Bioorganic & Medicinal Chemistry Letters 20 (2010) 7426–7428

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel osthole derivatives as potential anti-breast cancer treatment

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ARTICLE INFO

Article history:
Received 27 July 2010
Revised 20 September 2010
Accepted 6 October 2010
Available online 2 November 2010

Keywords:
Osthole
Antitumor
Breast cancer
Natural product
Structure modification

ABSTRACT

Osthole, an ingredient of Traditional Chinese Medicine (TCM) from natural product *Cnidium monnieri (L.) Cusson*, was used as a lead compound for structural modification. A series of osthole derivatives bearing aryl substituents at 3-position of coumarin, has been prepared and evaluated for their growth inhibitory activity against human breast cancer cell lines MCF-7 and MDA-MB-231. Interestingly, some derivatives exhibited good inhibition, among them compound **8e** was found to be the most potent compound with IC_{50} values of 0.24 μM, 0.31 μM against MCF-7 and MDA-MB-231, respectively, which was improved more than 100-folds compared with its parent compound osthole.

The coumarin(benzopyran-2-one, or chromen-2-one) ring system, present in natural products (such as the anticoagulant Warfarin, **1**), that display interesting pharmacological properties,[1,2] has intrigued chemists and medicinal chemists for decades to explore the natural coumarins or synthetic analogs for their applicability as drugs (Fig. 1). Many interesting molecules based on the coumarin ring system have been synthesized utilizing innovative synthetic techniques. Some new derivatives bearing coumarin ring including the furanocoumarins (e.g., Imperatorin, **2**), pyranocoumarins (e.g., Seselin, **3**), and coumarin sulfamates (Coumates) (**4**), have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy and others.[3,4] Among the diverse biological activities of coumarins, the most intriguing is the notable effect of, some of the coumarins against breast cancer, some coumarins and their active metabolite 7-hydroxycoumarin analogs have shown sulfatase and aromatase inhibitory activities.[5,6]

Coumarin based selective estrogen receptor modulators (SERMs) and coumarin estrogen conjugates have also been described as potential anti-breast cancer agents according some recently publications.[7] Currently, tamoxifen is most commonly used adjuvant drug for estrogen receptor (ER)-positive breast cancer,[8] which competes with estrogen and downregulates estrogenic actions in breast cancer, however, it is less effective in ER-negative breast cancer, and its safety is also controversial.[9–11] Therefore, there is a strong impetus to identify new anti-breast cancer agents with improved activity and reduced side effects.

Osthole, 7-methoxy-8-(3-methyl-2-butenyl) coumarin (**5**) (Fig. 1), is a coumarin derivative clinically ingested as an important component of medicinal plants and herbs[12,13] in Tradition Chinese Medicine (TCM), and it exhibits many pharmacological and biological activities,[14,15] The original study of osthole could be dated back to more than 100 years ago. It was first discovered from *Peucedanum ostruthium*. Now osthole is being found to be widely distributed in the plant kingdom. *Cnidium monnieri (L.) Cusson*, a kind of plant containing high percentage of osthole, which has been used in China since several hundred years before as an herbal medicine to treat male sexual dysfunction. It has been proved that osthole has some important therapeutic function and safe profile compared with other natural product, which makes it a very promising lead compound in drug discovery area. Many reviews have summarized its diverse biological activities and therapeutic application. In this paper, we utilized osthole as lead compound to modify in order to improve its anti-cancer activity and drug-likeness properties.

The studies on growth-inhibitory cytostatic activity in human cancer cell line: MCF-7 breast carcinoma cells revealed that osthole demonstrated some estrogenic activity by preventing the synthesis and action of estrogens (ER antagonists)[16,17] which indicated that osthole has the potential to become a breast cancer treatment reagents. However, osthole exhibited comparatively weak activity, low water solubility and limited permeability, and these properties may lower its absorption upon oral administration and bioavailability, which need improve for better drug-likeness.[3,12]

According to literature analysis[18], 3- and 4-position of osthole might play an important role in proliferation activity, therefore, we tried to introduce some substituents at these positions to improve its anti-cancer activity. Bromination and Vilsmeier–Haack

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doi:10.1016/j.bmcl.2010.10.027

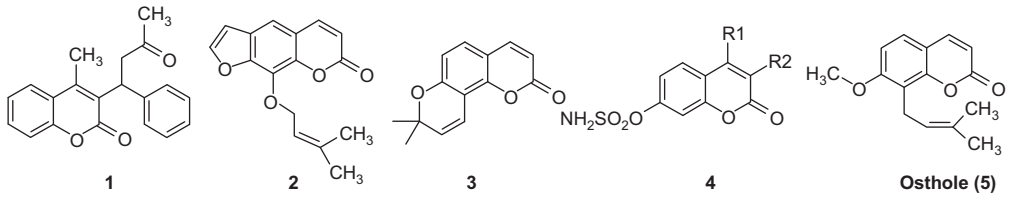
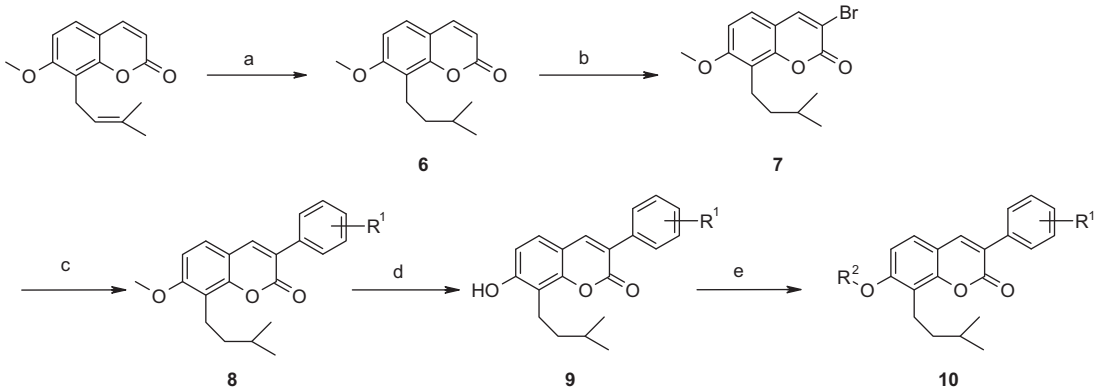


Figure 1. Structures of coumarin Warfarin (**1**), Imperatorin (**2**), Seselin (**3**), and Coumates (**4**).



Scheme 1. Reagents and conditions: (a) PtO_2, H_2, 24 h, 80%; (b) NBS, NaOAc, CH_3CN, MW, 2 h, 60%; (c) $Pd(PPh_3)_4$, phenylboronic acid, K_3PO_4, dioxane, 60–90%; (d) BBr_3, DCM, −78 °C, 40–70%; (e) bromide, DMF, NaH, 60–85%.

reaction were attempted to introduce bromo or formaldehyde at 3- or 4-position of osthole (Scheme 1). However due to the existing of double bond of isopentenyl, these efforts were failed finally; neither 3-position nor 4-position substituted products were obtained successfully. It was believed that the modification at 3- or 4-position could be realized after the hydrogenation of isopentenyl. It was proved that platinum oxide could catalyze the hydrogenation to afford reduced osthole derivative **6** with a good yield (more than 80%), the hydrogenated product **6** could carry out bromination under microwave irradiations selectively at 3-position to yield the important intermediate **7**, which could be employed to couple with various aryl boric acid and prepare a series of novel osthole derivatives **8**. After demethylation and nucleophilic substitution, a new class of osthole derivatives **9** and **10** were obtained too.

The anti-cancer activity tests in vitro of the above osthole analogs were carried on human breast cancer MCF-7 and MDA-MB-231 cell lines. The structure and activity relationship studies showed the methoxy at 7-position is critical for maintaining the antitumor activity, and the modification at 3-position and the hydrogenation on the double bond of isoamylene could obviously improve its antitumor activity (Fig. 2).

Preliminary experiments illustrated that the series of novel osthole derivatives with modification at 3-position exhibited improved inhibitory activity against breast cancer cell (Table 1). The

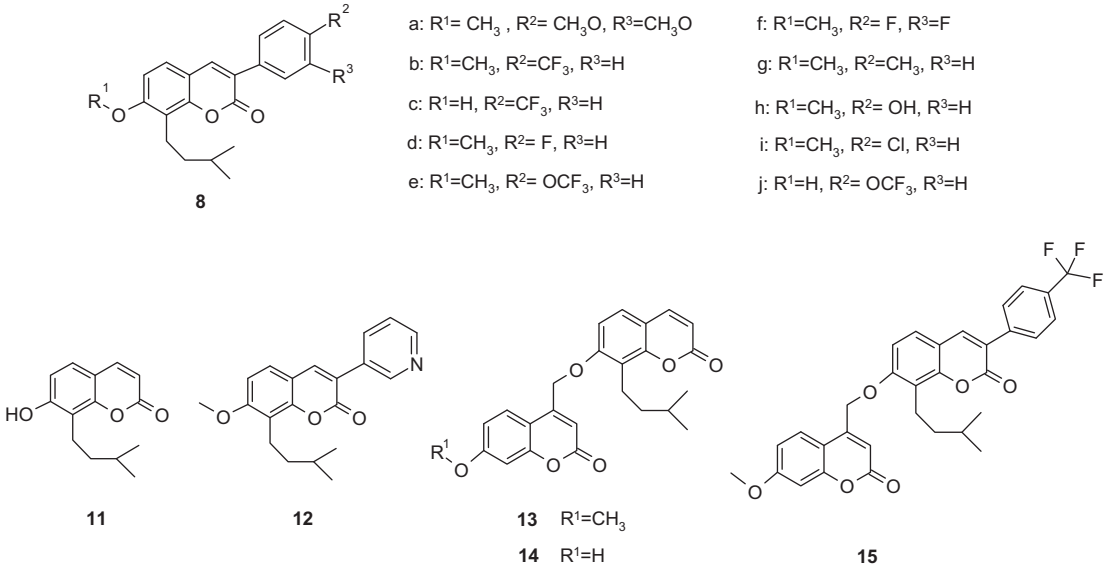


Figure 2. The novel derivatives of osthole.

Table 1
Cell growth inhibitory activity of osthole derivatives **8**–**15** in various cell lines with MTT assay

Compound no.	MCF-7 Cell growth inhibition IC_{50} (μM)[b]	MDA-MB-231 IC_{50} (μM)
Tamoxifen citrate[a]	10.3	12.8
Osthole (**1**)	25.8	30.2
6	47.0	NA[c]
8[a]	51.6	NA
8b	24.5	38.1
8c	42.9	NA
8d	52.6	NA
8e	0.24	0.31
8f	27.3	33.6
8g	50.2	NA
8h	3.23	4.15
8i	1.27	5.23
8j	5.70	7.42
11	NA	NA
12	NA	NA
13	NA	NA
14	NA	NA
15	45.9	NA

[a] Positive control.
[b] Mean IC_{50} (μM), from three or more independent tests.
[c] NA means not active and having IC_{50} >50 μM.

most potent analog investigated in this study appears to be trifluormethyl phenyl derivative **8e**, of which the cytotoxic activity against MCF-7 and MDA-MB-231 cell lines was improved more than 100-fold to 0.24 μM, 0.31 μM, respectively, compared with its parent compound osthole.

For the analog **8i** bearing *p*-chloro phenyl substituted at 3-position, the antitumor activity was improved significantly (1.27 μM vs 25.8 against MCF-7), about 20-fold higher than its parent compound. The inhibitory activity of compound **8h** containing *p*-phenol also had eight times increase (IC_{50} value is 3.23 μM). Compounds **8a**, **8b**, **8d** and **8f**, which incorporate dimethoxyl phenyl, trifluormethyl, fluoro, and difluoro phenyl at 3-position of the coumarin ring, respectively, showed slightly increase (two, threefold) or comparable cytotoxic activity with osthole. In contrast, methyl phenyl derivative **8g** was found to weakly inhibit human cancer cell growth in vitro with IC_{50} values around 50 μM.

The activity of demethylated compound **8j** declined more than 20-folds, **8c** is a 7-position analog prepared from **8b**, exhibited reduced activity (IC_{50} = 42.9 μM), it revealed that the 7-methyloxy group is a very sensitive substituent for maintaining the antitumor activity. Same impacts were also observed in compound **11**, which is a hydrogenated and demethylated osthole derivative, of which the activity was totally lost. The replacement of the phenyl ring **12** by a 3-pyridyl moiety also leads to the losing of cellular activity. Compounds **13** and **14** did not show even lowest activity.

Since **8e** and **8i** showed better cell growth inhibition for breast cancer cell lines than tamoxifen citrate and osthole, their cytotoxicity was also tested in normal cells, Human Embryonic Kidney (HEK)-293. Results show that **8e** and **8i** had no cell growth inhibitory effect in HEK-293 cells. The further MOA and in vivo study for **8e** is under going now.

In conclusion, we designed and prepared a series of novel osthole derivatives by structural modification and bio-isosteric replacement at 3-position of coumarin core, of which the in vitro anti-breast cancer activities against MCF-7 and MDA-MB-231 cell lines were investigated, and preliminary SAR around this scaffold was established. The results indicated that 7-methoxy and 3-aryl ring are critical for maintaining cytotoxicity potency. Compound **8e** were the most potent analogs (IC_{50} values of 0.24 and 1.27 μM, respectively, against the MCF-7 and MDA-MB-231 cell lines), and showed 100 higher antitumor activity compared with osthol, and lacked cytotoxicity to normal HERK-293 cells. Mechanism of action study is ongoing and the latest progress will be reported in due course. In summary, **8e** and **8i** are the two novel and promising lead compounds suitable for further development toward a potential clinical trials candidate.

Acknowledgments

Financial assistance from Project of Shanghai Educational Commission (08CZ08). 'Xinlin' scholars and outstanding team training plan of Shanghai University of Traditional Chinese Medicine.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.027.

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