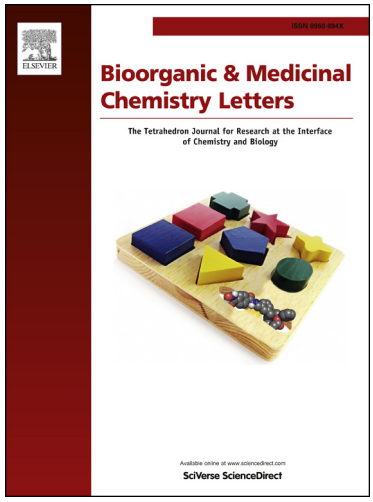

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Synthesis of biscoumarin and dihydropyran derivatives with promising antitumor and antibacterial activities

Jing Li[a§], Yun-Peng Sui[b, c§], Jia-Jia Xin[d§], Xin-Liang Du[e], Jiang-Tao Li[a], Hai-Ru Huo[b], Hai Ma[b], Wei-Hao Wang[b], Hai-Yu Zhou[b], Hong-Dan Zhan[b], Zhu-Ju Wang[b], Chun Li[b], Feng Sui[b*], Xia Li[f*]

[a] School of Chemical Engineering, the Key Laboratory for Surface Engineering and Remanufacturing in Shaanxi Province, Xi'an University, Xi'an 710065, China

[b] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

[c] Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China

[d] Department of Blood Transfusion, Xijing Hospital, the Fourth Military Medical University, Xi'an 710032, China

[e] Graduate School of China Academy of Chinese Medical Sciences, Beijing 100700, China

[f] Department of Neurosurgery, Xijing Hospital, the Fourth Military Medical University, Xi'an 710032, China

[§] These authors contributed equally to this work.

[*] Corresponding authors: fsui@icmm.ac.cn (Feng Sui); sjwklixia@sina.com (Xia Li)

Abstract

Two series of biscoumarin (1-3) and dihydropyran (4-12) derivatives were successfully synthesized as new antitumor and antibacterial agents. The molecular structures of four representative compounds 2, 4, 7 and 10 were confirmed by single crystal X-ray diffraction study. The synthesized compounds (1-12) were evaluated for their antitumor activities against human intestinal epithelial adenocarcinoma cell line (HuTu80), mammary adenocarcinoma cell line (4T1) and pancreatic cancer cell line (PANC1) and antibacterial activities against one drug-sensitive *S. aureus* (*S. aureus* ATCC 29213) strain and three MRSA strains (MRSA XJ 75302, Mu50, USA 300 LAC). The further mechanism study demonstrated that the most potent compound 1 could obviously inhibit the proliferation of cancer cells via the mechanism to induce apoptosis.

Key Words: Bisoumarin, Dihydropyran, Antitumor, Antibacterial

Cancer is one of the most serious threats against human health in the world [1, 2]. According to World Cancer Report from the International Agency for Research on Cancer, cases of cancer doubled globally between 1975 and 2000, will double again by 2020, and will nearly triple by 2030 [3]. In addition, *Staphylococcus aureus* is a main pathogen responsible for a number of diseases from serious hospital infections and community acquired infections, such as folliculitis, impetigo, and cellulitis, which is the main cause of in-hospital mortality as high as 15% to 60% [4-6]. However, most of clinical drugs used for tumor and infection treatment show poor curative effect, high toxicity, low selectivity and severe drug resistance [7].

Heterocyclic compounds attract special attention in chemical literature because of their abundance in natural products and the diverse biological properties associated with them [8-11]. There are a large variety of heterocycles known and among them coumarin and pyran ring systems are of particular importance [12-15]. Numerous interesting arrays of biological activities have been linked to natural and unnatural compounds possessing a substituted coumarin or pyran nucleus, making it a suitable building block for many therapeutic agents including antimicrobial activity [16-18], growth stimulating effects [19, 20], antifungal and plant growth regulation effects [21, 22],

antitumor activity [23, 24], central nervous system (CNS) activity [25] and hypotensive effect [26].

In the present studies, two novel series of biscoumarin (1-3) and pyran derivatives (4-12) were reported (Fig. 1), all of which were evaluated for their *in vitro* antitumor activities against intestinal epithelial adenocarcinoma cell line (HuTu80), mammary adenocarcinoma cell line (4T1) and pancreatic cancer cell line (PANC1); and for their *in vitro* antibacterial activities against one drug-sensitive *S. aureus* (*S. aureus* ATCC 29213) strain and three MRSA strains (MRSA XJ 75302, Mu50, USA 300 LAC). In addition, compound 1 with the most promising antitumor activity was further studied to demonstrate whether the growth inhibition induced by compound 1 in the cells derived from apoptosis or necrosis.

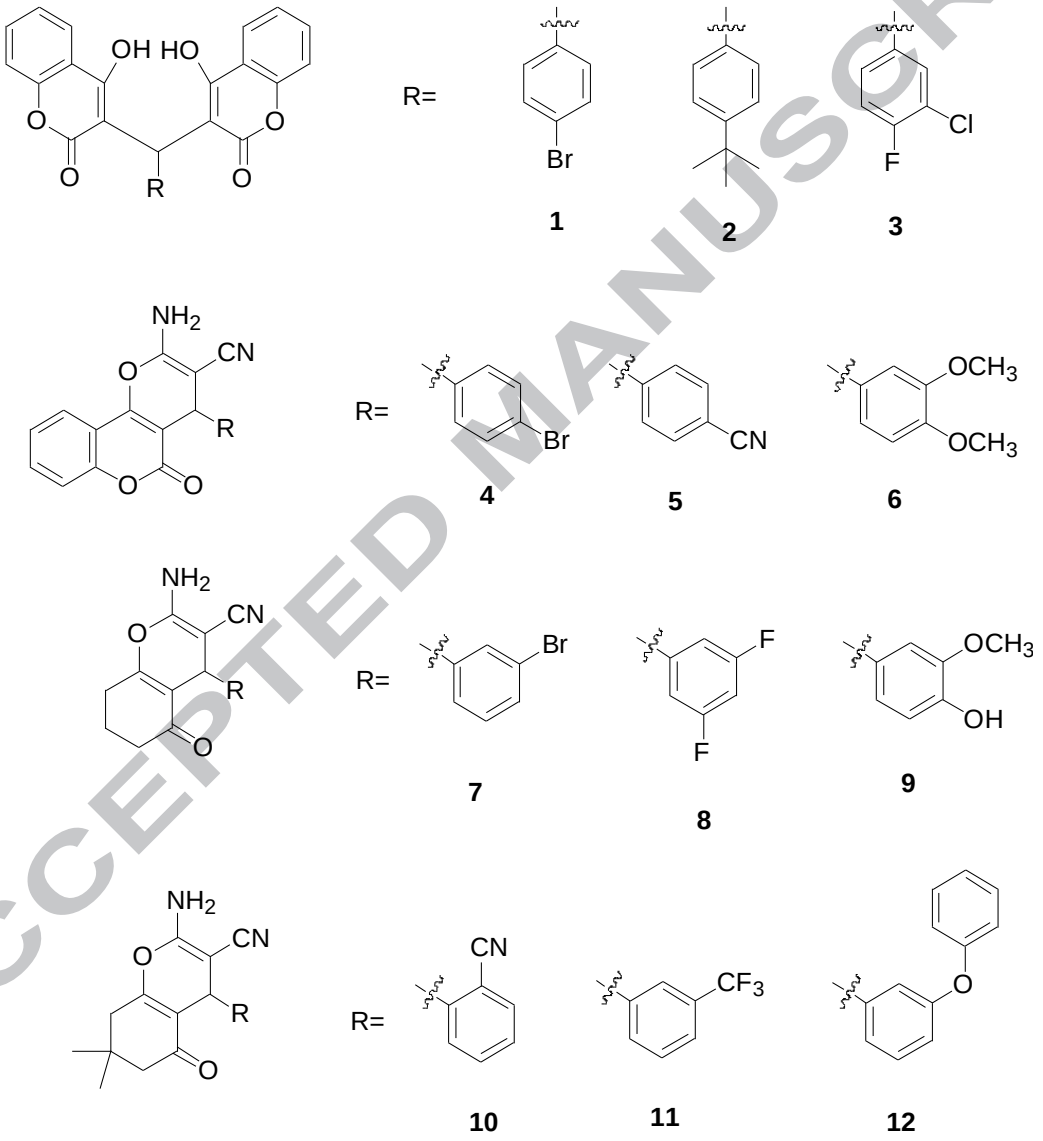
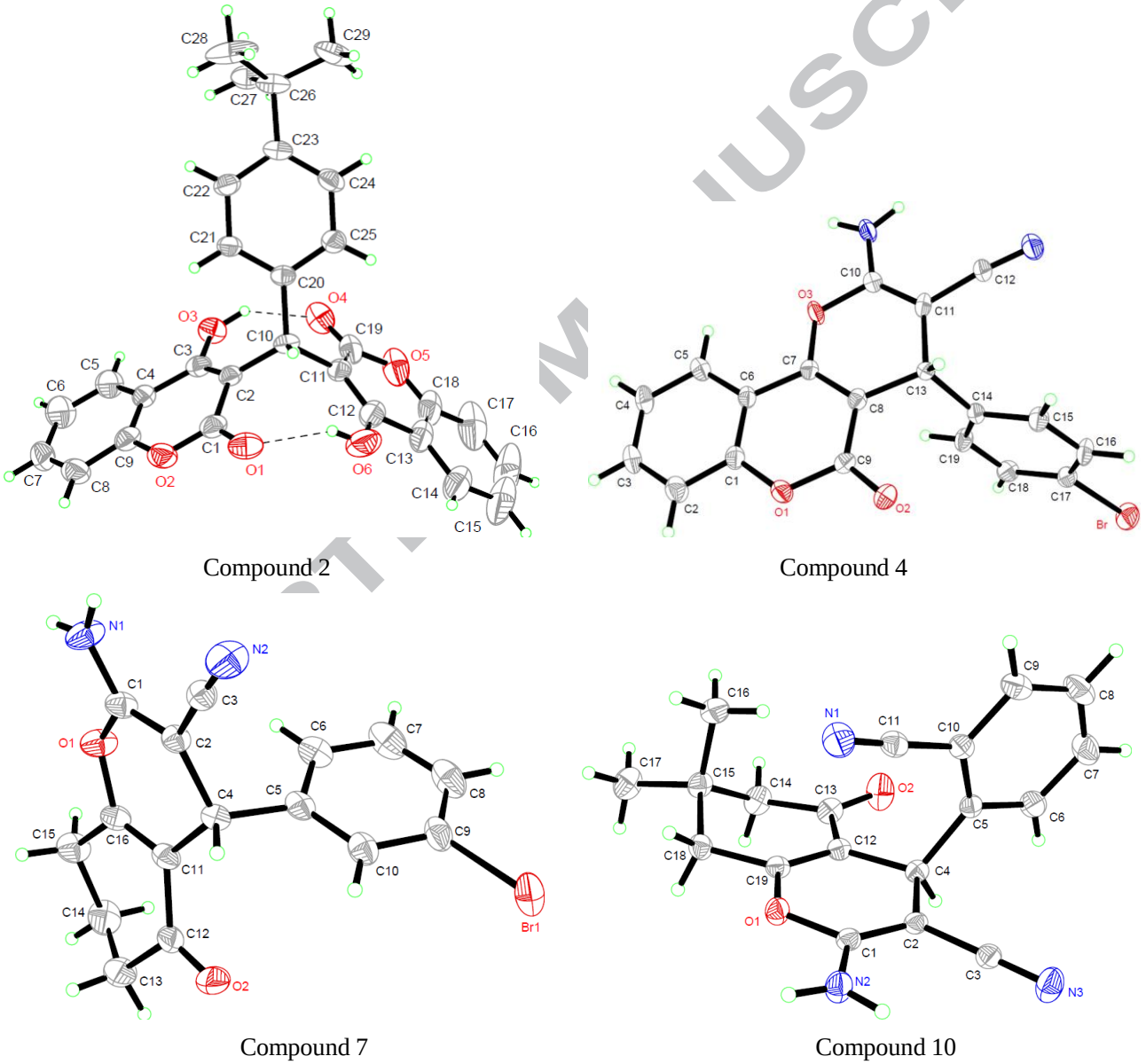


Fig. 1 Chemical structures of compounds 1-12

The compounds were synthesized according to general method as described in Scheme 1 and 2 (see Supplementary data). Biscoumarin 1-3 were synthesized via a one-pot two-component reaction by condensing aromatic aldehydes and 4-hydroxycoumarin in the presence of catalytic amount of piperidine in ethanol under reflux conditions. Pyran derivatives 4-12 were synthesized via a one-pot three-component reaction by condensing aromatic aldehydes, 4-hydroxycoumarin (3,5-cyclohexanedione, or 1,1-dimethyl-3,5-cyclohexanedione) and

malononitrile in the presence of 4-(dimethylamino)pyridine (DMAP) as a highly efficient homogenous catalyst. Additionally, chemical structures of all compounds were further characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS (see Supplementary data).

In order to further confirm the configuration of the products, single crystals of four representative compounds 2, 4, 7 and 10 were cultured for X-ray diffraction analysis. From Fig. 2 we can see that, in crystal structure of compound 2, in order to stabilize the whole structure, there are two classical intramolecular hydrogen bonds (O_3–H_3···O_4 and O_6–H_6···O_1) between a hydroxyl group of one coumarin fragment and a lacton carbonyl group of another coumarin fragment, which are further linked by a methylene bridge having a 4-tertbutylphenyl group on the center.



Compound 2 Compound 4

Compound 7 Compound 10

Fig. 2 Crystal structures of compounds 2, 4, 7 and 10.

In the crystal structures of compounds 4, 7 and 10, the new formed pyran ring and the adjacent coumarin (or ketone) ring are both basically planar, and the two planes are also essentially parallel each other. However, the aromatic ring makes a torsion angle to the pyran ring in the three compounds.

For compounds 1-12, the antitumor effect *in vitro* on the growth of three human tumor cell lines, namely, human intestinal epithelial adenocarcinoma cell line (HuTu80), mammary adenocarcinoma cell line (4T1) and pancreatic cancer cell line (PANC1), was evaluated after a continuous 48h exposure. For comparison purpose, the cytotoxicity of carboplatin, a standard antitumor drug, was evaluated under the same condition.

As shown in Table 1, all the tested compounds exhibited antitumor activity against the cancer cell lines HuTu80, 4T1 and PANC1 to a certain degree, and the degree of their inhibitory action get stronger accompanied with the increased concentration of these compounds. The related IC_{50} and IC_{90} values of compounds 1-12 (dose of the compound which cause a 50% and 90% reduction of survival values respectively), were calculated and shown in Table 1. The results showed that the differences in antitumor activity among the four different groups of the tested compounds is significant. Biscoumarins 1-3 from the first group showed more potent antitumor activity against all the tested cancer cell lines with the IC_{50} value ranged from 19.26 μg/mL to 32.60 μg/mL and the IC_{90} value ranged from 36.68 μg/mL to 64.45 μg/mL, which is much lower than the IC_{50} and IC_{90} values (45.85-65.62 μg/mL; 102.14-126.24 μg/mL) of the positive control drug carboplatin. However, the compounds 4-12 in other three groups showed lower antitumor activity with relatively higher IC_{50} and IC_{90} values.

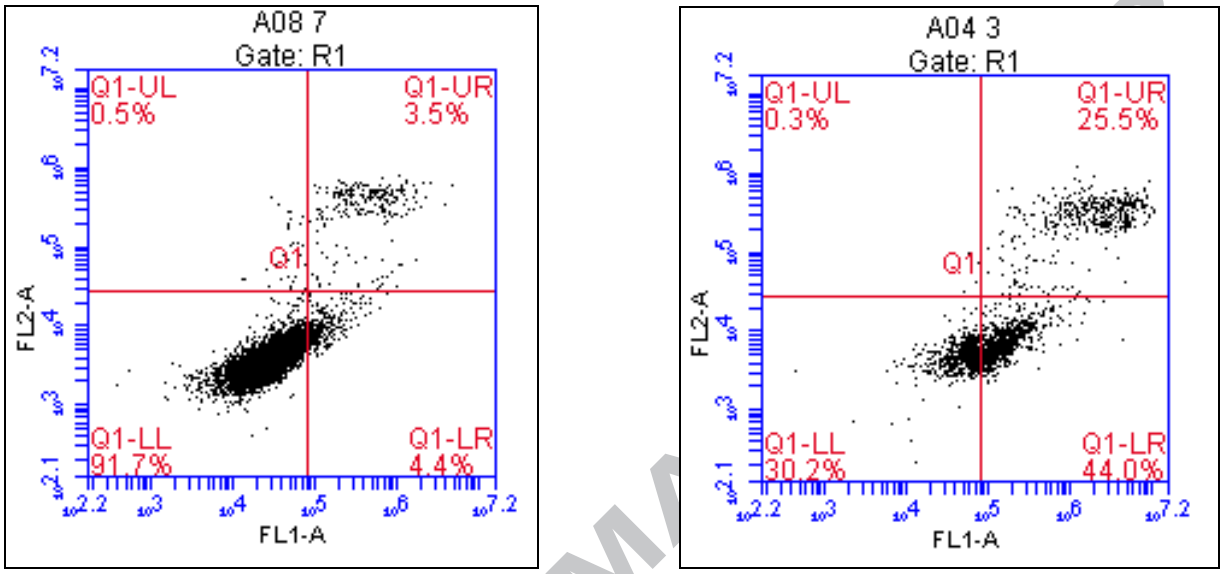
Table 1 IC_{50} and IC_{90} values of compounds 1-12 and carboplatin against three tumor cell lines (μg/mL)

Drugs	HUTU80		4T1		PANC1	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Compound 1	29.70	52.64	19.26	36.68	31.80	61.41
Compound 2	31.15	55.53	24.56	47.13	27.97	53.16
Compound 3	32.60	64.45	22.93	43.19	30.77	55.77
Compound 4	140.33	273.67	96.17	172.00	176.42	327.7
Compound 5	121.25	221.25	114.70	207.77	149.75	285.99
Compound 6	132.50	232.5	106.68	194.03	168.45	310.4
Compound 7	281.10	543.15	157.00	286.62	368.38	703.67
Compound 8	445.97	824.40	164.23	307.65	318.99	624.10
Compound 9	208.05	398.52	113.55	218.65	232.24	461.99
Compound 10	414.4	814.4	248.55	503.32	241.84	512.29
Compound 11	388.00	788.00	286.78	575.18	248.08	502.69
Compound 12	899.80	1699.8	480.95	927.38	383.11	759.05
Carboplatin	65.62	126.24	45.85	102.136	52.94	109.94

The IC_{50} (dose of the compound which caused a 50% reduction of survival.) and IC_{90} (dose of the compound which caused a 90% reduction of survival) values were calculated from dose-response curves done in triplicate for each compound. Carboplatin was used as positive control.

On the basis of the anti-proliferative data, the most potent compound 1 was selected for further mechanistic studies to determine whether the growth inhibition induced by compound 1 in the cells derived from apoptosis or necrosis. To assess the effect of compound 1 on apopsis of 4T1 (the most senstive cell line to compound 1), the flow cytometry technique was used and the cell culture was not only treated with vehicle alone as control but also dealt with compound 1 at the concentration of 5 μg/mL for 48 h and stained with Annexin V/Alexa Fuor 488 and

propidium iodide (PI). The percentages of apoptotic cells were determined by flow cytometry analysis. As shown in Fig. 3, compound 1 displayed a significant action on the induction of apoptosis to the tested cells; apoptotic 4T1 cells (early and late apoptosis) increased more than 8 folds after 48 h treatment with compound 1, as compared to the untreated vehicle control. Apparently, compound 1 mediated apoptosis of the tested cells, at least in part, contributes to its antiproliferative effects.



(a) (b)

Fig. 3 Apoptosis induction in 4T1 cell line after 48 h treatment with compound 1 at the concentration of 5 μg/mL (b) and no treatment (Control) (a).

The antibacterial activity of compounds 1-12 *in vitro* was investigated by one drug-sensitive *S. aureus* (*S. aureus* ATCC 29213) strain and three MRSA strains (MRSA XJ 75302, Mu50, USA 300 LAC) respectively. From Table 2 we can see that compared with pyran derivatives 4-12, biscoumarins 1-3 exerted the most bactericidal effects against nearly all types of *S. aureus* tested with the value of 8-32 μg/mL. In addition, the MIC values of levofloxacin, ceftazidime, ceftriaxone, gentamicin and piperacillin against *S. aureus* (ATCC 29213) strains were lower (less than 8 μg/mL) but were higher against resistant strains at varying degrees.

Table 2 MIC of compounds 1-12 and antibiotics in Mueller-Hinton Broth Culture.

Drugs	MIC (ug/mL)			
	S. aureas (ATCC 29213)	MRSA (XJ 75302)	Mu50 (ATCC 700699)	LAC (USA 300)
Compound 1	16	16	8	8
Compound 2	16	16	16	32
Compound 3	16	16	8	8
Compound 4	>256	>256	>256	>256
Compound 5	>256	>256	>256	>256

Compound 6	>256	>256	>256	>256
Compound 7	>256	>256	>256	>256
Compound 8	>256	>256	>256	>256
Compound 9	>256	>256	>256	>256
Compound 10	>256	>256	>256	>256
Compound 11	>256	>256	>256	>256
Compound 12	>256	>256	>256	>256
Levofloxacin	<0.125 (S)	4 (R)	4 (R)	8 (R)
Ceftazidime	8 (S)	>256 (R)	256 (R)	64 (R)
Ceftriaxone	2 (S)	>256 (R)	256 (R)	32 (R)
Gentamicin	0.12 (S)	64 (R)	32 (R)	0.25 (S)
Piperacillin	2 (S)	>128 (R)	>128 (R)	8 (R)

S means drug susceptibility, *R* means drug resistance.

In conclusion, we prepared biscoumarin and pyran derivatives using simple reaction conditions in a single pot and evaluated for their *in vitro* antitumor activities against intestinal epithelial adenocarcinoma cell line (HuTu80), mammary adenocarcinoma cell line (4T1) and pancreatic cancer cell line (PANC1); and for their *in vitro* antibacterial activities against one drug-sensitive *S. aureus* (*S. aureus* ATCC 29213) strain and three MRSA strains (MRSA XJ 75302, Mu50, USA 300 LAC). In the series of biscoumarin and pyran derivatives, biscoumarins 1-3 showed very potent antitumor and antibacterial activities, possibly because of two asymmetrical intramolecular O—H···O HBs in their structures in assisting the molecule to attain the correct configuration for biological activity [27]. The flow cell assay further demonstrated that the proportions of apoptosic cells in the groups treated with compound 1 increased significantly, indicating that the promising antitumor activity of compound 1 may be attributed to the induction of the cells to apoptosis. The above data is helpful to further exploit these compounds for antitumor and antibacterial activity mechanistic studies and also helpful to design and synthesize more such derivatives to be taken-up for further studies.

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Graphical abstract