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Core Scaffold-Inspired Concise Synthesis of Chiral Spirooxindole-Pyranopyrimidines with Broad-Spectrum Anticancer Potency

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Abstract: Due to the lack of tumor-specific anticancer agents, the discovery and development of new types of highly selective anticancer agents is still a very urgent topic. Herein, we present our contribution to concise construction of novel chiral spirooxindole-type pyranopyrimidines exhibiting a unique profile of biological activities. We have found that this new type of spiro alkaloid could inhibit the proliferation of various cancer cells in a preliminary biological evaluation. These findings suggested that spirooxindole-type pyranopyrimidines, developed by an asymmetric Michael/cyclization strategy, can potentially serve as a new kind of anticancer candidate.

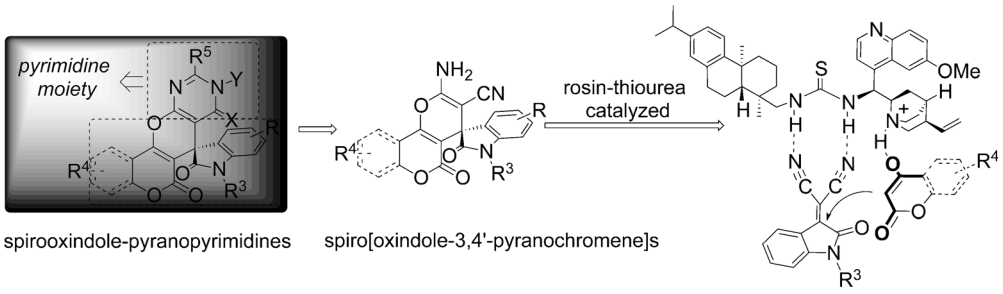
Keywords: anticancer activity; asymmetric synthesis; pyranopyrimidines; spiroheterocycles

Introduction

The synthesis of chiral molecular complexity around a biologically relevant framework has played a critical role in the discovery and development of drugs, contributing to drug innovation that is important both for improvements in health care and for the progress of target-oriented organic synthesis in medicinal chemistry.[1] In this process, particularly the involved efficient synthetic methodologies, and the significant preclinical studies on structure-activity relationships can finally contribute in guiding the discovery of new pharmaceuticals in the future. Significantly, chiral spiroheterocycles have also become important molecular probes for studying biological activities toward different cellular processes in recent years.[2] As a result of its importance and versatility in the synthesis of diverse natural alkaloids and drug candidates, the development of highly efficient synthetic methods to access optically active substituted spiroheterocycles would be of great utility for drug discovery.[3] However, this represents a considerable difficulty due to the synthetic challenges of the spiro-motifs including incorporating heterocycles and obtaining high enantioselectivity. The elegant advances for the enantioselective synthesis of bioactive spiroheterocycle-type alkaloids[4] by using a new rosin-derived thiourea catalytic system in our group are encouraging.[5] In this study, we present our contribution to the successful discovery of new potential anticancer candidates through concise construction of novel chiral spirooxindole-type[6] pyranopyrimidines exhibiting a unique profile of biological activities *via* an organocatalytic asymmetric Michael/cyclization reaction.[7] Furthermore, we hope that this new type of spiro alkaloid can serve as a bridge between chemistry, biology and medicine, providing a promising set of alternatives for antitumor drugs in the future.

Results and Discussion

We envisioned that the intramolecular asymmetric Michael/cyclization reaction of coumarins with isatylidenemalononitriles could be initiated in the presence of a chiral tertiary amine, leading to the generation of spiro[oxindole-3,4′-pyranochromene]s as precursors. The intriguing pyranopyrimidine skeletons could be constructed through subsequent further cyclization (Scheme 1). To the best of our knowledge, there is no precedent for the catalytic asymmetric synthesis of new types of highly optically active spirooxindole-pyr-



Scheme 1. Strategy for the synthesis of pyranopyrimidines.

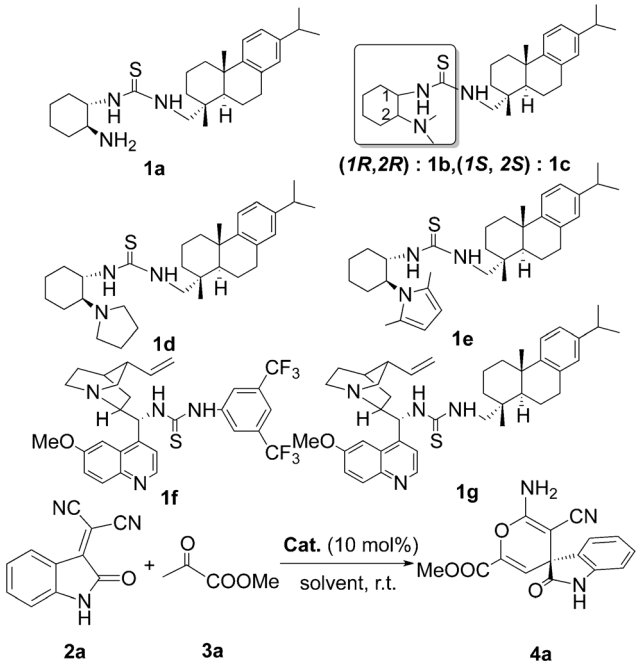
anopyrimidines. To explore the efficacy of the proposed Michael/cyclization process,[8] we initiated our studies by first evaluating the reaction between isatylidenemalononitriles and α-keto esters using the rosin-derived tertiary amine-thiourea[9]. It is worth noting that tertiary amine-thiourea-catalyzed variants have not been addressed, and to date there is no report concerning the Michael/cyclization reaction of α-keto esters with isatylidenemalononitriles.[10] We can envision that an efficient catalytic asymmetric tactic is not only particularly promising but also strongly desired.

Our investigation began with screening of several amine-thioureas with diversely structured scaffolds to firstly evaluate their ability to promote the Michael/cyclization reaction of isatylidenemalononitrile (**2a**) with α-keto ester (**3a**) in the presence of a 5.0 mol% catalyst loading at room temperature in CH_2Cl_2 (Table 1). While the desired product with excellent yield (90–95%) could be obtained in the presence of **1b**–**1d** and **1f**, only low to moderate enantioselectivities (25–78% *ee*) were observed in the reactions (entries 2–4 and 6). As shown in Table 1, the rosin-derived bifunctional catalyst **1g** was the most effective among catalysts **1a**–**1g**, furnishng **4a** with excellent yield and 85% *ee* (entry 7). Subsequently, we screened more parameters of the condition for the purpose of optimizing the reaction process. Surprisingly, a change of the solvent had a significant effect on the enantioselectivity. The enantiocontrol was dramatically enhanced, and an excellent *ee* value (95%) was obtained with nearly quantitative yield (99%) when the reaction was conducted in ether (entry 11). Gratifyingly, addition of the 4 Å molecular sieve further enhanced the enatioselectivity (98% *ee*) of the product while giving the same high yield (entry 14). Notably, a slight decrease in both yield (91%) and enantioselectivity (93% *ee*) was observed when the reaction was carried out at the lower temperature of 0 °C (entry 15).

Results of experiments under the optimized condition for probing the scope of the reaction are summarized in Table 2. A range of isatylidenemalononitriles was examined with various α-keto esters in the catalytic reactions for the construction of optically active spiro[oxindole-3,4′-pyran]s. The results showed that, in general, variation of the electronic properties of the substituent at different sites of the N-protected or unprotected isatylidenemalononitriles with different steric parameters was tolerated, giving the spiro[oxindole-3,4′-pyran]s in excellent yields and enantioselectivities (91–>99% *ee*, entries 1–5, and 9–16), albeit with a comparably low yield for the 4,7-di-Cl-substituted substrate (entry 17). Additionally, substrates substituted with electron-donating groups took a longer reaction time due to the increase of electron density of the Michael acceptors (entries 12 and 13). As expected, α-keto esters **3b** and **3c** also underwent the reaction smoothly affording excellent yields (97% and 99%, respectively) and enantioselectivities (99% and 98%, respectively, entries 6 and 7). It is worth noting that the α-substituted keto ester (**3d**) bearing steric repulsion in the reaction could also afford the desired product in 98% *ee* and 60% yield (entry 8). The relative and absolute configurations of the products were determined by an X-ray crystal structure analysis of **4j**.[11]

Encouraged by the successful construction of optically active spiro[oxindole-3,4′-pyran]s as described above, we hoped that this efficient asymmetric catalytic system could be applied to the diversity-oriented synthesis of various intriguing spiro[oxindole-3,4′-pyranochromene] frameworks considering their potential versatility as important precursors (Table 3). We initially carried out the model Michael/cyclization reaction of isatylidenemalononitrile (**2a**) with 4-hydroxycoumarin (**5a**) under the above optimized reaction conditions. However, the reaction resulted in the product with a low enantioselectivity (58% *ee*, entry 1). Increasing the loading of catalyst **1g** to 10 mol% led to an excellent yield (93%), but the *ee* value was still unsatisfactory (75% *ee*, entry 2). Gratifyingly, the later rescreening of the solvent uncovered that CH_2Cl_2 was the most suitable solvent in terms of both the yield (99%) and enantiochemical outcome (90% *ee*, entry 3). To the best of our knowledge, no asymmetric protocol to access optically active spiro[oxindole-3,4′-pyranochromene] frameworks has as yet been reported,[12] and the current precedent provides an alternative asymmetric access to these versatile compounds.

Table 1. Optimization of the reaction conditions.[a]



Entry	Cat.	Solvent	Yield [%][b]	*ee* [%][c]
1	**1a**	CH_2Cl_2	63	0
2	**1b**	CH_2Cl_2	95	78
3	**1c**	CH_2Cl_2	92	25
4	**1d**	CH_2Cl_2	90	47
5	**1e**	CH_2Cl_2	10	8
6	**1f**	CH_2Cl_2	95	64
7	**1g**	CH_2Cl_2	93	83
8	**1g**	toluene	96	73
9	**1g**	$CHCl_3$	90	86
10	**1g**	1,2-DCE	80	74
11	**1g**	Et_2O	99	95
12	**1g**	THF	91	35
13	**1g**	DME	72	39
14[d]	**1g**	Et_2O	99	98
15[e]	**1g**	Et_2O	91	93

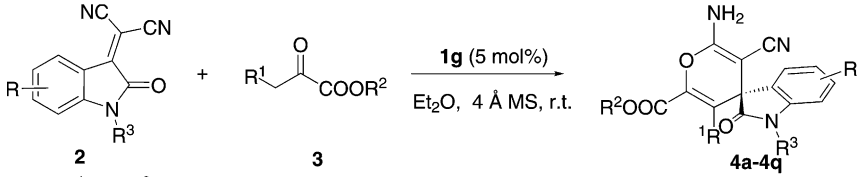
[a] Unless noted, the reaction was conducted with **2a** (0.20 mmol) and **3a** (0.22 mmol) for 12 h at room temperature.
[b] Isolated yield.
[c] The *ee* values were determined by chiral phase HPLC with a Chiralcel AD-H chiral column, and the configuration was assigned by comparison of HPLC data and X-ray crystal data of **4j**.
[d] 4 Å MS was used.
[e] The reaction was performed for 12 h at 0 °C.

Results of experiments in which a variety of diversely structured spirooxindole-type pyranochromenes were ingeniously constructed under the optimized conditions are summarized in Table 4. It showed that a variety of 4-hydroxycoumarins bearing substituents with different electron properties at C-6 or C-7, as well as various isatylidenemalononitriles were tolerated, affording the more complicated spiro[oxindole-3,4′-pyranochromene] complexes in good to excellent yield (85–99%) and excellent enantioselectivities (90–>99% *ee*, entries 1–11 and 14–17). To our delight, the spirooxindole complexities incorporating other electron-withdrawing groups, such as COOMe or COOBn substituted pyran scaffolds, were also favorably formed with 90% *ee* and excellent yields (**6l**: 90% and **6m**: 92%, respectively, entries 12 and 13). It was worth noting that the established asymmetric protocol was also proved to be efficient for 1-naphthol, which is a relatively inert substrate, again affording the corresponding pyranochromenes with excellent enantioselectivities (95–99% *ee*, entries 18–20) in *o*-xylene.

We proposed a possible model to account for the high enantioselectivity of the present reaction as shown in Figure 1. In light of the above results and recent studies,[13] the rosin-derived chiral tertiary amine-thiourea should act in a bifunctional fashion. The α-carbon atom of 4-hydroxycoumarin could be activated by an interaction between the tertiary amine moiety of the catalyst and the hydroxy group of 4-hydroxycoumarin, while the isatylidenemalononitrile is fixed and activated by the neighboring two thiourea hydrogen atoms through weak hydrogen bonds. The electron-rich α-carbon atom of 4-hydroxycoumarin predominantly attacks the *re*-face of the electron-deficient isatylidenemalononitrile to generate the Micheal adduct intermediate. Subsequent intramolecular cyclization reactions of the intermediates and tautomerization afforded the chiral spirocyclic products, which is consistent with the experimental results. In recent studies,[4,5c] we disclosed that the two chiral moieties of the thiourea are mutually reinforcing for the high efficacy of the catalyst, and the inherent property of the excellent structural backbone and well-defined stereocenters of the dehydroabietic amine moiety of the thiourea also have an important effect on the high enantioselectivity for the formation of adduct.

Having successfully constructed a range of spiro[oxindole-3,4′-pyranochromene] frameworks under the asymmetric protocol established, biological activitiy-oriented synthesis of diversely structured chiral spirooxindole-type pyranopyrimidines was smoothly carried out by the futher transformations as shown in Scheme 2. As expected, various chiral spirooxindole-type pyranopyrimidines **JP-7a** through **JP-9b** were successfully formed in yields ranging from 12% to 82%. We decided to evaluate their preliminary biological activities toward the anticancer orientation and the IC_{50} values as a measure of the relative cytotoxicity of chiral pyranopyrimidines to each cell line was adopted.[14] Excitingly, the anticancer activity studies showed that **JP-8a** displayed good cytotoxic effect against five different types of tumor cell lines

Table 2. Scope of the Michael/cyclization reaction of isatylidenemalononitriles with α-keto esters.[a]

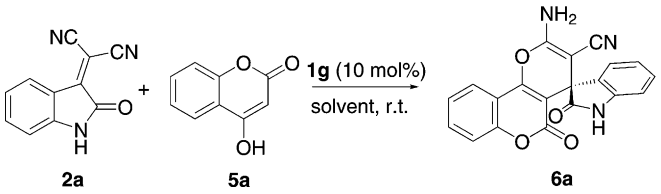


3a: R^1 =H, R^2 = Me
3b: R^1 = H, R^2 = Et
3c: R^1 = H, R^2 = Bn
3d: R^1 = Ac, R^2 =Me

Entry	2	3	Time [h]	Yield[b] [%]	*ee*[c] [%]
1	R = H, R^3 = H	**3a**	12	(**4a**) 99	98
2	R = H, R^3 = Me	**3a**	3	(**4b**) 91	99
3	R = H, R^3 = Ph	**3a**	4	(**4c**) 99	91
4	R = H, R^3 = Bn	**3a**	12	(**4d**) 96	93
5	R = H , R^3 = allyl	**3a**	0.5	(**4e**) 99	>99
6	R = H, R^3 = H	**3b**	2	(**4f**) 97	99
7	R = H, R^3 = H	**3c**	4	(**4g**) 99	98
8	R = H, R^3 = H	**3d**	96	(**4h**) 60	98
9	R = 5-Cl, R^3 = H	**3a**	0.5	(**4i**) 93	98
10	R = 5-Br, R^3 = H	**3a**	3.5	(**4j**) 93	96
11	R = 5-F, R^3 = H	**3a**	1.5	(**4k**) 96	96
12	R = 5-Me, R^3 = H	**3a**	48	(**4l**) 99	97
13	R = 5-OMe, R^3 = H	**3a**	96	(**4m**) 90	98
14	R = 7-Cl, R^3 = H	**3a**	4	(**4n**) 99	99
15	R = 7-F, R^3 = H	**3a**	4	(**4o**) 99	99
16	R = 5-Cl, 7-Me, R^3 = H	**3a**	12	(**4p**) 87	94
17	R = 4,7-diCl, R^3 = H	**3a**	12	(**4q**) 63	93

[a] For experimental details, see the Supporting Information.
[b] Isolated yield, and the configuration was assigned by comparison of HPLC data and X-ray crystal data of **4j**.
[c] The *ee* values were determined by chiral phase HPLC with a Chiralcel AD-H chiral column.

Table 3. The model reaction for synthesis of spiro[oxindole-3,4′-pyranochromene] frameworks.[a]



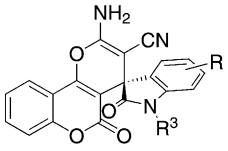
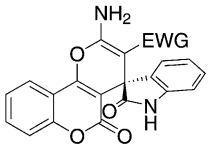
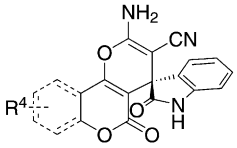
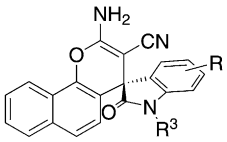
Entry	Solvent	Yield [%][b]	*ee* [%][c]
1[d]	Et_2O	81	58
2	Et_2O	93	75
3	CH_2Cl_2	99	90
4	$CHCl_3$	83	60
5	toluene	99	5
6	MeOH	91	0

[a] Unless noted, the reaction was conducted with **2a** (0.22 mmol) and **5a** (0.20 mmol) for 12 h at room temperature.
[b] Isolated yield.
[c] The *ee* values were determined by chiral phase HPLC with Chiralcel AD-H chiral column, and the configuration was assigned by comparison of HPLC data and X-ray crystal data of **4j**.
[d] Using 5 mol% catalyst loading.

(IC_{50}:7.711 ± 3.444 ~ 20.394 ± 7.143 μM, see the Supporting Information, supplementary Table 3).

On the basis of the above exciting biological results of **JP-8a**, we questioned whether other structurally substituted analogues (Scheme 3) would show more powerful anticancer activity to thereby provide an opportunity to discover novel anticancer agents. Therefore, new kinds of substituted chiral spirooxindole-pyranopyrimidines having various steric and electronic parameters (**JP-8b**–**JP-8h**), were synthesized in 57–81% yields. Subsequently, the further screening of anticancer activity was performed (see Supporting Information, supplementary Table 4). The results of cytotoxic activity evaluation were quite revealing in that **JP-8g** produced better potencies (IC_{50}: 6.296 ± 0.46–10.381 ± 3.008 μM) on these tumor cell lines (Table 5). Furthermore, we discovered that **JP-8g** exhibited a notable broad-spectrum anticancer activity in comparison with the classic cancer therapy drug CPT.[15] These findings also suggested that modest structural changes in the framework and optical purity of the compounds have a significant effect on anticancer activities of pyranopyrimidines.

Table 4. Diversity-oriented synthesis of optically active spiro[oxindole-3,4′-pyranochromene] frameworks.[a]

Entry	Time [h]	Product[b]	Yield [%][c]	*ee* (%) [d]
				
1	12	**6a:** R = H, R^3 = H	99	90%
2	4	**6b:** R = H, R^3 = allyl	99	>99%
3	3	**6c:** R = H, R^3 = Bn	99	93%
4	12	**6d:** R = H, R^3 = Me	99	93%
5	3	**6e:** R = 5-F, R^3 = H	95	98%
6	3	**6f:** R = 7-F, R^3 = H	99	97%
7	3	**6g:** R = 5-Cl, R^3 = H	86	94%
8	3	**6h:** R = 7-Cl, R^3 = H	99	>99%
9	3	**6i:** R = 5-OMe, R^3 = H	92	93%
10	3	**6j:** R = 5-NO_2, R^3 = H	99	94%
11	3	**6k:** R = 5-Cl, 7-Me, R^3= H	85	96%
				
12	12	**6l:** EWG = COOBn	90	90%
13	4	**6m:** EWG = COOMe	92	90%
				
14	3	**6n:** R^4 = 7-F	92	98%
15	3	**6o:** R^4 = 6-F	96	96%
16	5	**6p:** R^4 = 6-Cl	89	95%
17	12	**6q:** R^4 = 6-Me	99	90%
				
18[e]	96	**6r:** R = H, R^3 = Me	89	99%
19[e]	48	**6s:** R = 5-Me, R^3 = Me	74	96%
20[e]	48	**6t:** R = 5-F, R^3 = Me	82	95%

[a] Unless noted, the reaction of isatylidenemalononitrile (0.22 mmol) with 4-hydroxycoumarin or 1-naphthol (0.20 mmol) using 10 mol% catalyst **1g** was conducted for 12 h at room temperature.

[b] The configuration was assigned by comparison of HPLC data and X-ray crystal data of **4j**.

[c] Isolated yield.

[d] The *ee* values were determined by chiral phase HPLC with Chiralcel AD-H or OD-H chiral column.

[e] *o*-Xylene was used as reaction solvent.

Furthermore, in order to evaluate the cytotoxicity of these analogues on normal cells, we invesitgated the cytotoxic activities of these analogues on normal human peripheral blood lymphocytes (hPBLs), compared with human T-cell leukemia cell line (Jurkat).[16] To our delight, we found that **JP-8g** exhibited more selective activity (IC_{50}: 63.824 ± 4.115 compared with 6.373 ± 0.652, almost 10-fold) in hPBLs and Jurkat cells, in comparison with the index compound **JP-8a** (IC_{50}: 29.3 ± 4.502 compared with 8.225 ± 2.854, about 3.5-fold), as shown in Figure 2. These findings suggested that the novel pyranopyrimidine **JP-8g** showed an excellent selective activity, which is a very pivotal index for the development of anticancer drugs. In general, although it exhibited moderate anticancer activities in comparison with the cancer therapy drug CPT, the notable broad-spectrum anticancer activity with a low toxicity will provide a clue for scaffold-inspired synthesis and further development of new types of candidates for future tumor therapy.

Conclusions

In summary, we have disclosed the synthesis of diversely structured spirooxindole-type pyranopyrimidines through an asymmetric strategy *via* organocatalyzed Michael/cyclization reaction for the first time. Several of the new spiro alkaloids were found to significantly inhibit the proliferation of various cancer cells in a preliminary biological evaluation. These findings suggested that spirooxindole-type pyranopyrimidines can serve as a new kind of potential chemotherapeutic agents.

Experimental Section

General Methods

All reactions were carried out under an argon atmosphere unless otherwise noted and solvents were dried according to established procedures. Reactions were monitored by thin layer chromatography (TLC), column chromatography purifications were carried out using silica gel GF254. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker 300 MHz spectrometer in DMSO-d_6 unless otherwise noted and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker 300 MHz spectrometer in DMSO-d_6 unless otherwise noted. Data are presented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, cm = complex multiplet) and coupling constant in Hertz (Hz). Infrared (IR) spectra were recorded on an FT-IR spectrometer. HR-MS was measured with an APEX II 47e mass spectrometer. Melting points were measured on an XT-4 melting point apparatus and are uncorrected. The *ee* values determination was carried out using chiral high-performance liquid chromatography (HPLC) with a Daicel Chiracel column on a Waters instrument with a 2996 UV-detector.

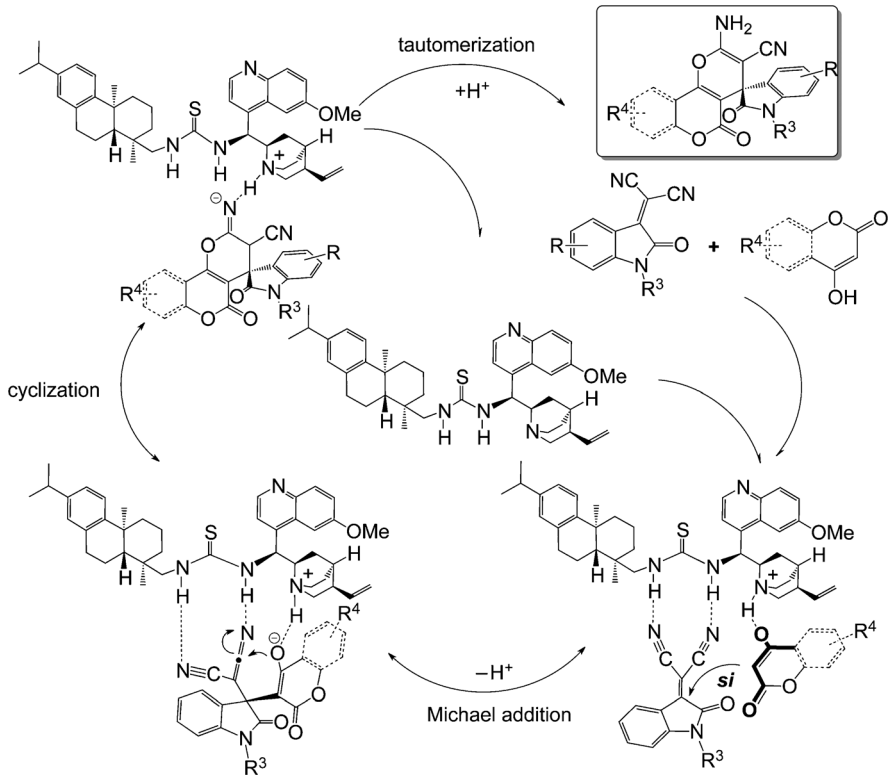
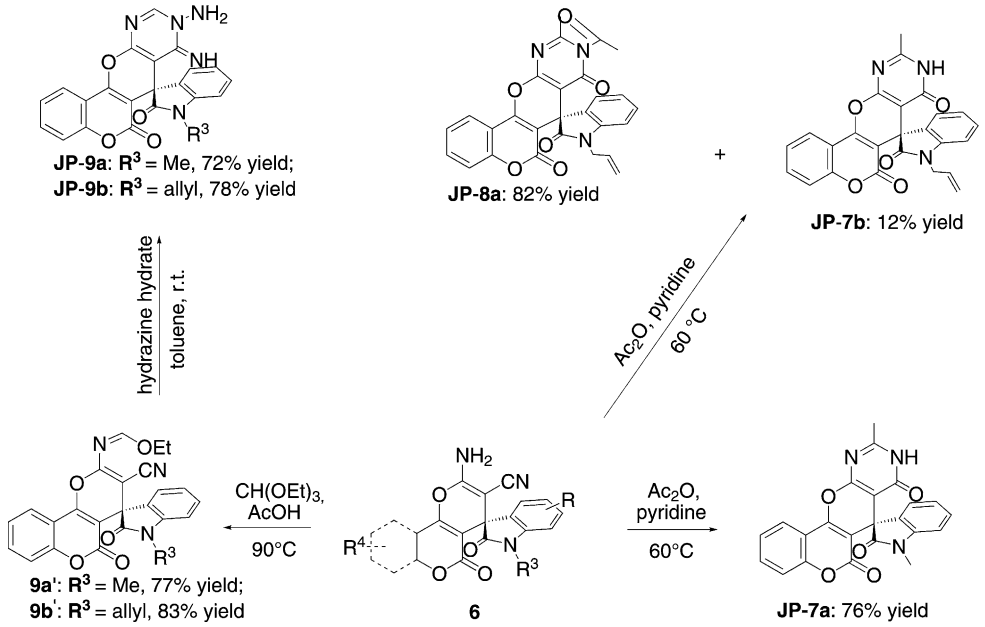


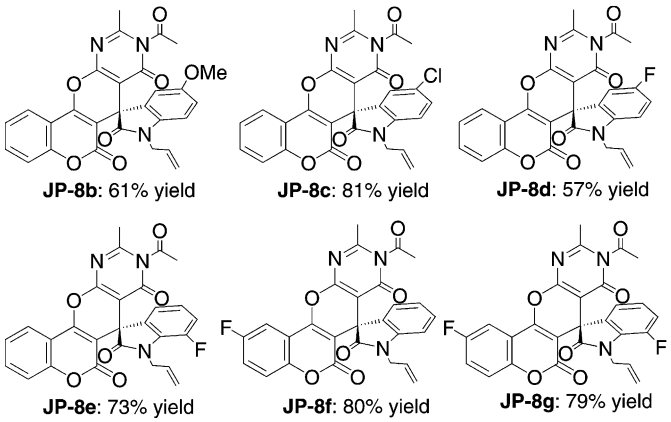
Figure 1. Proposed catalytic cycle for the Michael/cyclization reaction.



Scheme 2. Synthesis of diverse-structured chiral spirooxindole-type pyranopyrimidines.

General Procedure for the Asymmetric Synthesis of Spiro[oxindole-3,4′-pyran]s *via* the Michael/Cyclization Reaction of Isatylidenemalononitriles with α-Keto Esters

To a stirred solution of **1g** (0.01 mmol, 5.0 mol%), 4 Å MS (100 mg) and isatylidenemalononitrile (0.20 mmol) in dry diethyl ether (1.0 mL), the α-keto ester (0.22 mmol) was added under argon, The solution was stirred at room temperature. After the reaction was completed (monitored by TLC), the resulting mixture was concentrated under reduced pressure and the residue was purified through column chromatography on silica gel (eluent, EtOAc/CH_2Cl_2 4:100 to



Scheme 3. Synthesis of anticancer active chiral spirooxindole-pyranopyrimidines.

Table 5. *In vitro* anticancer activities of **JP-8a** and **JP-8g** in comparison with CPT.

	IC_{50} ($\mu M \pm$ error)[a]		
JPs[b]	**JP-8a**	**JP-8g**	**CPT**[c]
MDA	11.004 ± 2.677	6.925 ± 2.654	23.401 ± 3.21
U937	7.711 ± 3.444	6.296 ± 0.46	0.015 ± 0.004
Jurkat	8.225 ± 2.854	6.548 ± 1.228	0.021 ± 0.002
Hela	20.394 ± 7.143	6.373 ± 0.652	25.226 ± 3.987
EJ	11.844 ± 0.987	10.381 ± 3.008	0.026 ± 0.013

[a] IC_{50} is the 50% inhibitory concentration.
[b] Unless noted, the chiral pyranopyrimidines were generated from the corresponding precursors with 99% *ee*.
[c] Camptothecin.

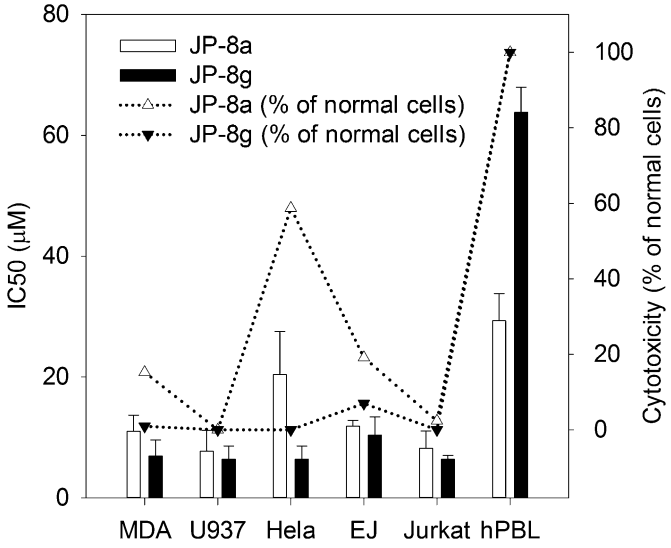


Figure 2. Cytotoxicity of **JP-8a** and **JP-8g** on normal cells compared with tumor cells.

20:100) to give the optically pure product. The enantiomeric purity of the product was determined by using HPLC.

Characterization of a representative compound – (*R*)-methyl 2′-amino-3′-cyano-2-oxospiro[indoline-3,4′-pyran]-6′-carboxylate (4a): White solid; mp 131 °C. ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 10.65$ (s, 1H), 7.33 (s, 1H), 7.19–7.30 (m, 3H), 7.01–7.06 (t, $J = 7.5$ Hz, 1H), 6.87–6.90 (d, $J = 7.5$ Hz, 1H), 6.01 (s, 1H), 3.76 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 177.1$, 160.7, 160.0, 141.1, 140.9, 140.0, 132.9, 129.4, 125.1, 122.4, 118.0, 111.8, 109.9, 53.2, 52.6, 49.1; IR (KBr): $\nu = 3202.05$, 2922.82, 2194.63, 1723.18, 1681.70, 1636.80, 1470.27, 1411.39, 1259.60, 1139.29, 936.60, 757.41, 681.37, 489.86 cm^{-1}; HR-MS (ESI): $m/z = 298.0829$, calcd. for $C_{15}H_{11}N_3O_4 + H^+$: 298.0822; $\Delta = 2.3$ ppm. HPLC: the *ee* was determined by HPLC analysis (Chiralcel AD-H, *i*-PrOH/hexane = 20/80, 1.0 mL min^{-1}, 254 nm; retention times): $t_{minor} = 12.30$ min, $t_{major} = 21.95$ min, *ee* = 98% (for the other compounds see the Supporting Information).

General Procedure for Diversity-Oriented Synthesis of Optically Active Spiro[oxindole-3,4′-pyranochromene] Frameworks *via* Michael/Cyclization Reaction of Isatylidenemalononitriles with 4-Hydroxycoumarins

To a stirred solution of **1g** (0.02 mmol, 10 mol%) and 4-hydroxycoumarin (0.20 mmol) in dry CH_2Cl_2 (1.0 mL), isatylidenemalononitrile (0.22 mmol) was added under argon, The solution was stirred at room temperature. After the reaction was completed (monitored by TLC), the resulting mixture was concentrated under reduced pressure and the residue was purified through column chromatography on silica gel (eluent, EtOAc/CH_2Cl_2 1:10) to give the optically pure product. The enantiomeric purity of the product was determined by using HPLC.

Characterization of a representative compound – (*R*)-2′-amino-2,5′-dioxo-5′*H*-spiro[indoline-3,4′-pyrano[3,2-*c*]chromene]-3′-carbonitrile (6a): White solid; mp 315 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.72 (s, 1H), 7.94–7.97 (d, $J = 7.8$ Hz, 1H), 7.70–7.80 (m, 3H), 7.49–7.58 (m, 2H), 7.21–7.24 (m, 2H), 6.86–7.98 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 177.1$, 158.4, 158.2, 155.0, 152.0, 142.1, 133.6, 133.0, 128.9, 125.0, 124.1, 122.6, 122.0, 116.9, 116.6, 112.4, 109.5, 101.4, 57.0, 47.6; IR (KBr): $\nu = 3356.58$, 3111.74, 2194.90, 1714.37, 1668.38, 1473.02, 1358.16, 1069.49, 968.60, 744.56, 679.27, 616.42 cm^{-1}; HR-MS (ESI): $m/z = 380.0647$, calcd. for $C_{20}H_{11}N_3O_4 + Na^+$: 380.0642; $\Delta = 1.3$ ppm. The *ee* was determined by HPLC analysis (Chiralcel AD-H, *i*-PrOH/hexane = 40/60, 1.0 mL min^{-1}, 254 nm; retention times): $t_{minor} = 16.05$ min, $t_{major} = 24.63$ min, *ee* = 90% (the the other compounds see the Supporting Information).

General Procedure for Diversity-Oriented Synthesis of Optically Active Spiro[oxindole-3,4′-pyranochromene] Frameworks *via* the Michael/Cyclization Reaction of Isatylidenemalononitriles with 1-Naphthol

To a stirred solution of **1g** (0.02 mmol, 10 mol%) and 1-naphthol (0.20 mmol) in dry *o*-xylene (1.0 mL), isatylidenemalononitriles (0.22 mmol) was added under argon, The solution was stirred at room temperature. After the reaction

was completed (monitored by TLC), the resulting mixture was concentrated under reduced pressure and the residue was purified through column chromatography on silica gel (eluent, EtOAc/CH_2Cl_2 1:10) to give the optically pure product. The enantiomeric purity of the product was determined by using HPLC.

Characterization of a representative compound: – (*S*)-2-amino-1′-methyl-2′-oxospiro[benzo[h]chromene-4,3′-indoline]-3-carbonitrile (6r): White solid; mp 265 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.25–8.28 (d, J = 7.5 Hz, 1H), 7.87–7.90 (d, J = 7.5 Hz, 1H), 7.52–7.68 (m, 5H), 7.37–7.41 (t, J = 6.3 Hz, 1H), 7.07–7.19 (m, 3H), 6.49–6.52 (d, J = 8.1 Hz, 1H), 3.23 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 177.5, 161.6, 144.1, 143.8, 134.3, 133.6, 129.8, 128.2, 127.9, 127.9, 125.1, 124.9, 123.9, 123.7, 123.2, 121.2, 118.9, 115.1, 109.6, 54.4, 50.9, 27.0; IR (KBr): ν = 3356.48, 2920.39, 2851.37, 2191.84, 1698.61, 1650.03, 1603.20, 1563.31, 1462.25, 1365.36, 1068.72, 1021.76, 808.08, 746.86 cm^{-1}; HR-MS (ESI): m/z = 376.1038, calcd. for $C_{22}H_{15}N_3O_2+Na^+$: 376.1056; found: Δ = 4.9 ppm. The *ee* was determined by HPLC analysis (Chiralcel AD-H, *i*-PrOH/hexane = 30/70, 1.0 mL min^{-1}, 254 nm; retention times): t_{minor} = 36.91 min, t_{major} = 11.34 min, *ee* = 99% (for the other compounds see the Supporting Information).

General Procedure for Synthesis of Diverse-Structured Chiral Spirooxindole-Type Pyranopyrimidines

Method A: The corresponding chiral spiro[oxindole-3,4′-pyranochromene] (0.3 mmol) was dissolved in 2.0 mL Ac_2O and 1.0 mL pyridine, and then stirred for about 3 h at 60 °C. When the reaction was completed, the solution was concentrated under vacuum. The residue was then dissolved in dry CH_2Cl_2, and washed with water several times. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified *via* flash chromalography.

Characterization of a representative compound – (*R*)-JP-8b: White solid; mp 180 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81–7.84 (d, J = 7.8 Hz, 1H), 7.65–7.72 (t, J = 7.5 Hz, 1H), 7.37–7.44 (m, 2H), 6.84–6.91 (m, 3H), 5.85–5.98 (m, 1H), 5.44–5.50 (d, J = 17.4 Hz, 1H), 5.28–5.31 (d, J = 10.2 Hz, 1H), 4.44–4.46 (d, J = 5.1 Hz, 2H), 3.76 (s, 3H), 2.66 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.1, 158.2, 156.8, 156.1, 152.8, 152.5, 136.2, 134.0, 130.7, 130.6, 125.0, 122.8, 118.1, 117.2, 115.3, 112.2, 111.6, 111.4, 110.7, 101.4, 95.9, 55.8, 50.1, 43.4; IR (KBr): ν = 3426.82, 2932.24, 2228.53, 1725.95, 1680.34, 1608.38, 1372.60, 1289.94, 1200.78, 1072.40, 965.39, 761.79 cm^{-1}; HR-MS(ESI): m/z = 534.1287 calcd. for $C_{28}H_{21}N_3O_7+Na^+$: 534.1272; Δ = 2.9 ppm (for the other compounds see the Supporting Information).

Method B: The corresponding chiral spiro[oxindole-3,4′-pyranochromene] (0.6 mmol) was added to 3.0 mL triethyl orthoformate and 1.0 mL acetic acid, and then stirred for about 5 h at 90 °C. When the reaction was completed, the mixture was concentrated under vacuum. The residue was then dissolved in dry CH_2Cl_2, and washed with water several times. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was used directly without further purification.

To a solution of the above crude product (0.3 mmol) in 3.0 mL toluene was added hydrazine hydrate (0.3 mmol, 1.0 equiv.), and the reaction mixture was stirred overnight at room temperature. When the reaction was completed, the mixture was concentrated under vacuum and applied to chromatography directly to afford the desired product.

Characterization of a representative compound – (*R*)-JP-9a: White solid; mp 307 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.17 (s, 1H), 7.98–8.00 (d, J = 6.6 Hz, 1H), 7.73–7.79 (m, 1H), 7.46–7.55 (m, 2H), 7.14–7.23 (m, 2H), 6.79–6.95 (m, 3H), 5.67 (s, 2H), 3.17 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 174.6, 157.1, 154.5, 153.9, 151.6, 151.0, 132.6, 127.7, 124.2, 122.4, 122.0, 120.8, 115.6, 111.7, 106.5, 101.8, 46.7, 25.7; IR (KBr): ν = 3336.07, 3204.11, 2921.26, 2851.71, 1713.29, 1662.40, 1609.03, 1490.18, 1362.95, 1242.31, 1170.97, 1069.67, 755.07, 496.92 cm^{-1}; HRMS (ESI): m/z = 414.1199, calcd. for $C_{22}H_{15}N_5O_4+H^+$: 414.1197, Δ = 0.5 ppm (for the other compounds see the Supporting Information).

General Methods for the Biological Studies

All compounds used in this study were synthesized in our laboratory, all of them were dissolved in deionized water with 5% DMSO (dimethyl sulfoxide) and further diluted with deionized water. Control trials were performed in the presence of corresponding concentration of DMSO to rule out any possible non-specific action of this solvent. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, Mo.). RPMI 1640, Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. Annexin V/PI apoptosis assay kit was purchased from Invitrogen-Vybrant.

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