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Synthesis and Cytotoxicity of 3,4-Diaryl-2(5*H*)-furanones

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Abstract—A series of 3,4-diaryl-2(5*H*)-furanone derivatives were synthesized and evaluated for their cytotoxicity in a small panel of cancer cell lines. Four out of 10 compounds in this series, for example 3-(3,4,5-trimethoxyphenyl)-4-(4-methoxyphenyl)-, 3-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-, 3-(3,4,5-trimethoxyphenyl)-4-(3-amino-4-methoxyphenyl)-, and 3-(3,4,5-trimethoxyphenyl)-4-(2-naphthyl)-2(5*H*)-furanones, were found to have potent cytotoxic activities with ED_{50} values of less than 20 nM in most of the cell lines tested.

Introduction

2(5*H*)-Furanone is a common moiety incorporated in a number of drugs with diverse biological activities such as antifungal, antibacterial and antiinflammatory.[1–4] A number of 2(5*H*)-furanone derivatives have also been reported from nature as cytotoxic and antitumor agents.[4–6] For example, gonbiobutenolide B6, isolated from the ethanol extract of the stem bark of *Goniothalamus giganteus*, exhibited significant cytotoxicity against human lung carcinoma (A549, ED_{50}, 0.9 μg/mL).[6] In a previous study, we reported the isolation of a furanone containing principle, deoxypodophyllotoxin (DPT, Fig. 1) from *Pulsatilla koreana*, one indigenous plant in Korea. This compound showed potent cytotoxicity in a variety of tumor cell lines. Strikingly, it was also shown to exhibit significant antiangiogenic activity at non-cytotoxic concentrations. In vivo, DPT was found to possess potent antitumor activity in several solid tumor models.[7]

Recently, we reported a series of diaryloxazolone derivatives with potent cytotoxicity and antitumor activity.[8] The dihedral angles (52.6°) between 3,4-diaryl groups in these 3,4-diaryloxazolones, especially that of compound **1** (Fig. 2) were found to be similar to that of DPT (70.3°). These results prompted us to design and synthesize compound 3-(3,4,5-trimethoxyphenyl)-4-(3,4-methylendioxyphenyl)-2(5*H*)-furanone **2f** and a series of its analogues, in which the two aromatic rings are tethered directly into the 2(5*H*)-furanone ring, a biomoiety found in a number of drugs with diverse biological activities, as mentioned above. Moreover, 3,4-diaryl-2(5*H*)-furanone derivatives could be considered as *cis*-restricted analogues of combretastatins (Fig. 1), the potent antimitotic and cytotoxic agents isolated from *Combretum caffrum*.[9]

In this paper, we would like to describe the synthesis and evaluation of the cytotoxicity of a series of such 3,4-diaryl-2(5*H*)-furanone derivatives.

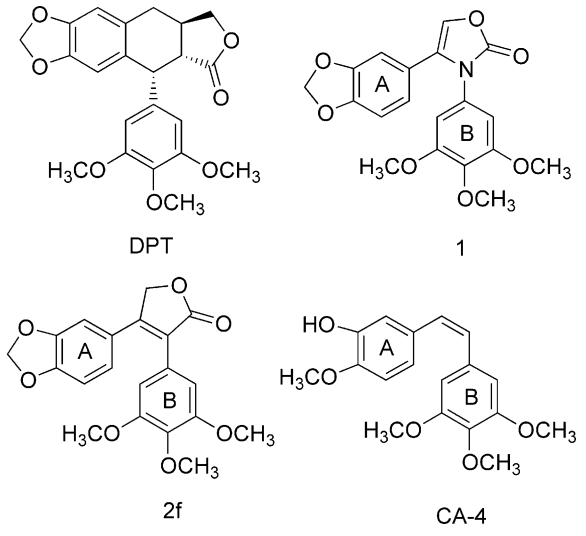

Figure 1. Deoxypodophyllotoxin (**DPT**), 3-(3,4,5-trimethoxy)-4-(3,4-methylenedioxyphenyl)oxazolone (**1**, 3,4-diaryloxazolone), 3-(3,4,5-trimethoxyphenyl)-4-(3,4-methylenedioxyphenyl)-2(5*H*)-furanone (**2f**, 3,4-diaryl-2(5*H*)-furanone), and combretastatin (**CA-4**).

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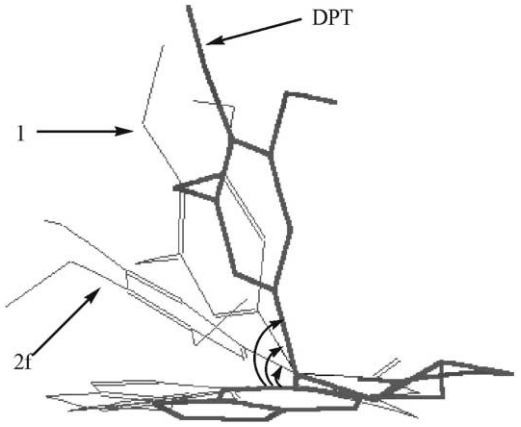
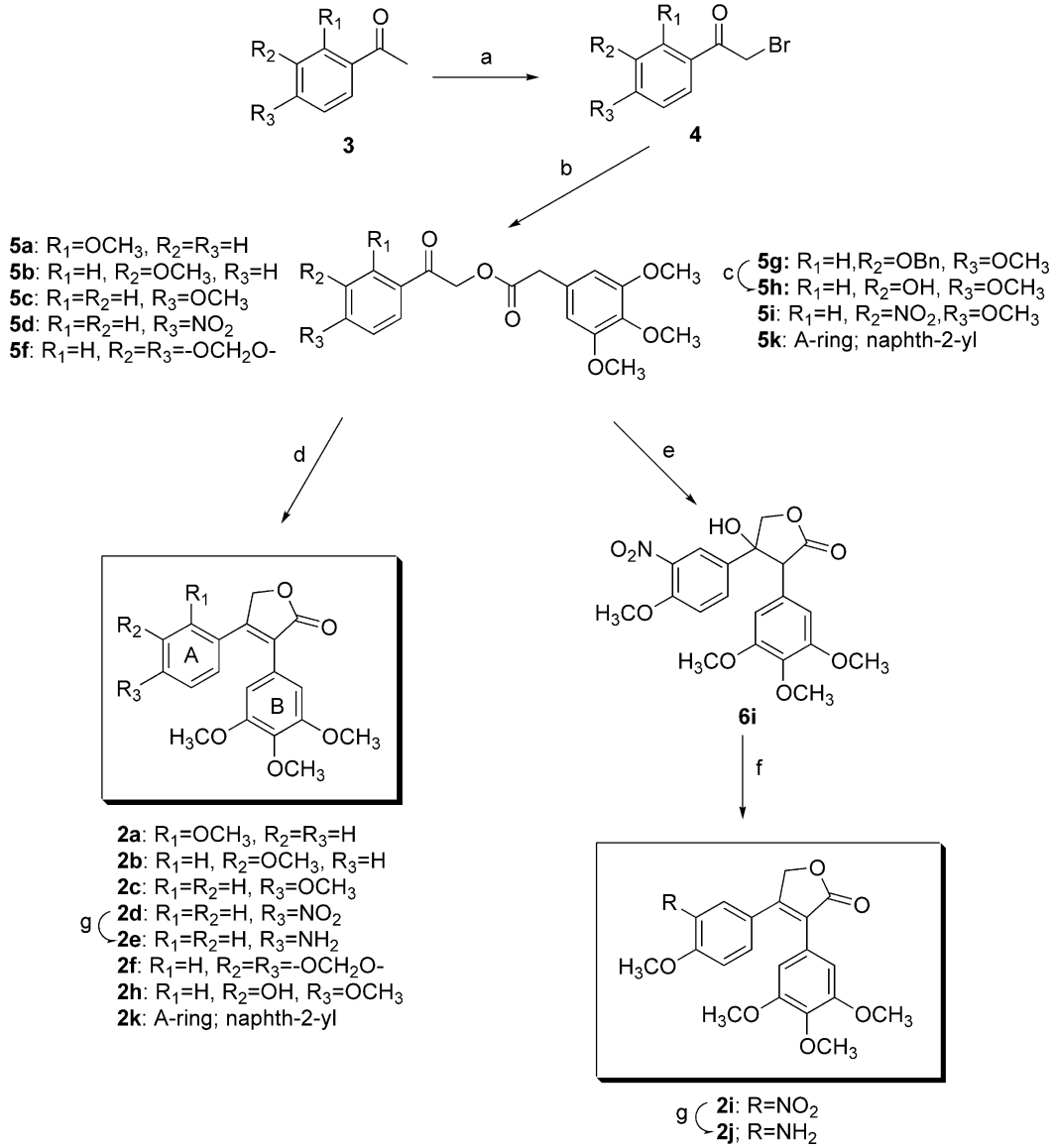


Figure 2. Superimposition of the lowest energy conformations of DPT (AC, 11.9 nM), diaryloxazolone (**1**, AC, 64.5 nM), and 3-(3,4,5-trimethoxyphenyl)-4-(3,4-methylenedioxyphenyl)-2(5*H*)-furanone (**2f**, AC, 83.8 nM).

Results and Discussion

Chemistry

The general methods for the preparation of 3,4-diaryl-2(5*H*)-furanone derivatives **2a–f** and **2 h–k** are outlined in Scheme 1. The appropriate acetophenones **3**[10,11] were brominated at the α carbonyl position. Reaction of 3,4,5-trimethoxyphenylacetic acid with the resulting α-bromo acetophenones **4** in the presence of triethylamine gave phenacylacetates **5a–d**, **5f**, **5i**, and **5k** in 59–75% yields. The aldol-type condensation and subsequent dehydration of the resulting phenacylacetates with triethylamine and *p*-toluenesulfonic acid (*p*-TsOH) gave the target compounds[12] **2a–d**, **2f**, and **2k** in 56–73% yields. Debenzylation of (3-benzyloxy-4-methoxy)phenacyl-3′,4′,5′-trimethoxyphenylacetate **5g** by catalytic hydrogenation with 10% Pd/C gave (3-hydroxy-4-methoxy) phenacyl-3′,4′,5′-trimethoxyphenylacetate **5h**. The phenacylacetate **5h** was then treated with triethylamine



Scheme 1. (a) Br_2, AcOH, HCl, rt; (b) 3,4,5-trimethoxyphenylacetic acid, NEt_3, CH_3CN, rt; (c) 10% Pd/C, THF, rt; (d) TEA, *p*-TsOH, 4 Å molecular sieve, CH_3CN, reflux; (e) DBU, CH_3CN, 0 °C; (f) *p*-TsOH, benzene, reflux; (g) Zn, AcOH, rt.

and *p*-TsOH to give **2h**[12] in 66% yield. Ring closure of **5i** was satisfactorily accomplished in a high yield by an aldol-type cyclization mediated by DBU to give **6i** that was dehydrated with *p*-TsOH in refluxing benzene to afford the target compound **2i**. The nitro groups on the A-ring of **2d** and **2i** were reduced by Zn/AcOH to give the corresponding aniline compounds[12] **2e** and **2j** in 81 and 83% yields, respectively.

Cytotoxic activity

Cytotoxic activities of the synthesized compounds were tested[13] against A549, SK-MEL-2, and MCF-7 cell lines. The results are shown in Table 1. For comparison purposes, the average cytotoxic activity (AC) of the compounds (average of the ED_{50} values) in three cancer cell lines was calculated and the results from this computation are included in Table 1. Previously, it was reported that the 3,4,5-trimethoxy group on the B-ring was essential for strong cytotoxic activities of the diaryloxazolones.[8] Taking into consideration these results and structural features of DPT, in the present study we fixed the B-ring as a trimethoxyphenyl group and modification was mainly focused on the variation of the substituents on the A ring.

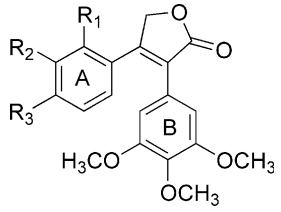
Compound **2c** (AC, 19.6 nM) possessing a 4-methoxy group in the A ring showed potent cytotoxic activity, meanwhile otherwise mono-substituted compounds were found to be inactive (**2a**, AC >5000 nM; **2b**, AC $=3200$ nM). This result indicated that location of the substituent at 4-position was important for cytotoxic activity.

In attempts to discern the electronic effects of the substituent in the A ring, a nitro group and an amino group were introduced into 4-position, resulting in compounds **2d** and **2e**. As shown in Table 1, compound **2d**, possessing the electron-withdrawing nitro group suffered a substantial loss of cytotoxicity in all three cell lines (AC, 3767 nM), meanwhile compound **2e**, bearing the electron-donating amino group, showed much stronger cytotoxicity (AC, 45.7 nM) compared to **2d**. These results indicated that the introduction of the electron-withdrawing group in the A-ring strongly reduced the activity of these types of compound.

Next, since the 4-methoxy group proved to be more favorable for bioactivity, we maintained this substituent at 4-position and introduced additional substituents at position 3 in the A ring. In overall, the introduction of electron-donating groups, as in compounds **2h**, **2j**, and **2k**, improved the cytotoxicity of these compounds by 1.5-, 4-, and 2-fold, respectively, compared to that of **2c**, meanwhile the presence of the electron-withdrawing NO_2 group reduced the activity by more than 2-fold compared to that of **2c** (compound **2i**, AC value of 42.2 nM versus compound **2c**, AC value of 19.6 nM). These results again indicated that the electron-withdrawing group on the A-ring was not favorable for the cytotoxicity of this compound type.

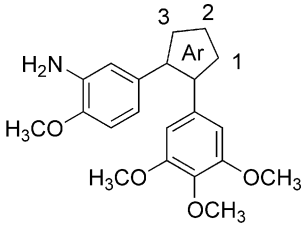
However, compound **2f** (AC, 83.8 nM) possessing a 3,4-methylendioxy group in the A-ring was found to be 4 times less potent than **2c** (AC, 19.6 nM). Compared to the bioactivity of DPT, it was found that **2f** was nearly 8-fold less active than DPT, meanwhile the AC values of **2h**, **2j**, and **2k** were comparable to that of DPT. Furthermore, **2f** was found to be less potent than the 3,4-diaryloxazolone **1**[8] (AC, 83.8 and 64.5 nM, respectively). To gain some insights into the causes of diminished activity observed with **2f**, we performed molecular modeling studies of these three compounds using Hyperchem® software and calculated the dihedral

Table 1. Cytotoxic activities of 3,4-diaryl-2(5*H*)-furanone analogues against some cancer cell lines[a]



Compd	R_1	R_2	R_3	ED_{50} (nM)[b]			AC[c]
				A549	SK-MEL-2	MCF-7	
2a	OCH_3	H	H	>5000	>5000	>5000	>5000
2b	H	OCH_3	H	3200	4200	2500	3300
2c	H	H	OCH_3	19.1	17.5	22.2	19.6
2d	H	H	NO_2	4800	3200	3300	3767
2e	H	H	NH_2	54.7	49.3	33.0	45.7
2f	H	–OCH_2–		100.2	70.8	80.4	83.8
2h	H	OH	OCH_3	16.3	10.2	11.4	12.6
2i	H	NO_2	OCH_3	44.6	40.2	41.9	42.2
2j	H	NH_2	OCH_3	5.3	3.3	4.7	4.4
2k	Naphthyl (A-ring)			9.8	8.1	9.1	9.0
DPT[d]				15.5	8.4	11.7	11.9

[a]A549, human lung cancer; SK-MEL-2, human melanoma, MCF-7, human breast cancer.
[b]The sample concentration produces a 50% reduction in cell growth.
[c]Averages of the ED_{50} values in three cancer cell lines.
[d]Deoxypodophyllotoxin.



(Ar; pyrazole, thiazole, triazole, tetrazole)

Figure 3. *cis*-Restricted five-membered heterocyclic combratastatins.[9]

angles between the two 3,4-aryl rings in each compound. As shown in Figure 1, the dihedral angles between the A and B rings in DPT, diaryloxazolone **1** and diarylfuranone **2f** were 70.3, 52.6, and 15.4°. The potencies of the three compounds were found to be in the same order (AC values of 11.9, 64.5, and 83.8 nM, respectively). Thus it seemed that the magnitude of the dihedral angle between A and B rings positively influenced the bioactivity of the compounds in this series.

Previously, Oshumi et al. reported a series of *cis*-restricted 5-membered heterocyclic combratastatins, and observed that substituents as small as $-NH_2$,$-CH_3$, carbonyl group on position 1 or 3 of the five-membered heterocycles substantially decreased their bioactivity (Fig. 3).[9] In contrast, it was noteworthy that most compounds in the present series of 3,4-diaryl-2(5*H*)-furanones which possess a carbonyl group on position 1 showed very potent cytotoxic activity.

In conclusion, we have synthesized a series of 3,4-diaryl-2(5*H*)-furanone derivatives with potent cytotoxic activity in tumor cell lines. This is the first report revealing compound of 3,4-diaryl-2(5*H*)-furanone type, a class of compounds well known previously as selective COX-II inhibitors, with potent cytotoxic activity in tumor cells. Four compounds in this series including **2c**, **2h**, **2j** and **2k** were found to have ED_{50} value of less than 20 nM in most of the cell lines assayed. Evaluation of a representative compound from this series, compound **2j**, in in vivo model is underway in our laboratory and the results will be disclosed in due course.

References and Notes

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10. Starting material of **2h**, 3-benzyloxy-4-methoxyacetophenone was synthesized as follows: commercially available 3-hydroxy-4-methoxybenzaldehyde was treated with benzylbromide in the presence of anhydrous K_2CO_3 to give 3-benzyloxy-4-methoxybenzaldehyde, which was then condensed with methylmagnesium bromide to give 1-(3-benzyloxy-4-methoxy)-1-ethanol in 92% yield. Oxidation of the alcohol group of 1-(3-benzyloxy-4-methoxy)-1-ethanol by PDC gave a 3-benzyloxy-4-methoxy-acetophenone in 98% yield.
11. Starting material of **2i**, 3-nitro-4-methoxyacetophenone was synthesized as follows: commercially available 3-nitro-4-hydroxyacetophenone was refluxed with dimethylformamide (DMF) in the presence of anhydrous K_2CO_3 to give 3-nitro-4-methoxyacetophenone in 92% yield.
12. All newly synthesized compounds gave satisfactory analytical and spectroscopic data. **2j**: ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.71 (dd, $J=8.77$, 2.15 Hz, 1H), 6.58 (d, $J=8.77$ Hz, 1H), 6.51 (d, $J=2.15$ Hz, 1H), 6.49 (s, 2H), 5.25 (s, 2H), 3.99 (s, 3H), 3.89 (s, 3H), 3.78 (s, 6H). Anal. ($C_{20}H_{21}NO_6$) calcd: C, 64.68; H, 5.70; N, 3.77; found: C, 64.42; H, 5.64; N, 3.71. **2k**: ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.88–7.76 (m, 4H), 7.57–7.53 (m, 2H), 7.40 (dd, $J=1.78$, 8.60 Hz, 1H), 6.72 (s, 2H), 5.30 (s, 2H), 3.89 (s, 3H), 3.70 (s, 6H). Anal. ($C_{23}H_{20}O_5$) calcd: C, 73.39; H, 5.36; found: C, 73.13; H, 5.31.
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