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Synthesis and Biological Evaluation of 1-Benzylidene-3, 4-dihydronaphthalen-2-one as a New Class of Microtubule-Targeting Agents

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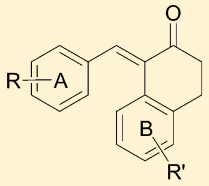
ABSTRACT: A series of 1-benzylidene-3,4-dihydronaphthalen-2-one derivatives were designed and synthesized, and their biological activities in vitro and in vivo were evaluated. The results showed a number of the title compounds exhibiting potent nanomolar activity in several human cancer cell lines. Of these, compound **22b** showed the strongest inhibitory activity against human CEM, MDA-MBA-435, and K562 cells (IC_{50} = 1 nM), displayed in vitro inhibition of tubulin polymerization (IC_{50} = 3.93 μM), and significantly induced cell cycle arrest in G2/M phase. In addition, compound **22b** could inhibit the tumor growth in colon nude mouse xenograft tumor model significantly and seemed safer than CA-4 when achieving a similar tumor suppression. This study provided a new molecular scaffold for the further development of antitumor agents that target tubulin.



22b R = 3,4,5-$(OCH_3)_3$, R' = 6-OCH_3
CEM, MDA-MBA-435, K562, IC_{50} = 1 nM
Tubulin assembly, IC_{50} = 3.93 ± 0.40 μM

INTRODUCTION

Microtubule arrangement and rearrangement play important roles in many aspects of cellular structure and function. Investigations into the polymerization dynamics that regulate microtubule efficacy and in turn cellular replication may prove useful in the development of anticancer therapeutics.[1] This is one of the reasons why microtubules are an attractive molecular target for anticancer agents. Compounds target microtubules bind at three distinct binding sites:[2] the taxane site for microtubule-stabilizing agents,[3,4] the vinca site,[5] and the colchicine site[6,7] for microtubule-destabilizing agents. The taxanes and vinca alkaloids have been successfully used in clinical oncology;[8−10] however, none of the colchicine site inhibitors (CSIs) have been approved in clinic as antitumor agents.

The first CSI colchicine, which inhibits cancer cell proliferation powerfully, has limited its applications in clinic as antitumor agent because of its narrow therapeutic index.[11] Recent studies show that another CSI combretastatin A-4 (CA-4, Figure 1) with strong cytotoxicities has activity as vascular disrupting agents (VDAs) targeting already established vasculature of solid tumors, causing rapid and catastrophic vascular shutdown to induce widespread necrosis in solid tumors.[12−14] Then a large number of structurally diverse CA-4 analogues have been developed[15−18] as VDAs in the past decade, and some of them have entered clinical trials.[19−26] (Figure 1).

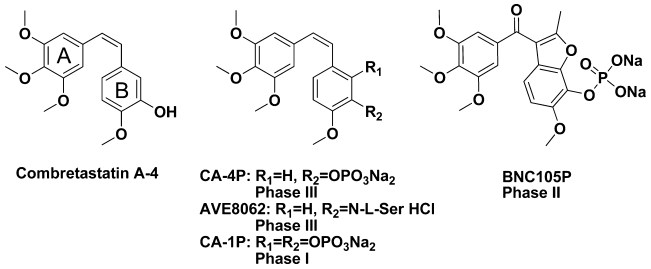


Figure 1. Structures of CA-4 and its analogues that entered clinical trials.

The early phase clinical trials in many solid tumors have demonstrated that these CA-4 analogues have promising therapeutic benefits.[27,28] However, many of them were also hampered by dose-limiting toxicities[21] or cumulative toxicities.[20] Some of these toxicities came from the effect on other tissues of these drugs (lack of specificity of tumor-related vasculature)[29] or from the systemic effects of cytokines and other factors produced in compensatory response of tumors.[30] Currently, CA4P in combination with antiangiogenic drugs, metronomic chemotherapy, or radiotherapy has been investigated to improve the therapeutic index in preclinical and clinical studies.[31−34] Furthermore, developing novel CA-4 analogues of improved specificity is another important approach to reduce their toxicity.[35−38]

It is well-known that CSIs have three important pharmacophore components, a linking bridge and two hydrophobic rings (rings A and B, Figure 1), and the appropriate dihedral angle and cis-configuration of the two rings are important. As the two hydrophobic rings of the CA-4 analogues are essential to their activities,[39−42] a large number of modifications of CA-4 focused on the linking bridge.[43−48] There also some modifications focused on combining the linking bridge with the B ring, which provided a potential advantage in promoting the acitivities,[49−53] enhancing the tumor selectivity, and minimizing the toxicity.[36−38,54] Therefore, we designed a new CA-4 analogue with the 1-benzylidene-3,4-dihydronaphthalen-2-one skeleton, in which the relatively flexible six-membered ring connects the

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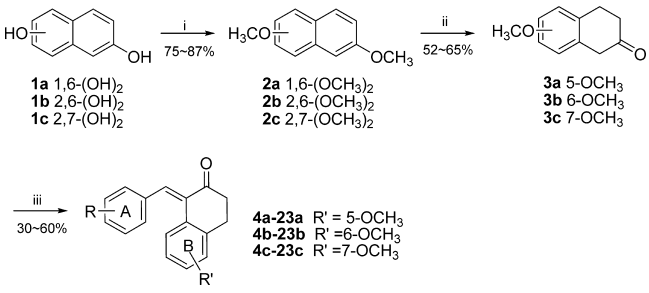
linking bridge and B ring. It provided proper restriction to the dihedral angle of the two aromatic rings, and the carbonyl group near the linking bridge can improve its solubility and may be favorable for activity because of its polar interactions with the β-Ala250−Leu255 loop at the tubulin colchicine site.[55−57]

In this study, a series of 1-benzylidene-3,4-dihydronaphthalen-2-one derivatives were synthesized, and their cytotoxicity levels were evaluated. The effects on tubulin polymerization and the tumor cell cycle distribution of selected compounds were investigated to determine their exact mechanism, and their modes of binding to tubulin were studied by molecular docking. The most promising compound was further tested for antitumor activity and toxicity in vivo.

RESULTS AND DISCUSSION

Chemistry. The general route for the synthesis of the target 1-benzylidene-3,4-dihydronaphthalen-2-one derivatives is depicted in Scheme 1. The appropriate dimethoxynaphthalenes

Scheme 1. Synthesis of the Target Compounds[a]



[a]Reagents: (i) $(CH_3)_2SO_2$, NaOH; (ii) Na, EtOH; (iii) substituted benzylidene, AcOH/piperidine, CH_2Cl_2.

were prepared by methylation with dimethyl sulfate. The intermediates **2a−c** were chemoselectively reduced at the β-position of the naphthalene ring using sodium in refluxing ethanol to yield derivatives **3a−c**. The Knoevenael reaction with the appropriate benzylidene catalyzed by piperidine and acetic acid at room temperature furnished 1-benzylidene-3,4-dihydronaphthalen-2-one derivatives.[58] The structures of 60 targeting compounds are listed in Scheme 1.

The steric hindrance of the carbonyl group on naphthalen-2-one was found to favor the *E* isomer. Though the final compounds could exist as either *E* or *Z* isomers because of the presence of the exocyclic double bond, the synthesis gave rise to a single (predominant) isomer. To distinguish the configuration (*E* or *Z*) of these compounds, we focused on the chemical shifts of the H-2′ or H-6′ protons on the benzylidene A ring. In the literature,[59] deshielding by the carbonyl group on the naphthalen-2-one would make chemical shifts of the H-2′ or H-6′ protons downfield from 7.45−7.84 ppm (*E* isomer) to 7.85−8.53 ppm (*Z* isomer). All the chemical shifts of the aromatic protons were below 7.84 ppm, consistent with the literature and indicating that the products were *E* isomers. To unequivocally confirm the configuration of these 1-benzylidene-3,4-dihydronaphthalen-2-one analogues, compound **22b** was subjected to single-crystal X-ray analysis, as shown in Figure 2A, which also verified the *E* configuration.

X-ray crystal analysis of one compound indicated a low-energy conformation. In order to determine whether the 1-benzylidene-3,4-dihydronaphthalen-2-one skeleton could provide a proper restriction to the dihedral angle of the two aromatic rings, the X-ray crystal structure of compound **22b** was superimposed onto the binding conformation to tubulin of CA-4. As shown in Figure 2B, the conformation of compound **22b** fits that of CA-4 very well. The 1-benzylidene-3,4-dihydronaphthalen-2-one, via its flexible nonplanar structure incorporating an olefin linking bridge, restricted the appropriate dihedral angle of the two rings in a proper range.

Biological Activity. The in vitro antitumor activity of all the synthesized 1-benzylidene-3,4-dihydronaphthalen-2-one analogues was tested in three human tumor cell lines by MTT assay: the CEM human leukemia, HCT-116 human colon cancer, and MDA-MB-435 human breast carcinoma cell lines. These cell lines were selected from the solid tumors[19,32] and non-solid tumors[60] used in preclinical and clinical research of CA-4 analogues. CA-4 was used as a positive control. The results showed that all of the target compounds exhibited anticancer activity (Table 1). Compound **22b** displayed the strongest antiproliferative activity against CEM (IC_{50} = 1 nM), MDA-MBA-435 (IC_{50} = 1 nM), and HCT-116 cells (IC_{50} = 0.1 μM). The compounds' cytotoxicities against CEM and MDA-MBA-435 cells were compared to those of CA-4. In a follow-up assay, compound **22b** also showed potent cytotoxicity against other cell lines of these three tumors: the K562 human leukemia (IC_{50} = 1 nM), HT-29 human colon cancer

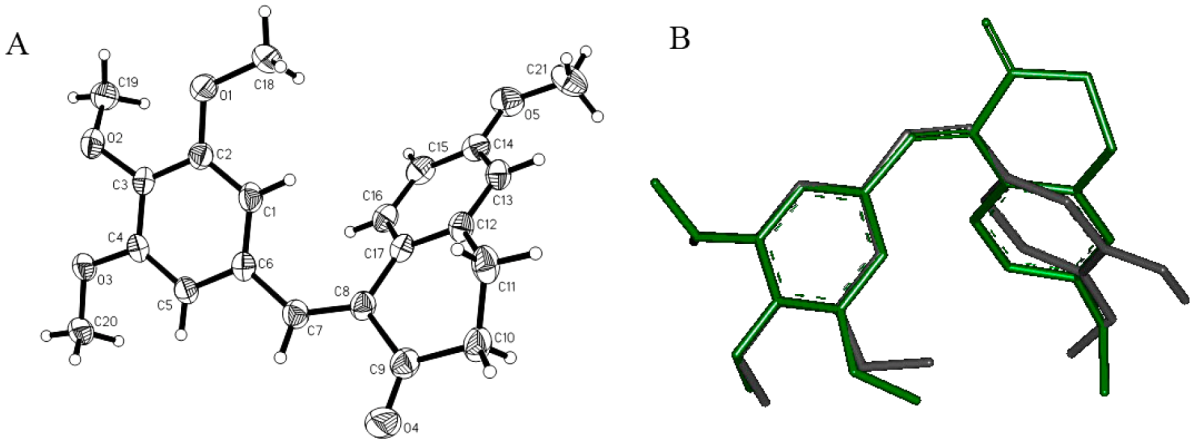


Figure 2. X-ray structure of compound **22b**: (A) ORTEP drawing of compound **22b**; (B) superposition of **22b** (green) with the docking modes of CA4 (gray) bound to the colchicine of tubulin.

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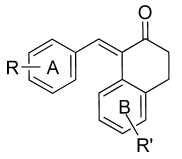
Table 1. In Vitro Antiproliferative Effects of the Target Compounds[a]



4a-23a R'=5-OCH_3
4b-23b R'=6-OCH_3
4c-23c R'=7-OCH_3

		IC_{50} (μM)					IC_{50} (μM)					IC_{50} (μM)		
compd	R	CEM	HCT-116	MDA-MB-435	compd	R	CEM	HCT-116	MDA-MB-435	compd	R	CEM	HCT-116	MDA-MB-435
4a	H	8.69 ± 0.81	>10	6.31 ± 0.84	**4b**	H	0.92 ± 0.16	0.47 ± 0.06	0.94 ± 0.18	**4c**	H	1.53 ± 0.12	3.21 ± 0.30	1.45 ± 0.087
5a	4-OCH_3	2.64 ± 0.32	3.58 ± 0.30	1.37 ± 0.13	**5b**	4-OCH_3	0.11 ± 0.021	0.86 ± 0.11	0.21 ± 0.04	**5c**	4-OCH_3	0.89 ± 0.051	1.91 ± 0.10	0.73 ± 0.14
6a	4-CH_3	>10	>10	>10	**6b**	4-CH_3	2.31 ± 0.44	2.56 ± 0.32	1.33 ± 0.24	**6c**	4-CH_3	3.77 ± 0.30	3.68 ± 0.37	2.62 ± 0.18
7a	4-$C(CH_3)_3$	>10	>10	>10	**7b**	4-$C(CH_3)_3$	3.10 ± 0.16	6.70 ± 0.85	1.77 ± 0.25	**7c**	4-$C(CH_3)_3$	>10	>10	>10
8a	4-OH	4.32 ± 0.34	2.69 ± 0.28	1.54 ± 0.26	**8b**	4-OH	0.96 ± 0.10	1.13 ± 0.11	0.45 ± 0.058	**8c**	4-OH	1.05 ± 0.20	3.24 ± 0.58	0.86 ± 0.12
9a	4-NO_2	>10	8.68 ± 0.68	7.68 ± 0.80	**9b**	4-NO_2	0.68 ± 0.13	2.23 ± 0.086	0.54 ± 0.067	**9c**	4-NO_2	>10	8.95 ± 0.79	>10
10a	4-CF_3	>10	5.38 ± 0.64	5.89 ± 0.16	**10b**	4-CF_3	1.21 ± 0.17	2.32 ± 0.30	0.75 ± 0.15	**10c**	4-CF_3	>10	6.16 ± 0.78	6.45 ± 0.76
11a	4-Br	>10	6.80 ± 0.48	7.59 ± 1.02	**11b**	4-Br	1.52 ± 0.11	2.94 ± 0.54	1.12 ± 0.067	**11c**	4-Br	>10	>10	>10
12a	4-Cl	8.53 ± 0.48	7.52 ± 1.00	6.86 ± 0.43	**12b**	4-Cl	0.95 ± 0.16	1.25 ± 0.16	0.89 ± 0.13	**12c**	4-Cl	>10	4.69 ± 0.69	5.83 ± 0.23
13a	2-Cl	8.91 ± 0.57	5.77 ± 1.06	3.35 ± 0.51	**13b**	2-Cl	0.58 ± 0.11	1.70 ± 0.30	0.46 ± 0.049	**13c**	2-Cl	5.26 ± 0.51	2.35 ± 0.36	1.68 ± 0.10
14a	2,4-Cl_2	>10	7.14 ± 0.75	9.32 ± 0.38	**14b**	2,4-Cl_2	1.20 ± 0.08	2.15 ± 0.24	0.33 ± 0.064	**14c**	2,4-Cl_2	>10	>10	>10
15a	3,4-Cl_2	>10	>10	>10	**15b**	3,4-Cl_2	1.86 ± 0.32	2.52 ± 0.34	1.23 ± 0.11	**15c**	3,4-Cl_2	>10	>10	>10
16a	2,6-Cl_2	8.64 ± 0.87	6.71 ± 0.98	>10	**16b**	3-OH	1.46 ± 0.26	1.76 ± 0.28	0.76 ± 0.12	**16c**	3-OH	2.57 ± 0.13	4.91 ± 0.84	2.07 ± 0.33
17a	4-$N(CH_3)_2$	>10	9.78 ± 0.15	7.76 ± 0.93	**17b**	4-$N(CH_3)_2$	8.03 ± 1.28	8.73 ± 0.47	5.68 ± 0.55	**17c**	4-$N(CH_3)_2$	>10	>10	>10
18a	3-OH-4-OCH_3	6.31 ± 1.13	>10	5.62 ± 0.98	**18b**	3-OH-4-OCH_3	0.12 ± 0.020	0.32 ± 0.049	0.63 ± 0.062	**18c**	3-OH-4-OCH_3	1.42 ± 0.16	1.37 ± 0.18	1.05 ± 0.16
19a	3-OCH_3-4-OH	>10	>10	8.63 ± 0.39	**19b**	3-OCH_3-4-OH	5.81 ± 0.47	7.50 ± 0.82	4.51 ± 0.54	**19c**	3-OCH_3-4-OH	6.93 ± 1.07	>10	4.35 ± 0.78
20a	3,4-$O(CH_3)_2$	1.35 ± 0.26	2.12 ± 0.36	1.54 ± 0.22	**20b**	3,4-$O(CH_3)_2$	0.072 ± 0.013	0.13 ± 0.020	0.20 ± 0.023	**20c**	3,4-$O(CH_3)_2$	0.27 ± 0.036	1.32 ± 0.16	0.89 ± 0.14
21a	3,5-$O(CH_3)_2$	>10	5.67 ± 0.71	3.95 ± 0.44	**21b**	3,5-$O(CH_3)_2$	0.078 ± 0.013	0.15 ± 0.027	0.39 ± 0.078	**21c**	3,5-$O(CH_3)_2$	0.26 ± 0.044	0.50 ± 0.071	0.85 ± 0.080
22a	3,4,5-$O(CH_3)_3$	2.19 ± 0.42	1.24 ± 0.18	0.61 ± 0.10	**22b**	3,4,5-$O(CH_3)_3$	0.001	0.10 ± 0.014	0.001	**22c**	3,4,5-$O(CH_3)_3$	0.001	0.51 ± 0.092	0.34 ± 0.062
23a	2,3,4-$O(CH_3)_3$	>10	>10	>10	**23b**	2,3,4-$O(CH_3)_3$	0.85 ± 0.13	1.68 ± 0.24	0.95 ± 0.14	**23c**	2,3,4-$O(CH_3)_3$	4.21 ± 0.48	2.68 ± 0.23	1.35 ± 0.14
CA-4		0.001	0.001	0.001										

[a] IC_{50} values are the mean of at least three independent determinations.

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(IC_{50} = 0.01 μM), and ZR-75-30 human breast carcinoma cell lines (IC_{50} = 0.01 μM).

The hydrophobic B ring was fundamental for antiproliferative activity.[40,61] The position of the methoxy substituent on the 3,4-dihydronaphthalen-2-one moiety (B ring) had a critical influence on their cytotoxicities. The SAR information indicated that the methoxy group located at the C-6 position exhibited the best activity; shifting it to the C-5 or C-7 position resulted in moderate activity. Most of the 6-methoxy substituted compounds were 2- to 60- fold more potent than 5- or 7-methoxy counterparts against all the test cancer cells. Notably, the most active compound, **22b**, caused a distinct increase in cytotoxicity by 2−3 orders of magnitude (**22b** vs **22a**, **22c**). The 6-methoxy group on the tetralone ring in 1-benzylidene-3,4-dihydronaphthalen-2-ones may mimic the para-methoxy on the B ring of CA-4, exerting maximum activity.

In the series of 6-methoxy-3,4-dihydronaphthalen-2-one analogues, compound **22b**, which has a 3,4,5- trimethoxy group on its A ring, showed the most pronounced antiproliferative activity. To determine the role of SAR substitutions on the 1-benzylidene ring (A ring of CA-4), we introduced various groups to the A ring. As shown in Table 1, compounds with a methoxy group were generally more cytotoxic than the other moieties. Neither replacement with a smaller group (**5b** vs **6b**) nor replacement with a larger (**5b** vs **7b**, **17b**) caused any decrease in potency. Replacement with more polar groups (**5b** vs **8b**, **9b**, **10b**) resulted in a slight loss of cytotoxic activity.

The number and location of methoxy substituted on A ring played a profound role on antiproliferative activity. A comparison between the antiproliferative activities of compounds **22b**, **20b**, **21b**, and **5b** illustrated the cytotoxic SAR of the number of methoxy groups substituted on the A ring (3 > 2 > 1). However, introducing a methoxy group at the C-2 position on the A ring showed a deleterious effect on activity. It was supported by compound **23b** (2,3,4- trimethoxy), which was around 5- to 10-fold less active than the dimethoxy substituent (**20b** and **21b**) and about 2- to 8-fold less potent than the methoxy substituent (**5b**). Replacing any methoxy on compound **20b** with a hydroxyl moiety (resulting in compounds **18b** and **19b**) was found to cause a 3- to 80-fold decrease in antiproliferative activity against all cell lines, further indicating that the presence of polar substituents on the A ring is unfavorable to potency.

To determine whether these compounds exert their activities by direct interaction with microtubules, selected compounds (**5b**, **20b**, **21b**, **22a**, **22b**, **22c**, and **23b**) were examined for their effects on the in vitro polymerization of tubulin (Table 2).

Table 2. In Vitro Antitubulin Effects of the Target Compounds

compd	inhibition of tubulin assembly, IC_{50} ± SD (μM)
22a	18.68 ± 2.32
22b	3.93 ± 0.40
22c	9.63 ± 1.1
20b	6.42 ± 0.55
21b	6.88 ± 1.03
5b	25.82 ± 2.96
23b	25.78 ± 2.29
CA-4	1.77 ± 0.42

CA-4 was also examined for comparative purposes. The results showed that compound **22b**, which had shown the strongest cytotoxicity, has the strongest antitubulin activity (IC_{50} of 3.93 μM compared to 1.77 μM for CA-4). This trend for the inhibition of tubulin polymerization of the compounds was in good qualitative agreement with their cytotoxic SAR. Compound **22b** (6-methoxy on the B ring), which has the same 3,4,5-trimethoxy group on the A ring, was found to be more potent than **22a** (5-methoxy) and **22c** (7-methoxy) against cancer cell lines. It was also more active against tubulin polymerization. This is consistent with the conclusion that methoxy substituted at the C-6 position of 3,4-dihydronaphthalen-2-one is more effective than at C-5 or C-7. A gradual decrease in antitubulin activity was observed as we moved from compounds **22b**, **20b**, **21b** to **5b**. This was also consistent with their antiproliferative tendencies, which appear to be based on the number of methoxy groups on the A ring. In addition, the antitubulin abilities (**23b** vs **5b**) also illustrated that a methoxy group at the C-2 position on the A ring had a negative effect. This was consistent with the observation of cytotoxicities. On the basis of this correlation between the antitubulin and anticancer activities, we concluded that the antiproliferative effects of these compounds were mediated by direct interaction with tubulin.

Analysis of Cell Cycle Effects. Because the inhibition of tubulin polymerization is often implicated in G2/M-phase cell cycle arrest in various cancer cell lines, the effects of different concentrations of compound **22b** on cell cycle progression were examined with HCT-116 cells. After 24 h of exposure, compound **22b** was found to cause an obvious increase in the number cells in the G2/M phase with a concomitant decrease of cells in the G1 and S phases. This effect appeared to be dose dependent. As shown in Figure 3, lower concentrations of **22b**

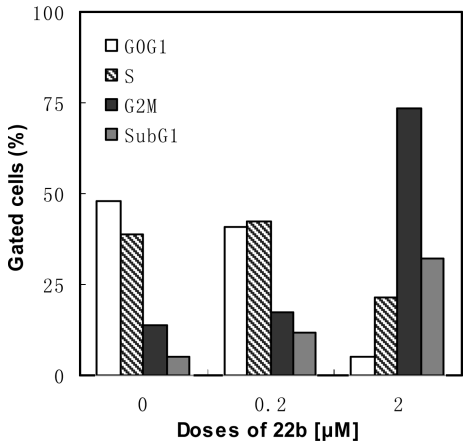

Figure 3. Cell cycle distribution of human cancer HCT-116 cells treated for 24 h with different amounts of **22b**.

(0.2 μM) induced a slight alteration (17% of cells were in the G2/M phase, compared to 13% of untreated cells) in the distribution of HCT-116 cells throughout the cell cycle, whereas higher concentrations (2 μM) induced a massive accumulation (74%).

Molecular Modeling Studies. To make sense of the cytotoxic and antitubulin data observed, molecular docking simulations of the relative compounds to tubulin were carried out. The X-ray crystal structure of the DAMA−colchicine−tubulin complex (PDB code 1SA0) was used as the tubulin

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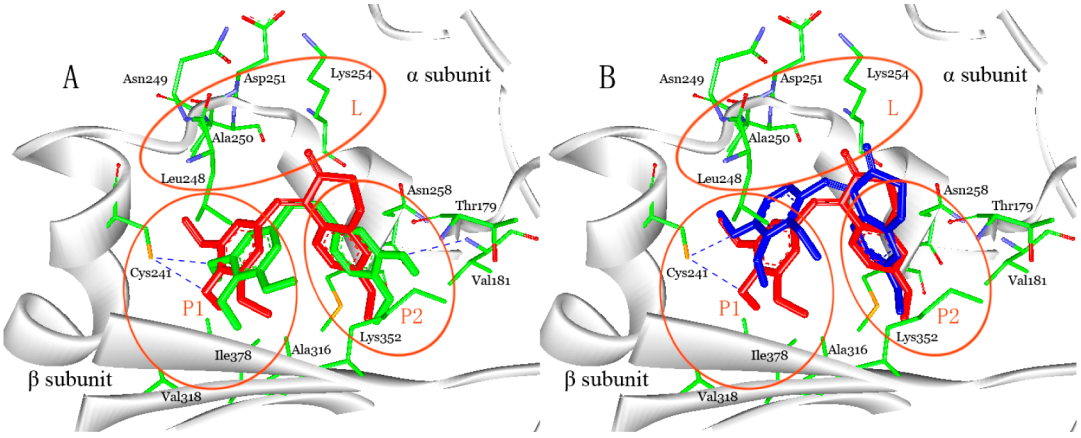

Figure 4. Predicted binding modes with DAMA-colchicine at the β-tubulin binding site: (A) CA-4 (green) and **22b** (red); (B) **22b** (red) and **23b** (blue). P1 and P2 are two hydrophobic pockets in the interface between α- and β-tubulin.

protein template. The result shows that most of the compounds bound to the colchicine binding site of tubulin in the same orientation with CA-4. Compound **22b** bound to tubulin nicely, and the binding mode is shown in Figure 4A. The trimethoxyphenyl moiety can foster van der Waals interactions with the hydrophobic P1 pocket bounded by Leu248, Ala316, Val318, Lys352, and Ile378 of β-tubulin, while the tetralone ring can foster van der Waals interactions with the hydrophobic P2 pocket consisting of Thr179, Val181 of α-tubulin and Asn258, Met259, Lys352 of β-tubulin. Two methoxy O atoms on the A ring form a hydrogen bond with the thiol group of Cys241 of β-tubulin. The carbonyl group on tetralone remains in proximity to the polar region L (Figure 4) with large flexibility (Leu248-Lys254 of β-tubulin), which has the possibility to form hydrogen bonds directly or through the structured water molecules.[57,62]

The methoxy on the B ring is placed in the hydrophobic P2 pocket and enhances the van der Waals interactions with it. However, the methoxy at the C-6 position was buried deeper in the pocket than C-5 or C-7 position when binding, leading to compounds bearing this group to have relatively higher binding abilities, which is consistent with their biological activity levels observed. In addition, the methoxy at C-3, C-4, and C-5 positions of the A ring forms van der Waals interactions well with the hydrophobic P1 pocket. This may be the reason why adding methoxy groups to these positions can enhance their cytotoxic activity, and the presence of polar substituents is unfavorable to activity. The methoxy is the group with optimal volume for interaction with this pocket, which can explain why replacement with neither a smaller group nor a larger group is unfavorable to activity. However, introducing a methoxy group at the C-2 position on the A ring showed a dramatic loss in activity, which can be rationalized by the docking result of compound **23b** compared to compound **22b** (Figure 4B). The A ring cannot occupy the P1 pocket well because of steric hindrance with tubulin of the C-2 position methoxy.

In Vivo Antitumor Activity. Compound **22b** and CA-4 were preliminarily evaluated for toxicity against male ICR mice, using ip administration once on day 1. Mice were randomly divided into the control group and intervention groups (150, 300, 600 mg/kg for compound **22b**; 50 and 100 mg/kg for CA-4) with 20 mice in each group and half male and half female. The dose 100 mg/kg for CA-4 caused one mouse (1/20) to die on day 2 and another two mice to die on day 5, and the other doses for CA-4 and compound **22b** did not cause any mouse to die. The body weight change of the mice over the course of the experiment is shown in Figure 5. Compared to

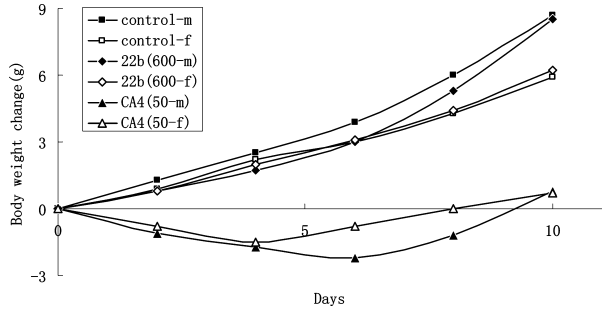

Figure 5. Acute toxicity testing for compound **22b** and CA-4.

the control vehicle-treated mice, compound **22b**, even at a dose of 600 mg/kg, did not cause significant body weight loss, which is indicative of negligible systemic toxicity over 10 days. However, significant body weight loss was observed at 50 mg/kg CA-4, suggesting some systemic toxicity.

Guided by the preliminary toxicity experiment, the doses (100, 150, 300 mg/kg for compound **22b**; 30 and 50 mg/kg for CA-4) were chosen to evaluate their in vivo antitumor activity. Human colon carcinoma xenografts were established by subcutaneous implantation of HT-29 cells (5×10^6) on the right flanks of BALB/C nude mice. Once the HT-29 xenografts reached a size of around 100 mm^3, mice were randomly assigned into control group (12 mice) and intervention groups (6 mice per group). Antitumor activities of compound **22b** and CA-4 (ip once a day) were studied with cisplatin (ip the first 2 days) as a positive control. The results (Figure 6 and Table 3) showed that compound **22b** at 100, 150, and 300 mg/kg dose-dependently decreased tumor volumes on day 21 (T/C = 52%, 45%, and 34%, respectively), while the T/C of CA-4 at 50 mg/kg was 47%. Compared to the control vehicle-treated mice, compound **22b** at diverse doses did not cause obvious body weight loss, and the vital organs (kidney, liver, and spleen) examined after euthanization at the end of the experiment appear the same in terms of color, weight, and size, indicating negligible toxicity to the mice. However, obvious body weight loss was observed at every dose of CA-4, and lost weight of the vital organs was observed at 50 mg/kg CA-4.

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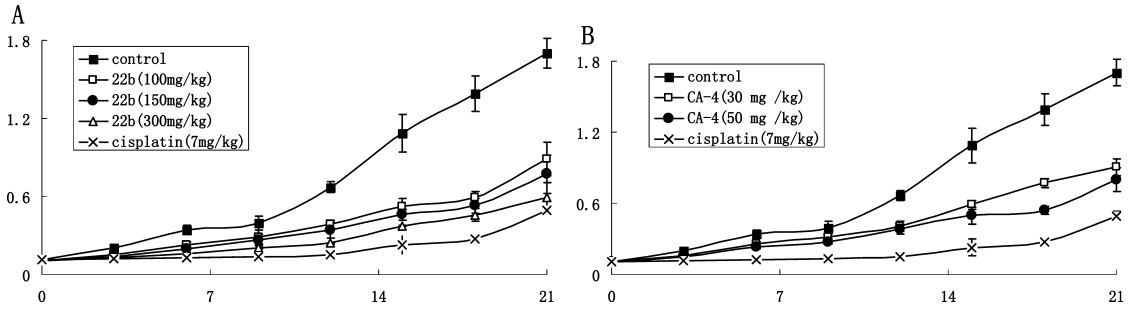

Figure 6. Inhibition of human xenograft growth in vivo by compound **22b** and CA-4. HT-29 tumor-bearing nude mice were administered vehicle alone or 100, 150, 300 mg/kg compound **22b** or 30, 50 mg/kg CA-4 intraperitoneally once a day. The figure shows the tumor volume of **22b** (A) and CA-4 (B) recorded at the indicated days after treatments. Data are expressed as mean ± SEM of tumor volume at each time point.

Table 3. Blood Analysis after in Vivo Antitumor Activity against Human Colon Carcinoma Model[a]

compd (mg/kg)	mice (*n*)	toxic death	tumor volume, mean ± SD (mm^3)	*T*/*C* (%)	body wt change, mean (g)	WBC ($\times10^3$ mm^{-3})	LY ($\times10^3$ mm^{-3})	PLT ($\times10^3$ mm^{-3})	ALT (IU/L)	ALP (IU/L)
control	12	0/12	1702 ± 114	100	2.7	25.38 ± 0.22	15.88 ± 0.41	1509 ± 34	28 ± 0.4	107.6 ± 0.8
22b (100)	6	0/6	888 ± 126**	52	2.3	24.42 ± 0.27**	11.08 ± 0.27**	1440 ± 4	24 ± 0.6*	112 ± 1.3*
22b (150)	6	0/6	772 ± 149**	45	3	20.18 ± 0.10**	10.03 ± 0.85*	1375 ± 21	28 ± 0.6	116.3 ± 0.8*
22b (300)	6	0/6	595 ± 73**	34	2.6	19.96 ± 0.49**	8.87 ± 0.61**	1240 ± 34*	26 ± 0.6	107.6 ± 0.8
CA-4 (30)	6	0/6	905 ± 98**	53	1.7	19.34 ± 0.38**	10.62 ± 2.01	1923 ± 38*	25 ± 0.6*	105.3 ± 1.1
CA-4 (50)	6	0/6	799 ± 98**	47	1.8	12.09 ± 0.20**	4.14 ± 0.04**	2206 ± 38**	66.3 ± 0.4**	149.3 ± 1.1**
cisplatin (7)	6	0/6	493 ± 41**	29	1.1	7.34 ± 0.24**	4.11 ± 0.14**	902 ± 14**	29.6 ± 1.1	127.6 ± 2.8*

[a]WBC = white blood cell. LY = lymphocyte. PLT = platelet. ALT = alanine transaminase. ALP = alkaline phosphatase. Data are expressed as the mean ± SEM. (*) $P < 0.05$, (**) $P < 0.01$ vs control.

The biochemical parameters (Table 3) examined at the end of the experiment showed that, after compound treatment, white blood cells and lymphocytes may decrease and alanine transaminase and alkaline phosphatase may increase compared to the vehicle control, which indicated some toxicity to liver and bone marrow. However, at a dose (compound **22b**, 150 mg/kg; CA-4, 50 mg/kg) achieving a similar *T*/*C* ratio, the change of the biochemical parameters of compound **22b** was less obvious than that of CA-4. Even at a greater dose achieving a larger *T*/*C* ratio (300 mg/kg, *T*/*C* = 34%), compound **22b** still displayed this advantage.

All these results showed that this CA-4 analogue compound **22b** could inhibit tumor growth significantly and seemed safer than CA-4 when achieving a similar tumor suppression, which is probably attributed to its negligible effect on normal tissues and good specificity of tumor-related vasculature. The exact mechanism still needs further research.

CONCLUSION

A series of 1-benzylidene-3,4-dihydronaphthalen-2-one derivatives were designed and synthesized, and their biological activities were evaluated in vitro and in vivo. The results showed all the title compounds to exhibit inhibitory activity against the three human cancer cell lines. Preliminary structure−activity relationship studies indicated that substitution at the C-6 position of the B ring of the title molecule was beneficial to cytotoxic activity. Among all analogues synthesized in this study, (*E*)-1-(3,4,5-trimethoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (**22b**) was the most potent inhibitor of human tumor cell growth (IC_{50} of 1 nM against several human tumor cell lines) and tubulin polymerization (IC_{50} of 3.93 μM). A flow cytometric study showed that a significant percentage of HCT-116 cells treated with the most potent compound, **22b**, became arrested in the G2/M phases of the cell cycle. These results showed that the antiproliferative effects of these compounds were mediated by direct interaction with tubulin. Then molecular docking simulations of these compounds to tubulin were carried out. The results show that they are nicely bound to the colchicine binding site of tubulin with a very similar binding mode of CA-4 and can rationalize the experimental SAR well. The antitumor activity in vivo of compound **22b** was tested, and the results showed that compound **22b** could inhibit the tumor growth in colon nude mouse xenograft tumor model (HT-29 cells) significantly and seemed safer than CA-4 when achieving a similar tumor suppression. This study provides a new molecular scaffold for the further development of antitumor agents that target tubulin.

EXPERIMENTAL SECTION

Chemistry. General Methods. Melting points were measured on an electrically heated RK-Z melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a PE Spectrum One FI-IR spectrometer as KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300P spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are given in ppm (δ), and the spectral data are consistent with the assigned structures. The mass spectra were recorded on a Micromass Qtof-Micro LC−MS instrument. Crystal structure was determined on a Bruker SMART CCD (Bruker Company, Germany). Silica gel thin-layer chromatography was performed on precoated plates GF-254. Silica gel column chromatography was performed with silica gel 60 G. All compounds were routinely checked by TLC by using silica gel plates GF-254. All solvents and reagents were analytically pure and, when necessary, were purified and dried by standard protocols. All starting materials were commercially available unless otherwise indicated. Yields of purified products were not optimized. The purity of each compound (>95%) was determined on an Agilent 1100 series LC system (column, ZORBAX

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Eclipse XDB C8, 4.6 mm × 150 mm, 5 μm; mobile phase, methanol (80%)/H_2O; flow rate, 1.0 mL/min; UV wavelength, absorbance at 254 nm; temperature, ambient; injection volume, 20 μL).

General Method for the Synthesis of Methoxy-2-tetralones 3a–c. The method to synthesize 5-methoxy-2-tetralone (**3a**) is described. To a 1 L round-bottom flask fitted with mechanical stirrer, 1,6-dimethoxynaphthalene (20.0 g, 0.11 mol) was added to the refluxing anhydrous ethanol (200 mL). Sodium (19 g, 0.83 mol), cut into small pieces, was added as rapidly as possible to the solution. Refluxing was continued until all sodium disappeared. The reaction mixture was cooled to 10 °C, and then 2 N hydrochloric acid (about 330 mL) was added dropwise until the pH was changed to 3. The reaction mixture was extracted with acetic ether (50 mL × 3). The ethyl acetate layers were combined and washed with saturated brine solution (50 mL × 3), dried over anhydrous sodium sulfate, and filtrated, and the solvent was evaporated. The residual oil was purified by distillation under reduced pressure to yield 11.8 g (63%) of **3a** as yellow oil, which solidified under room temperature: bp 160–165 °C (10 mmHg). Recrystallization from petroleum ether (bp 60–90 °C) gave **3a** as a white needle: mp 38–40 °C

Similarly, **3b** and **3c** were synthesized from the appropriate dimethoxynaphthalenes. Compound **3b**: mp 36–38 °C (lit. mp 36 °C). Compound **3c**: mp 28–30 °C (lit. mp 27–28 °C)

General Method for the Synthesis of (*E*)-1-Benzylidenemethoxy-2-tetralone Analogues (4–23). (*E*)-1-(3,4,5-Trimethoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (22b). A solution of compound **3b** (1.76 g, 10 mmol) and 3,4,5-trimethoxybenzaldehyde (1.96 g, 10 mmol) in dichloromethane (30 mL) was stirred under room temperature for 24 h. The reaction mixture was washed with water, saturated brine solution. The combined organic layers were dried over anhydrous sodium sulfate, filtered, evaporated in vacuo, and purified by column chromatography on silica gel (eluent, petroleum ether/acetic ether, 50:1 to 10:1 v/v) or recrystallization to afford the pure compound **22b** as a yellow crytstal. Yield 47%. Mp: 102–104 °C. HPLC: 99.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.44 (d, *J* = 8.6 Hz, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 6.72 (s, 2H), 6.64 (dd, *J* = 8.6, 2.2 Hz, 1H), 3.85 (s, 6H), 3.71 (s, 6H), 3.01 (t, *J* = 6.4 Hz, 2H), 2.62 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.78, 159.42, 152.91, 140.23, 138.68, 133.21, 132.59, 130.60, 130.46, 124.86, 112.73, 111.81, 106.86, 60.89, 55.93, 55.25, 36.98, 28.05; HRMS (ES+) *m*/*z* found 354.1466; $C_{21}H_{22}O_5$(M^+) requires 354.1467.

The synthetic methods for the following compounds were similar to the synthesis of compound **22b**.

(*E*)-1-Benzylidene-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (4a). Yield 58%, yellow crystal. Mp: 110–112 °C. HPLC: 96.2%. IR (KBr) ν (cm^{-1}): 3355, 3072, 3020, 2995, 2958, 2904, 2831, 1688, 1606, 1569, 1488, 1439, 1403, 1359, 1303, 1278, 1241, 1190, 1167, 1096, 1038, 991, 934, 899, 874, 850, 808, 756, 710, 690, 586, 553, 504, 462, 425. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.41–7.44 (m, 2H), 7.24–7.29 (m, 3H), 7.17 (d, *J* = 8.4 Hz, 1H), 6.83 (s, 1H), 6.77 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.45 (s, 3H), 2.99 (t, *J* = 6.3 Hz, 2H), 2.62 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.50, 157.54, 135.37, 135.27, 133.84, 130.73, 129.56, 128.90, 128.87, 128.40, 115.33, 113.16, 55.00, 37.32, 26.90; HRMS (ES+) *m*/*z* found 264.1152; $C_{18}H_{16}O_2$ (M^+) requires 264.1150.

(*E*)-1-Benzylidene-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (4b). Yield 52%, yellow crystal. Mp: 105–107 °C. HPLC: 95.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.40–7.47 (m, 2H), 7.24–7.29 (m, 4H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.57 (dd, *J* = 8.7, 2.4 Hz, 1H), 3.83 (s, 3H), 3.02 (t, *J* = 6.6 Hz, 2H), 2.63 (t, *J* = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.78, 159.40, 140.07, 135.55, 133.31, 133.24, 130.38, 129.40, 128.60, 128.34, 124.94, 112.78, 112.03, 55.20, 37.01, 28.04; HRMS (ES+) *m*/*z* found 264.1166; $C_{18}H_{16}O_2$ (M^+) requires 264.1150.

(*E*)-1-Benzylidene-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (4c). Yield 58%, yellow crystal. Mp: 118–120 °C. HPLC: 97.4%. IR (KBr) ν (cm^{-1}): 3077, 3043, 3021, 2996, 2966, 2933, 2873, 2837, 2795, 1686, 1599, 1574, 1473, 1442, 1408, 1365, 1342, 1301, 1272, 1253, 1181, 1159, 1138, 1067, 1020, 962, 929, 910, 841, 783, 756, 732, 697, 662, 564, 537, 471, 441. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.30–7.36 (m, 2H), 7.23–7.27 (m, 3H), 6.98 (d, *J* = 7.8 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.79 (d, *J* = 7.8 Hz, 1H), 3.88 (s, 3H), 3.10 (t, *J* = 6.3 Hz, 2H), 2.58 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.09, 156.19, 135.44, 135.29, 134.00, 133.91, 129.65, 128.85, 128.28, 127.02, 126.54, 121.45, 109.69, 55.56, 36.55, 19.33; HRMS (ES+) *m*/*z* found 264.1151; $C_{18}H_{16}O_2$ (M^+) requires 264.1150.

(*E*)-1-(4-Methoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (5a). Yield 55%, yellow crystal. Mp: 106–108 °C. HPLC: 96.6%. IR (KBr) ν (cm^{-1}): 3075, 2994, 2953, 2906, 2842, 1682, 1606, 1588, 1510, 1490, 1462, 1442, 1409, 1364, 1295, 1251, 1208, 1171, 1136, 1110, 1028, 946, 904, 871, 826, 720, 590, 527, 502. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.18 (d, *J* = 8.1 Hz, 1H), 6.99 (s, 1H), 6.81 (d, *J* = 7.8 Hz, 2H), 6.76 (s, 1H), 3.82 (s, 3H), 3.55 (s, 3H), 2.96 (s, 2H), 2.60 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.69, 160.38, 157.52, 135.30, 133.76, 131.93, 131.63, 130.70, 128.81, 127.19, 114.83, 113.78, 112.93, 55.25, 55.14, 37.36, 26.92; HRMS (ES+) *m*/*z* found 294.1255; $C_{19}H_{18}O_3$ (M^+) requires 294.1256.

(*E*)-1-(4-Methoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (5b). Yield 52%, yellow crystal. Mp: 118–120 °C. HPLC: 97.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.47 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 2H), 6.87 (d, *J* = 8.1 Hz, 2H), 6.80 (d, *J* = 2.7 Hz, 1H), 6.60 (dd, *J* = 8.7, 2.1 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.00 (t, *J* = 6.6 Hz, 2H), 2.61 (t, *J* = 6.6 Hz, 2H); HRMS (ES+) *m*/*z* found 294.1257; $C_{19}H_{18}O_3$ (M^+) requires 294.1256.

(*E*)-1-(4-Methoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (5c). Yield 60%, yellow crystal. Mp: 128–130 °C. HPLC: 95.8%. IR (KBr) ν (cm^{-1}): 3091, 3072, 3002, 2955, 2932, 2906, 2882, 2835, 1687, 1604, 1568, 1507, 1466, 1438, 1367, 1340, 1305, 1255, 1168, 1140, 1110, 1067, 1031, 964, 911, 825, 798, 779, 732, 517, 428. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.98–7.07 (m, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.76 (s, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.07 (t, *J* = 6.3 Hz, 2H), 2.56 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.17, 160.33, 156.20, 135.40, 134.44, 132.05, 131.61, 127.41, 126.96, 126.48, 121.17, 113.72, 109.46, 55.55, 55.23, 36.64, 19.34; HRMS (ES+) *m*/*z* found 294.1254; $C_{19}H_{18}O_3$ (M^+) requires 294.1256.

(*E*)-1-(4-Methylbenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (6a). Yield 40%, yellow crystal. Mp: 104–106 °C. HPLC: 96.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.1 Hz, 2H), 6.92 (s, 1H), 6.77 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.83 (dd, *J* = 8.4 Hz, *J* = 2.2 Hz, 1H), 3.52 (s, 3H), 2.98 (t, *J* = 6.6 Hz, 2H), 2.61 (t, *J* = 6.6 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.63, 157.49, 139.26, 135.56, 133.57, 133.03, 132.08, 130.68, 129.70, 129.06, 128.76, 114.96, 113.17, 55.06, 37.31, 26.89, 21.38; HRMS (ES+) *m*/*z* found 278.1330; $C_{19}H_{18}O_2$(M^+) requires 278.1307.

(*E*)-1-(4-Methylbenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (6b). Yield 46%, yellow crystal. Mp: 120–122 °C. HPLC: 96.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.35 (dd, *J* = 8.1, 2.7 Hz, 3H), 7.07 (d, *J* = 8.1 Hz, 2H), 6.80 (d, *J* = 2.7 Hz, 1H), 6.60 (dd, *J* = 8.7, 2.7 Hz, 1H), 3.83 (s, 3H), 3.00 (t, *J* = 6.3 Hz, 2H), 2.62 (t, *J* = 6.3 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.88, 159.34, 140.05, 138.94, 133.57, 132.53, 130.29, 129.52, 129.11, 125.23, 112.76, 112.03, 55.23, 37.10, 28.10, 21.41; HRMS (ES+) *m*/*z* found 278.1307; $C_{19}H_{18}O_2$(M^+) requires 278.1307.

(*E*)-1-(4-Methylbenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (6c). Yield 48%, yellow crystal. Mp: 160–162 °C. HPLC: 97.9%. IR (KBr) ν (cm^{-1}): 3092, 3022, 2959, 2889, 2855, 2835, 1690, 1608, 1594, 1573, 1503, 1474, 1438, 1400, 1365, 1340, 1303, 1264, 1249, 1183, 1163, 1141, 1109, 1068, 1020, 986, 965, 910, 887, 844, 813, 789, 780, 731, 693, 669, 573, 499, 472, 430. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.06 (d, *J* = 8.1 Hz, 2H), 6.99 (d, *J* = 4.5 Hz, 2H), 6.78 (t, *J* = 4.5 Hz, 1H), 3.89 (s, 3H), 3.08 (t, *J* = 6.6 Hz, 2H), 2.57 (t, *J* = 6.6 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.19, 156.16, 139.22, 135.65, 134.25, 133.12, 132.22, 129.78, 129.02, 126.95, 126.50, 121.35, 109.54,

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55.55, 36.60, 21.43, 19.33; HRMS (ES+) m/z found 278.1306; $C_{19}H_{18}O_2(M^+)$ requires 278.1307.

(*E*)-1-(4-*tert*-Butylbenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (7a). Yield 53%, yellow crystal. Mp: 140−142 °C. HPLC: 99.3%. IR (KBr) ν (cm^{-1}): 3027, 2955, 2952, 2906, 2863, 2836, 1693, 1612, 1594, 1571, 1489, 1402, 1362, 1302, 1273, 1244, 1192, 1166, 1093, 1040, 993, 904, 828, 800, 709, 591, 549, 492, 420. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 1H), 6.89 (d, J = 2.7 Hz, 1H), 6.77 (dd, J = 8.4, 2.7 Hz, 1H), 3.47 (s, 3H), 2.98 (t, J = 6.6 Hz, 1H), 2.62 (t, J = 6.6 Hz, 1H), 1.31 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.68, 157.45, 152.43, 135.52, 133.57, 133.29, 132.25, 130.70, 129.49, 128.79, 125.30, 115.39, 112.92, 54.94, 37.37, 34.76, 31.11, 26.92; HRMS (ES+) m/z found 320.1778; $C_{22}H_{24}O_2$ (M^+) requires 320.1776.

(*E*)-1-(4-*tert*-Butylbenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (7b). Yield 56%, yellow crystal. Mp: 146−148 °C. HPLC: 97.1%. IR (KBr) ν (cm^{-1}): 3445, 3360, 3027, 2972, 2952, 2864, 1688, 1600, 1494, 1460, 1406, 1362, 1308, 1259, 1229, 1191, 1163, 1141, 1108, 1089, 1029, 912, 873, 843, 824, 714, 664, 621, 561, 470. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.40 (s, 3H), 7.28 (d, J = 6.6 Hz, 2H), 6.81 (s, 1H), 6.62 (d, J = 8.4 Hz, 1H), 3.84 (s, 3H), 3.00 (s, 2H), 2.61 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.02, 159.33, 152.21, 140.01, 133.44, 132.57, 132.37, 130.30, 129.41, 125.28, 112.71, 112.06, 55.23, 37.06, 34.74, 31.14, 28.07; HRMS (ES+) m/z found 320.1776; $C_{22}H_{24}O_2$ (M^+) requires 320.1776.

(*E*)-1-(4-*tert*-Butylbenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (7c). Yield 46%, yellow powder. Mp: 150−152 °C. HPLC: 96.3%. IR (KBr) ν (cm^{-1}): 3079, 3002, 2960, 2869, 2838, 1688, 1595, 1572, 1505, 1471, 1440, 1403, 1364, 1342, 1250, 1176, 1158, 1139, 1105, 1072, 1019, 962, 912, 827, 777, 727, 674, 576, 440. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 7.8 Hz, 2H), 6.80 (d, J = 7.5 Hz, 1H), 3.89 (s, 3H), 3.08 (t, J = 6.6 Hz, 2H), 2.57 (t, J = 6.6 Hz, 2H), 1.31 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.33, 156.16, 152.48, 135.49, 134.31, 133.19, 132.12, 129.68, 126.94, 126.52, 125.20, 121.40, 109.60, 55.56, 36.58, 34.76, 31.15, 19.34; HRMS (ES+) m/z found 320.1779; $C_{22}H_{24}O_2$ (M^+) requires 320.1776.

(*E*)-1-(4-Hydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (8a). Yield 40%, yellow crystal. Mp: 150−152 °C. HPLC: 95.4%. IR (KBr) ν (cm^{-1}): 3377, 3021, 2995, 2940, 2908, 2834, 1679, 1612, 1586, 1512, 1488, 1432, 1403, 1367, 1303, 1275, 1249, 1191, 1167, 1100, 1039, 994, 961, 906, 831, 803, 706, 627, 587, 549, 525, 477, 446; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 6.98−7.12 (m, 2H), 6.82 (d, J = 7.2 Hz, 2H), 6.70−6.76 (m, 1H), 5.50 (s, 1H), 3.82 (s, 3H), 2.99 (t, J = 6.3 Hz, 2H), 2.59 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 280.1098; $C_{18}H_{16}O_3$ (M^+) requires 280.1099.

(*E*)-1-(4-Hydroxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (8b). Yield 42%, yellow crystal. Mp: 146−148 °C. HPLC: 95.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.41 (dd, J = 8.4, 2.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 6.77 (dd, J = 8.1, 2.7 Hz, 2H), 6.70 (d, J = 2.7 Hz, 1H), 6.58 (dd, J = 8.7, 2.4 Hz, 2H), 5.62 (s, 1H) 3.88 (s, 3H), 3.83 (s, 3H), 3.00 (t, J = 6.6 Hz, 2H), 2.61 (t, J = 6.6 Hz, 2H); HRMS (ES+) m/z found 280.1086; $C_{18}H_{16}O_3$ (M^+) requires 280.1099.

(*E*)-1-(4-Hydroxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (8c). Yield 42%, yellow crystal. Mp: 153−155 °C. HPLC: 99.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.98−7.07 (m, 2H), 6.78 (d, J = 7.2 Hz, 1H), 6.73 (d, J = 8.4 Hz, 2H), 5.58 (s, 1H), 3.88 (s, 3H), 3.06 (t, J = 6.3 Hz, 2H), 2.56 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 203.02, 156.91, 156.21, 135.85, 134.31, 131.90, 127.26, 126.89, 126.55, 121.18, 115.36, 109.52, 55.59, 36.70, 19.31; HRMS (ES+) m/z found 280.1119; $C_{18}H_{16}O_3$ (M^+) requires 280.1099.

(*E*)-1-(4-Nitrobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (9a). Yield 52%, yellow crystal. Mp: 131−133 °C. HPLC: 95.2%. IR (KBr) ν (cm^{-1}): 3097, 3021, 2998, 2969, 2943, 2907, 2835, 1698, 1608, 1520, 1485, 1462, 1343, 1305, 1282, 1247, 1209, 1165, 1107, 1038, 889, 866, 848, 812, 751, 709, 689, 586, 558, 498, 441; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 9 Hz, 2H), 7.65 (s, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.27 (s, 1H), 7.24 (d, J = 9 Hz, 1H), 6.83 (dd, J = 8.4, 2.2 Hz, 1H), 3.51 (s, 3H), 3.03 (t, J = 6.3 Hz, 2H), 2.66 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 309.0998; $C_{18}H_{15}NO_4$ (M^+) requires 309.1001.

(*E*)-1-(4-Nitrobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (9b). Yield 50%, yellow crystal. Mp: 150−152 °C. HPLC: 95.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 8.7 Hz, 2H), 7.63 (s, 1H), 7.50 (d, J = 8.7 Hz, 2H), 7.24 (s, 1H), 7.18 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 8.4, 2.4 Hz, 1H), 3.51 (s, 3H), 3.03 (t, J = 6.3 Hz, 2H), 2.66 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 309.1002; $C_{18}H_{15}NO_4$ (M^+) requires 309.1001.

(*E*)-1-(4-Nitrobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (9c). Yield 50%, yellow crystal. Mp: 156−158 °C. HPLC: 96.4%. IR (KBr) ν (cm^{-1}): 3112, 3082, 3029, 2989, 2966, 2931, 2906, 2859, 2833, 1697, 1594, 1516, 1471, 1434, 1405, 1342, 1296, 1247, 1176, 1133, 1108, 1066, 962, 914, 841, 787, 749, 688, 566, 478, 417. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, J = 11.1 Hz, 2H), 7.62 (s, 1H), 7.48 (d, J = 11.1 Hz, 2H), 6.98 (t, J = 8.1 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.72 (d, J = 7.5 Hz, 1H), 3.88 (s, 3H), 3.11 (t, J = 6.6 Hz, 2H), 2.65 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.47, 156.42, 147.34, 142.49, 136.73, 132.81, 131.99, 130.21, 127.44, 127.00, 123.58, 121.31, 110.48, 55.58, 36.17, 19.27; HRMS (ES+) m/z found 309.1004; $C_{18}H_{15}NO_4$ (M^+) requires 309.1001.

(*E*)-1-(4-Trifluoromethylbenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (10a). Yield 46%, yellow crystal. Mp: 130−131 °C. HPLC: 96.8%. IR (KBr) ν (cm^{-1}): 3063, 2994, 2965, 2944, 2907, 2847, 1699, 1614, 1599, 1568, 1489, 1412, 1356, 1322, 1247, 1165, 1129, 1104, 1063, 1042, 1015, 885, 833, 803, 703, 645, 602, 564. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.48−7.55 (m, 4H), 7.19 (d, J = 8.4 Hz, 1H), 6.78 (dd, J = 8.4, 1.8 Hz, 1H), 6.67 (d, J = 2.1 Hz, 1H), 3.43 (s, 3H), 3.00 (t, J = 6.3 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.16, 157.63, 139.21, 135.55, 133.07, 132.55, 130.92, 129.68, 129.12, 125.33, 125.29, 115.57, 113.27, 54.89, 37.05, 26.77; HRMS (ES+) m/z found 332.1021; $C_{19}H_{15}F_3O_2$ (M^+) requires 332.1024.

(*E*)-1-(4-Trifluoromethylbenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (10b). Yield 48%, yellow crystal. Mp: 123−125 °C. HPLC: 97.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.51 (s, 4H), 7.15 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 2.7 Hz, 1H), 6.58 (dd, J = 8.7, 2.7 Hz, 1H), 3.83 (s, 3H), 3.03 (t, J = 6.3 Hz, 2H), 2.65 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.02, 159.87, 140.41, 139.52, 134.96, 131.09, 130.49, 129.55, 125.37, 124.25, 113.17, 112.25, 55.29, 36.86, 28.00; HRMS (ES+) m/z found 332.1018; $C_{19}H_{15}F_3O_2$ (M^+) requires 332.1024.

(*E*)-1-(4-Trifluoromethylbenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (10c). Yield 53%, yellow crystal. Mp: 123−125 °C. HPLC: 96.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.4 Hz, 1H), 6.95 (s, 1H), 6.67 (d, J = 2.7 Hz, 1H), 3.62 (s, 3H), 3.05 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 332.1028; $C_{19}H_{15}F_3O_2$ (M^+) requires 332.1024.

(*E*)-1-(4-Bromobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (11a). Yield 43%, yellow powder. Mp: 126−128 °C. HPLC: 96.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.42 (d, J = 8.7 Hz, 2H), 7.36 (s, 1H), 7.30 (s, 1H), 7.07 (d, J = 8.7 Hz, 2H), 7.00 (dd, J = 8.4, 2.2 Hz, 1H), 3.51 (s, 3H), 3.05 (t, J = 6.3 Hz, 2H), 2.59 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.42, 157.64, 134.31, 134.10, 133.76, 132.95, 131.63, 131.15, 130.84, 129.03, 123.01, 115.24, 113.24, 55.13, 37.16, 26.84; HRMS (ES+) m/z found 342.0257; $C_{18}H_{15}BrO_2$ (M^+) requires 342.0255.

(*E*)-1-(4-Bromobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (11b). Yield 47%, yellow crystal. Mp: 126−128 °C. HPLC: 97.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.7 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.57 (dd, J = 8.7, 2.4 Hz, 1H), 3.83 (s, 3H), 2.99 (t, J = 6.3 Hz, 2H), 2.61 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.53, 159.62, 140.24, 134.52, 133.73, 131.69, 131.60, 130.95, 130.27, 124.52, 122.59, 113.03, 112.15, 55.24, 36.91,

28.00; HRMS (ES+) m/z found 342.0251; $C_{18}H_{15}BrO_2$ (M^+) requires 342.0255.

(*E*)-1-(4-Bromobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (11c). Yield 56%, yellow crystal. Mp: 130−132 °C. HPLC: 96.5%. IR (KBr) ν (cm^{-1}): 3089, 2955, 2836, 1688, 1599, 1573, 1474, 1460, 1436, 1394, 1362, 1340, 1298, 1266, 1252, 1164, 1100, 1067, 1012, 1010, 986, 967, 906, 889, 846, 817, 797, 783, 733, 711, 697, 669, 647, 568, 545, 499, 476, 441. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.38 (d, J = 8.7 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.1 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 3.88 (s, 3H), 3.08 (t, J = 6.6 Hz, 2H), 2.56 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.81, 156.27, 134.38, 134.21, 133.86, 133.58, 131.54, 131.17, 127.12, 126.74, 122.92, 121.23, 109.88, 55.56, 36.43, 19.29; HRMS (ES+) m/z found 342.0258; $C_{18}H_{15}BrO_2$ (M^+) requires 342.0255.

(*E*)-1-(4-Chlorobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (12a). Yield 46%, yellow powder. Mp: 140−142 °C. HPLC: 97.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.39 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 8.4 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 6.84 (dd, J = 8.4, 2.4 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 3.52 (s, 3H), 3.00 (t, J = 6.3 Hz, 2H), 2.58 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 299.0835; $C_{18}H_{15}ClO_2$ (M + H^+) requires 299.0839.

(*E*)-1-(4-Chlorobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (12b). Yield 42%, yellow powder. Mp: 142−144 °C. HPLC: 97.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.31 (d, J = 8.7 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.7 Hz, 1H), 6.78 (d, J = 2.7 Hz, 1H), 6.57 (dd, J = 8.7, 2.7 Hz, 1H), 3.83 (s, 3H), 2.99 (t, J = 6.6 Hz, 2H), 2.62 (t, J = 6.6 Hz, 2H); HRMS (ES+) m/z found 298.0758; $C_{18}H_{15}ClO_2$ (M^+) requires 298.0761.

(*E*)-1-(4-Chlorobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (12c). Yield 46%, yellow powder. Mp: 148−150 °C. HPLC: 95.9%. IR (KBr) ν (cm^{-1}): 3089, 3046, 3009, 2989, 2958, 2936, 2901, 2857, 2835, 1691, 1600, 1574, 1480, 1438, 1397, 1363, 1339, 1301, 1269, 1250, 1164, 1093, 1068, 1009, 965, 909, 823, 800, 781, 733, 668, 566, 501. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 6.99 (t, J = 8.1 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 3.88 (s, 3H), 3.08 (t, J = 6.3 Hz, 2H), 2.57 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.87, 156.26, 134.60, 133.82, 133.74, 133.60, 130.93, 128.57, 127.13, 126.70, 121.22, 109.87, 55.55, 36.43, 19.29; HRMS (ES+) m/z found 299.0832; $C_{18}H_{15}ClO_2$ (M + H^+) requires 299.0839.

(*E*)-1-(2-Chlorobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (13a). Yield 46%, yellow powder. Mp: 98−100 °C. HPLC: 96.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.20−7.26 (m, 2H), 7.09−7.18 (m, 2H), 6.76 (dd, J = 8.1, 2.4 Hz, 1H), 6.56 (d, J = 2.7 Hz, 1H), 3.40 (s, 3H), 3.00 (t, J = 6.3 Hz, 2H), 2.66 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 200.72, 157.58, 135.18, 134.77, 134.49, 132.98, 132.30, 130.68, 130.08, 129.87, 129.65, 128.89, 126.43, 115.44, 113.10, 54.91, 37.46, 26.91; HRMS (ES+) m/z found 298.0783; $C_{18}H_{15}ClO_2$ (M^+) requires 298.0761.

(*E*)-1-(2-Chlorobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (13b). Yield 50%, yellow crystal. Mp: 122−124 °C. HPLC: 97.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.20−7.24 (m, 2H), 7.15−7.26 (m, 2H), 6.56 (dd, J = 8.4, 2.4 Hz, 1H), 6.56 (d, J = 2.4 Hz, 1H), 3.63 (s, 3H), 2.99 (t, J = 6.3 Hz, 2H), 2.68 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.72, 160.01, 140.24, 135.41, 134.52, 133.86, 132.01, 130.48, 129.93, 128.86, 128.35, 126.88, 124.36, 113.09, 112.30, 55.40, 38.93, 28.54; HRMS (ES+) m/z found 298.0754; $C_{18}H_{15}ClO_2$ (M^+) requires 298.0761.

(*E*)-1-(2-Chlorobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (13c). Yield 50%, yellow crystal. Mp: 124−126 °C. HPLC: 95.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.04 (dd, J = 8.1, 2.4 Hz, 2H), 6.96 (s, 1H), 6.72−6.80 (m, 1H), 6.54−6.58 (m, 1H), 3.76 (s, 3H), 3.09 (t, J = 6.6 Hz, 2H), 2.63 (t, J = 6.6 Hz, 2H); HRMS (ES+) m/z found 298.0760; $C_{18}H_{15}ClO_2$ (M^+) requires 298.0761.

(*E*)-1-(2,4-Dichlorobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (14a). Yield 42%, yellow powder. Mp: 118−120 °C. HPLC: 95.1%. IR (KBr) ν (cm^{-1}): 3088, 3064, 3023, 3000, 2965, 2945, 2908, 2855, 2832, 1693, 1608, 1574, 1543, 1492, 1465, 1435, 1357, 1324, 1301, 1279, 1235, 1193, 1169, 1099, 1046, 904, 867, 826, 808, 748, 719, 592. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.31 (dd, J = 8.4, 1.8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.89 (dd, J = 8.4, 2.7 Hz, 1H), 6.77 (dd, J = 8.1, 2.4 Hz, 1H), 6.56 (d, J = 2.7 Hz, 1H), 3.48 (s, 3H), 3.02 (t, J = 6.6 Hz, 2H), 2.65 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 200.53, 158.96, 136.76, 135.60, 134.82, 133.19, 132.01, 130.81, 129.41, 128.86, 126.37, 114.39, 110.59, 55.44, 39.02, 27.28; HRMS (ES+) m/z found 332.0368; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(2,4-Dichlorobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (14b). Yield 40%, yellow powder. Mp: 122−124 °C. HPLC: 96.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.49 (dd, J = 7.8, 1.8 Hz, 1H), 7.42−7.47 (m, 1H), 7.39 (s, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.20−7.25 (m, 1H), 6.61 (dd, J = 8.7, 2.4 Hz, 1H), 3.84 (s, 3H), 3.02 (t, J = 6.6 Hz, 2H), 2.67 (t, J = 6.6 Hz, 2H); HRMS (ES+) m/z found 332.0370; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(2,4-Dichlorobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (14c). Yield 47%, yellow powder. Mp: 126−128 °C. HPLC: 97.3%. IR (KBr) ν (cm^{-1}): 3089, 3066, 2998, 2958, 2901, 2829, 1695, 1603, 1577, 1475, 1453, 1359, 1294, 1262, 1226, 1098, 1080, 1049, 1019, 996, 881, 861, 823, 771, 733, 579, 555, 448. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.22 (dd, J = 8.1, 1.5 Hz, 1H), 7.15 (s, 1H), 7.09 (d, J = 8.4 Hz, 1H), 6.54−6.62 (m, 1H), 3.80 (s, 3H), 3.01 (t, J = 6.3 Hz, 2H), 2.58 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.05, 156.29, 137.01, 135.59, 134.74, 133.63, 131.93, 130.94, 129.65, 128.86, 127.10, 126.76, 125.63, 121.31, 109.95, 55.60, 38.48, 20.71; HRMS (ES+) m/z found 332.0370; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(3,4-Dichlorobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (15a). Yield 35%, yellow powder. Mp: 118−120 °C. HPLC: 96.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.35 (s, 1H), 7.18−7.26 (m, 2H), 7.04−7.12 (m, 2H), 6.64−6.72 (m, 1H), 3.69 (s, 3H), 2.96 (t, J = 6.3 Hz, 1H), 2.67 (t, J = 6.3 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 200.53, 158.96, 136.76, 135.60, 134.82, 133.19, 132.01, 130.81, 129.41, 128.86, 126.37, 114.39, 110.59, 55.44, 39.02, 27.28; HRMS (ES+) m/z found 332.0370; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(3,4-Dichlorobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (15b). Yield 42%, yellow crystal. Mp: 124−126 °C. HPLC: 96.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.42 (dd, J = 11.1, 2.1 Hz, 2H), 6.99−7.07 (m, 1H), 6.78 (s, 1H), 6.55 (dd, J = 8.7, 2.7 Hz, 1H), 3.86 (s, 3H), 3.04 (t, J = 6.6 Hz, 2H), 2.62 (t, J = 6.6 Hz, 2H); HRMS (ES+) m/z found 332.0374; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(3,4-Dichlorobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (15c). Yield 52%, yellow crystal. Mp: 124−126 °C. HPLC: 95.8%. IR (KBr) ν (cm^{-1}): 3445, 2998, 2961, 2914, 2831, 1686, 1542, 1473, 1453, 1385, 1351, 1290, 1262, 1226, 1182, 1131, 1078, 1022, 978, 897, 855, 810, 770, 704, 663, 581, 554, 491, 432. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.37 (s, 1H), 7.23 (dd, J = 7.8, 2.4 Hz, 1H), 7.05 (dd, J = 8.4, 2.4 Hz, 1H), 6.89 (d, J = 8.1 Hz, 1H), 7.02 (dd, J = 8.4, 1.8 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 3.88 (s, 3H), 3.09 (t, J = 6.3 Hz, 2H), 2.64 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 332.0370; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(2,6-Dichlorobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (16a). Yield 30%, yellow powder. Mp: 120−122 °C. HPLC: 95.8%. IR (KBr) ν (cm^{-1}): 3081, 3026, 2993, 2963, 2915, 2861, 2839, 1698, 1615, 1571, 1487, 1428, 1348, 1301, 1276, 1236, 1191, 1165, 1096, 1036, 992, 879, 860, 819, 779, 742, 700. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 7.2 Hz, 1H), 7.14 (d, J = 8.7 Hz, 1H), 6.74 (dd, J = 8.4, 2.7 Hz, 1H), 6.30 (d, J = 2.7 Hz, 1H), 3.36 (s, 3H), 2.99 (t, J = 6.3 Hz, 1H), 2.69 (t, J = 6.3 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 200.37, 157.87, 137.78, 134.66, 134.23, 133.81, 130.20, 129.27, 129.11, 128.75, 128.17, 115.48, 111.28, 54.79, 37.38, 26.76; HRMS (ES+) m/z found 332.0372; $C_{18}H_{14}Cl_2O_2$ (M^+) requires 332.0371.

(*E*)-1-(3-Hydroxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (16b). Yield 35%, yellow crystal. Mp: 168−170 °C. HPLC: 96.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.30 (d, J = 8.7 Hz, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 6.92 (s, 1H), 6.78 (d, J = 7.8 Hz, 2H), 6.59 (dd, J = 8.7, 2.7 Hz, 1H), 3.86 (s, 3H), 3.00 (t, J = 6.3 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 200.37, 157.87, 137.78, 134.66, 134.23, 133.81, 130.20, 129.27, 129.11, 128.75, 128.17, 115.48, 111.28, 54.79, 37,38, 26.76; HRMS (ES+) m/z found 280.1036; $C_{18}H_{16}O_3$ (M^+) requires 280.1099.

(*E*)-1-(3-Hydroxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (16c). Yield 35%, yellow crystal. Mp: 160−162 °C. HPLC: 97.7%. IR (KBr) ν (cm^{-1}): 3174, 2976, 2361, 1673, 1591, 1571, 1451, 1364, 1343, 1286, 1261, 1196, 1162, 1067, 1022, 1001, 974, 942, 906, 880, 867, 792, 732, 692, 592, 564, 532, 482, 462, 430. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.94−6.98 (m, 2H) 6.89 (s, 1H), 6.74−6.80 (m, 2H), 5.22 (s, 1H), 3.88 (s, 3H), 3.08 (t, J = 6.3 Hz, 2H), 2.58 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.37, 156.14, 155.50, 136.76, 135.19, 134.19, 133.80, 129.62, 126.98, 126.61, 122.27, 121.57, 116.07, 109.78, 55.57, 36.54, 19.29; HRMS (ES+) m/z found 280.1096; $C_{18}H_{16}O_3$ (M^+) requires 280.1099.

(*E*)-1-(4-Dimethylaminobenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (17a). Yield 58%, yellow crystal. Mp: 118−119 °C. HPLC: 99.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.23 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 6.76 (dd, J = 8.1, 2.4 Hz, 1H), 6.56 (d, J = 2.7 Hz, 2H), 3.68 (s, 3H), 3.10 (s, 6H), 2.89 (t, J = 6.3 Hz, 1H), 2.65 (t, J = 6.3 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.16, 157.50, 151.00, 136.59, 134.70, 132.09, 130.59, 129.44, 128.63, 114.30, 112.74, 111.42, 55.28, 40.18, 37.56, 27.07; HRMS (ES+) m/z found 307.1576; $C_{20}H_{21}NO_2$ (M^+) requires 307.1572.

(*E*)-1-(4-Dimethylaminobenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (17b). Yield 52%, yellow crystal. Mp: 128−130 °C. HPLC: 97.5%. IR (KBr) ν (cm^{-1}): 2905, 2360, 1676, 1607, 1582, 1522, 1490, 1431, 1363, 1301, 1264, 1229, 1191, 1147, 1136, 1101, 1066, 1031, 1015, 945, 900, 867, 847, 821, 807, 718, 668, 662, 564, 510, 474. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.54 (s, 1H), 7.47 (s, 1H), 7.44 (s, 1H), 6.79 (d, J = 2.7 Hz, 1H), 6.65 (dd, J = 8.7, 2.7 Hz, 1H), 6.58 (d, J = 8.7 Hz, 2H), 3.84 (s, 3H), 3.00 (s, 6H), 2.96 (t, J = 6.3 Hz, 2H), 2.57 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.98, 158.87, 150.74, 139.82, 134.69, 131.64, 129.83, 129.15, 126.28, 112.60, 111.91, 111.44, 55.26, 40.14, 37.32, 28.24; HRMS (ES+) m/z found 307.1575; $C_{20}H_{21}NO_2$ (M^+) requires 307.1572.

(*E*)-1-(4-Dimethylaminobenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (17c). Yield 48%, yellow crystal. Mp: 98−100 °C. HPLC: 97.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.56(d, J = 8.1 Hz, 2H), 7.37 (d, J = 7.8 Hz, 1H), 6.79 (d, J = 2.7 Hz, 1H), 6.65 (dd, J = 8.7, 2.7 Hz, 3H), 3.92 (s, 3H), 3.12 (s, 6H), 3.06 (t, J = 6.3 Hz, 2H), 2.53 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 307.1589; $C_{20}H_{21}NO_2$ (M^+) requires 307.1572.

(*E*)-1-(3-Hydroxy-4-methoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (18a). Yield 42%, yellow crystal. Mp: 170−172 °C. HPLC: 95.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.16 (d, J = 7.6 Hz, 2H), 7.11 (s, 1H), 7.01 (s, 2H), 6.77 (d, J = 7.8 Hz, 2H), 5.71 (s, 1H), 3.89 (s, 3H), 3.57 (s, 3H), 2.95 (s, 1H), 2.59 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.91, 157.52, 147.55, 145.21, 135.37, 133.54, 132.31, 130.72, 128.74, 128.06, 123.32, 115.38, 114.97, 113.18, 110.32, 55.88, 55.24, 37.33, 26.89; HRMS (ES+) m/z found 310.1211; $C_{19}H_{18}O_4$ (M^+) requires 310.1205.

(*E*)-1-(3-Hydroxy-4-methoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (18b). Yield 48%, yellow crystal. Mp: 170−172 °C. HPLC: 96.4%. IR (KBr) ν (cm^{-1}): 3556, 2999, 2939, 2902, 2839, 1681, 1613, 1585, 1570, 1510, 1484, 1436, 1367, 1332, 1277, 1245, 1196, 1161, 1129, 1094, 1039, 1021, 999, 926, 901, 873, 816, 796, 760, 727, 622, 562, 487, 424; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 2.1 Hz, 1H), 7.01 (dd, J = 8.7, 2.1 Hz, 1H), 6.78 (d, J = 2.1 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 6.62 (dd, J = 8.7, 2.7 Hz, 1H), 5.55 (s, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 2.98 (t, J = 6.3 Hz, 2H), 2.59 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.01, 159.30, 147.25, 145.21, 139.99, 133.34, 131.97, 130.22, 128.63, 125.18, 122.98, 115.05, 112.72, 112.07, 110.35, 55.86, 55.22, 37.08, 28.08; HRMS (ES+) m/z found 310.1204; $C_{19}H_{18}O_4$ (M^+) requires 310.1205.

(*E*)-1-(3-Hydroxy-4-methoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (18c). Yield 43%, yellow crystal. Mp: 178−180 °C. HPLC: 99.7%. IR (KBr) ν (cm^{-1}): 3315, 3005, 2961, 2940, 2905, 2882, 2840, 2733, 1681, 1590, 1506, 1471, 1441, 1370, 1344, 1283, 1251, 1173, 1130, 1068, 1027, 997, 971, 944, 908, 806, 780, 757, 730, 673, 615, 503, 434. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.05 (d, J = 7.8 Hz, 2H), 7.02 (dd, J = 7.8, 2.4 Hz, 2H), 6.97 (dd, J = 8.1, 2.1 Hz, 1H), 6.76 (dd, J = 8.4, 2.7 Hz, 2H), 5.61 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.05 (t, J = 6.6 Hz, 2H), 2.54 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.32, 156.13, 147.46, 145.12, 135.38, 134.20, 132.54, 128.30, 126.92, 126.50, 123.28, 121.31, 115.37, 110.24, 109.60, 55.86, 55.55, 36.59, 19.30; HRMS (ES+) m/z found 310.1204; $C_{19}H_{18}O_4$ (M^+) requires 310.1205.

(*E*)-1-(4-Hydroxy-3-methoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (19a). Yield 49%, yellow crystal. Mp: 158−160 °C. HPLC: 99.3%. IR (KBr) ν (cm^{-1}): 3359, 3002, 2945, 2841, 1677, 1612, 1573, 1516, 1489, 1444, 1383, 1306, 1284, 1247, 1230, 1204, 1166, 1121, 1096, 1044, 1025, 934, 801, 626, 548, 481, 422. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.09 (s, 1H), 7.04 (d, J = 2.7 Hz, 2H), 6.83 (d, J = 8.4 Hz, 1H), 6.76 (dd, J = 8.4, 2.7 Hz, 1H), 5.97 (s, 1H), 3.71 (s, 3H), 3.64 (s, 3H), 2.96 (t, J = 6.3 Hz, 2H), 2.60 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.81, 157.46, 147.01, 146.15, 135.63, 133.70, 131.82, 130.81, 128.85, 126.79, 124.76, 114.65, 114.46, 113.31, 112.02, 55.77, 55.21, 37.36, 26.92; HRMS (ES+) m/z found 310.1209; $C_{19}H_{18}O_4$ (M^+) requires 310.1205.

(*E*)-1-(4-Hydroxy-3-methoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (19b). Yield 52%, yellow crystal. Mp: 153−155 °C. HPLC: 98.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.06 (s, 1H), 7.02 (s, 1H), 6.83 (s, 1H), 6.80 (s, 1H), 6.63 (dd, J = 8.7, 2.7 Hz, 1H), 5.85 (s, 1H), 3.83 (s, 3H), 3.72 (s, 3H), 2.99 (t, J = 6.6 Hz, 2H), 2.60 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.67, 159.26, 146.65, 146.11, 140.16, 133.70, 131.36, 130.27, 127.33, 125.31, 124.44, 114.42, 112.73, 111.88, 111.60, 55.77, 55.27, 37.11, 28.13; HRMS (ES+) m/z found 310.1206; $C_{19}H_{18}O_4$ (M^+) requires 310.1205.

(*E*)-1-(4-Hydroxy-3-methoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (19c). Yield 50%, yellow crystal. Mp: 160−162 °C. HPLC: 98.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.11 (d, J = 7.8 Hz, 1H), 7.02 (d, J = 7.8, 2.1 Hz, 2H), 6.99 (s, 1H), 6.80 (dd, J = 8.1, 2.1 Hz, 2H), 5.98−6.02 (m, 1H), 3.88 (s, 3H), 3.67 (s, 3H), 3.06 (t, J = 6.3 Hz, 2H), 2.55 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.38, 156.17, 146.96, 146.05, 135.77, 134.32, 131.79, 127.03, 126.91, 126.27, 124.97, 121.33, 114.40, 111.84, 109.46, 55.72, 55.53, 36.61, 19.32; HRMS (ES+) m/z found 310.1209; $C_{19}H_{18}O_4$ (M^+) requires 310.1205.

(*E*)-1-(3,4-Dimethoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (20a). Yield 50%, yellow solid. Mp: 84−86 °C. HPLC: 97.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.00 (dd, J = 8.4, 2.4 Hz, 1H), 6.95 (s, 1H), 6.68 (d, J = 2.4 Hz, 2H), 6.62 (dd, J = 8.7, 2.4 Hz, 1H), 3.85(s, 6H), 3.63 (s, 3H), 2.92 (t, J = 6.3 Hz, 2H), 2.61 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 324.1360; $C_{20}H_{20}O_4$(M^+) requires 324.1362.

(*E*)-1-(3,4-Dimethoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (20b). Yield 50%, yellow powder. Mp: 114−116 °C. HPLC: 95.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.07 (dd, J = 8.7, 2.2 Hz, 1H), 7.03 (s, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.62 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 3.90 (s, 3H), 3.85 (s, 6H), 2.99 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 324.1361; $C_{20}H_{20}O_4$(M^+) requires 324.1362.

(*E*)-1-(3,4-Dimethoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (20c). Yield 43%, yellow crystal. Mp: 114−116 °C. HPLC: 95.5%. IR (KBr) ν (cm^{-1}): 3005, 2950, 2835, 2361, 1686, 1595, 1579, 1509, 1471, 1440, 1418, 1370, 1342, 1250, 1157, 1137, 1070, 1016, 968, 903, 860, 807, 793, 781, 768, 735, 695, 674,

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623, 604, 553, 460, 437. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.00−7.10 (m, 4H), 6.78 (d, J = 8.4 Hz, 2H), 3.89 (s, 6H), 3.64 (s, 3H), 3.07 (t, J = 6.3 Hz, 2H), 2.56 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.08, 156.17, 150.04, 148.26, 135.43, 134.31, 132.08, 127.47, 127.05, 126.27, 124.48, 121.41, 112.02, 110.70, 109.47, 55.80, 55.57, 55.53, 36.59, 19.34; HRMS (ES+) m/z found 324.1364; $C_{20}H_{20}O_4(M^+)$ requires 324.1362.

(*E*)-1-(3,5-Dimethoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (21a). Yield 48%, yellow crystal. Mp: 126−128 °C. HPLC: 95.7%. IR (KBr) ν (cm^{-1}): 2955, 2833, 2360, 1691, 1584, 1491, 1459, 1361, 1341, 1318, 1299, 1279, 1233, 1201, 1163, 1099, 1068, 1038, 995, 914, 874, 843, 816, 805, 744, 714, 689, 546, 484, 423. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.17 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 6.77 (dd, J = 8.4, 2.7 Hz, 1H), 6.58 (d, J = 2.1 Hz, 2H), 6.39 (d, J = 2.1 Hz, 1H), 3.68 (s, 6H), 3.53 (s, 3H), 2.99 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.61, 160.62, 157.45, 136.90, 135.22, 134.16, 133.14, 130.76, 128.79, 115.32, 113.51, 107.32, 101,64, 55.29, 55.15, 37.28, 26.87; HRMS (ES+) m/z found 324.1365; $C_{20}H_{20}O_4(M^+)$ requires 324.1362.

(*E*)-1-(3,5-Dimethoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (21b). Yield 53%, yellow crystal. Mp: 126−128 °C. HPLC: 98.8%. IR (KBr) ν (cm^{-1}): 2959, 2836, 2362, 1687, 1594, 1498, 1448, 1424, 1373, 1336, 1309, 1263, 1252, 1222, 1205, 1154, 1093, 1053, 1027, 904, 850, 834, 694, 672 619, 563, 537. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.33 (d, J = 8.7 Hz, 1H), 6.78 (d, J = 2.4 Hz, 1H), 6.62 (d, J = 8.7 Hz, 1H), 6.58 (d, J = 8.7 Hz, 1H), 6.38 (d, J = 2.4 Hz, 1H), 3.83 (s, 3H), 3.69 (s, 6H), 3.01 (t, J = 6.3 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.61, 160.59, 159.47, 140.09,137.32, 133.59, 133.23, 130.79, 124.82, 112.68, 111.98, 107.17, 101.37, 55.27, 37.00, 28.05; HRMS (ES+) m/z found 324.1363; $C_{20}H_{20}O_4$ (M^+) requires 324.1362.

(*E*)-1-(3,5-Dimethoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (21c). Yield 50%, yellow powder. Mp: 126−128 °C. HPLC: 99.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 6.97−7.04 (m, 2H), 6.78 (dd, J = 6.6, 2.4 Hz, 1H), 6.54 (s, 2H), 6.39 (d, J = 2.2 Hz, 1H), 3.87 (s, 3H), 3.67 (s, 6H), 3.08 (t, J = 6.6 Hz, 2H), 2.58 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.09, 160,49, 156.07, 136.94, 135.27, 134.24, 133.79, 127.02, 126.38, 121.76, 109.74, 107.40, 101.67, 55.52, 55.24, 36.49, 19.30; HRMS (ES+) m/z found 324.1381; $C_{20}H_{20}O_4$ (M^+) requires 324.1362.

(*E*)-1-(3,4,5-Trimethoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (22a). Yield 1.89 g (53%). Mp: 115−117 °C. HPLC: 99.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.00 (s, 1H), 6.78 (dd, J = 8.4, 2.7 Hz, 1H), 6.73 (s, 2H), 3.86 (s, 3H), 3.70 (s, 6H), 3.56 (s, 3H), 2.98 (t, J = 6.6 Hz, 2H), 2.60 (t, J = 6.6 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.60, 157.39, 152.91, 138.97, 135.12, 133.22, 130.84, 129.99, 128.89, 114.89, 113.52, 107.16, 60.89, 55.95, 55.15, 37.25, 26.86; HRMS (ES+) m/z found 354.1466; $C_{21}H_{22}O_5$ (M^+) requires 354.1467.

(*E*)-1-(3,4,5-Trimethoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (22c). Yield 53%, yellow crystal. Mp: 120−123 °C. HPLC: 99.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.04−7.10 (m, 2H), 6.78 (d, J = 8.7 Hz, 1H), 6.69 (s, 2H), 3.90 (s, 6H), 3.69 (s, 6H), 3.08 (t, J = 6.3 Hz, 2H), 2.57 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 202.17, 156.17, 152.84, 138.92, 135.26, 133.93, 133.22, 130.10, 127.15, 126.27, 121.63, 109.65, 107.22, 60.93, 55.94, 55.54, 36.52, 19.32; HRMS (ES+) m/z found 354.1469; $C_{21}H_{22}O_5(M^+)$ requires 354.1467.

(*E*)-1-(2,3,4-Trimethoxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (23a). Yield 50%, yellow crystal. Mp: 118−120 °C. HPLC: 96.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 9 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 6.75 (dd, J = 8.7, 2.7 Hz, 1H), 6.50 (d, J = 8.7 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.53 (s, 3H), 2.97 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.09, 157.47, 154.61, 153.49, 142.15, 134.05, 132.78, 131.26, 130.61, 128.73, 124.33, 121.93, 114.43, 113.16, 106.87, 61.45, 60.93, 55.99, 55.04, 37.57, 26.99; HRMS (ES+) m/z found 354.1464; $C_{21}H_{22}O_5(M^+)$ requires 354.1467.

(*E*)-1-(2,3,4-Trimethoxybenzylidene)-6-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (23b). Yield 43%, yellow powder. Mp: 112−114 °C. HPLC: 96.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.03 (d, J = 8.7 Hz, 1H), 6.77 (s, 1H), 6.57 (dd, J = 8.7, 2.2 Hz, 1H), 6.37 (d, J = 8.7 Hz, 1H), 3.90 (s, 6H), 3.85 (s, 6H), 2.99 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 354.1464; $C_{21}H_{22}O_5(M^+)$ requires 354.1467.

(*E*)-1-(2,3,4-Trimethoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-2(1*H*)-one (23c). Yield 46%, yellow solid. Mp: 78−80 °C. HPLC: 97.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 6.93−6.99 (m, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.73 (s, 1H), 6.68 (dd, J = 8.7, 2.4 Hz, 1H), 6.50 (d, J = 8.4 Hz, 1H), 3.92 (s, 6H), 3.73 (s, 6H), 2.99 (t, J = 6.3 Hz, 2H), 2.61 (t, J = 6.3 Hz, 2H); HRMS (ES+) m/z found 354.1466; $C_{21}H_{22}O_5(M^+)$ requires 354.1467.

Biology. Cytotoxicity Assay. Cytotoxic effects were examined in the MDA-MB-435 human breast carcinoma, HCT-116 human colon carcinoma, and CEM human leukemia cell lines. Cells in logarithmic phase were diluted to a density of 40000−50000 cells/mL in culture medium based on growth characteristics. For each well of a 96-well microplate, 100 μL of cell dilution was seeded, allowed to attach overnight, and then exposed to varying concentrations (10^{-4}−10^{-9} M) of compounds for 72 h (37 °C, 5% CO_2 atmosphere). The number of living cells was estimated by the MTT assay. Absorbance at 570 nm was recorded on a Wellscan MK-2 multifunction microplate reader (Labsystems Inc.). Compounds were tested in triplicate in at least three independent assays. The IC_{50} values were determined by a nonlinear regression analysis. Average values were reported.

Tubulin Polymerization Assay.[63,64] Procine brain tubulin was purchased from cytoskeleton. Tubulin (>99% pure, 3 mg/mL) in 100 μL of general tubulin buffer (80 mM PIPES, pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$, 1 mM GTP, and 5% glycerol) at 0 °C was placed in a prewarmed half area 96-well plate at 37 °C in the presence of tested compounds at varying concentrations. The reaction was started by warming the samples at 37 °C. The mass of polymer formed was monitored by turbidimetry at 340 nm every 1 min for 60 min with a BioTek's Synergy 4 multifunction microplate reader.

Cell Cycle Analysis. For flow cytometric analysis of DNA content, 10^6 HCT-116 cells in exponential growth were treated with varying concentrations of compound **22b** for 24 h. After centrifugation, the cell pellet was fixed in 2 mL of citrate buffer. Fixed cells were treated with 1.5 mL of RNase A for 20 min. Samples were analyzed on a BD FACStar Plus cell sorter flow cytometer. The percentage of each phase of the cell cycle (G1, S, G2/M) was calculated on living cells. The percentage of apoptotic cells is referred to the cell populations characterized by the appearance of a sub-G1-peak.

In Vivo Antitumor Activity. Four-week-old female BALB/c nude mice (18−20 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The animals were maintained under specific pathogen-free conditions in Shanghai Institute of Pharmaceutical Industry. Once the HT-29 xenografts reached a size of around 100 mm^3, mice were randomly assigned into corresponding groups and injected intraperitoneally at 0.1 mL per 20 g of body weight.[65] The drugs or vehicle were administered once a day. After completing the treatment schedule and the evaluation period, tumor-bearing mice were euthanized. Tumor volume (TV) was calculated by the formula: TV= $(ab^2)/2$ where a is the length and b is the width of the tumor nodules determined once per 4 days by caliper measurements and recorded along with body weights. The study was approved by Shanghai Institute of Pharmaceutical Industry.

Molecular Docking. The X-ray structure of the DAMA−colchicine−tubulin complex (PDB code 1SA0) was used as the tubulin protein template. All calculations were performed on an Origin 300 server. Docking was carried out with AutoDock program. A grid of 45 × 40 × 40 was used to ensure that the area probed was adequate for the ligands to explore all possible binding modes. Docking runs were performed using the Larmarckian genetic algorithm (LGA) with some modifications of the docking parameters. Each docking experiment was derived from 50 different runs that was set to terminate after a maximum of 10 000 000 energy evaluations or 370 000 generations, yielding 50 docked conformations. At the end of a docking job with multiple runs,

AutoDock performed cluster analysis. Docking solutions with ligand all-atom rmsd values within 0.5 Å of each other were clustered together and ranked by the lowest energy representative. Low energy binding modes and the lowest energy conformation from clusters of five or more occurrences were selected for further refinement. These were then refined by SYBYL, with the binding mode that had the lowest complex energy after minimization being selected as the final binding mode.

X-ray Crystallography. Crystal data for **22b** are as follows: $C_{21}H_{22}O_5$, MW 354.39; crystal size, 0.06 × 0.05 × 0.04 mm^3; yellow, prism; space group $P2(1)/c$; monoclinic, a = 11.13(3) Å, b = 12.69(3) Å, c = 14.47(3) Å, $\alpha = \gamma = 90°$, β = 111.59(3) °; V = 1900(8) Å; Z = 4; Dc = 1.239 g/cm^3; $F(000)$ = 752; m = 0.088 mm^{-1}; range for data collection, 2.53−26.16; reflections collected 6490; unique reflections 3326 [R_{int} = 0.1003]; data/restraints/parameters 3326/0/240; goodness-of-fit on F^2 1.056; R indices (all data), R1 = 0.0824, wR2 = 0.1960; final R indices [$I > 2\sigma(I)$], R1 = 0.0628, wR2 = 0.1785; CCDC, 761109.

Statistical Analysis. The differences between different treatments were analyzed, using the two-sided Student's t test. P values lower than 0.05 were considered significant.

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Notes

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ABBREVIATIONS USED

VDA, vascular disrupting agent; CA-4, combretastatin A-4; CA-4P, combretastatin A-4 phosphate; CSI, colchicine site inhibitor; TLC, thin layer chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PIPES, piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]sequisodium salt; EGTA, ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; WBC, white blood cell; LY, lymphocyte; PLT, platelet; ALT, alanine transaminase; ALP, alkaline phosphatase

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