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Short communication

Design and synthesis of 2-phenyl-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-ones as potential anticancer agents starting from cytotoxic spiromamakone A

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ABSTRACT

The spirocycle is a key structure found in many bioactive compounds. From the cytotoxic and spirocyclic natural product, spiromamakone A (**1**) and its analogues, a more synthetically accessible spiroacetal template **4** was designed based on structural similarity analysis. A total of 50 compounds were rapidly synthesized in only one or two synthetic steps from the starting compound, and their cytotoxicity was evaluated. As a result, (±)-(2*R**,5*R**)-2-(4-iodophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (**7d-II**) was discovered and found to be fifteen-fold more cytotoxic than **1**. The easily accessible spiroacetal **7d-II** appeared to act in a manner similar to the highly oxidized natural product, spiromamakone A (**1**).

1. Introduction

Spirocyclic compounds have garnered considerable recent attention due to their unique three-dimensional orientation and proven biological activities [1–3]. Spiromamakone A (**1**) is a potent cytotoxic natural product (IC_{50} value, 0.33 μM against the murine leukemia cell line P388) that was first isolated in 2006 by Munro and co-workers [4]. It has an unprecedented spiro-nonadiene skeleton with a high degree of oxidation and unsaturation. Two analogues, 4-oxo-spiromamakone A (**2**) and spiropreussione (**3**), are reportedly also cytotoxic (**2**: IC_{50} value, 1.13 μM against the murine leukemia cell line P388, **3**: IC_{50} values, 2.4 μM against the human ovarian carcinoma cell line A2780, 3.0 μM against the human liver carcinoma cell line BEL-7404) [4,5]. Although spiromamakone A and its analogues are attractive candidates for anticancer agents, their total synthesis is yet to be reported, and it is difficult to establish a short-step synthetic method for **1**–**3** [6]. Moreover, the structures of spiromamakone A and its analogues are not easy to modify, which is needed in order to tune its properties for drug development. The development of more synthetically accessible molecules would be very valuable, particularly if they could retain the same, or higher, biological activity as that of spiromamakone A and its analogues [7–9].

Starting from spiromamakone A and its analogues, 2-phenyl-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-ones **4**, the spiroacetal template for a rapid synthesis of analogues was designed based on structural similarity analysis. A total of 50 compounds were prepared in one or two synthetic steps from the starting compound **5**, and their cytotoxicity was evaluated. As a result, an easily

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accessible spiroacetal, (±)-(2*R**,5*R**)-2-(4-iodophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (**7d-II**) was discovered and found to be a 15-fold more cytotoxic agent than **1**.

2. Results and discussion

From the structural similarity analysis of spiromamakone A (**1**) and its analogues **2** and **3** (Fig. 1b–d) [10], we speculated that a common structure (emphasized in Fig. 1a) is pivotal for inducing the cytotoxicity of these compounds. Synthetically accessible, 2-phenyl-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one **4** was designed as a template for the rapid synthesis of analogues. From a comparison of the molecular shapes between **1** and the template **4**, the structure of template **4** overlapped well with the pivotal structure in **1** (Fig. 1d).

Forty-three compounds retaining various substituents were rapidly synthesized in only one or two synthetic steps from the starting compound **5**, as shown in Fig. 2 (see Supporting information). Aryl halides **7** were prepared from the acetal

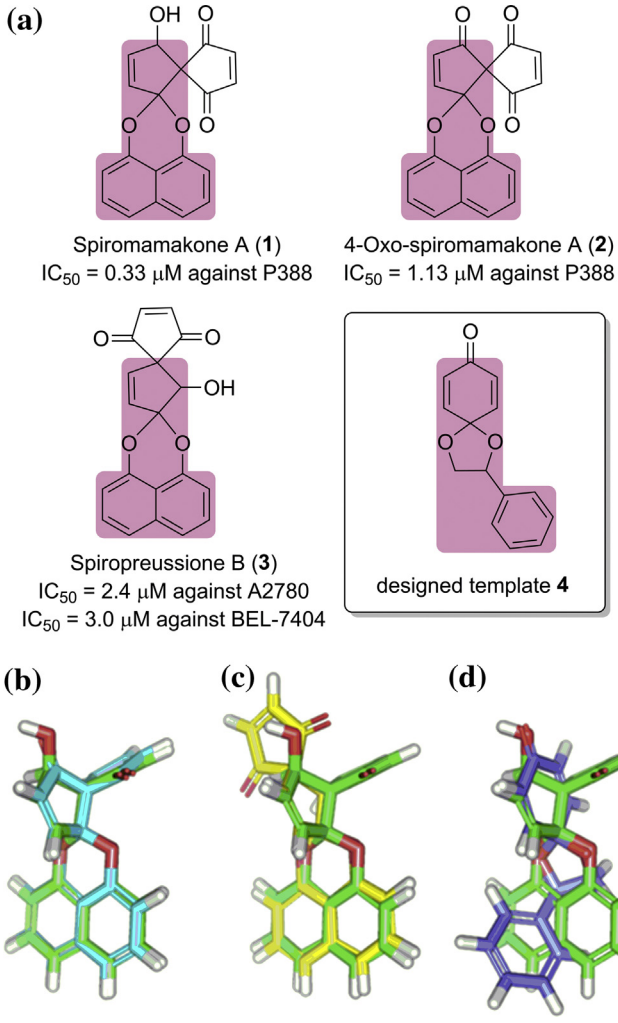

Fig. 1. (a) Structures of spiromamakone A (**1**), 4-oxo-spiromamakone A (**2**), spiropreussione B (**3**), and the designed spiroacetal template **4**. The common structure is emphasized. (b) Structural similarity analysis of spiromamakone A (**1**) and **2**. (c) Structural similarity analysis of **1** and **3**. (d) Structural similarity analysis of **1** and the designed template **4**. Carbon atoms for **1**, **2**, **3**, and **4** are shown in green, cyan, yellow and purple, respectively. Construction of 3D models and energy minimization was performed using the OPLS-AA force field in the Conformational Search algorithm in the MacroModel program (Schrödinger, LLC). All compounds are aligned by a shape similarity score with **1**, which was calculated using the ROCS (OpenEye Scientific Software, Inc).

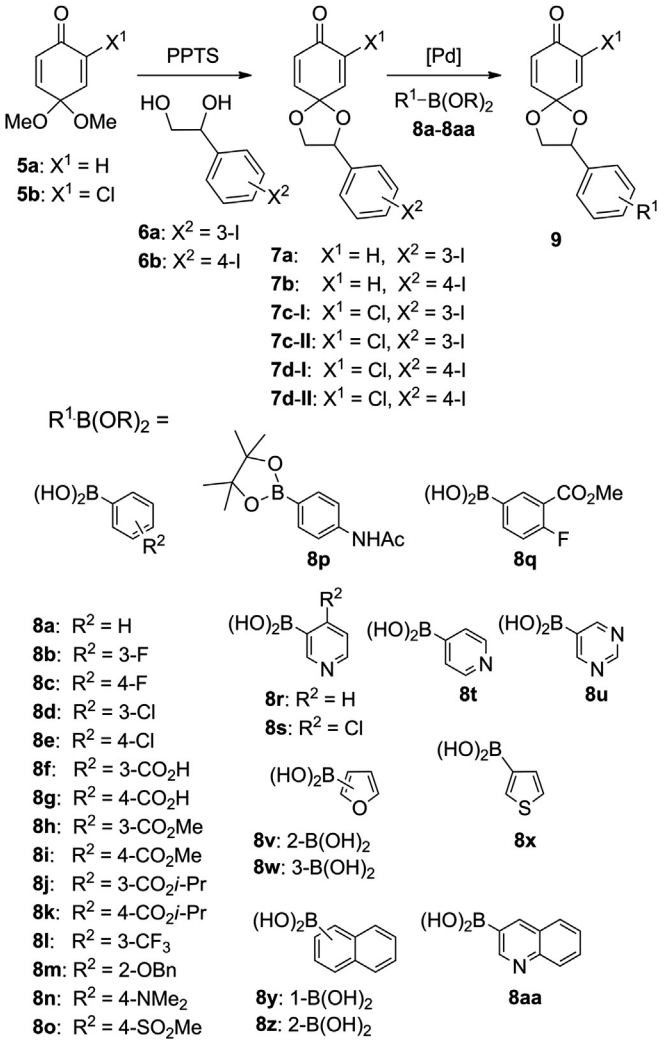

Fig. 2. Rapid synthesis of 43 spiroacetals **7** and **9**.

exchange reaction between diols **6** and dimethyl acetal **5** in the existence of pyridinium *p*-toluenesulfonate (PPTS). The dimethyl acetal **5** was derived from the corresponding 4-methoxy phenols in one step [11]. Two diastereomers, **I** and **II**, were obtained from the compounds of **7c** and **7d**. Although the relative stereochemistries of the obtained diastereomers were not determined at this point, these diastereomers were isolated by silica gel column chromatography. **7a**, **7b** and a diastereomer of **7d-II**, were used for further modification based on a Suzuki–Miyaura coupling reaction [12,13] with various aryl building blocks **8** [14,15].

The cytotoxicity of synthesized compounds **7** and **9** against cervical carcinoma HeLa cells was evaluated (Table 1). Twelve compounds, **7d-II**, **9c**, **9e–g**, **9j**, **9s–v**, **9y**, and **9ag** (Table 1, entries 7, 10, 12–14, 17, 26–29, 32 and 40) exhibited higher cytotoxicity than that of spiromamakone A (**1**). In particular, **7d-II** showed a 15-fold more potent cytotoxicity than **1** (Table 1, entry 7). Interestingly, both diastereomers **7c-I** and **7c-II** exhibited a comparable degree of cytotoxicity (Table 1, entries 4 and 5). On the other hand, the diastereomer, **7d-II** was 15-fold more cytotoxic than its diastereomer **7d-I** (Table 1, entries 6 and 7). This result clearly indicates that the relative stereochemistry of **7d-II** is important to its cytotoxicity. From the comparison of the cytotoxicity between **7b** and **7d-II**, and between **7c** and **7d**, it was indicated that the chloro and 4-iodo groups in **7d** were also important for the potency of its

Table 1
Cytotoxicity of the synthesized compounds **7**, **9**, and spiromamakone A (**1**) against cervical carcinoma HeLa cells.

Entry	Substrate **7**	Substrate **8**	Evaluated compounds	IC_{50} (μM)[a]
1	–	–	**1**	2.2
2	–	–	**7a**	1.6
3	–	–	**7b**	1.1
4	–	–	**7c-I**	1.5
5	–	–	**7c-II**	1.6
6	–	–	**7d-I**	2.4
7	–	–	**7d-II**	0.14
8	**7a**	**8a**	**9a**	1.4
9	**7a**	**8b**	**9b**	1.6
10	**7a**	**8c**	**9c**	0.67
11	**7a**	**8d**	**9d**	1.8
12	**7a**	**8e**	**9e**	0.85
13	**7a**	**8o**	**9f**	0.79
14	**7a**	**8p**	**9g**	0.88
15	**7a**	**8s**	**9h**	1.7
16	**7a**	**8v**	**9i**	3.2
17	**7a**	**8x**	**9j**	0.91
18	**7a**	**8y**	**9k**	1.3
19	**7a**	**8z**	**9l**	1.3
20	**7a**	**8aa**	**9m**	2.4
21	**7b**	**8d**	**9n**	1.4
22	**7b**	**8f**	**9o**	3.1
23	**7b**	**8g**	**9p**	1.7
24	**7b**	**8h**	**9q**	1.5
25	**7b**	**8i**	**9r**	2.6
26	**7b**	**8j**	**9s**	0.80
27	**7b**	**8k**	**9t**	0.82
28	**7b**	**8l**	**9u**	0.80
29	**7b**	**8m**	**9v**	0.96
30	**7b**	**8n**	**9w**	3.5
31	**7b**	**8o**	**9x**	1.7
32	**7b**	**8p**	**9y**	0.90
33	**7b**	**8q**	**9z**	1.8
34	**7b**	**8r**	**9aa**	1.5
35	**7b**	**8s**	**9ab**	1.3
36	**7b**	**8t**	**9ac**	4.5
37	**7b**	**8u**	**9ad**	3.2
38	**7b**	**8w**	**9ae**	2.5
39	**7b**	**8x**	**9af**	2.5
40	**7b**	**8aa**	**9ag**	0.79
41	**7d-II**	**8r**	**9ah**	9.4
42	**7d-II**	**8t**	**9ai**	2.3
43	**7d-II**	**8u**	**9aj**	4.6
44	**7d-II**	**8aa**	**9ak**	2.7

DMSO was employed as a negative control and no cytotoxicity was observed.
[a] The IC_{50} value was defined as the concentration at which 50% survival of cells was observed. The results are listed in the table.

cytotoxicity (Table 1, entries 3–7). In the case of **9a**–**m**, which were derived from **7a** (Table 1, entries 8–20), the top 4 cytotoxic compounds **9c** and **9e**–**g** retained *p*-substituted phenyl rings at the ends of their structures (Table 1, entries 10, 12, 13 and 14). On the other hand, in the case of compounds **9n**–**ag**, which were derived from **7b** (Table 1, entries 21–40), highly cytotoxic compounds **9s**–**v** and **9y** (IC_{50} < 1 μM) contained *o*-, *m*-, or *p*-substituted phenyl rings at the ends of their structures (Table 1, entries 26–29 and 32). Generally, it seemed that the basic aromatic ring decreased the cytotoxicity because all less cytotoxic compounds **9ac**, **9ah**, **9aj** (IC_{50} > 4 μM) contain a pyridine or pyrimidine ring (Table 1, entries 36, 41 and 43).

To elucidate the detailed structure–activity relationship, 7 analogues of **7d** with different halogen atoms at different positions were prepared (Fig. 3). The cytotoxicity of synthesized analogues against the HeLa cells was evaluated, and the results are shown in Table 2. From a comparison of the cytotoxicities of **7d-II**, **7f** and **7h**, the importance of the 4-iodo group for potent cytotoxicity was confirmed (Table 2, entries 2, 4, 5, 8 and 9). Interestingly, in the case of aryl bromides **7g** and **7h**, 3-bromo analogues **7g** were more

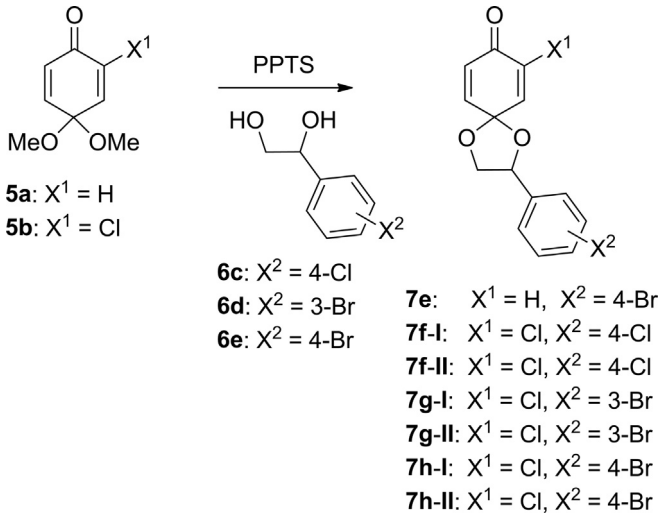

Fig. 3. Synthesis of seven analogues of **7d**.

cytotoxic than 4-bromo analogues **7h** (Table 2, entries 6–9) in contrast with 4-aryl iodides **7c** and **7d** (Table 1, entries 4–7).

At this point, we embarked on the structural determination of **7d-II**. Fortunately, a single crystal of the less cytotoxic diastereomer **7d-I** was obtained, and the X-ray crystal structure revealed its relative stereochemistry, as shown in Fig. 4. Thus, the relative stereochemistry of **7d-II** was unambiguously determined, as shown in Fig. 4.

Finally, the panel assay of 39 human cancer cell lines for spiromamakone A (**1**) and **7d-II** was performed (see Supporting information), because there reportedly is a significant correlation between a pattern of differential cytotoxicity against the cell lines by a drug and its mode of action [16]. The obtained results indicated that **7d-II** exerts higher cytotoxicity against most cell lines than **1**, but the cytotoxic patterns between **1** and **7d-II** show high similarity. In addition, these patterns were weak similar (correlation coefficient = 0.339–0.461) to those of purine antagonists such as 6-mercaptopurine and 6-thioguanine and were predicted that **1** and **7d-II** possess a unique mechanism of action. These results indicate that our originally developed, synthetically accessible spiroacetal **7d-II** acts in a manner similar to the highly oxidized spirocyclic natural product, spiromamakone A (**1**). It is conceivable that both **1** and **7d-II** act as a nucleoside mimetic to exert their cytotoxicity.

3. Conclusion

In summary, starting from the cytotoxic, spirocyclic natural product, spiromamakone A (**1**) and its analogues, 2-phenyl-1,4-

Table 2
Cytotoxicity of the synthesized seven analogues of **7d**, spiromamakone A (**1**) and **7d-II** against cervical carcinoma HeLa cells.

Entry	Evaluated compounds	IC_{50} (μM)[a]
1	**1**	2.2
2	**7d-II**	0.14
3	**7e**	2.4
4	**7f-I**	2.9
5	**7f-II**	2.7
6	**7g-I**	0.64
7	**7g-II**	0.61
8	**7h-I**	4.5
9	**7h-II**	2.1

DMSO was employed as a negative control and no cytotoxicity was observed.
[a] The IC_{50} value was defined as the concentration at which 50% survival of cells was observed. The results are listed in the table.

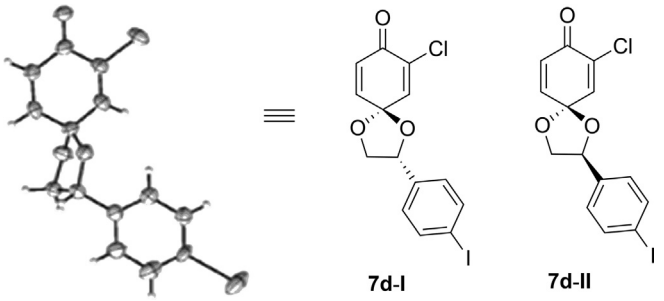

Fig. 4. X-ray single crystal structure of **7d-I**, and the determined relative stereochemistries of **7d-I** and **7d-II**.

dioxa-spiro[4.5]deca-6,9-dien-8-ones **4**, the synthetically accessible spiroacetal template was designed for a synthesis of analogues based on structural similarity analysis. A total of 50 compounds were rapidly synthesized in only one or two synthetic steps from the starting compound **5**, and its cytotoxicity was evaluated. As a result, a total of 12 compounds exerted higher cytotoxicity ($IC_{50} < 1$ μM) than **1**. In particular, (±)-(2*R**,5*R**)-2-(4-iodophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (**7d-II**) was discovered as a 15-fold more cytotoxic agent than **1**. The panel assay for various cancer cell lines suggested that the spiroacetal **7d-II** which can be prepared in only one synthetic step from **5b** acts in a manner similar to the highly oxidized natural product, spiromamakone A (**1**). It is conceivable that both **1** and **7d-II** act as a nucleoside mimetic to exert their cytotoxicity. The synthetically accessible, cytotoxic spiroacetal **7d-II** would be a valuable aid in the drug discovery process.

4. Experimental

4.1. Chemistry

NMR spectra were recorded on either a JEOL Model ECP-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) or on a JEOL Model EX-270 (270 MHz for ^{1}H, 67.5 MHz for ^{13}C) instrument for the indicated solvent. Chemical shifts were reported in units of parts per million (ppm) relative to the signal for internal tetramethylsilane (0.00 ppm for ^{1}H) for solutions in $CDCl_3$. The ^{1}H NMR spectral data were reported as follows: $CDCl_3$ (7.26 ppm). ^{13}C NMR spectral data were reported as follows: $CDCl_3$ (77.1 ppm). Multiplicities were reported using the following abbreviations: s; singlet, d; doublet, t; triplet, m; multiplet, *J*; coupling constants in Hertz. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrophotometer. Only the strongest and/or structurally important absorption is reported as the IR data given in cm^{-1}. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, visualized by *p*-anisaldehyde solution, ceric sulphate or 10% ethanolic phosphomolybdic acid. Column chromatography was performed on Merck silica gel 60 (0.063–0.200 mm). ESI-TOF Mass spectra were measured using a Waters LCT Premier™ XE. The HRMS (ESI-TOF) were calibrated using leucine enkephalin.

4.2. General procedure for the synthesis of compounds *7d–h*

A diol **6c** or **6d** or **6e** (2 equiv) and PPTS (0.1 equiv) was added to a toluene (25.0 mL/1 mmol of substrate) solution of a substituted-4,4-dimethoxycyclohexa-2,5-dienone **5a** or **5b** (1 equiv) at room temperature. After stirring at 40 °C for 18 h, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ and the product was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried over anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure. The residue was purified by preparative TLC to give the title compound.

4.2.1. (±)-(2R*,5S*)-2-(4-Iodophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7d-I)

Mp. 101–103 °C; yield: 19%; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.75 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 6.88 (d, *J* = 2.7 Hz, 1H), 6.78 (dd, *J* = 2.7, 8.1 Hz, 1H), 6.31 (d, *J* = 8.1 Hz, 1H), 5.23 (dd, *J* = 5.4, 8.1 Hz, 1H), 4.52 (dd, *J* = 5.4, 8.1 Hz, 1H), 3.86 (t, *J* = 8.1 Hz, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 177.9, 143.3, 139.3, 138.0, 136.2, 133.8, 127.9, 127.8, 100.7, 94.4, 78.7, 71.9; IR (neat) 3060, 2889, 1685, 1653, 1615, 1487, 1333, 1132, 1020, 1006, 976, 822; HRMS (ESI-TOF): calcd. for $[C_{14}H_{10}ClIO_3 + H]^+$ 388.9441, found 388.9463.

4.2.2. (±)-(2R*,5R*)-2-(4-Iodophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7d-II)

Yield: 13%; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.75 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.1 Hz, 2H), 6.93 (d, *J* = 2.7 Hz, 1H), 6.73 (dd, *J* = 2.7, 8.1 Hz, 1H), 6.31 (d, *J* = 8.1 Hz, 1H), 5.23 (t, *J* = 8.1 Hz, 1H), 4.51 (t, *J* = 8.1 Hz, 1H), 3.88 (t, *J* = 8.1 Hz, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 177.9, 143.7, 138.9, 138.0, 136.4, 133.4, 128.3, 127.9, 100.7, 94.4, 78.6, 71.9; IR (neat) 3058, 2887, 1686, 1652, 1615, 1487, 1334, 1278, 1216, 1132, 1020, 1006, 975, 822; HRMS (ESI-TOF): calcd. for $[C_{14}H_{10}ClIO_3 + H]^+$ 388.9441, found 388.9461.

4.2.3. (±)-2-(4-Bromophenyl)-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7e)

Yield: 46%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.1 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 6.69–6.79 (m, 2H), 6.19–6.26 (m, 2H), 5.25 (dd, *J* = 5.4, 8.1 Hz, 1H), 4.51 (dd, *J* = 5.4, 8.1 Hz, 1H), 3.87 (t, *J* = 8.1 Hz, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 185.1, 143.2, 142.7, 136.1, 132.0, 129.4, 128.9, 127.8, 122.6, 99.3, 72.0, 78.4.

4.2.4. (±)-2-(4-Chlorophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7f-I)

Yield: 29%; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.39 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 6.88 (d, *J* = 2.7 Hz, 1H), 6.74 (dd, *J* = 2.7, 10.8 Hz, 1H), 6.33 (d, *J* = 10.8 Hz, 1H), 5.27 (dd, *J* = 5.4, 8.1 Hz, 1H), 4.52 (dd, *J* = 5.4, 8.1 Hz, 1H), 3.89 (t, *J* = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 143.9, 139.0, 135.3, 134.8, 133.5, 129.2, 128.5, 127.6, 100.8, 72.1, 78.6.

4.2.5. (±)-2-(4-Chlorophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7f-II)

Yield: 17%; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.40 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 6.89 (d, *J* = 2.7 Hz, 1H), 6.79 (dd, *J* = 2.7, 10.8 Hz, 1H), 6.31 (d, *J* = 10.8 Hz, 1H), 5.26 (dd, *J* = 5.4, 8.1 Hz, 1H), 4.52 (dd, *J* = 5.4, 8.1 Hz, 1H), 3.86 (t, *J* = 8.1 Hz, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 178.0, 143.3, 139.4, 135.0, 134.8, 133.8, 129.1, 127.8, 127.5, 100.7, 78.7, 72.0.

4.2.6. (±)-2-(3-Bromophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7g-I)

Yield: 39%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (s, 1H), 7.49–7.53 (m, 1H), 7.29–7.31 (m, 2H), 6.89 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 2.9, 10.2 Hz, 1H), 6.31 (d, *J* = 10.2 Hz, 1H), 5.24 (dd, *J* = 6.1, 8.3 Hz, 1H), 4.51 (dd, *J* = 6.1, 8.3 Hz, 1H), 3.89 (t, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 143.2, 139.3, 138.8, 133.8, 131.9, 130.4, 129.1, 127.8, 124.6, 123.0, 100.7, 78.4, 72.0.

4.2.7. (±)-2-(3-Bromophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (7g-II)

Yield: 29%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (s, 1H), 7.49–7.53 (m, 1H), 7.26–7.29 (m, 2H), 6.95 (d, *J* = 2.9 Hz, 1H), 6.75 (dd, *J* = 2.9,

10.2 Hz, 1H), 6.34 (d, J = 10.2 Hz, 1H), 5.26 (dd, J = 6.1, 8.3 Hz, 1H), 4.53 (dd, J = 6.1, 8.3 Hz, 1H), 3.90 (t, J = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 143.7, 139.1, 138.8, 133.4, 131.9, 130.4, 129.1, 128.4, 124.7, 123.0, 100.8, 77.3, 72.0.

*4.2.8. (±)-2-(4-Bromophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (**7h-I**)*

Yield: 20%; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.55 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 6.88 (d, J = 2.7 Hz, 1H), 6.79 (dd, J = 2.7, 10.8 Hz, 1H), 6.31 (d, J = 10.8 Hz, 1H), 5.25 (dd, J = 5.4, 8.1 Hz, 1H), 4.52 (dd, J = 5.4, 8.1 Hz, 1H), 3.86 (t, J = 8.1 Hz, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 178.4, 143.8, 139.8, 136.6, 134.3, 132.5, 128.2, 128.2, 123.3, 101.2, 79.2, 72.4.

*4.2.9. (±)-2-(4-Bromophenyl)-7-chloro-1,4-dioxa-spiro[4.5]deca-6,9-dien-8-one (**7h-II**)*

Yield: 19%; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.54 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 6.95 (d, J = 2.7 Hz, 1H), 6.74 (dd, J = 2.7, 10.8 Hz, 1H), 6.33 (d, J = 10.8 Hz, 1H), 5.26 (dd, J = 5.4, 8.1 Hz, 1H), 4.52 (dd, J = 5.4, 8.1 Hz, 1H), 3.88 (t, J = 8.1 Hz, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 178.4, 144.2, 139.4, 136.2, 133.9, 132.5, 128.8, 128.2, 123.3, 101.21, 79.00, 72.5.

4.3. Crystallographic studies for (±)-7d-II

Single X-ray diffraction data were collected on a Rigaku R-AXIS RAPID imaging plate diffractometer with Mo$K\alpha$ radiation (λ = 0.71075 Å). Empirical formula: $C_{14}H_{10}ClIO_3$, formula weight: 388.57, temperature: 123(2) K, crystal system: triclinic, space group: $P\bar{1}$ (no. 2), unit cell dimensions: a = 4.8452(8) Å, b = 15.210(3) Å, c = 18.506(3) Å, α = 86.232(4)°, β = 88.992(4)°, γ = 87.240(4)°, volume: 1359.1(4) Å^3, Z = 4, density (calculated): 1.899 Mg/m^3, absorption coefficient: 2.552 mm^{-1}, F(000): 752, crystal size: 0.16 × 0.06 × 0.05 mm^3, theta range for data collection: 3.31–27.46°, index ranges: $-6 \le h \le 6$, $-19 \le k \le 19$, $-23 \le l \le 21$, reflections collected: 20,622, independent reflections: 6167 [R(int) = 0.0772], completeness to theta = 27.46°, 99.3%, Absorption correction: semi-empirical from equivalents, max. and min. transmission: 0.9600 and 0.6342, refinement method: full-matrix least-squares on F^2, data/restraints/parameters: 6167/0/343, Goodness-of-fit on F^2: 1.051, final R indices [$I > 2$sigma(I)]: R_1 = 0.0570, wR_2 = 0.1090, R indices (all data): R_1 = 0.1244, wR_2 = 0.1391, largest diff. peak and hole: 1.927 and −1.699 e Å^{-3}. CCDC 905767 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.4. Cytotoxicity assay

The human cancer cell line used in this study was cervical carcinoma HeLa cells. The cells were maintained in Dulbecco's modified Eagle medium (Sigma) supplemented with 10% fetal bovine serum (Gibco), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. The cytotoxicity of HeLa cells was assayed with WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] colorimetric assay (Cell Counting Kit; Dojindo). The 384-well plates were seeded with aliquots of a 20 μL medium containing 1.0 × 10^3 cells per well and were incubated overnight before being treated with compounds dissolved in DMSO at various concentrations for 48 h. Plates were incubated for 1 h at 37 °C after the addition of 2 μL WST-8 reagent solution per well. The absorption of the formazan dye formed was measured at 450 nm. The vehicle solvent (DMSO) was used as a negative control.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.05.030. These data include MOL files and InChiKeys of the most important compounds described in this article.

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