezerra, D. P.; Pessoa, C.; de Moraes, M. O.; Saker-Neto, N.; Silveira, E. R.; Costa-Lotufo, L. V. *Eur. J. Pharm. Sci.* **2012**, *48*, 453−463.
13 Boskovic, Z. V.; Hussain, M. M.; Adams, D. J.; Dai, M.; Schreiber, S. L. *Tetrahedron*, **2013**, *69*.
14 Randhawa, H.; Kibble, K.; Zeng, H.; Moyer, M. P.; Reindl, K. M. *Toxicol. In Vitro* **2013**, *27*, 1626−1633.
15 Wang, Y.; Wang, J.; Li, J.; Zhang, Y.; Huang, W.; Zuo, J.; Liu, H.; Xie, D.; Zhu, P. *Chem. Biol. Drug Des.* **2015**, Doi: 10.1111/cbdd.12714.
16 Punganuru, S. R.; Madala, H. R.; Venugopal, S. N.; Samala, R.; Mikelis, C.; Srivenugopal, K. S. *Eur. J. Med. Chem.* **2016**, *107*, 233-244.
17 Aras, D.; Cinar, O.; Cakar, Z.; Ozkavukcu, S.; Can, A. *Mol. Hum. Reprod.* **2016**, *22*, 57-67.
18 Cai, W.; Zhang, B.; Duan, D.; Wu, J.; Fang, J. *Toxicol. Appl. Pharmacol.* **2012**, *262*, 341-348.
19 Duan, D.; Zhang, B.; Yao, J.; Liu, Y.; Fang, J. *Free Radic. Biol. Med.* **2014**, *70*, 182-193.
20 Duan, D.; Zhang, B.; Yao, J.; Liu, Y.; Sun, J.; Ge, C.; Peng, S.; Fang, J. *Free Radic. Biol. Med.* **2014**, *69*, 15-25.
21 Zhang, B.; Duan, D.; Ge, C.; Yao, J.; Liu, Y.; Li, X.; Fang, J. *J. Med. Chem.* 2**015**, *58*, 1795-1805.
22 Duan, D.; Zhang, J.; Yao, J.; Liu, Y.; Fang, J. *J. Biol. Chem.* **2016**, *291*, 10021-10031.
23 Zhang, J.; Li, Y.; Duan, D.; Yao, J.; Gao, K.; Fang, J. *Biochem. Pharmacol.* **2016**, *102*, 34-44.