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Meeting Organocatalysis with Drug Discovery: Asymmetric Synthesis of 3,3′-Spirooxindoles Fused with Tetrahydrothiopyrans as Novel p53-MDM2 Inhibitors

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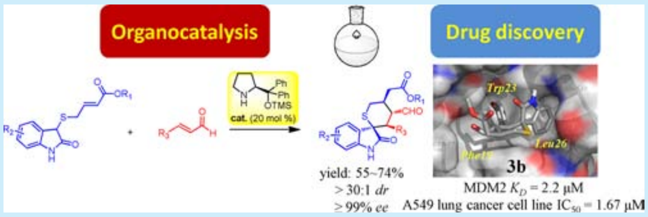
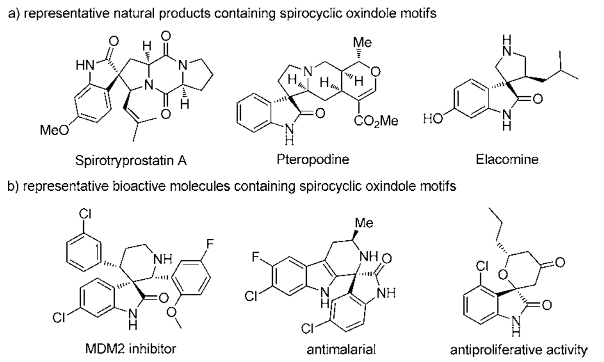
Supporting Information

ABSTRACT: An organocatalytic enantioselective Michael−Michael cascade reaction is developed for the synthesis of chiral spirotetrahydrothiopyrans. This highly functionalized scaffold was assembled in moderate to good yield (55−74%) and excellent diastereo- and enantioselectivities (>30:1 dr, ≥ 99% ee) with the creation of four consecutive stereogenic centers. The novel spiro-oxindole scaffold is validated as a new class of p53-MDM2 protein−protein interaction inhibitors with good antitumor activity.



Spirocyclic oxindole scaffolds are widely distributed in a number of natural products[1] and pharmaceutically important compounds (Figure 1). These scaffolds always exhibit interesting pharmacological activities, such as contraceptive,[2] anti-HIV,[3] anticancer,[4] antituberculosis,[5] and antimalarial.[6] Owing to their wide distribution and diverse activities, considerable efforts have been devoted to developing efficient asymmetric protocols to assemble these interesting scaffolds.[7]


a) representative natural products containing spirocyclic oxindole motifs

b) representative bioactive molecules containing spirocyclic oxindole motifs

Figure 1. Representative natural products and bioactive molecules containing the spirocyclic oxindole motifs.

For the construction of chiral spirooxindole six-membered heterocycles, the organo- or organometallic−catalytic asymmetric cascade strategies have been successfully applied for assembling highly functionalized chiral δ-lactones,[8] 4*H*-pyran,[9] tetrahydropyranone,[4b,10] dihydropyran,[11] tetrahydropyran-γ-methylidene,[12] dihydropyranone,[13] piperidine,[14] tetrahydropyridine,[15] and dihydropyridone[16] (Figure 2). Nevertheless, to the best of our knowledge, no asymmetric method has been reported for the preparation of the chiral spirocyclic oxindole−tetrahydrothiopyran scaffold, which is an intriguing combination of pharmacologically interesting tetrahydrothiopyran[17] and oxindole motifs. Herein, we aim to develop an organocatalyzed asymmetric approach to this novel framework. Notably, a highly functionalized chiral spirocyclic oxindole−tetrahydrothiopyran scaffold with four consecutive stereogenic centers is constructed in a "one-pot" operation under mild reaction conditions.

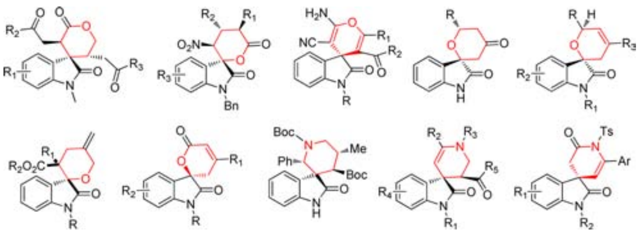


Figure 2. Organo- or organometallic−catalytic asymmetric cascade reactions to assemble chiral spirooxindole six-membered heterocycles.

Previously, our group developed novel organocatalytic cascade reactions for the construction of highly functionalized chiral 3,4-dihydro-2*H*-thiopyrans[18] and tetrahydrothiopyrans.[19]

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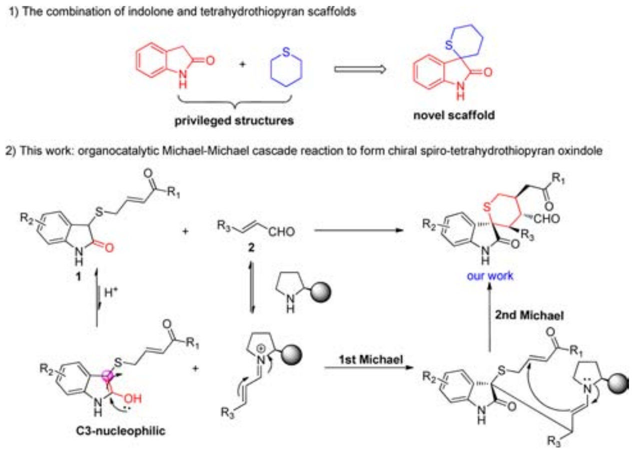
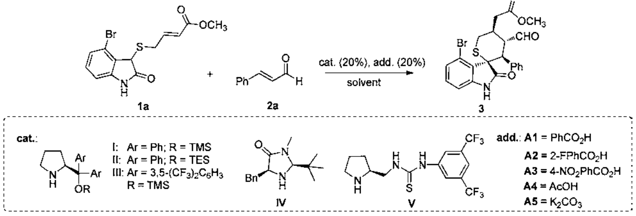
Considering the importance of oxindole and tetrahydrothiopyran as privileged scaffolds in drug discovery, we envisioned that a novel spirocycled-3,3′-oxindole scaffold containing spirotetrahydrothiopyran core structure could be efficiently constructed by developing new organocatalytic asymmetric cascade reactions (Scheme 1). Toward this end, we designed a new Michael—Michael cascade process using a new substrate **1** bearing the nucleophilic oxindole-C3 as the Michael donor and *α*,*β*-unsaturated ester as the Michael acceptor (Scheme 1).

Scheme 1. Organocatalytic Enantioselective Access to Chiral Spiro-tetrahydrothiopyran—Oxindole



To probe the validity of the proposed organocatalytic Michael—Michael cascade, we probed a model reaction by using substrate **1a** with *trans*-cinnamaldehyde **2a** catalyzed by diphenylprolinyl silyl ether (**I**) in the presence of $PhCO_2H$ as the additive (Table 1). To our delight, the reaction proceeded smoothly to afford the desired oxindole—spiro-tetrahydrothiopyran **3a** in good yield (62%, entry 1), with excellent diastereo- (dr > 30:1) and enantioselectivities (ee > 99%). Encouraged by this result, we further screened other secondary amine catalysts **II**—**V**. A similar result was obtained with catalyst **II**, while catalysts **III**—**V** failed to do so (entries 3—5). Accordingly, catalyst **I** was used for further studies. Solvent screening indicated the reaction media had a significant impact on the reaction efficiency (entries 6—10). Good yields were obtained with solvents $CHCl_3$ and DCE (entries 9 and 10). In contrast, no reaction was observed with solvent Et_2O. Moreover, toluene and acetonitrile offered inferior outcomes (entries 6 and 8). Acid additives screening revealed that $PhCO_2H$ was the best choice (entries 11—13), while a base was unfavored (entry 14). Furthermore, upon increasing the amount of cinnamaldehyde **2a** to 3 equiv and increasing the reaction temperature (35 °C), the reaction time was reduced (1.5 d), while the yield was slightly decreased (yield 58%, entry 15). These results led us to select the protocol consisting of catalyst **I** and $PhCO_2H$ as the additive in DCM to probe the scope the Michael—Michael cascade reaction (entry 1).

Table 1. Optimization of Reaction Conditions[a]



entry	cat.	add.	solvent	time (d)	yield[b] (%)	dr[c]	% ee[d]
1	**I**	**A1**	DCM	3	62	>30:1	>99
2	**II**	**A1**	DCM	3	56	>30:1	>99
3	**III**	**A1**	DCM	3	ND[e]		
4	**IV**	**A1**	DCM	3	ND		
5	**V**	**A1**	DCM	3	ND		
6	**I**	**A1**	PhMe	3	35	>30:1	>99
7	**I**	**A1**	Et_2O	3	ND		
8	**I**	**A1**	ACN	3	44		
9	**I**	**A1**	$CHCl_3$	3	61	>30:1	>99
10	**I**	**A1**	DCE	3	58	>30:1	>99
11	**I**	**A2**	DCM	3	54	>30:1	>99
12	**I**	**A3**	DCM	3	40	>30:1	>99
13	**I**	**A4**	DCM	3	<10		
14	**I**	**A5**	DCM	3	ND		
15[f]	**I**	**A1**	DCM	1.5	58	>30:1	>99

[a] Reaction conditions (unless otherwise specified): solvent (2.0 mL), substrate **1a** (0.11 mmol, 1.0 equiv), cinnamaldehyde **2a** (0.13 mmol, 1.2 equiv), catalyst (20 mol %), additive (20 mol %), room temperature. [b] Yield of isolated product after column chromatography. [c] Determined by ^1H NMR analysis. [d] Determined by chiral HPLC analysis. [e] No reaction. [f] Cinnamaldehyde **2a** (3 equiv), reaction performed at 35 °C.

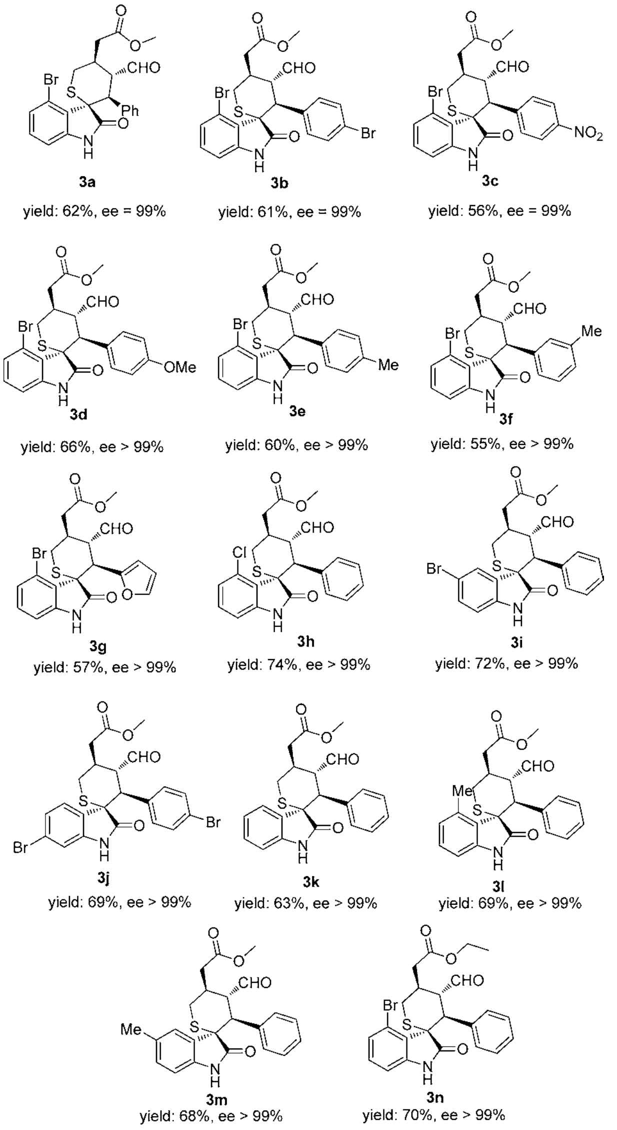
With the optimized conditions in hand, the substrate scope and the generality of the reaction were explored (Scheme 2). To our delight, a variety of substrates **1** participated in the reaction with structurally diverse enals **2**. It appeared that aldehydes bearing electron-withdrawing (**3b**,**c**) or -donating (**3d**—**f**) groups were both well tolerated and afforded the desired products with moderate to good yield (55—68%) and stereoselectivities (dr > 30:1, ee ≥ 99%). Besides phenyl-substituted enals, heterocyclic-substituted enals such as furanyl (**3g**) were also well tolerated and gave the products in moderate yield (57%) and excellent enantioselectivity (ee > 99%). No reactions were observed between substrate **1a** and the aliphatic enals, such as pent-2-enal and 4-methylpent-2-enal, which might be due to the lower reactivity of substrate **1a**.

Next, the structural variation of substrates **1** was probed under the optimized conditions (Scheme 2, **3h**—**n**). It appeared that electron-withdrawing (such as bromo and chloro groups, **3g**—**j** and **3n**) or electron-donating groups (such as methyl group, **3l** and **3m**) on the aromatic ring were both well tolerated and afforded the desired products with high yields (57—74%) and stereoselectivities (dr > 30:1, ee > 99%). Moreover, the position of the R group on substrate **1** had little effect on reaction yield and stereocontrol. For the part of the Michael acceptor, it appeared that methyl esters (**3a**—**m**) and ethyl ester (**3n**) were well tolerated. When the R1 group was *tert*-butyl ether, no reaction occurred, indicating steric hindrance hampered the reaction (data not shown).

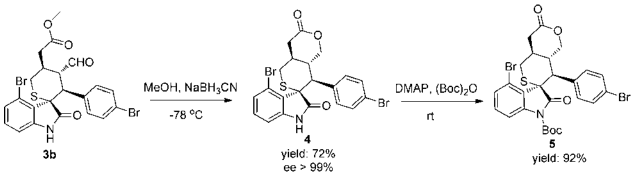
Starting from compound **3b**, adduct **4** with increased molecular complexity and diversity was obtained with good yield (72%) and excellent enantioselectivity (ee > 99%) via a one-step reduction—condensation reaction (Scheme 3).

The absolute configuration of the products obtained in the Michael—Michael cascade reaction was determined by X-ray crystallography analysis of derivative **5**, whose absolute configuration was determined to be (3*R*,4a′*S*,8′*S*,8a′*S*)[20] (Figure 3).

Scheme 2. Reaction of Various 3-Substituted Indolin-2-one Derivatives with α,β-Unsaturated Aldehydes



Scheme 3. Synthetic Elaboration of Michael–Michael Adducts 3b



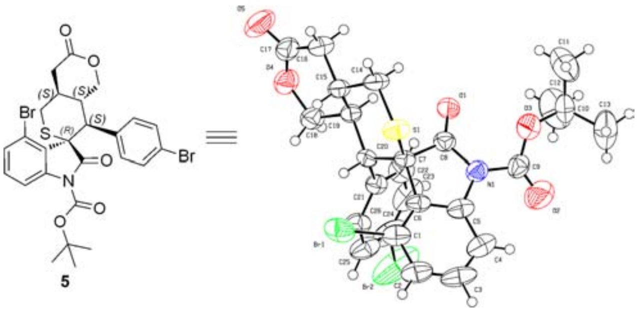


Figure 3. X-ray structure of compound **5**.

Table 2. In Vitro Antitumor Activities of the Oxindole–Spiro-tetrahydrothiopyrans (IC_{50}, μM)

compd	A549	HCT116	MDA-MB-231
3a	35.14	34.34	21.34
3b	1.67	1.57	3.55
3c	30.82	29.20	20.92
3d	34.86	27.43	24.91
3g	30.92	25.86	19.39
3h	12.55	12.21	13.77
3i	12.26	11.70	12.72
3j	6.20	4.39	2.82
3k	86.40	32.36	29.88
3l	13.82	5.00	11.65
3m	17.15	11.23	21.12
3n	25.67	11.88	12.37
4	17.57	13.37	12.69
nutlin-3	2.22	1.16	4.68

The oxindole–spiro-tetrahydrothiopyran derivatives were assayed for in vitro antitumor activity against three types of human cancer cell lines with p53 in its wild type (A549 lung carcinoma cell, HCT116 colon cancer cell, and MDA-MB-231 breast cancer cell) using the standard MTT method.[21] As shown in Table 2, most derivatives exhibited moderate to good inhibitory activity against all tested cancer cell lines. Among them, compound **3b** showed the best antitumor activity with IC_{50} values in the range of 1.57 to 3.55 μM. We then investigated potential target of this new type of spirooxindole scaffold. Previously, spiro-oxindoles have been reported as inhibitors of p53-MDM2 protein–protein inhibitors.[4c,22] Inspired by the results, we tested the MDM2 inhibitory activity of compound **3b** using the fluorescence polarization assay.[23] Interestingly, compound **3b** was identified as a potent MDM2 inhibitor with a K_D value of 2.2 μM (Figure 4a). Furthermore, we investigated whether **3b** inhibited the p53-MDM2 interaction in the cancer cells by Western blotting analysis. Similar to nutlin-3 (a classical MDM2 inhibitor),[24] compound **3b** showed a dose-dependent up-regulation of p53 and MDM2 expressions in A549 cells (Figure 4c). The binding mode of compound **3b** with MDM2 was clarified by our previously reported molecular docking protocols.[23,25] As depicted in Figure 4b, the oxindole, methyl ester, and bromophenyl groups interacted with the Leu26, Phe19, and Trp23 hot spots of MDM2, respectively.

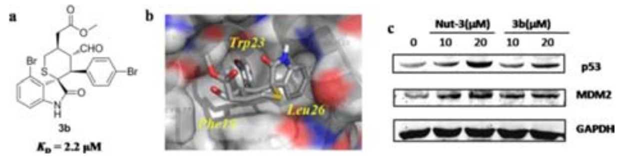


Figure 4. (a) Chemical structure and MDM2 inhibitory activity of **3b**. (b) Predicted binding mode of **3b** in three hot spots of MDM2. (c) Cellular activity of nutlin-3 and -3b for the p53 pathway activation to up-regulate p53 and MDM2 detected by Western Blot in A549 cells.

In summary, we have developed an organocatalytic Michael–Michael cascade reaction for the preparation of a novel oxindole–spiro-tetrahydrothiopyran scaffold in moderate to good yield and with excellent diastereo- (dr > 30:1) and enantioselectivies (ee ≥ 99%). Biological assays indicated that the spirooxindole derivative **3b** was a potent p53-MDM2 inhibitor with good antitumor activity, which represents a promising lead compound for antitumor drug discovery. Further exploration of the biological properties of these compounds is currently underway in our laboratories.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00155.

Experimental details and analytical data (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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