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Diversity-oriented synthesis of spiro-oxindole-based 2,5-dihydropyrroles *via* three-component cycloadditions and evaluation on their cytotoxicity†

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The construction of a spiro-oxindole-based 2,5-dihydropyrrole scaffold with potential bioactivity has been established *via* an efficient three-component reaction of isatin, amino-ester and alkyne. This protocol represents the first 1,3-dipolar cycloaddition of electron-deficient alkynes with isatin-derived azomethine ylides, providing an easy access to spiro-oxindole-based 2,5-dihydropyrroles with structural diversity in high yields (up to 99%). In addition, the bioscreen of these new spiro-dihydropyrroles has led to the finding of sixteen compounds with promising cytotoxicity to MCF-7 cells.

Introduction

The spiro-oxindole core is a privileged heterocyclic ring system, which not only constitutes the core structural element of pharmaceutically relevant natural products,[1] but also is featured in a large family of unnatural compounds exhibiting a wide spectrum of important bioactivities such as antitumor,[2] antimycobacterial,[3] antimicrobial[4] and antitubercular[5] properties (Fig. 1).

On the other hand, the 2,5-dihydropyrrole skeleton also exists in a variety of biologically significant compounds possessing antioxidant,[6] antimicrobial,[7] anti-tumoral,[8] and anti-inflammatory[9] activities (Fig. 2).

Considering the versatile bioactivities of the two structures of spiro-oxindole and 2,5-dihydropyrrole, we hypothesize that the integration of the two scaffolds into a spiro-oxindole-based 2,5-dihydropyrrole (in Scheme 1) may result in the discovery of new drug candidates with unknown bioactivities. However, hardly any approaches have been available to access this spiro-architecture, yet.[10] Therefore, the design of an efficient protocol to access this novel target with structural diversity is highly desirable and valuable for medicinal chemistry and drug discovery.

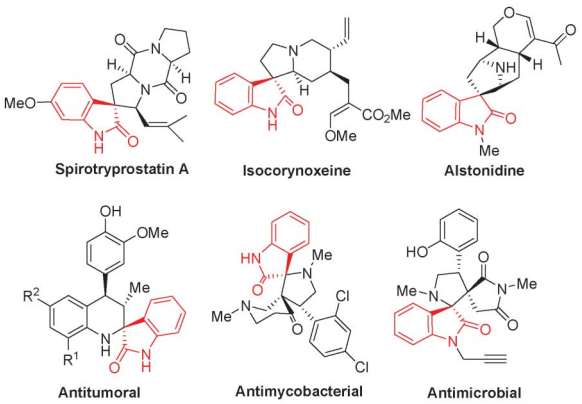

Fig. 1 Biologically important molecules containing a spiro-oxindole core.

1,3-Dipolar cycloadditions of azomethine ylides to electron-deficient olefins have offered a robust method to construct a pyrrolidine skeleton.[11] Inspired by this success, we envision that the 1,3-dipolar cycloaddition of electron-deficient alkynes

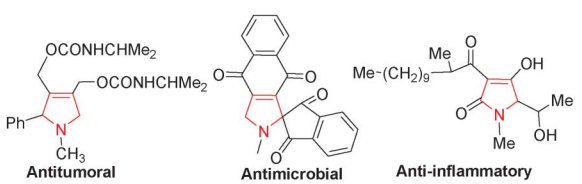

Fig. 2 Bioactive compounds containing a 2,5-dihydropyrrole core.

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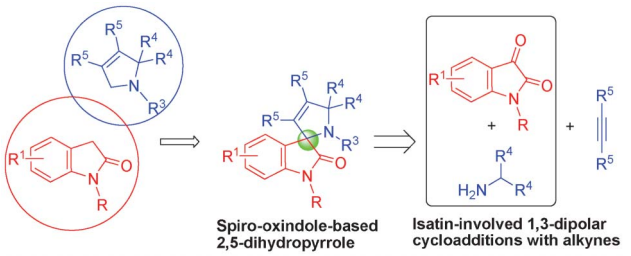
‡ These two authors contributed equally to the work.



Scheme 1 Design of the reaction to access a spiro-oxindole-based 2,5-dihydropyrrole scaffold.

to isatin-derived azomethine ylides will provide a straightforward entry to the spiro-oxindole-based 2,5-dihydropyrrole scaffold (Scheme 1). Besides, the designed three-component reactions of isatins, amino-esters and alkynes will facilitate the diversity-oriented synthesis,[12] which plays an important role in offering a source of compounds for screening experiments with the attempt to discover novel biologically active entities.[13] However, electron-deficient alkynes have scarcely been employed as dipolarophiles to react with azomethine ylides,[14] especially with isatin-derived ones, which are more difficult to form and have lower reactivity in subsequent cycloadditions than their aldehyde-derived counterparts. Thus, the development of 1,3-dipolar cycloadditions of electron-deficient alkynes with isatin-derived azomethine ylides is of great importance with regard to the chemistry of 1,3-dipolar cycloadditions and the construction of the spiro-oxindole-based 2,5-dihydropyrrole skeleton.

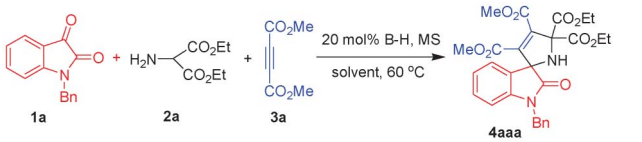
As a continuation of our efforts in developing new multi-component reactions (MCRs) and synthesizing bioactive heterocycles *via* MCRs,[15] we herein report the efficient diversity-oriented synthesis of novel spiro-oxindole-based 2,5-dihydropyrroles (up to 99% yield, 36 examples) *via* an unprecedented three-component 1,3-dipolar cycloaddition of electron-deficient alkynes with isatin-derived azomethine ylides. Additionally, the bioscreen of the products has led to the finding of sixteen compounds with promising cytotoxicity to MCF-7 cells.

Results and discussion

The initial attempt to validate our hypothesis examined a three-component reaction of *N*-benzylisatin **1a**, diethyl 2-aminomalonate **2a** and dimethyl but-2-ynedioate **3a**, which was performed in the presence of 20 mol % Brønsted acid (B–H) in toluene at 60 °C (Table 1).

However, a preliminary screening of the catalysts led to disappointing results (Table 1, entries 1–3). Benzoic acid and 4-methylbenzenesulfonic acid (TsOH) could hardly catalyze the reaction (entries 1–2), and trifluoroacetic acid could catalyze the reaction only with a low yield of 16% (entry 3). Subsequent screening of solvents revealed that 1,2-dichloroethane was the most suitable for increasing the yield to 48% (entry 7 *vs.* 3–6). Further evaluation of molecular sieves (MS)

Table 1 Optimization of reaction conditions[a]



Entry	B–H	Solvent	MS	**1a : 2a : 3a**	Yield (%)[b]
1	$PhCO_2H$	$PhCH_3$	3 Å	1.2 : 1 : 2	—[c]
2	TsOH	$PhCH_3$	3 Å	1.2 : 1 : 2	—[c]
3	CF_3CO_2H	$PhCH_3$	3 Å	1.2 : 1 : 2	16
4	CF_3CO_2H	1,4-dioxane	3 Å	1.2 : 1 : 2	45
5	CF_3CO_2H	CH_3CN	3 Å	1.2 : 1 : 2	33
6	CF_3CO_2H	EtOH	3 Å	1.2 : 1 : 2	—[c]
7	CF_3CO_2H	$ClCH_2CH_2Cl$	3 Å	1.2 : 1 : 2	48
8	CF_3CO_2H	$ClCH_2CH_2Cl$	4 Å	1.2 : 1 : 2	42
9	CF_3CO_2H	$ClCH_2CH_2Cl$	—[d]	1.2 : 1 : 2	—[c]
10	CF_3CO_2H	$ClCH_2CH_2Cl$	3 Å	1.2 : 1 : 8	56
11	CF_3CO_2H	$ClCH_2CH_2Cl$	3 Å	1.2 : 1 : 12	73
12	CF_3CO_2H	$ClCH_2CH_2Cl$	3 Å	1.2 : 1 : 20	70

[a] Unless indicated otherwise, the reaction was carried out in 0.1 mmol scale in solvent (1 mL) with MS (100 mg) at 60 °C for 36 h. [b] Isolated yield. [c] Trace product was observed. [d] In the absence of MS.

disclosed that 3 Å MS played an important role in controlling the reactivity since trace product was observed in the absence of MS (entry 9 *vs.* 7–8), which may be ascribed to the action of 3 Å MS in efficiently removing water molecules produced by the condensation of **1a** with **2a**, thereby promoting the generation of the isatine-derived azomethine ylide. Finally, the mole ratio of **1a** : **2a** : **3a** was fine tuned to improve the yield (entries 10–12). To our delight, properly increasing the stoichiometry of **3a** rendered the reaction to proceed in a much higher yield of 73% (entry 11).

With the optimal conditions in hand, the diversity-oriented synthesis of spiro-oxindole-based 2,5-dihydropyrroles **4** was then carried out by three-component reactions of various isatins **1**, diethyl 2-aminomalonate **2a** and but-2-ynedioate **3** (Table 2). Firstly, the effect of *N*-substituents of isatin **1** on the reaction was investigated. As shown in entries 1–7, this protocol was amenable to a wide scope of isatins with different types of *N*-substituents, including a hydrogen atom and alkyl, phenyl or benzyl groups in high yields (64–99%). Obviously, *N*-unsubstituted isatin **1b** gave the highest yield of 99% among different *N*-substituted isatins (entry 1). Basically, *N*-alkyl substituted isatins gave a higher yield than *N*-phenyl and *N*-benzyl substituted ones (entries 2–4 *vs.* 5–7). It seemed that the size of the *N*-substituents also imposed some effect on the reactivity. For instance, smaller *N*-substituents such as a methyl group led to higher reactivity (entry 2), while larger *N*-substituents as exemplified by a 1-naphthyl-methylene group resulted in decreased reactivity (entry 7).

The influence of substituents at the phenyl moiety of isatins **1** on the reaction was studied next (entries 8–24). Generally, the introduction of an electronically different substituent at the C4, C5, C6 or C7 position of isatins was

Table 2 Diversity-oriented synthesis of spiro-compounds **4**[a]

Entry	4	R/R^1 (1)	R^2 (3)	Yield (%)[b]
1	**4baa**	H/H (**1b**)	Me (**3a**)	99 (87)[c]
2	**4caa**	Me/H (**1c**)	Me (**3a**)	90
3	**4daa**	*i*-Pr/H (**1d**)	Me (**3a**)	76
4	**4eaa**	cyclopentyl/H (**1e**)	Me (**3a**)	87
5	**4faa**	Ph/H (**1f**)	Me (**3a**)	79
6	**4aaa**	Bn/H (**1a**)	Me (**3a**)	73
7	**4gaa**	1-naphthyl-CH_2/H (**1g**)	Me (**3a**)	64
8	**4haa**	Me/4-Cl (**1h**)	Me (**3a**)	62
9	**4iaa**	Me/5-Me (**1i**)	Me (**3a**)	92
10	**4jaa**	Me/5-OMe (**1j**)	Me (**3a**)	87
11	**4kaa**	Me/5-Br (**1k**)	Me (**3a**)	85
12	**4laa**	Me/5-Cl (**1l**)	Me (**3a**)	86
13	**4maa**	Me/5-F (**1m**)	Me (**3a**)	81
14	**4naa**	Bn/5-Me (**1n**)	Me (**3a**)	61
15	**4oaa**	Bn/5-OMe (**1o**)	Me (**3a**)	62
16	**4paa**	Bn/5-Br (**1p**)	Me (**3a**)	67
17	**4qaa**	Bn/6-Br (**1q**)	Me (**3a**)	63
18	**4raa**	Bn/7-Br (**1r**)	Me (**3a**)	79
19	**4saa**	H/5-Me (**1s**)	Me (**3a**)	99 (82)[c]
20	**4taa**	H/5-OMe (**1t**)	Me (**3a**)	99 (63)[c]
21	**4uaa**	H/5-F (**1u**)	Me (**3a**)	99 (55)[c]
22	**4vaa**	H/7-Br (**1v**)	Me (**3a**)	76
23	**4waa**	H/7-CF_3 (**1w**)	Me (**3a**)	58
24	**4xaa**	H/5,6-F_2 (**1x**)	Me (**3a**)	52
25	**4bab**	H/H (**1b**)	Et (**3b**)	99 (51)[c]
26	**4cab**	Me/H (**1c**)	Et (**3b**)	94
27	**4eab**	cyclopentyl/H (**1e**)	Et (**3b**)	87
28	**4fab**	Ph/H (**1f**)	Et (**3b**)	84
29	**4aab**	Bn/H (**1a**)	Et (**3b**)	85
30	**4iab**	Me/5-Me (**1i**)	Et (**3b**)	86
31	**4jab**	Me/5-OMe (**1j**)	Et (**3b**)	71
32	**4kab**	Me/5-Br (**1k**)	Et (**3b**)	79
33	**4lab**	Me/5-Cl (**1l**)	Et (**3b**)	85
34	**4yab**	Me/6-Br (**1y**)	Et (**3b**)	73
35	**4wab**	H/7-CF_3 (**1w**)	Et (**3b**)	73
36	**4vab**	H/7-Br (**1v**)	Et (**3b**)	65

[a] Unless indicated otherwise, the reaction was carried out in 0.1 mmol scale in the presence of 20 mol% trifluoroacetic acid in 1,2-dichloroethane (1 mL) with 3 Å MS (100 mg) at 60 °C for 36 h, and the mole ratio of **1** : **2a** : **3** was 1.2 : 1 : 12. [b] Isolated yield. [c] The reaction time is 16 h.

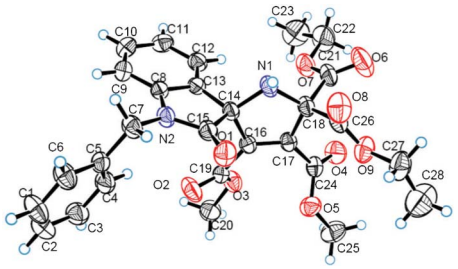


Fig. 3 The molecular structure of compound **4aaa**.

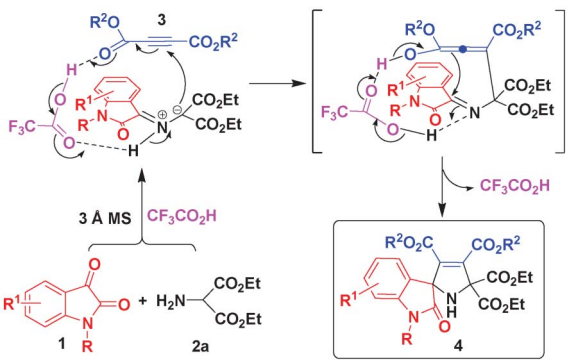
tolerable to the reaction with good to excellent yields in most cases. But in some cases, the position of the substituents appeared to exert some impact on the reactivity. For *N*-methyl isatins, there was no significant difference in reactivity when varying the C5-substituent, and high yields of 81–92% were obtained regardless of the electronic nature of the substituents (entries 9–13). However, C4-substituted *N*-methyl isatin was inferior to its C5-substituted counterpart in terms of yield (entry 8 *vs.* 12). For *N*-benzyl isatins, C7-substituted isatin delivered a higher yield of 79% than C5 or C6-substituted ones (entry 18 *vs.* 14–17), while for *N*-unsubstituted isatins, C5-substituted isatins exhibited a much superior reactivity of delivering a quantitative yield of 99% to C7-substituted or C5,C6-disubstituted ones (entries 19–21 *vs.* 22–24).

Moreover, ethyl but-2-ynedioate **3b** was also employed in the reaction with some representative isatins to demonstrate the applicability of this protocol and to provide a variety of spiro-oxindole-based 2,5-dihydropyrroles **4** for further bioassays. As indicated in entries 25–36, ethyl but-2-ynedioate **3b** served as an appropriate substrate to furnish corresponding spiro-compounds in high yields ranging from 65% to 99%. Similarly, a wide range of isatins with different types of *N*-substituents (entries 25–29) or with electronically different substituents at various positions of the phenyl moiety (entries 30–36) could smoothly participate in the reactions to provide the desired products with structural diversity.

In order to show the real difference of products with quantitative yields, we further performed the reactions for **4baa**, **4saa**, **4taa**, **4uaa** and **4bab** with a shorter reaction time of 16 h. The results indicated that products **4baa** and **4saa** were easier to form than **4taa**, **4uaa** and **4bab** in terms of yield (entries 1 and 19 *vs.* 20–21 and 25, in parentheses).

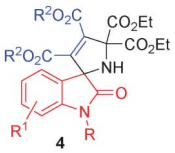
The structures of all these new spiro-compounds **4** were unambiguously determined by IR, ^{1}H NMR, ^{13}C NMR and HRMS (ESI†).[16] Besides, the structure of compound **4aaa** was further confirmed by X-ray crystallographic analysis (Fig. 3).[17]

On the basis of our experimental results and previous studies on the reaction mechanism,[15b,c] we proposed a possible pathway and transition state of the reaction (Scheme 2). Initially, the condensation of isatins **1** and diethyl 2-aminomalonate **2a** in the presence of trifluoroacetic acid



Scheme 2 Possible reaction pathway and transition state.

Table 3 Preliminary evaluation on the cytotoxicity of compounds **4**[a]



4

Entry	4	R/R^1	R^2	Inhibition rate (%) 1 μg mL^{-1}	10 μg mL^{-1}	100 μg mL^{-1}
1	**4baa**	H/H	Me	6.4 ± 1.9	12.7 ± 3.1	18.6 ± 3.4
2	**4caa**	Me/H	Me	—[b]	5.2 ± 1.5	23.5 ± 3.3
3	**4daa**	*i*-Pr/H	Me	6.0 ± 2.0	10.0 ± 2.7	17.5 ± 2.0
4	**4aaa**	Bn/H	Me	—[b]	—[b]	12.2 ± 2.7
5	**4naa**	Bn/5-Me	Me	—[b]	—[b]	12.4 ± 2.6
6	**4oaa**	Bn/5-OMe	Me	—[b]	7.8 ± 1.3	13.1 ± 2.4
7	**4paa**	Bn/5-Br	Me	5.2 ± 1.6	7.9 ± 1.9	15.8 ± 3.0
8	**4raa**	Bn/7-Br	Me	—[b]	5.6 ± 3.0	11.0 ± 2.7
9	**4saa**	H/5-Me	Me	—[b]	6.0 ± 2.1	21.6 ± 2.9
10	**4taa**	H/5-OMe	Me	4.0 ± 1.2	10.6 ± 2.8	13.0 ± 2.9
11	**4uaa**	H/5-F	Me	—[b]	—[b]	10.3 ± 2.6
12	**4waa**	H/7-CF_3	Me	—[b]	—[b]	13.3 ± 3.1
13	**4bab**	H/H	Et	6.3 ± 1.7	11.5 ± 2.9	**28.0 ± 3.3**
14	**4iab**	Me/5-Me	Et	—[b]	**20.8 ± 2.7**	24.8 ± 2.6
15	**4jab**	Me/5-OMe	Et	5.4 ± 2.9	9.7 ± 3.1	20.7 ± 2.7
16	**4yab**	Me/6-Br	Et	—[b]	9.6 ± 2.8	15.3 ± 2.7
17	PC[c]			—[b]	12.9 ± 2.5	35.3 ± 2.6

[a] The cytotoxicity of the tested compounds was represented as inhibition rate (mean ± S.D., $n = 4$) on MCF-7 cells. [b] No inhibition effect was observed. [c] Doxorubicin hydrochloride was used as a positive control (PC).

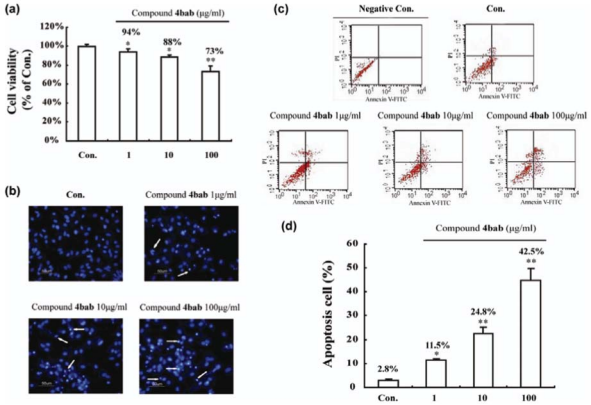


Fig. 4 Compound **4bab** inhibited cell viability and induced apoptosis in MCF-7 cells. (a) Effect of compound **4bab** on MCF-7 cell viability. (b) The morphological features of apoptosis were monitored by fluorescence microscopy after staining with Hoechst 33 342. Scale bar: 50 μm. (c) The apoptosis rate was measured by flow cytometry analysis after staining with Annexin V-FITC and PI. (d) Compound **4bab** (1, 10 and 100 μg mL^{-1}) induced MCF-7 apoptosis in a concentration-dependent manner when incubated for 24 h. The data are presented as mean ± SEM of four individual experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with the control group.

generated the corresponding azomethine ylides, which then participated in 1,3-dipolar cycloadditions with but-2-ynedioates **3** to afford products **4** *via* a sequential Michael addition and Mannich-type cyclization rather than a concerted pathway.[15b] The trifluoroacetic acid served as a Brønsted acid/Lewis base bifunctional catalyst to simultaneously activate both the isatin-derived azomethine ylides and but-2-ynedioates by H-bonding interactions, thereby accelerating the desired reaction to build up the structurally rigid spiro-skeleton.

In order to survey the possible bioactivity of these novel spiro-oxindole-based 2,5-dihydropyrroles, all the synthesized compounds **4** were subjected to a preliminary evaluation on their *in vitro* cytotoxic activity, which was represented as the inhibition rate of the tested compounds to mammary carcinoma cell line MCF-7 at three concentrations of 1, 10 and 100 μg mL^{-1}. Among the tested compounds **4**, sixteen compounds exhibited an inhibition effect on MCF-7 cells at different concentrations (Table 3). In detail, all sixteen bioactive compounds and the positive control (PC) showed a tendency of concentration-dependent cytotoxicity because their inhibition rates were greatly enhanced with increasing concentration. Although only six compounds slightly inhibited the growth of MCF-7 cells at a concentration of 1 μg mL^{-1}, these results were better than the positive control (doxorubicin hydrochloride), a powerful anticancer drug, which did not show any inhibition effect at this concentration. At a concentration of 10 μg mL^{-1}, five compounds exhibited considerable cytotoxicity to MCF-7 cells (inhibition rate > 10%, entries 1, 3, 10, 13–14, column 6). Notably, compound **4iab** showed the highest inhibition rate of 20.8% (entry 14, column 6), which was much higher than the positive control. At a concentration of 100 μg mL^{-1}, all sixteen compounds displayed promising cytotoxicity to MCF-7 cells with inhibition rates varying from 10.3% to 28.0%. Five compounds exhibited remarkable cytotoxic activity (inhibition rate > 20%, entries 2, 9, 13–15, column 7). Among them, compound **4bab** was the most powerful to inhibit the growth of MCF-7 cells to 28.0% (entry 13, column 7), which was comparable to the positive control.

With the aim of gaining some insight into the possible mechanism for the cytotoxicity of these compounds, we then carried out an in-depth study on the cytotoxic mechanism of representative compound **4bab**. The effects of compound **4bab** on MCF-7 cell line viability were examined using the CCK8 assay. As shown in Fig. 4a, a typical dose-dependent inhibitory effect on cell viability was discovered in MCF-7 cells that were treated with different doses of compound **4bab** (1, 10 and 100 μg mL^{-1}) for 24 h. To test whether the inhibition of cell growth of compound **4bab** was related to cell apoptosis, compound **4bab**-induced MCF-7 cell apoptosis was determined using Hoechst 33 324 staining. It was found that the percentage of early apoptotic cells was markedly elevated in a dose-dependent manner. As shown in Fig. 4b, untreated cells exhibited regular and round-shaped nuclei. In contrast, the condensation and fragmentation of nuclei, characteristic of apoptotic cells, was evident in cells treated with different concentrations of compound **4bab** for 24 h. The results of flow cytometry further confirmed the effects of compound **4bab** on

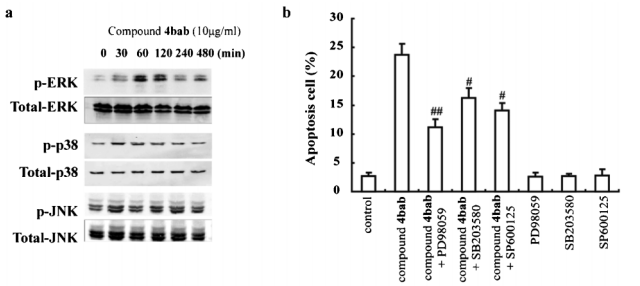


Fig. 5 Role of MAPKs in compound **4bab**-induced apoptosis. (a) MCF-7 cells were treated with 10 μg mL^{-1} compound **4bab** for the indicated time periods. ERK1/2, p38 and JNK MAPKs and their phosphorylated forms were determined by western blotting using specific antibodies. (b) Effects of MAPK inhibitors on apoptosis induced by compound **4bab**. MCF-7 cells were pretreated with 10 mM PD98059, SB203580 or SP600125 for 1 h and then exposed to 10 μg mL^{-1} compound **4bab** for 24 h (controls were not exposed to compound **4bab**). After the treatment, annexin V-PI double staining was used to analyze apoptotic cell death. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* compound **4bab** (10 μg mL^{-1}) treatment groups. Data are presented as the mean ± SEM of three independent experiments.

MCF-7 cell apoptosis. Annexin-V binding and PI uptake were detected in MCF-7 cells (Fig. 4c). The portion of annexin-V positive cells increased from 2.8% in control cells to 11.5%, 24.8% and 42.5% in the cells treated with 1, 10 and 100 μg mL^{-1} of compound **4bab** for 24 h, respectively (Fig. 4d).

The balance between survival and apoptosis signal pathways controls the cancer pathogenesis. Mitogen-activated protein kinase (MAPK) signaling is a signaling pathway which has received increasing attention as a target for cancer prevention and treatment. MAPK includes JNK (c-Jun NH2-terminal kinase), p38 MAPK (mitogen-activated protein kinase) and ERK (extracellular signal-regulated kinase), leading to the activation of NF-κB, cell growth, and cell survival.[19] An extracellular signal-regulated kinase (ERK)/MAPK pathway has been demonstrated to be involved in the regulation of cell apoptosis.[20] P38 kinase has been demonstrated to be an essential regulator of sustained G2 arrest.[21] A previous study has demonstrated that the duration and intensity of JNK activation is associated with cell death.[22] It has been reported that MAPK is activated in various cancers and that the activation of the MAPKs selectively phosphorylate cellular targets, leading to regulation of gene expression and biological events such as proliferation, differentiation, and apoptosis.[23] To elucidate the mechanism of apoptosis that was induced by compound **4bab**, ERK1/2, p38 and JNK levels and their phosphorylation states were measured. As shown in Fig. 5a, phosphorylated ERK1/2 was significantly increased 60 min after treatment of compound **4bab**. Phosphorylated p38 and JNK were increased 30 min after treatment of compound **4bab** and remained at a high level for up to 8 h. The total ERK 1/2, p38 MAPK and JNK protein levels in the **4bab**-treated group were similar to the control group.

To examine the functional consequences of the ERK 1/2, p38 MAPK and JNK phosphorylation changes, cells were pre-treated with MAPK inhibitors 1 h prior to compound **4bab** exposure. PD98059 is a potent, cell-permeable, and selective inhibitor of MEK1, which results in inhibition of the phosphorylation and activation of ERK1/2. SB203580 is a pyridinyl imidazole compound which acts as a competitive inhibitor of ATP binding on the p38 kinase, and thus serves as a specific inhibitor of p38 MAPKs. SP600126 is a potent, selective, reversible, and cell-permeable inhibitor of JNK. As shown in Fig. 5b, PD98059, SB203580 and SP600125 alone failed to affect the MCF-7 apoptosis. However, PD98059, SB203580 and SP600125 significantly reduced compound **4bab**-induced apoptosis of MCF-7. These results suggested that compound **4bab** induced MCF-7 apoptosis by the MAPK pathway.

Conclusions

In summary, we have established the construction of a novel spiro-oxindole-based 2,5-dihydropyrrole scaffold with potential bioactivity *via* efficient three-component reactions of isatins, amino-ester and alkynes. In addition, this protocol also represents the first 1,3-dipolar cycloaddition of electron-deficient alkynes with isatin-derived azomethine ylides, providing an easy access to spiro-oxindole-based 2,5-dihydropyrroles with structural diversity. More importantly, the bioscreen of these new spiro-dihydropyrroles has led to the finding of sixteen compounds with promising cytotoxicity to MCF-7 cells. This approach not only provides a valuable tool to synthesize spiro-oxindole-based 2,5-dihydropyrroles with high yields, atom-economy and operational simplicity, but also greatly enriches the chemistry of 1,3-dipolar cycloadditions. Significantly, the preliminary bioassay of these compounds will cast light on their medicinal applications after further structural modulation and biological studies.

Experimental

General information

NMR spectra were measured respectively at 400 and 100 MHz on a Brucker-400 MHz spectrometer. The solvent used for NMR spectroscopy was $CDCl_3$, using tetramethylsilane as the internal reference. HRMS (Bio TOF Q) spectra were recorded with an ESI resource on *P*-SIMS-Gly of Bruker Daltonics Inc. Infrared spectra were recorded on a Nicolet MX-1E FT-IR spectromter.

Analytical grade solvents for the column chromatography and commercially available reagents were used as received. All commercially available starting materials were used directly. Substrates **1a**, **1c–1r** and **1y** were synthesized according to the literature methods.[15c,18]

General procedure for the synthesis of spiro-oxindole-based 2,5-dihydropyrroles 4

A solution of isatins **1** (0.12 mmol), 2-aminomalonate **2a** (0.1 mmol), trifluoroacetic acid (0.02 mmol), and 3 Å molecular

sieves (100 mg) in 1,2-dichloroethane (0.5 mL) was stirred at rt for 20 min. Then, this resultant mixture was added to but-2-ynedioate **3** (1.2 mmol) and another 0.5 mL 1,2-dichloroethane. The reaction mixture was stirred at 60 °C for 36 h. Then the reaction mixture was filtered to remove the molecular sieves, and the solid powder was washed with ethyl acetate. The resultant solution was evaporated under the reduced pressure, and the residue was purified through flash column chromatography on silica gel to yield pure products **4**.

Characterization of new compounds 4

5′,5′-Diethyl 3′,4′-dimethyl 2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4baa). (Flash column chromatography eluent, petroleum ether–acetone = 3 : 1); reaction time = 36 h; yield: 99%; yellow solid; m.p. 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.43 (s, 1H), 7.39 (d, J = 7.4 Hz, 1H), 7.26–7.20 (m, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 4.34 (dd, J = 14.1, 7.0 Hz, 4H), 3.84 (s, 3H), 3.75 (s, 1H), 3.55 (s, 3H), 1.34 (dt, J = 14.4, 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.5, 168.6, 168.0, 162.6, 161.3, 141.2, 140.0, 139.7, 130.3, 128.9, 125.0, 123.2, 110.3, 80.0, 75.5, 63.1, 62.8, 52.5, 13.92, 13.89; IR (KBr): γ 3334, 2981, 2940, 1742, 1674, 1610, 1479, 1439, 1297, 1252, 1113, 1031, 871, 814, 709 cm^{-1}; ESI FTMS exact mass calcd for $(C_{21}H_{22}N_2O_9 + H)^+$ requires m/z 447.1404, found m/z 447.1376.

5′,5′-Diethyl 3′,4′-dimethyl 1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4caa). (Flash column chromatography eluent, petroleum ether–acetone = 6 : 1); reaction time = 36 h; yield: 90%; light yellow solid; m.p. 132–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.41 (dd, J = 7.4, 0.8 Hz, 1H), 7.31 (td, J = 7.8, 1.3 Hz, 1H), 7.06 (td, J = 7.6, 0.9 Hz, 1H), 6.80 (d, J = 7.8 Hz, 1H), 4.42–4.27 (m, 4H), 3.83 (s, 3H), 3.73 (s, 1H), 3.52 (s, 3H), 3.21 (s, 3H), 1.35 (t, J = 6.2 Hz, 3H), 1.31 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.3, 168.7, 168.0, 162.6, 161.1, 142.6, 140.2, 139.7, 129.9, 128.9, 125.0, 122.8, 109.7, 79.9, 75.1, 63.0, 53.0, 52.2, 27.3, 25.1, 13.9, 13.8; IR (KBr): γ 3444, 3349, 2984, 2939, 2859, 1742, 1660, 1607, 1469, 1429, 1297, 1239, 1183, 1031, 871, 804, 749, 698 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{24}N_2O_9 + Na)^+$ requires m/z 483.1374, found m/z 483.1353.

5′,5′-Diethyl 3′,4′-dimethyl 1-isopropyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4daa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 76%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.43 (dd, J = 7.4, 1.0 Hz, 1H), 7.27 (td, J = 7.8, 1.3 Hz, 1H), 7.02 (td, J = 7.6, 0.8 Hz, 1H), 6.95 (d, J = 7.9 Hz, 1H), 4.62–4.49 (m, 1H), 4.42–4.29 (m, 4H), 3.82 (s, 3H), 3.77 (s, 1H), 3.51 (s, 3H), 1.49 (d, J = 5.8 Hz, 3H), 1.47 (d, J = 5.7 Hz, 3H), 1.38–1.33 (m, 3H), 1.31 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.0, 168.7, 168.0, 162.6, 161.2, 142.7, 140.1, 139.7, 130.0, 129.0, 125.0, 122.7, 109.9, 79.9, 75.1, 63.0, 62.8, 52.4, 52.3, 44.5, 19.1, 13.9, 13.8; IR (KBr): γ 3458, 3339, 2964, 2926, 2852, 1729, 1660, 1607, 1453, 1443, 1289, 1252, 1117, 1035, 871, 772 cm^{-1}; ESI FTMS exact mass calcd for $(C_{24}H_{28}N_2O_9 + Na)^+$ requires m/z 511.1687, found m/z 511.1662.

5′,5′-Diethyl 3′,4′-dimethyl 1-cyclopentyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4eaa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 87%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.43 (dd, J = 7.4, 1.0 Hz, 1H), 7.27 (td, J = 7.8, 1.3 Hz, 1H), 7.03 (td, J = 7.6, 0.8 Hz, 1H), 6.88 (d, J = 7.9 Hz, 1H), 4.69 (t, J = 8.7 Hz, 1H), 4.40–4.29 (m, 4H), 3.82 (s, 3H), 3.76 (s, 1H), 3.50 (s, 3H), 2.08 (dt, J = 15.0, 7.4 Hz, 2H), 1.92 (d, J = 2.9 Hz, 4H), 1.69 (s, 2H), 1.35 (t, J = 7.0 Hz, 3H), 1.31 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.3, 168.7, 168.0, 162.6, 161.1, 142.6, 140.2, 139.7, 129.9, 128.9, 125.0, 122.8, 109.7, 79.9, 75.1, 63.0, 62.8, 53.0, 52.4, 52.2, 27.6, 27.3, 25.1, 25.1, 13.9, 13.8; IR (KBr): γ 3334, 2998, 2954, 2859, 1742, 1673, 1607, 1443, 1307, 1239, 1157, 1127, 1035, 874, 735, 700 cm^{-1}; ESI FTMS exact mass calcd for $(C_{26}H_{30}N_2O_9 + Na)^+$ requires m/z 537.1844, found m/z 537.1833.

5′,5′-Diethyl 3′,4′-dimethyl 2-oxo-1-phenylspiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4faa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 79%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.54–7.48 (m, 3H), 7.46–7.37 (m, 3H), 7.24 (td, J = 7.8, 1.6 Hz, 1H), 7.10 (td, J = 7.6, 0.9 Hz, 1H), 6.79 (d, J = 7.8 Hz, 1H), 4.46–4.28 (m, 4H), 3.88 (s, 1H), 3.85 (s, 3H), 3.59 (s, 3H), 1.65 (s, 1H), 1.37 (t, J = 7.1 Hz, 3H), 1.30 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.0, 168.6, 167.9, 162.6, 161.3, 144.0, 139.9, 139.9, 134.3, 130.2, 129.6, 128.2, 126.3, 125.0, 123.7, 109.6, 80.1, 75.4, 63.1, 62.9, 52.5, 29.7, 13.9, 13.8; IR (KBr): γ 3342, 2988, 2954, 2869, 1742, 1660, 1620, 1446, 1307, 1252, 1183, 1117, 1035, 939, 857, 789, 749, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{27}H_{26}N_2O_9 + Na)^+$ requires m/z 545.1531, found m/z 545.1519.

5′,5′-Diethyl 3′,4′-dimethyl 1-benzyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4aaa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 73%; light yellow solid; m.p. 149–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45–7.36 (m, 3H), 7.36–7.23 (m, 3H), 7.20 (td, J = 7.8, 1.2 Hz, 1H), 7.02 (td, J = 7.6, 0.8 Hz, 1H), 6.71 (d, J = 7.8 Hz, 1H), 5.12 (d, J = 15.6 Hz, 1H), 4.66 (d, J = 15.6 Hz, 1H), 4.41–4.31 (m, 4H), 3.85 (s, 3H), 3.80 (s, 1H), 3.40 (s, 3H), 1.36 (t, J = 5.2 Hz, 3H), 1.33 (t, J = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.7, 168.6, 168.0, 162.6, 161.2, 143.2, 140.5, 139.6, 135.5, 130.2, 128.7, 128.6, 127.7, 124.7, 123.3, 109.3, 79.9, 75.0, 63.1, 62.9, 52.5, 52.3, 44.5, 13.9, 13.9; IR (KBr): γ 3349, 2984, 2954, 2859, 1729, 1660, 1613, 1498, 1429, 1262, 1035, 926, 854, 809, 690, 598 cm^{-1}; ESI FTMS exact mass calcd for $(C_{28}H_{28}N_2O_9 + H)^+$ requires m/z 537.1873, found m/z 537.1843.

5′,5′-Diethyl 3′,4′-dimethyl 1-(naphthalen-1-ylmethyl)-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4gaa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 64%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.15 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 8.2 Hz, 1H), 7.59 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.56–7.37 (m, 4H), 7.15 (td, J = 7.8, 1.2 Hz, 1H), 7.03 (td, J = 7.6, 0.7 Hz, 1H), 6.67 (d, J = 7.8 Hz, 1H), 5.57 (d, J = 16.3 Hz, 1H), 5.22 (d, J = 16.3 Hz, 1H), 4.38 (qd, J = 7.1, 1.7 Hz, 4H), 3.87 (s, 4H), 3.40 (s, 3H), 1.38 (t, J = 5.6 Hz, 3H), 1.34 (t, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.9, 168.6, 167.9, 162.7, 161.2, 143.6, 140.7, 139.4, 133.8, 131.0, 130.3, 130.2, 128.8, 128.7, 128.3, 126.6, 125.9,

125.3, 125.0, 124.7, 123.4, 123.0, 109.8, 80.0, 75.1, 63.2, 62.9, 52.6, 52.4, 42.7, 13.9, 13.8; IR (KBr): γ 3461, 3334, 2994, 2923, 2854, 1729, 1660, 1620, 1501, 1469, 1361, 1294, 1249, 1183, 1117, 1031, 854, 817, 690 cm^{-1}; ESI FTMS exact mass calcd for $(C_{32}H_{30}N_2O_9 + Na)^+$ requires *m*/*z* 609.1844, found *m*/*z* 609.1836.

5′,5′-Diethyl 3′,4′-dimethyl 4-chloro-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4haa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 62%; light yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.27 (t, *J* = 8.0 Hz, 1H), 6.98 (dd, *J* = 8.2, 0.6 Hz, 1H), 6.72 (d, *J* = 7.4 Hz, 1H), 4.40–4.28 (m, 4H), 3.89 (s, 1H), 3.85 (s, 3H), 3.58 (s, 3H), 3.19 (s, 3H), 1.37–1.33 (m, 3H), 1.33–1.30 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.7, 168.2, 167.6, 162.6, 161.5, 146.3, 141.6, 138.0, 131.8, 131.6, 124.0, 123.4, 106.8, 80.0, 75.2, 63.1, 62.8, 52.5, 26.8, 13.8; IR (KBr): γ 3334, 2981, 2926, 2859, 1742, 1657, 1617, 1484, 1439, 1371, 1252, 1130, 1034, 871, 749, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{28}H_{28}N_2O_9 + H)^+$ requires *m*/*z* 495.1170, found *m*/*z* 495.1146.

5′,5′-Diethyl 3′,4′-dimethyl 1,5-dimethyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4iaa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 92%; light yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.22 (s, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 6.69 (d, *J* = 7.9 Hz, 1H), 4.40–4.30 (m, 4H), 3.83 (s, 3H), 3.73 (s, 1H), 3.53 (s, 3H), 3.19 (s, 3H), 2.30 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.5, 168.7, 168.0, 162.7, 161.2, 141.6, 139.8, 139.8, 132.9, 130.6, 128.4, 125.4, 108.0, 80.0, 75.3, 63.1, 62.8, 52.5, 52.4, 26.6, 21.0, 13.9, 13.8; IR (KBr): γ 3334, 2981, 2939, 2864, 1742, 1633, 1511, 1456, 1426, 1262, 1143, 1031, 871, 772, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{23}H_{26}N_2O_9 + H)^+$ requires *m*/*z* 475.1717, found *m*/*z* 475.1689.

5′,5′-Diethyl 3′,4′-dimethyl 5-methoxy-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4jaa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 87%; light yellow solid; m.p. 127–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.07 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 4.39–4.31 (m, 4H), 3.83 (s, 3H), 3.77 (s, 3H), 3.75 (s, 1H), 3.54 (s, 3H), 3.19 (s, 3H), 1.38–1.33 (m, 3H), 1.31 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.3, 168.6, 167.9, 162.6, 161.2, 156.5, 139.8, 139.7, 137.4, 129.8, 114.9, 111.7, 108.7, 80.0, 75.5, 63.0, 62.9, 55.8, 52.5, 52.4, 26.7, 13.9, 13.8; IR (KBr): γ 3344, 2981, 2926, 2872, 1742, 1660, 1607, 1456, 1361, 1294, 1239, 1183, 1117, 1035, 871, 745, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{23}H_{26}N_2O_{10} + Na)^+$ requires *m*/*z* 513.1479, found *m*/*z* 513.1476.

5′,5′-Diethyl 3′,4′-dimethyl 5-bromo-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4kaa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 85%; light yellow solid; m.p. 148–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.56 (d, *J* = 1.9 Hz, 1H), 7.44 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.69 (d, *J* = 8.3 Hz, 1H), 4.41–4.30 (m, 4H), 3.84 (s, 3H), 3.76 (s, 1H), 3.57 (s, 3H), 3.20 (s, 3H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.0, 168.3, 167.6, 162.5, 160.9, 143.0, 140.5, 138.6, 133.1, 130.9, 128.0, 115.9, 109.7, 80.1, 75.0, 63.2, 63.0, 52.6, 52.5, 26.7, 13.9, 13.8; IR (KBr): γ 3352, 2994, 2954, 2859, 1742, 1647, 1603, 1469, 1429, 1252, 1133, 1035, 939, 857, 749, 680 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{23}BrN_2O_9 + Na)^+$ requires *m*/*z* 563.0462, found *m*/*z* 563.0476.

5′,5′-Diethyl 3′,4′-dimethyl 5-chloro-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4laa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 86%; light yellow solid; m.p. 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.43 (d, *J* = 2.1 Hz, 1H), 7.29 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 1H), 4.36 (m, 4H), 3.84 (s, 3H), 3.77 (s, 1H), 3.56 (s, 3H), 3.20 (s, 3H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.1, 168.4, 167.6, 162.5, 160.9, 142.5, 140.5, 138.7, 130.5, 130.1, 128.6, 125.3, 109.3, 80.1, 75.0, 63.2, 63.0, 52.6, 52.5, 26.8, 13.9, 13.8; IR (KBr): γ 3334, 2954, 2844, 1729, 1660, 1620, 1456, 1294, 1252, 1180, 1113, 1088, 1048, 867, 806, 749, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{23}ClN_2O_9 + H)^+$ requires *m*/*z* 495.1170, found *m*/*z* 495.1186.

5′,5′-Diethyl 3′,4′-dimethyl 5-fluoro-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4maa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 81%; light yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.22 (dd, *J* = 7.5, 2.6 Hz, 1H), 7.01 (td, *J* = 8.8, 2.6 Hz, 1H), 6.74 (dd, *J* = 8.5, 4.0 Hz, 1H), 4.39–4.31 (m, 4H), 3.83 (s, 3H), 3.77 (s, 1H), 3.55 (s, 3H), 3.21 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.3, 168.5, 167.7, 162.5, 161.0, 160.7, 158.3, 140.3, 139.9, 139.0, 130.4, 116.6, 116.3, 113.1, 112.9, 108.9, 108.8, 80.1, 75.3, 75.3, 63.1, 63.0, 52.5, 26.8, 13.9, 13.8; IR (KBr): γ 3334, 2959, 2913, 2854, 1739, 1660, 1634, 1494, 1443, 1294, 1249, 1035, 864, 809, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{23}FN_2O_9 + H)^+$ requires *m*/*z* 479.1466, found *m*/*z* 479.1463.

5′,5′-Diethyl 3′,4′-dimethyl 1-benzyl-5-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4naa). (Flash column chromatography eluent, petroleum ether–acetone = 6 : 1); reaction time = 36 h; yield: 61%; light yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.38 (d, *J* = 7.0 Hz, 2H), 7.34–7.28 (m, 2H), 7.28–7.21 (m, 2H), 6.99 (dd, *J* = 7.9, 0.9 Hz, 1H), 6.59 (d, *J* = 7.9 Hz, 1H), 5.10 (d, *J* = 15.6 Hz, 1H), 4.63 (d, *J* = 15.6 Hz, 1H), 4.37 (m, 4H), 3.85 (s, 3H), 3.80 (s, 1H), 3.41 (s, 3H), 2.27 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.6, 168.6, 168.0, 162.7, 161.2, 140.8, 140.3, 139.7, 135.6, 133.0, 130.5, 128.7, 128.5, 127.7, 127.6, 125.4, 109.1, 79.9, 75.2, 63.1, 62.9, 52.5, 52.3, 44.5, 21.0, 13.9, 13.8; IR (KBr): γ 3334, 2978, 2936, 2872, 1732, 1647, 1620, 1469, 1443, 1389, 1255, 1117, 1035, 933, 857, 745, 690 cm^{-1}; ESI FTMS exact mass calcd for $(C_{29}H_{30}N_2O_9 + H)^+$ requires *m*/*z* 551.2030, found *m*/*z* 551.2040.

5′,5′-Diethyl 3′,4′-dimethyl 1-benzyl-5-methoxy-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4oaa). (Flash column chromatography eluent, petroleum ether–acetone = 6 : 1); reaction time = 36 h; yield: 62%; light yellow solid; m.p. 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.41–7.35 (m, 2H), 7.35–7.27 (m, 3H), 7.07 (d, *J* = 2.6 Hz, 1H), 6.72 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.60 (d, *J* = 8.5 Hz, 1H), 5.09 (d, *J* =

15.5 Hz, 1H), 4.63 (d, J = 15.6 Hz, 1H), 4.37 (m, 4H), 3.84 (s, 3H), 3.82 (s, 1H), 3.74 (s, 3H), 3.42 (s, 3H), 1.36 (t, J = 6.0 Hz, 3H), 1.33 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.5, 168.6, 167.9, 162.6, 161.1, 156.5, 140.4, 139.6, 136.4, 135.6, 129.9, 128.7, 127.7, 114.9, 111.6, 109.9, 79.9, 75.4, 63.1, 62.9, 55.7, 52.5, 52.3, 44.6, 13.9, 13.9; IR (KBr): γ 3444, 3334, 2984, 2923, 2859, 1729, 1610, 1498, 1469, 1371, 1296, 1252, 1170, 1120, 1018, 857, 745 cm^{-1}; ESI FTMS exact mass calcd for $(C_{29}H_{30}N_2O_{10} + H)^+$ requires m/z 567.1979, found m/z 567.1973.

5′,5′-Diethyl 3′,4′-dimethyl 1-benzyl-5-bromo-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4paa). (Flash column chromatography eluent, petroleum ether–acetone = 6 : 1); reaction time = 36 h; yield: 67%; light yellow solid; m.p. 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.56 (d, J = 2.0 Hz, 1H), 7.39–7.26 (m, 6H), 6.57 (d, J = 8.3 Hz, 1H), 5.09 (d, J = 15.6 Hz, 1H), 4.66 (d, J = 15.6 Hz, 1H), 4.37 (m, 4H), 3.86 (s, 3H), 3.83 (s, 1H), 3.46 (s, 3H), 1.39 (t, J = 7.1 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.2, 168.3, 167.6, 162.5, 160.8, 142.2, 141.1, 138.5, 135.0, 133.0, 131.0, 128.8, 128.0, 127.6, 116.0, 110.8, 80.1, 74.9, 63.3, 63.1, 62.8, 52.6, 44.6, 13.9, 13.8; IR (KBr): γ 3458, 3346, 2988, 2923, 2862, 1739, 1657, 1607, 1484, 1364, 1297, 1235, 1103, 1028, 867, 817, 680 cm^{-1}; ESI FTMS exact mass calcd for $(C_{28}H_{27}BrN_2O_9 + Na)^+$ requires m/z 639.0776, found m/z 639.0770.

5′,5′-Diethyl 3′,4′-dimethyl 1-benzyl-6-bromo-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4qaa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 6 : 1); reaction time = 36 h; yield: 63%; light yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.29 (dd, J = 7.1, 3.8 Hz, 3H), 7.26–7.17 (m, 3H), 7.08 (dd, J = 7.9, 1.6 Hz, 1H), 6.77 (d, J = 1.6 Hz, 1H), 5.01 (d, J = 15.6 Hz, 1H), 4.54 (d, J = 15.6 Hz, 1H), 4.34–4.22 (m, 4H), 3.76 (s, 3H), 3.68 (s, 1H), 3.35 (s, 3H), 1.26 (dd, J = 15.3, 7.2 Hz, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.6, 168.5, 167.8, 162.5, 161.0, 144.5, 140.9, 138.8, 134.9, 128.9, 127.9, 127.7, 127.6, 126.3, 126.1, 123.9, 112.7, 79.9, 74.6, 63.2, 63.0, 52.6, 52.5, 44.6, 13.9, 13.8; IR (KBr): γ 3334, 2981, 2954, 2859, 1742, 1660, 1617, 1484, 1429, 1294, 1252, 1113, 1035, 857, 817, 693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{28}H_{27}BrN_2O_9 + Na)^+$ requires m/z 639.0776, found m/z 639.0786.

5′,5′-Diethyl 3′,4′-dimethyl 1-benzyl-7-bromo-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4raa). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 6 : 1); reaction time = 36 h; yield: 79%; light yellow solid; m.p. 134–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.43–7.38 (m, 2H), 7.36–7.27 (m, 4H), 7.24 (s, 1H), 6.93 (dd, J = 8.1, 7.4 Hz, 1H), 5.47–5.25 (m, 2H), 4.40–4.31 (m, 4H), 3.84 (s, 3H), 3.80 (s, 1H), 3.51 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H), 1.31 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.6, 168.5, 167.7, 162.5, 161.0, 141.0, 140.8, 138.9, 137.2, 136.1, 132.14, 128.4, 127.1, 126.8, 124.7, 124.1, 102.5, 79.9, 74.3, 63.2, 63.0, 52.6, 52.5, 45.2, 13.9, 13.8; IR (KBr): γ 3334, 2968, 2872, 1729, 1660, 1607, 1474, 1443, 1307, 1239, 1173, 1031, 871, 749 cm^{-1}; ESI FTMS exact mass calcd for $(C_{28}H_{27}BrN_2O_9 + Na)^+$ requires m/z 639.0776, found m/z 639.0779.

5′,5′-Diethyl 3′,4′-dimethyl 5-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4saa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 99%; yellow solid; m.p. 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.28 (s, 1H), 7.20 (s, 1H), 7.08–7.00 (m, 1H), 6.74 (d, J = 7.9 Hz, 1H), 4.36 (m, 4H), 3.85 (s, 3H), 3.75 (s, 1H), 3.57 (s, 3H), 2.29 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.5, 168.6, 168.0, 162.6, 161.3, 139.9, 139.8, 138.7, 132.8, 130.6, 128.9, 125.6, 110.0, 80.0, 75.6, 63.1, 62.8, 52.5, 52.5, 21.0, 13.9, 13.8; IR (KBr): γ 3339, 2992, 2943, 2923, 2846, 1736, 1610, 1493, 1474, 1448, 1299, 1254, 1127, 1037, 864, 751 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{24}N_2O_9 + Na)^+$ requires m/z 483.1374, found m/z 483.1364.

5′,5′-Diethyl 3′,4′-dimethyl 5-methoxy-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4taa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 99%; light yellow solid; m.p. 140–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.21 (s, 1H), 7.03 (d, J = 2.0 Hz, 1H), 6.84–6.68 (m, 2H), 4.43–4.27 (m, 4H), 3.84 (s, 3H), 3.76 (s, 4H), 3.57 (s, 3H), 1.36 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.3, 168.6, 167.9, 162.6, 161.2, 156.3, 140.0, 139.6, 134.3, 130.2, 115.4, 111.6, 110.7, 80.0, 75.9, 63.1, 62.9, 55.7, 52.5, 52.5, 13.9, 13.8; IR (KBr): γ 3321, 2984, 2929, 2859, 1742, 1660, 1607, 1469, 1443, 1371, 1294, 1239, 1183, 1143, 1103, 1035, 857, 779, 735 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{24}N_2O_{10} + H)^+$ requires m/z 477.1509, found m/z 477.1517.

5′,5′-Diethyl 3′,4′-dimethyl 5-fluoro-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4uaa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 99%; light yellow solid; m.p. 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.42 (s, 1H), 7.18 (dd, J = 7.6, 2.6 Hz, 1H), 6.95 (td, J = 8.8, 2.6 Hz, 1H), 6.80 (dd, J = 8.5, 4.1 Hz, 1H), 4.35 (m, 4H), 3.85 (s, 3H), 3.80 (s, 1H), 3.58 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.31 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.5, 168.4, 167.7, 162.4, 161.1, 160.5, 158.1, 140.4, 139.0, 137.1, 130.8, 116.8, 116.6, 113.2, 112.9, 111.1, 111.0, 80.0, 75.8, 63.2, 63.0, 52.6, 13.9, 13.8; IR (KBr): γ 3468, 3349, 2971, 2939, 2862, 1742, 1670, 1607, 1484, 1443, 1347, 1294, 1249, 1183, 1130, 1035, 857, 755, 709 cm^{-1}; ESI FTMS exact mass calcd for $(C_{21}H_{21}FN_2O_9 + H)^+$ requires m/z 465.1309, found m/z 465.1301.

5′,5′-Diethyl 3′,4′-dimethyl 7-bromo-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4vaa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 76%; light yellow solid; m.p. 95–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (s, 1H), 7.37 (dd, J = 12.4, 4.1 Hz, 2H), 6.94 (t, J = 7.8 Hz, 1H), 4.41–4.30 (m, 4H), 3.85 (s, 3H), 3.78 (s, 1H), 3.58 (s, 3H), 1.35 (t, J = 6.7 Hz, 3H), 1.30 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.8, 168.4, 167.6, 162.5, 160.9, 140.6, 140.4, 138.7, 132.8, 130.5, 124.5, 124.04, 103.0, 80.1, 76.5, 63.2, 63.0, 52.6, 13.9, 13.8; IR (KBr): γ 3334, 2981, 2923, 2859, 1732, 1607, 1479, 1456, 1374, 1294, 1254, 1117, 1035, 857 cm^{-1}; ESI FTMS exact mass calcd for $(C_{21}H_{21}BrN_2O_9 + Na)^+$ requires m/z 549.0305, found m/z 549.0320.

5′,5′-Diethyl 3′,4′-dimethyl 2-oxo-7-(trifluoromethyl)spiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4waa). (Flash column chromatography eluent, petroleum ether–acetone = 6 : 1); reaction time = 36 h; yield: 58%; light yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.87 (s, 1H), 7.62 (d, J = 7.4 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 4.36 (dd, J = 7.0, 5.0 Hz, 4H), 3.85 (s, 3H), 3.80 (s, 1H), 3.56 (s, 3H), 1.34 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.3, 168.4, 167.6, 162.4, 160.7, 141.1, 138.5, 138.3, 131.0, 128.7, 126.9, 126.8, 125.0, 123.1, 122.3, 112.5, 112.2, 80.1, 74.2, 63.2, 63.0, 52.6, 52.5, 13.9, 13.8; IR (KBr): γ 3349, 2981, 2949, 2859, 1739, 1660, 1607, 1498, 1446, 1299, 1235, 1130, 1031, 871, 814, 680 cm^{-1}; ESI FTMS exact mass calcd for $(C_{22}H_{21}F_3N_2O_9 + H)^+$ requires m/z 515.1277, found m/z 515.1286.

5′,5′-Diethyl 3′,4′-dimethyl 5,6-difluoro-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4xaa). (Flash column chromatography eluent, petroleum ether–acetone = 5 : 1); reaction time = 36 h; yield: 52%; light yellow solid; m.p. 175–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.50 (s, 1H), 7.35–7.27 (m, 1H), 6.73 (dd, J = 9.7, 6.3 Hz, 1H), 4.35 (dt, J = 12.1, 7.1 Hz, 4H), 3.85 (s, 3H), 3.78 (s, 1H), 3.61 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.31 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.5, 168.4, 167.7, 162.4, 161.0, 140.6, 138.5, 137.4, 124.7, 115.0, 114.7, 100.7, 100.5, 80.0, 75.3, 63.3, 63.1, 52.7, 52.6, 13.9, 13.8; IR (KBr): γ 3344, 2954, 2841, 1729, 1660, 1607, 1484, 1443, 1265, 1209, 1143, 1601, 1031, 871, 807, 693, 598 cm^{-1}; ESI FTMS exact mass calcd for $(C_{21}H_{20}F_2N_2O_9 + Na)^+$ requires m/z 505.1029, found m/z505.1020.

Tetraethyl 2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4bab). (Flash column chromatography eluent, petroleum ether–acetone = 4 : 1); reaction time = 36 h; yield: 99%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.28 (s, 1H), 7.41 (d, J = 7.4 Hz, 1H), 7.23 (dd, J = 7.7, 1.2 Hz, 2H), 7.03 (td, J = 7.6, 0.9 Hz, 1H), 6.84 (d, J = 7.7 Hz, 1H), 4.49–4.17 (m, 6H), 4.11–3.83 (m, 2H), 3.76 (s, 1H), 1.37–1.30 (m, 9H), 0.98 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.5, 168.7, 168.1, 162.1, 160.8, 141.2, 139.9, 139.8, 130.2, 129.2, 125.1, 123.2, 110.1, 80.0, 75.5, 63.0, 62.8, 61.8, 61.6, 13.9, 13.9, 13.4; IR (KBr): γ 3362, 3444, 2964, 2876, 1739, 1670, 1610, 1469, 1389, 1374, 1294, 1239, 1180, 1130, 1103, 1035, 857, 749 cm^{-1}; ESI FTMS exact mass calcd for $(C_{23}H_{26}N_2O_9 + Na)^+$ requires m/z 497.1531, found m/z 497.1531.

Tetraethyl 1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4cab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 94%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.43 (d, J = 7.3 Hz, 1H), 7.31 (td, J = 7.7, 1.1 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 6.79 (d, J = 7.8 Hz, 1H), 4.41–4.15 (m, 6H), 4.00–3.88 (m, 2H), 3.74 (s, 1H), 3.20 (s, 3H), 1.37–1.29 (m, 9H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.7, 168.7, 168.1, 162.1, 160.7, 144.0, 139.9, 139.8, 130.2, 128.7, 124.7, 123.3, 108.1, 79.9, 75.1, 63.0, 62.8, 61.7, 61.3, 29.6, 26.5, 13.9, 13.8, 13.5; IR (KBr): γ 3334, 2994, 2954, 2859, 1739, 1660, 1484, 1429, 1262, 1035, 857, 804, 680, 598 cm^{-1}; ESI FTMS exact mass calcd for $(C_{24}H_{28}N_2O_9 + Na)^+$ requires m/z 511.1687, found m/z 511.1687.

Tetraethyl 1-cyclopentyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4eab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 6 : 1); reaction time = 36 h; yield: 87%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45 (dd, J = 7.4, 1.0 Hz, 1H), 7.27 (td, J = 7.8, 1.3 Hz, 1H), 7.03 (td, J = 7.6, 0.7 Hz, 1H), 6.88 (d, J = 7.9 Hz, 1H), 4.73–4.64 (m, 1H), 4.39–4.25 (m, 6H), 4.06–3.87 (m, 2H), 3.77 (s, 1H), 2.14–1.88 (m, 6H), 1.71–1.62 (m, 2H), 1.37–1.29 (m, 9H), 0.93 (t, J = 7.1 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.4, 168.8, 168.2, 162.1, 160.7, 142.7, 140.3, 139.6, 129.8, 129.2, 125.1, 122.8, 109.6, 79.9, 75.1, 62.9, 62.8, 61.7, 61.3, 53.0, 27.7, 27.3, 25.2, 25.1, 13.9, 13.9, 13.8, 13.6; IR (KBr): γ 3484, 3344, 2981, 2939, 2859, 1739, 1663, 1607, 1456, 1439, 1294, 1249, 1130, 1038, 867, 749 cm^{-1}; ESI FTMS exact mass calcd for $(C_{28}H_{34}N_2O_9 + Na)^+$ requires m/z 565.2157, found m/z 565.2131.

Tetraethyl 2-oxo-1-phenylspiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4fab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 6 : 1); reaction time = 36 h; yield: 84%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.56–7.34 (m, 6H), 7.23 (dd, J = 7.8, 1.2 Hz, 1H), 7.10 (td, J = 7.6, 0.7 Hz, 1H), 6.81 (d, J = 7.9 Hz, 1H), 4.43–4.21 (m, 6H), 4.12–3.94 (m, 2H), 3.88 (s, 1H), 1.42–1.27 (m, 9H), 0.98 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.1, 168.8, 168.0, 162.1, 160.8, 144.0, 140.0, 139.8, 134.3, 130.1, 129.5, 128.4, 128.1, 126.2, 125.1, 123.7, 109.5, 80.1, 75.4, 63.0, 62.8, 61.8, 61.6, 13.9, 13.9, 13.9, 13.7; IR (KBr): γ 3458, 3336, 2981, 2951, 2861, 1739, 1663, 1608, 1484, 1439, 1307, 1257, 1183, 1133, 1048, 861, 821, 740, 710, 640 cm^{-1}; ESI FTMS exact mass calcd for $(C_{29}H_{30}N_2O_9 + H)^+$ requires m/z 551.2030, found m/z 551.2030.

Tetraethyl 1-benzyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4aab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 6 : 1); reaction time = 36 h; yield: 85%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.24 (m, 6H),7.19 (td, J = 7.8, 1.2 Hz, 1H) 7.02 (td, J = 7.6, 0.8 Hz, 1H), 6.69 (d, J = 7.8 Hz, 1H), 5.02 (d, J = 15.6 Hz, 1H), 4.74 (d, J = 15.6 Hz, 1H), 4.39–4.24 (m, 6H),4.01–3.95 (m, 1H), 3.82–3.77 (m, 2H), 1.38–1.28 (m, 9H), 0.81 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.8, 168.7, 168.1, 162.1, 160.7, 143.3, 140.2, 139.8, 135.5, 130.1, 128.8, 128.7, 127.7, 124.8, 123.3, 109.2, 79.9, 75.1, 63.0, 62.8, 61.8, 61.4, 44.5, 13.9, 13.9, 13.4; IR (KBr): γ 3349, 2939, 2859, 1729, 1660, 1607, 1484, 1443, 1294, 1249, 1130, 1035, 857, 804, 680 cm^{-1}; ESI FTMS exact mass calcd for $(C_{30}H_{32}N_2O_9 + Na)^+$ requires m/z 587.2000, found m/z 587.2009.

Tetraethyl 1,5-dimethyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4iab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 3 : 1); reaction time = 36 h; yield: 86%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.25 (m, 1H), 7.09 (m, 1H), 6.67 (d, J = 7.9 Hz, 1H), 4.38–4.26 (m, 6H), 4.01–3.89 (m, 2H), 3.74 (s, 1H), 3.18 (s, 3H), 2.30 (s, 3H), 1.38–1.29 (m, 9H), 0.96 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.6, 168.8, 168.1, 162.1, 160.7, 141.6, 140.0, 139.7, 132.9, 130.4, 128.6, 125.5, 107.9, 79.9, 75.3, 63.0, 62.8, 61.7, 61.3, 26.6, 21.0, 13.9, 13.8, 13.5; IR (KBr): γ 3461, 3352, 2981, 2923, 2859, 1739, 1667, 1607, 1501, 1456, 1361, 1294, 1252, 1130, 1035, 939, 844, 762,

693 cm^{-1}; ESI FTMS exact mass calcd for $(C_{25}H_{30}N_2O_9 + Na)^+$ requires *m/z* 525.1844, found *m/z* 525.1840.

Tetraethyl 5-methoxy-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4jab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 3 : 1); reaction time = 36 h; yield: 71%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.09 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 1H), 4.38–4.26 (m, 6H), 4.02–3.90 (m, 2H), 3.77 (s, 3H), 3.76 (s, 1H),3.17 (s, 3H), 1.37–1.29 (m, 9H), 0.97 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.4, 168.7, 168.0, 162.1, 160.6, 156.5, 139.9, 139.8, 137.4, 130.0, 114.9, 111.7, 108.6, 79.9, 75.5, 62.9, 62.8, 61.7, 61.4, 55.8, 26.6, 13.9, 13.9, 13.8, 13.5; IR (KBr): γ 3334, 2991, 2926, 1742, 1660, 1607, 1456, 1374, 1252, 1198, 1127, 1035, 857, 762, 697 cm^{-1}; ESI FTMS exact mass calcd for $(C_{25}H_{30}N_2O_{10} + Na)^+$ requires *m/z* 541.1793, found *m/z* 541.1780.

Tetraethyl 5-bromo-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4kab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 3 : 1); reaction time = 36 h; yield: 79%; light yellow solid; m.p. 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.58 (d, *J* = 2.0 Hz, 1H), 7.43 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 4.40–4.26 (m, 6H), 4.04–3.92 (m, 2H), 3.77 (s, 1H), 3.19 (s, 3H), 1.40–1.30 (m, 9H), 1.01 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.2, 168.4, 167.8, 161.9, 160.4, 143.0, 140.5, 138.8, 132.9, 131.12, 128.0, 115.8, 109.6, 80.1, 75.0, 63.1, 63.0, 61.9, 61.6, 26.7, 13.9, 13.8, 13.5; IR (KBr): γ 3349, 2994, 2958, 2872, 1742, 1660, 1633, 1488, 1443, 1265, 1035, 923, 814, 732, 690 cm^{-1}; ESI FTMS exact mass calcd for $(C_{24}H_{27}BrN_2O_9 + Na)^+$ requires *m/z* 589.0792, found *m/z* 589.0781.

Tetraethyl 5-chloro-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4lab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 3 : 1); reaction time = 36 h; yield: 85%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45 (d, *J* = 2.1 Hz, 1H), 7.28 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 1H), 4.40–4.24 (m, 6H), 4.04–3.92 (m, 2H), 3.78 (s, 1H), 3.19 (s, 3H), 1.40–1.30 (m, 9H), 1.01 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.3, 168.4, 167.8, 161.9, 160.4, 142.5, 140.5, 138.8, 130.7, 130.0, 128.6, 125.3, 109.1, 80.1, 75.0, 63.1, 63.0, 61.9, 61.6, 29.6, 26.7, 13.9, 13.8, 13.5; IR (KBr): γ 3459, 3334, 2981, 2929, 2862, 1725, 1660, 1607, 1501, 1473, 1361, 1284, 1252, 1120, 1031, 861, 819 cm^{-1}; ESI FTMS exact mass calcd for $(C_{24}H_{27}ClN_2O_9 + Na)^+$ requires *m/z* 545.1297, found *m/z* 545.1296.

Tetraethyl 6-bromo-1-methyl-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4yab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 5 : 1); reaction time = 36 h; yield: 73%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.31 (d, *J* = 7.9 Hz, 1H), 7.19 (dd, *J* = 7.9, 1.6 Hz, 1H), 6.95 (d, *J* = 1.6 Hz, 1H), 4.38–4.26 (m, 6H), 4.03–3.92 (m, 2H), 3.72 (s, 1H), 3.18 (s, 3H), 1.36–1.29 (m, 9H), 1.01 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.5, 168.6, 167.9, 162.0, 160.5, 145.3, 140.4, 138.9, 127.8, 126.1, 123.9, 111.7, 80.0, 74.7, 63.1, 62.9, 61.8, 61.5, 29.6, 26.7, 13.9, 13.8, 13.6; IR (KBr): γ 3349, 2981, 2926, 2872, 1745, 1660, 1617, 1498, 1456, 1357, 1289, 1249, 1031, 953, 871, 817, 698 cm^{-1}; ESI FTMS exact mass calcd for $(C_{24}H_{27}BrN_2O_9 + Na)^+$ requires *m/z* 589.0792, found *m/z* 589.0799.

Tetraethyl 2-oxo-7-(trifluoromethyl)spiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4wab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 4 : 1); reaction time = 36 h; yield: 73%; yellow sticky oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.78 (s, 1H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.15 (t, *J* = 7.7 Hz, 1H), 4.38–4.26 (m, 6H), 4.05–3.95 (m, 2H), 3.81 (s, 1H), 1.61 (s, 1H), 1.37–1.29 (m, 9H), 0.98 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.4, 168.5, 167.7, 161.9, 160.2, 141.3, 138.5, 138.3, 131.2, 128.8, 126.8, 125.0, 123.1, 122.3, 112.4, 112.0, 80.0, 74.2, 63.1, 63.0, 62.7, 62.0, 61.7, 13.9, 13.8, 13.3; IR (KBr): γ 3331, 2926, 2852, 1732, 1663, 1617, 1469, 1449, 1361, 1294, 1252, 1184, 1130, 1031, 857, 789, 762 cm^{-1}; ESI FTMS exact mass calcd for $(C_{24}H_{25}F_3N_2O_9 + Na)^+$ requires *m/z* 565.1404, found *m/z* 565.1420.

Tetraethyl 7-bromo-2-oxospiro[indoline-3,2′-pyrrole]-3′,4′,5′,5′(1′H)-tetracarboxylate (4vab). (Flash column chromatography eluent, petroleum ether–ethyl acetate = 3 : 1); reaction time = 36 h; yield: 65%; light yellow solid; m.p. 191–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.58 (s, 1H), 7.39–7.37 (m, 2H), 6.95 (dd, *J* = 8.2, 7.5 Hz, 1H), 4.37–4.28 (m, 6H), 4.13–3.93 (m, 2H), 3.79 (s, 1H), 1.37–1.30 (m, 9H), 1.01 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.9, 168.5, 167.8, 161.9, 160.3, 140.6, 140.4, 138.8, 132.7, 130.8, 124.5, 124.1, 102.9, 80.1, 76.5, 63.1, 62.9, 61.9, 61.7, 13.9, 13.9, 13.8, 13.4; IR (KBr): γ 3349, 2968, 2913, 2859, 1739, 1647, 1610, 1494, 1443, 1297, 1249, 1130, 1035, 857, 804, 690 cm^{-1}; ESI FTMS exact mass calcd for $(C_{23}H_{25}BrN_2O_9 + Na)^+$ requires *m/z* 575.0636, found *m/z* 575.0639.

Cytotoxic evaluation of compounds 4 to mammary carcinoma cell line MCF-7

The cytotoxicity of the tested compounds to MCF-7 cells was assayed using the CCK8 (Cell Counting Kit-8) method. MCF-7 cells were seeded in 96-well plates at a density of 10^4 cells per well with 200 μL complete culture medium. After adhesion for 24 h, the medium was changed to 1640 supplemented without FBS and the compounds were added to the medium to final concentrations ranging from 1 μg mL^{-1} to 100 μg mL^{-1}. The cells were then cultured for another 24 h. Cells that were not exposed to compounds were used as controls and the wells to which only culture medium was added served as blanks. At the end of compound stimulation, the supernatant was removed, and 100 μL 1640 medium containing 10 μL CCK8 was added to each well for another 3 h at 37 °C. The culture plates were then shaken for 10 min and the optical density (OD) values were read at 450 nm. The inhibition rate was calculated according to the following formula: Inhibition rate = $(OD_{450\ control} - OD_{450\ sample})/OD_{450\ control} \times 100\%$, where OD stands for optical density at 450 nm.

Morphological assessment and quantification of apoptotic astrocytes

Hoechst staining. To quantify apoptotic cells, a MCF-7 cell monolayer was fixed and stained with Hoechst 33324 (Sigma, USA). The morphological features of apoptosis (cell shrinkage,

chromatin condensation, and fragmentation) were monitored by fluorescence microscopy (Olympus BX 60, Japan). At least 400 cells from 12 randomly selected fields per dish were counted, and each treatment was performed in triplicate.

Flow cytometry. MCF-7 cell apoptosis was estimated using the Annexin-V Fluorescein (FITC) apoptosis detection kit (Oncogene, USA) according to the kit instructions. The cell samples were analyzed in a flow cytometry apparatus (Becton Dickinson FACSVantage SE, USA). Annexin V binds to phosphatidylserine that is translocated during apoptosis from the inner to the outer leaflet of the plasma membrane. Live cells with intact membranes are distinguished by their ability to exclude propidium iodide (PI), which readily penetrates dead or damaged cells. Dual analysis was introduced using a quadrant dot plot, in which necrotic cells were identified as single PI-positive, early apoptotic cells were annexin V-FITC-positive only, and cells in late apoptosis were recognized as double-positive for annexin V-FITC and PI. Cells that stained negative for both annexin V-FITC and PI were classified as live cells. Finally, the number of cells in each category was expressed as a percentage of the total number of stained cells counted.

Western blotting. Cells were washed twice with ice-cold PBS and homogenized in 200 μL lysis buffer. After incubation for 20 min on ice, cell lysates were centrifuged (10 000 × g for 10 min at 4 °C) and the protein concentration in the extracts was determined by the Bradford assay.[24] Proteins in cell extracts (50 μg) were denatured with SDS sample buffer and separated by 10% SDS-PAGE. Proteins were transferred to nitrocellulose membranes using a Bio-Rad miniprotein-III wet transfer unit. The membranes were incubated with 5% BSA dissolved in TBST (pH 7.5, 10 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween-20) at room temperature for 1 h, washed three times and incubated with different antibodies (JNK, phosphor-JNK, ERK, phosphor-ERK p38 and phosphor-p38 at 1 : 1000) overnight at 4 °C. The membranes were washed three times with TBST buffer and incubated with the secondary antibody (1 : 2000) for 1 h followed by four washings. Signal detection was performed with an enhanced chemiluminescence kit.

Statistical analysis. All values were expressed as mean ± standard error of the mean (SEM). The significance of the difference between the control and samples treated with various drugs was determined by one-way ANOVA followed by the post-hoc least significant difference (LSD) test. Differences were considered significant at $P < 0.05$.

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