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Comparative in vivo evaluation of polyalkoxy substituted 4*H*-chromenes and oxa-podophyllotoxins as microtubule destabilizing agents in the phenotypic sea urchin embryo assay

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ABSTRACT

A series of polyalkoxy substituted 7-hydroxy- and 7-methoxy-4-aryl-4*H*-chromenes were evaluated using the sea urchin embryo model to yield several compounds exhibiting potent antimitotic microtubule destabilizing activity. Data obtained by the assay were further confirmed in the NCI60 human cancer cell screen. The replacement of methylenedioxy ring A and lactone ring D in podophyllotoxin analogues by 7-methoxy, 2-NH_2, and 3-CN groups in 4-aryl-4*H*-chromenes resulted in potent antimitotic microtubule destabilizing agents. Feasible synthesis and high yields render 7-methoxy-4*H*-chromenes to be a promising series for further anticancer drug development.

There are multiple classes of antitumor agents that target tubulin/microtubules.[1–5] However, numerous current cytotoxic drugs have been described to show clinical limitations including systemic toxicity and multidrug resistance of cancer cells. Many of these agents also exhibit suboptimal bioavailability and pharmacokinetics. Structure–activity relationship (SAR) studies and syntheses of taxane, vinca alkaloid, and podophyllotoxin (PT) derivatives pose significant challenges due to their structural complexity. Considering the above, there is still the need for safer and more efficacious therapies to treat cancer.

In an attempt to streamline screening for novel anti-tubulin agents, we have developed a robust, reproducible, and quantifiable phenotypic assay based on the sea urchin embryo.[6] It should be pointed out that this in vivo model has several advantages over the existing 'canonical' multi-step protocol. In addition to yielding information on antiproliferative and microtubule destabilizing activity, this 'one-pot' assay also suggests general cytotoxicity and cell permeability potential for a molecule. Specific measurements include: (i) the antimitotic activity of an agent as displayed by cleavage alteration/arrest of the fertilized egg, and (ii) the effect of a molecule on a free-swimming blastulae when treated immediately after hatching (~9 h after fertilization). It was concluded that there are very specific changes in embryo swimming pattern that unequivocally point to a microtubule destabilizing activity of a compound. Several specific examples include lack of forward movement and rapid spinning around the animal–vegetal axis.[7]

In our search for novel antimitotic agents, we turned our attention to the natural lignan PT and its synthetic analogues. The parent molecule is a well-characterized potent antimitotic microtubule destabilizing agent interacting with the colchicine site of tubulin.[8–11] However, therapeutic application of PT as tumor suppressor is limited due to its significant side effects in the human trials. On the contrary, semisynthetic PT analogues etoposide, teniposide, and etopophos are widely used as clinical drugs targeting DNA topoisomerase II.[9,12] Four contiguous chiral centers play the central role

Abbreviations: PT, podophyllotoxin; SAR, structure–activity relationship.

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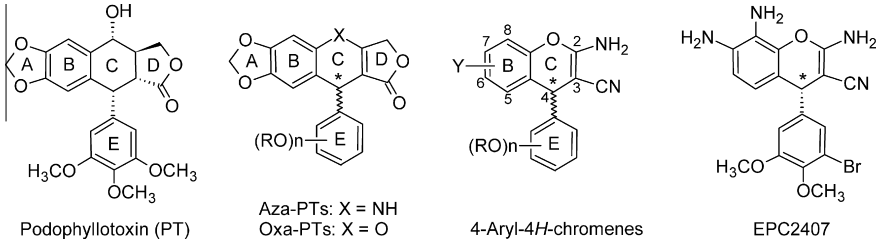


Figure 1. Structure of podophyllotoxin and its heterocyclic analogues.

in anticancer activity of PT (Fig. 1).[8] The complex stereochemistry of PT renders SAR studies of its derivatives to be challenging. Introduction of a heteroatom in ring C allowed several research groups to address both epimerization and SAR issues associated with this compound class. For instance, the 'simplified' aza- and oxa-PT analogues (Fig. 1) are potent microtubule destabilizing agents exhibiting significant cytotoxicity.[13,14] It was shown that methylenedioxy ring A could be successfully replaced by a 7-methoxy moiety. Such modification together with the derivatization of the ring E with 3,5-dimethoxy and 3,4,5-trialkoxy groups proved to be advantageous for compound activity.[13,14] Notably, oxa-PTs were reported to be more stable towards oxidation as compared to the respective aza-derivatives.[14] However, the synthetic approach to 7-methoxy-oxa-PTs (Fig. 1) resulted in low overall yields of the targeted molecules (3–6%), presumably due to the ambiguous reactivity of tetronic acid with 3-methoxyphenol.[14,15]

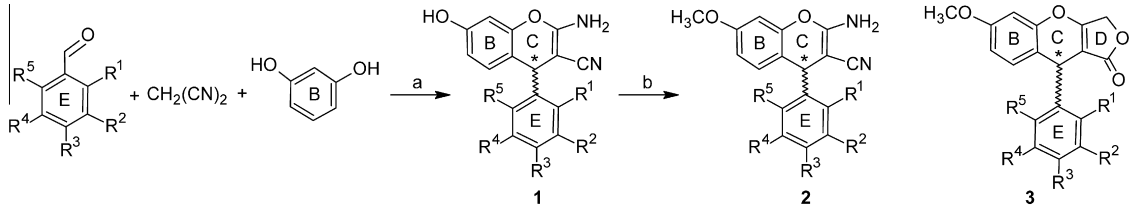
4-Aryl-4*H*-chromenes (Fig. 1) could be regarded as structural analogues of oxa-PTs where the lactone ring D is replaced with CN and NH_2-groups (Fig 1). These compounds were reported to be microtubule destabilizing agents interacting with or adjacent to the colchicine binding site, and cytotoxic against a panel of human cancer cells.[16] Their effects on cancer cells, including mitotic arrest, caspase-dependent apoptotic cell death, and tumor vasculature disruption were described in detail.[16–23] 4-Aryl-chromene EPC2407 (Fig. 1) is currently in the phase I/II clinical trials as a vasculature targeting anticancer agent and apoptosis inducer for the treatment of patients with advanced solid tumors.[23–25]

4-Aryl-4*H*-chromenes are easily accessed via the three-component reaction of aldehydes, malononitrile, and phenols (Scheme 1).[26–29] Interestingly, this procedure involving 3-methoxyphenol affords low yields of the targeted chromenes **2** (7–24%),[30] whereas application of resorcinol followed by methylation of the 7-hydroxy group in **1** furnishes **2** in 70–90% yields.[26–29]

Diversity of starting materials, synthetic feasibility of the condensation protocol, and potential for antimitotic properties turned our attention to 7-methoxy-4-aryl-4*H*-chromenes **2** as analogues of highly active 7-methoxy-PT derivatives.[14] It was speculated that the optimized substitution pattern for ring E may further improve antiproliferative activity of the targeted compounds.[8,31,32] In addition, small hydrophobic moieties at C7 in ring B (methoxy, amino, dimethylamino, and ethylamino groups) were found to be beneficial for inhibition of cancer cell growth and caspase-dependent apoptosis.[19,30] Considering the above, our initial aim was the biological evaluation of 4-aryl-4*H*-chromenes **1** and **2** with different alkoxy substituents in ring E[33] as antimitotic microtubule destabilizing agents in the phenotypic sea urchin embryo assay.[6] The results are presented in Table 1. Typical effects of 7-methoxy-4-aryl-4*H*-chromenes on the sea urchin embryos, as exemplified by compound **2a**, are demonstrated in Figure 2.

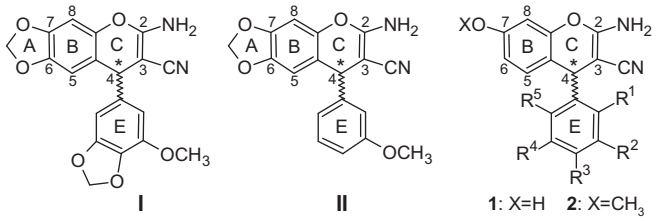
It was found that 7-hydroxy-4*H*-chromenes (**1a**–**t**) displayed weak to no activity. Only chromene **1g** with a 3-methoxy-4,5-methylenedioxy ring E caused embryo spinning, indicating antimitotic microtubule destabilizing activity. A similar compound with an ethylenedioxy group in ring E (**1h**) as well as molecules containing an unsubstituted (**1t**) or a 3-methoxyphenyl ring E (**1o**) were identified as weak antitubulin agents, as suggested by the formation of tuberculate eggs. In contrast, 7-methoxy-4*H*-chromenes (**2**) markedly influenced sea urchin embryo development, producing cleavage alteration/arrest and embryo spinning. They exhibited significant antimitotic microtubule destabilizing activity in the low nanomolar concentration range, and were 100-fold or more potent than the corresponding 7-hydroxychromenes (**1**). Methylenedioxy ring A in 4*H*-chromenes, as well as in aza-PTs,[13] was not essential and could be substituted by a 7-methoxy group yielding active molecules (compare **I** and **2g**, **II** and **2o**). Our tests further demonstrated that the 3-methoxy substituent in ring E was critical to the antitubulin effect of 7-methoxy-4*H*-chromenes. The replacement of the 3-methoxy moiety with an $-NO_2$ or especially an $-NH_2$ group (**2h** vs **2i** and **2ii**) markedly reduced the activity. Compounds **2m** and **2n** featuring a 2-methoxyphenyl ring E showed moderate activity. The increase of the number of methoxy substituents from three to four (**2g** and **2h** vs **2a**) was unfavourable. However, it was impossible to establish precisely the relationship between the number of methoxy groups in ring E and antimitotic potency, since chromenes with one (**2o**) or three (**2g** and **2h**) substituents exhibited similar effects. Instead, the position of the methoxy groups in the ring E apparently played the more important role (compare **2j** and **2n**, **2k** and **2m**, **2o** and **2p**). Chromene **2j** with a 3,5-dimethoxyphenyl ring E was identified as the most active antitubulin agent in the sea urchin embryo assay.

The stereochemistry of microtubule destabilizing agents based on the PT core was reported to significantly affect their antiproliferative activity.[8,34] For example, the *R*(−)-isomer of 4-aryl-4*H*-chromene EPC2407 (Fig. 1) was shown to activate caspases, induce



Scheme 1. Synthesis of 7-substituted 4-aryl-4*H*-chromenes (**1** and **2**). Reagents and conditions: (a) MW, DBU (5 mol %), EtOH, 50 °C, 2–4 min; (b) CH_3CN, CH_3I, K_2CO_3, 20 °C, 48 h. (**3**): Respective 7-oxa-podophyllotoxins.

Table 1
Effects of 4-aryl-4*H*-chromenes on sea urchin embryos and human cancer cells with PT and oxaPTs **I** and **II** as reference compounds



Compd	R^1	R^2	R^3	R^4	R^5	Sea urchin embryo effects, EC (μM)[a]			NCI60 screen	
						Cleavage alteration	Cleavage arrest	Embryo spinning	Mean GI_{50}, μM[b]	Mean cell growth, %[c]
PT						0.02	0.05	0.5	0.021[d]	
I[e]						0.005	0.05	0.2	0.389	
II[e]						0.005	0.05	0.2	0.065	
1a	OCH_3	$-OCH_2O-$	OCH_3	H	ND[f]					
1b	OCH_3	OCH_3	$-OCH_2O-$	H	4	>4	>4	ND[f]		
1c	OCH_3	OCH_3	OCH_3	OCH_3	H	>4	>4	>4	ND[f]	
1d	H	OCH_3	OCH_3	OCH_3	H	4	>4	>4	0.692	
1e	OCH_3	H	OCH_3	H	OCH_3	2	>4	>4	ND[f]	
1f	OCH_3	OCH_3	OCH_3	H	H	>4	>4	>4	ND[f]	
1g	H	OCH_3	$-OCH_2O-$	H	0.5	4	10	ND[f]		
1h	H	OCH_3	$-OCH_2CH_2O-$	H	1	4 (TE)[g]	>5	ND[f]		
1i	H	NO_2	$-OCH_2CH_2O-$	H	4	>4	>4		99.3	
1j	H	OCH_3	H	OCH_3	H	ND[f]				
1k	H	OCH_3	OCH_3	H	H	>4	>4	>4		99.4
1l	OCH_3	H	H	OCH_3	H	1	>4	>4		94.5
1m	OCH_3	OCH_3	H	H	H	>4	>4	>4	ND[f]	
1n	OCH_3	H	OCH_3	H	H	>4	>4	>4	ND[f]	
1o	H	OCH_3	H	H	H	0.1	2 (TE)[g]	>5	ND[f]	
1p	H	H	OCH_3	H	H	>4	>4	>4		96.0
1q	OCH_3	H	H	H	H	0.5	>4	>5	ND[f]	
1r	I	H	H	H	H	4	>4	>4	ND[f]	
1s	F	H	H	H	H	1	4	>5	ND[f]	
1t	H	H	H	H	H	0.5	2 (TE)[g]	>5	ND[f]	
2a	H	OCH_3	$-OCH_2O-$	OCH_3	0.1	1	4	ND[f]		
2d[e]	H	OCH_3	OCH_3	OCH_3	H	0.01	0.05	0.5	0.045	
2d-R	H	OCH_3	OCH_3	OCH_3	H	0.005	0.04	0.2	ND[f]	
2d-S	H	OCH_3	OCH_3	OCH_3	H	2	>4	>5	ND[f]	
2g	H	OCH_3	$-OCH_2O-$	H	0.005	0.02	0.05	ND[f]		
2h	H	OCH_3	$-OCH_2CH_2O-$	H	0.005	0.02	0.1	ND[f]		
2i	H	NO_2	$-OCH_2CH_2O-$	H	0.02	0.1	1	ND[f]		
2ii	H	NH_2	$-OCH_2CH_2O-$	H	0.1	0.5	5	ND[f]		
2j	H	OCH_3	H	OCH_3	H	0.0005	0.005	0.02	0.014 (T-47D)[h] 0.009 (ZR-75–1)[h] 0.032 (H1299)[i]	
2k	H	OCH_3	OCH_3	H	H	0.05	0.2	1	ND[f]	
2m	OCH_3	OCH_3	H	H	H	0.2	2	4	ND[f]	
2n	OCH_3	H	OCH_3	H	H	0.1	1	4	ND[f]	
2o	H	OCH_3	H	H	H	0.002	0.01	0.05	0.055	
2o-R	H	OCH_3	H	H	H	0.001	0.01	0.05	ND[f]	
2o-S	H	OCH_3	H	H	H	0.05	0.5	4	ND[f]	
2p	H	H	OCH_3	H	H	0.1	1 (TE)[g]	>5	ND[f]	
2q	OCH_2CH_3	H	H	H	H	0.02	0.2	0.5	ND[f]	

[a] The sea urchin embryo assay was conducted as described previously.[6] Fertilized eggs and hatched blastulae were exposed to 2-fold decreasing concentrations of compounds. Duplicate measurements showed no differences in effective threshold concentration (EC) values. Typical cleavage alteration and cleavage arrest phenotypes are presented in Fig. 2.
[b] GI_{50}: concentration required for 50% cell growth inhibition.
[c] Cell growth percent at 10 μM concentration.
[d] NCI data for PT NSC 24818.
[e] Data from Ref. 34.
[f] ND: not determined.
[g] TE: arrested eggs obtained tuberculate shape typical for microtubule destabilizing agents.
[h] Data on two human breast cancer cell lines.[30]
[i] Data on human non-small cell lung cancer cell line.[19]

apoptosis, inhibit growth of human cancer cells, and caused pronounced tumor vascular disruption with potencies 50–100 higher than the *S*(+)-isomer.[23,35] Similar results have been reported for the antimitotic effect of enantiomers of 4-aryl-4*H*-chromene **II** (structure presented in Table 1) using the sea urchin embryo model.[34] Therefore, in the next series of experiments we studied the effect of stereochemistry on the antitubulin activity of compounds **2d** and **2o** in the sea urchin embryo assay. A racemic mixture was separated by chiral HPLC to yield pure enantiomers, as reported previously.[13,34] The isomer **2d**–***R*** exhibited a significant antimitotic effect, inducing cleavage alteration and cleavage arrest at concentration of 0.005 and 0.04 μM, respectively (Table 1). This enantiomer was twice as potent as racemic **2d** and ca. 400 times more potent than the **2d**–***S*** isomer. There was a 10-fold difference

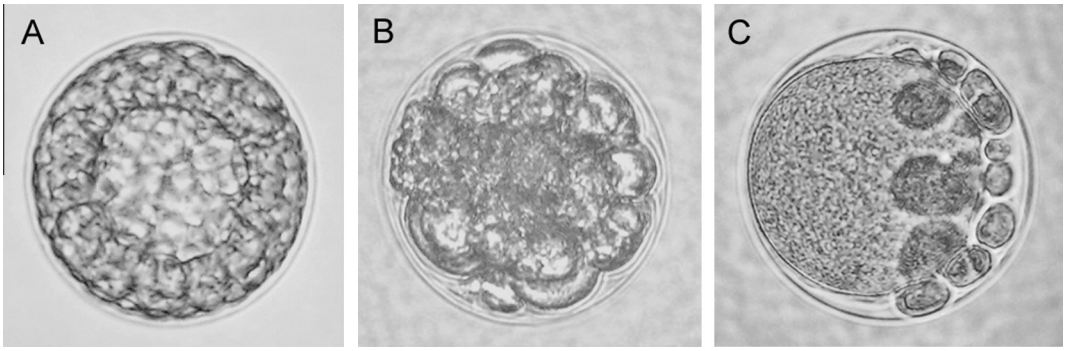
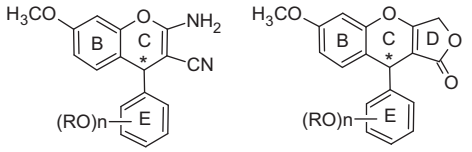


Figure 2. Effect of 7-methoxy-4-aryl-4*H*-chromene (**2a**) on sea urchin embryo development. Time after fertilization (20 °C): 6 h. (A) Intact embryo at early blastula stage. Fertilized eggs were exposed continuously to **2a** at 0.2 μM (B) and 1 μM (C). Note abnormal embryo with different size and irregular position of cells (B). Arrested egg (C) acquired tuberculate shape typical for microtubule destabilizing agents. The average embryo diameter is 115 μ.

Table 2
Comparative effects of 7-methoxy-4-aryl-4*H*-chromenes **2** and respective 7-methoxy-oxa-PTs **3** on sea urchin embryos[a]



2 3

Compd	Sea urchin embryo effects, EC (μM)[b]		
	Cleavage alteration	Cleavage arrest	Embryo spinning
2a	0.1	1	4
3a[c]	0.1	0.5	4
2d[d]	0.01	0.05	0.5
3d[c]	0.005	0.05	0.2
2h	0.005	0.02	0.1
3h[c]	0.01	0.05	0.5
2g	0.005	0.02	0.05
3g[c]	0.002	0.01	0.1
2j	0.0005	0.005	0.02
3j[c]	0.001	0.005	0.05

[a] Substitution patterns in the ring E are presented in Table 1.
[b] The sea urchin embryo assay was conducted as described previously.[6] Fertilized eggs and hatched blastulae were exposed to 2-fold decreasing concentrations of compounds. Duplicate measurements showed no differences in effective threshold concentration (EC) values. Typical cleavage alteration and cleavage arrest phenotypes are presented in Fig. 2.
[c] Data for 7-methoxy-oxa-PTs from Ref. 14.
[d] Data from Ref. 34.

in the antimitotic potency between the enantiomers **2o**–***R*** and **2o**–***S*** as well (EC of 0.001 and 0.01 μM, respectively). Notably, both enantiomers caused embryo spinning, suggesting their microtubule destabilizing properties. Determination of the absolute configuration of these chiral isomers is in progress.

The next step of the present study was the comparative biological evaluation of 7-methoxy-4*H*-chromenes (**2**) and 7-methoxy-oxa-PTs (**3**) with matching alkoxy-substituents in the ring E (Table 2). These series displayed similar antimitotic microtubule destabilizing activity in the phenotypic sea urchin embryo assay. It could be concluded that the lactone ring in the parent PT could be replaced with 2-NH_2 and 3-CN groups without significantly affecting antimitotic potency derived from the microtubule destabilization.

Several 4-aryl-4*H*-chromenes were selected for evaluation in the NCI60 anticancer drug screen (Table 1; Table S1, Supplementary data). Similar to the other PT analogues, the effect of 4*H*-chromenes on the sea urchin embryo correlated well with their growth inhibitory activity against human tumor cell lines. Specifically, compounds **1i**, **1k**, **1l**, and **1p** were found to be inactive whereas molecules **2d** and **2o** exhibited potent antiproliferative properties in both assay systems. The molecule **1d** substituted with a 3,4,5-trimethoxyphenyl group was more potent in the tumor cell assay as compared to the sea urchin embryo assay, paralleling the aza-PT series.[13] Furthermore, 4*H*-chromenes **2d**, **2j**, and **2o** showed cytotoxicity comparable to that of EPC2407 (EC_{50} of 0.081 and 0.034 μM for H1299 human lung cancer cells and T-47D human breast cancer cells, respectively) (Table 1).[23] Notably, chromene **2d** has been identified previously as a potent proapoptotic agent.[19] It exhibited markedly higher cytotoxicity against the panel of human cancer cells than the respective 7-methoxy-oxa-PT (**3d**) (Table S1, Supplementary data), suggesting the favorable substitution of the lactone ring D with –CN and –NH_2 groups. As for sea urchin embryos, in the cytotoxicity screen the replacement of the methylenedioxy ring A by a 7-methoxy moiety yielded 4*H*-chromene with similar cell growth inhibition potency (compare **2o** and **II**, Table 1, Table S1, Supplementary data). Molecule **2j** containing a 3,5-dimethoxyphenyl ring E exhibited the highest potency both in the sea urchin embryo model and in the cellular assay. Compound **2d** was more active than **2o** in the NCI panel (GI_{50} of 0.045 μM and 0.055 μM, respectively), as well as in T47D breast cancer cells (GI_{50} of 0.026 μM and 0.052 μM, respectively) and H1299 lung carcinoma cells (GI_{50} of 0.057 μM and 0.089 μM, respectively).[19] However, relative activities were reversed in the sea urchin assay (Table 1), namely, **2o** was more potent. One of the conceivable reasons for this discrepancy could be the significantly shorter mitotic cycle of sea urchin embryo blastomers (35–45 min) than of cultured cells (20–24 h). As a consequence, the main target of microtubule destabilizing agents could be the mitotic spindle in the sea urchin embryo, or presumably interphase microtubules in cancer cells.

In conclusion, a series of 4-aryl-4*H*-chromenes were assayed in the cellular and phenotypic sea urchin embryo assays for antimitotic microtubule destabilizing activity. The resulting data correlated well between these two test systems. Notably, neither the methylenedioxy nor lactone functionalities (rings A and D, respectively) were essential for antimitotic microtubule destabilizing activity, and could be successively replaced by 2-NH_2, 3-CN, and 7-methoxy groups. 7-Methoxy-4-aryl-4*H*–chromenes (**2**) and their respective oxa-PT analogues (**3**) displayed similar potencies, however the chromene series were more synthetically accessible with markedly higher overall yields. The most active molecules in both series featured a 3,5-dimethoxyphenyl ring E (**2j** and **3j**). The difference between *R*- and *S*-enantiomers of compounds **2o** and **2d** was 10 and 400 times, respectively. Considering significant antimitotic activity, synthetic feasibility, and high yields of 7-methoxy-4-aryl-4*H*-chromenes, they represent a promising starting point in the development of novel anticancer therapies.

Acknowledgments

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Supplementary data

Supplementary data (sea urchin embryo assay; cytotoxicity of selected chromenes in NCI60 human cancer cell screen.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Kingston, D. G. I. *J. Nat. Prod.* **2009**, *72*, 507.
2. Perez, E. A. *Mol. Cancer Ther.* **2009**, *8*, 2086.
3. Dumontet, C.; Jordan, M. A. *Nat. Rev. Drug Disc.* **2010**, *9*, 790.
4. Stanton, R. A.; Gernert, K. M.; Nettles, J. H.; Aneja, R. *Med. Res. Rev.* **2011**, *31*, 443.
5. Lu, Y.; Chen, J.; Xiao, M.; Li, W.; Miller, D. D. *Pharm. Res.* **2012**, *29*, 2943.
6. Semenova, M. N.; Kiselyov, A. S.; Semenov, V. V. *BioTechniques* **2006**, *40*, 765. Sea urchin embryo assay. Adult sea urchins, *Paracentrotus lividus* L. (Echinidae), were collected from the Mediterranean Sea on the Cyprus coast and kept in an aerated seawater tank. Gametes were obtained by intracoelomic injection of 0.5 M KCl. Eggs were washed with filtered seawater and fertilized by adding drops of diluted sperm. Embryos were cultured at room temperature under gentle agitation with a motor-driven plastic paddle (60 rpm) in filtered seawater. The embryos were observed with a Biolam light microscope (LOMO, St. Petersburg, Russia). For treatment with the test compounds, 5 mL aliquots of embryo suspension were transferred to six-well plates and incubated as a monolayer at a concentration up to 2000 embryos/mL. Stock solutions of compounds were prepared in DMSO at 10 mM concentration followed by a 10-fold dilution with 96% EtOH. This procedure enhanced the solubility of the test compounds in the salt-containing medium (seawater), as evidenced by microscopic examination of the samples. The maximal tolerated concentrations of DMSO and EtOH in the in vivo assay were determined to be 0.05% and 1%, respectively. Higher concentrations of either DMSO ($\geqslant$0.1%) or EtOH (>1%) caused nonspecific alteration and retardation of the sea urchin embryo development independent of the treatment stage. The antiproliferative activity was assessed by exposing fertilized eggs (8–20 min after fertilization, 45–55 min before the first mitotic cycle completion) to 2-fold decreasing concentrations of the compound. Cleavage alteration and arrest were clearly detected at 2.5–5.5 h after fertilization. The effects were estimated quantitatively as an effective threshold concentration, resulting in cleavage alteration and embryo death before hatching or full mitotic arrest. At these concentrations all tested microtubule destabilizers caused 100% cleavage alteration and embryo death before hatching, whereas at 2-fold lower concentrations the compounds failed to produce any effect. For microtubule-destabilizing activity, the compounds were tested on free-swimming blastulae just after hatching (8–10 h after fertilization), which originated from the same embryo culture. Embryo spinning was observed after 15 min to 20 h of treatment, depending on the structure and concentration of the compound. Both spinning and lack of forward movement were interpreted to be the result of the microtubule-destabilizing activity of a molecule.
7. Video illustrations are available at http://www.chemblock.com.
8. Desbene, S.; Giorgi-Renault, S. *Curr. Med. Chem.: Anti-Cancer Agents* **2002**, *2*, 71.
9. Gordaliza, M.; Garcia, P. A.; Miguel del Corral, J. M.; Castro, M. A.; Gomez-Zurita, M. A. *Toxicon* **2004**, *44*, 441.
10. Ravelli, R. B.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198.
11. Gupta, S.; Das, L.; Datta, A. B.; Poddar, A.; Janik, M. E.; Bhattacharyya, B. *Biochemistry* **2006**, *45*, 6467.
12. Meresse, P.; Dechaux, E.; Monneret, C.; Bertounesque, E. *Curr. Med. Chem.* **2004**, *11*, 2443.
13. Semenova, M. N.; Kiselyov, A. S.; Tsyganov, D. V.; Konyushkin, L. D.; Firgang, S. I.; Semenov, R. V.; Malyshev, O. R.; Raihstat, M. M.; Fuchs, F.; Stielow, A.; Lantow, M.; Philchenkov, A. A.; Zavelevich, M. P.; Zefirov, N. S.; Kuznetsov, S. A.; Semenov, V. V. *J. Med. Chem.* **2011**, *54*, 7138.
14. Chernysheva, N. B.; Tsyganov, D. V.; Philchenkov, A. A.; Zavelevich, M. P.; Kiselyov, A. S.; Semenov, R. V.; Semenova, M. N.; Semenov, V. V. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2590.
15. Frackenpohl, J.; Adelt, I.; Antonicek, H.; Arnold, C.; Behrmann, P.; Blaha, N.; Bohmer, J.; Gutbrod, O.; Hanke, R.; Hohmann, S.; van Houtdreve, M.; Losel, P.; Malsam, O.; Melchers, M.; Neufert, V.; Peschel, E.; Reckmann, U.; Schenke, T.; Thiesen, H. P.; Velten, R.; Vogelsang, K.; Weiss, H.-C. *Bioorg. Med. Chem.* **2009**, *17*, 4160.
16. Kasibhatla, S.; Gourdeau, H.; Meerovitch, K.; Drewe, J.; Reddy, S.; Qiu, L.; Zhang, H.; Bergeron, F.; Bouffard, D.; Yang, Q.; Herich, J.; Lamothe, S.; Cai, S. X.; Tseng, B. *Mol. Cancer Ther.* **2004**, *3*, 1365.
17. Gourdeau, H.; Leblond, L.; Hamelin, B.; Desputeau, C.; Dong, K.; Kianicka, I.; Boudreau, C.; Geerts, L.; Cai, S.-X.; Drewe, J.; Labrecque, D.; Tseng, B. *Mol. Cancer Ther.* **2004**, *3*, 1375.
18. Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Wang, Y.; Zhao, J.; Jia, S.; Herich, J.; Labreque, D.; Storer, R.; Meerovitch, K.; Bouffard, D.; Rej, R.; Denis, R.; Blais, C.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2004**, *47*, 6299.
19. Kemnitzer, W.; Kasibhatla, S.; Jiang, S.; Zhang, H.; Zhao, J.; Jia, S.; Xu, L.; Crogan-Grundy, C.; Denis, R.; Barriault, N.; Vaillancourt, L.; Charron, S.; Dodd, J.; Attardo, G.; Labrecque, D.; Lamothe, S.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4745.
20. Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Zhao, J.; Crogan-Grundy, C.; Xu, L.; Lamothe, S.; Gourdeau, H.; Denis, R.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2007**, *50*, 2858.
21. Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Crogan-Grundy, C.; Labreque, D.; Bubenick, M.; Attardo, G.; Denis, R.; Lamothe, S.; Gourdeau, H.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2008**, *51*, 417.
22. Kemnitzer, W.; Jiang, S.; Wang, Y.; Kasibhatla, S.; Crogan-Grundy, C.; Bubenik, M.; Labrecque, D.; Denis, R.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 603.
23. Cai, S. X.; Drewe, J.; Kemnitzer, W. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 456.
24. http://www.epicept.com/Products_&_Pipeline/Product_Pipeline/Cancer/EPC2407/.
25. ClinicalTrials.gov Identifier: NCT01240590.
26. Raghuvanshi, D. S.; Singh, K. N. *ARKIVOC* **2010**, *x*, 305.
27. Khaksar, S.; Rouhollahpour, A.; Talesh, S. M. *J. Fluorine Chem.* **2012**, *141*, 11.
28. Safari, J.; Zarnegar, Z.; Heydarian, M. B. *Chem. Soc. Jpn.* **2012**, *85*, 1332.
29. Patra, A.; Mahapatra, T. *Synth. Commun.* **2013**, *43*, 1602.
30. Cytovia, Inc. US Patent US2003065018 A1, 2003.
31. Petcher, T. J.; Weber, H. P.; Kuhn, M.; Von Wartburg, A. *J. Chem. Soc., Perkin Trans. 2* **1973**, 288.
32. Rithner, C. D.; Bushweller, C. H.; Gensler, W. J.; Hoogasian, S. *J. Org. Chem.* **1983**, *48*, 1491.
33. Polyalkoxy substituted 4*H*-chromenes **1a**–**t** and **2a**,**d**,**g**–**k**,**m**–**q** were purchased from Chemical Block Ltd. Library. Purity of the materials for the biological testing exceeded 97% as evidenced by NMR, LCMS, and combustion analyses.
34. Shestopalov, A. M.; Litvinov, Y. M.; Rodinovskaya, L. A.; Malyshev, O. R.; Semenova, M. N.; Semenov, V. V. *ACS Comb. Sci.* **2012**, *14*, 484.
35. Cai, S. X.; Drewe, J. A.; Kasibhatla, S.; Kemnitzer, W. D.; Tseng, B. Y.; Blais, C.; Labrecque, D.; Gourdeau, H. US Patent 7,968,595 B2, 2011.