Synthesis and Antitumor Activity Evaluation of Regioselective Spiro [pyrrolidine-2,3'-oxindole] Compounds

Gang Chen,[1] Hong-ping He,[2] Jiang Ding[3] and Xiao-jiang Hao[2,*]

[1]*College of Chemistry and Chemical Engineering, Xi'an Shiyou University, Xi'an 701165, P. R.China*
[2]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China*
[3]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences Shanghai 201203, P. R. China*

Abstract: A series of spiro [pyrrolidine-2,3'-oxindole] derivatives were synthesized by 1,3-dipolar cycloaddition reaction of isatin, α-amino acid and (E)-β-substituted-styrene. Four kinds of trapping dipolarophiles were introduced into this reaction, and the regioselectivity of these reactions proved to be the same fashion. Bioactivity screening showed these compounds were active on anti-tumor in A549 and P388 cell line, and several compounds were found to be active under the condensation of 10^{-4}M.

Introduction

The indole derivatives fall into an important class of organic compounds, and they have received the attention of biochemists because of their therapeutic and biochemical activities.[1,2] Several oxindole derivatives are known to possess antibacterial, antiprotozoal, and anti-inflammatory activities.[3] Besides, the spiro [pyrrolidine-2,3'- oxindole] ring system is a structural feature found in a wide variety of oxindole alkaloids, such as horsfiline[4] and spirotryprostatin B,[5,6] which have been reported to behave as aldose reductase,[7] poliovirus, and rhinovirus 3C-proteinase inhibitors.[8]

The 1,3-dipolar cycloaddition reaction provides a simple and direct entry into a number of five-membered heterocyclic compounds, such as pyrrolidines, pyrrolines, and pyrroles. This reaction has also been employed for the construction of spiro compounds, and by this way many spiro [pyrrolidine-2,3'-oxindoline] derivatives have been synthesized.[9,10] Alkene derivatives, such as chalcone,[11] acrylate, maleimide,[9] maleic anhydride 2-arylidene-1-tetralone and arylidenemalononitrile derivatives,[10] have been efficiently used as trapping dipolarophiles. Almost all these reactions are in good yield and high regio- and stereoselectivity. The synthesis and bioactivity research of this kind of compounds have been one of the hot topic in organic chemistry and bioorganic medical chemistry.

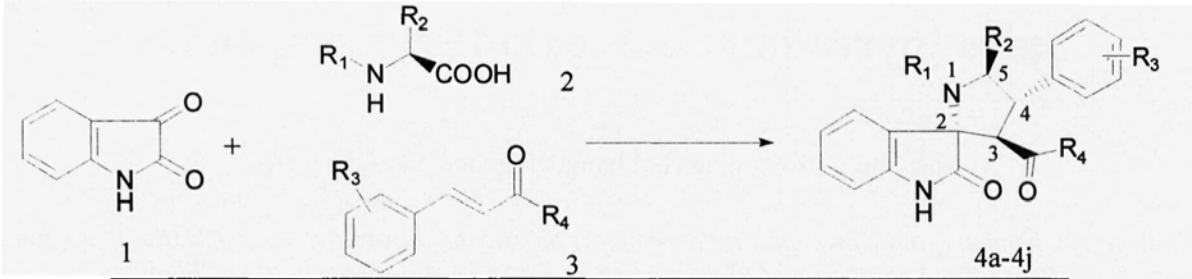
For a extended research of the regio-chemistry in 1,3-dipolar cycloaddition reaction and the bioactivity of spiro [pyrrolidine-2,3'-oxindoline] compounds, four kinds of trapping dipolarophiles, chalcone, cinnamic ester, cinnamic acyl amine, 1,5-diphenyl- penta-1,4-dien-3-one and 3-benzylidene-1,3-dihydro-indol-2-one, were introduced in this reaction to synthesis a series of spiro [pyrrolidine-2,3'-oxindole] derivatives, with which the bioactivity on anti-tumor in A549 and P388 cell line was evaluated.

Results and Discussion

Chemistry

Firstly, a series of (*E*)-β-substituted-styrene with different electron-withdrawing group (containing carboxyl group) were designed and synthesized for the effect of the substitute on the regio-chemistry. In the 1,3-dipolar cycloaddition reactions, isatin, α-amino acid and (*E*)-β-substituted-styrene were refluxed in a methanol / water (3: 1) medium. The reaction mixture refluxed and was monitored by TLC. The solvent was distilled off under reduced pressure and the products were purified by silica gel column chromatography and characterized by NMR and MS.

Scheme 1



Compd	R_1	R_2	R_3	R_4	Yield (%)
4a		-$(CH_2)_3$-	H	OCH_3	84.2
4b		-$(CH_2)_3$-	H	$OCH(CH_3)_2$	65.1
4c		-$(CH_2)_3$-	*p*-OCH_3	OCH_3	78.4
4d		-$(CH_2)_3$-	H	$N(CH_2CH_2)_2O$	66.4
4e		-$(CH_2)_3$-	H	Ph	80.9
4f	H	Bn	H	Ph	74.5
4g	H	CH_2OH	H	Ph	82.1
4h	H	$CH_2CH(CH_3)_2$	*o*-Cl	Ph	83.8
4i		-$(CH_2)_3$-	H	CH=CHPh	60.3
4j		-$(CH_2)_3$-	*o*-Cl	3-indol-2-one	36.5

The ^{1}H-NMR spectrum of **4a** exhibited multiplets signals in the region δ 1.76-3.96 for the hexahydro-pyrrolizine ring protons, the signals in the region δ 6.95-7.44 are for protons of aryl groups, and the N-H signal at 9.47. In the HMBC and HMQC spectrum, the doublet signal (H_a) at δ 3.96 (1H, d, J = 12.0 Hz) demonstrates the correlation of ester group and C-3 of the newly-constructed pyrrolidine; furthermore, the signal (H_b) at δ, 3.92 (1H, m) showed the correlation of phenyl group and C-4. In the ^{13}C-NMR spectrum, the signals of δ 179.2, 169.7 are due to the ester and amide carbonyl; the signal of δ 110.0-142.6 are due to the phenyl carbon; the other signals δ 27.3-72.6 are due to the hexahydro-pyrrolizine and the methoxy carbon, among which δ72.6 is due to the spiro carbon. Moreover the EI-MS spectrum exhibited a molecular peak at *m/z* 362 (M^+). These observed values confirmed the structure of **4a**.

The 1D-NMR and 2D-NMR spectrum of **4d** showed a doublet at δ 3.98 (1H, m) and a multiplet at δ 3.92 (1H, d, J = 9.6 Hz) for H_a and H_b, respectively. The conclusion also can be drawn that the acyl amide connected with C-3 and phenyl group connected with C-4. In the ^{1}H-NMR spectrum of **4i**, it is also can be found there is a signal at δ 4.51 (1H, d, J = 11.6 Hz), which is due to the α proton of 3-phenyl-propenal. The double perk of this signal indicate the correlation of 3-phenyl-propenal and C-3 of the newly-constructed pyrrolidine. In the ^{1}H-NMR spectrum of **4j**, the signal of δ 5.89 (1H, d, J = 9.6 Hz) is due to the α proton of 2-chloro-phenyl, The double perk of this signal indicate the correlation of 2-chloro-phenyl and C-4 of the newly-constructed pyrrolidine.

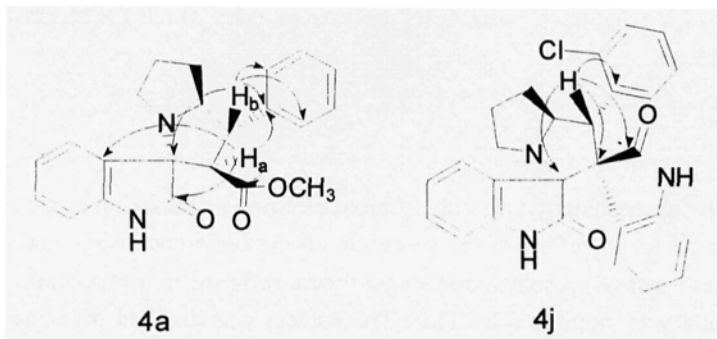


Figure 1 The main C-H correlations of **4a** and **4j**

Based on the results above, a conclusion can be drawn that the regioslectivity of the reactions are the same: the electron-withdrawing groups of (*E*)-β-substituted-styrene, ester, acyl amine, 3-phenyl-propenal and oxindole, always connect with the C-3 of the newly-constructed pyrrolidine.

Steric effect could be found in these reactions. For an example, isatin, L-phenylalanine and cinnamic ester were refluxed for hours, and only little new points were found by TLC detection, but the products were too complex to determine whether there as an expected compounds. As the cinnamic ester was replaced by chalcone, the yield of the target molecule (**4f**) was 74.5%, or the L-phenylalanine was replaced by L-proline, the yield of the target molecule (**4a**) was 84.2%.

Biological Activity

These synthesized spiro [pyrrolidine-2,3'-oxindole] derivatives were a series of natural product analogue of oxindole alkaloids which have aroused a great deal of synthetic effort and with significant biological acitivity.[12,13] In view of these observations and our continued interest in the research of indole alkaloids,[14-16] it was considered worthwhile to evaluate their bioactivity. Herein we disclose the screening results of their ant-tumor activity of some of the compounds in Table 1.

Table 1 In *vitro* Cytotoxicity against A549 and P388 Cell Line

Compd	*P388*		*A-549*	
	100μM	*10 μM*	*100μM*	*10 μM*
4a	35.4	10.1	57.1	3.0
4b	91.9	14.3	74.8	0
4d	19.9	11.0	34.4	0
4e	91.4	5.0	95.9	5.0
4f	96.9	7.3	100	6.6
4g	14.5	8.9	60.7	11.6
4h	100	0	96.5	0
4i	100	42.9	96.6	69.8

From the results, it was found that almost all compounds could inhibit both cancer cells effectively at the concentration of 100 μM, except **4d** and **4g**. Compounds **4e**, **4f**, **4h** and **4i** showed potent activity, with the inhibition percentage more than 90 for P388 and A-549. But the activities of these compounds decline as the concentration decreased to 10 μM, except **4i**, the inhibition percentage of which is 42.9 for P388 and 69.8 for A-549. Compared with the structure of **4e**, the C=C bond of 3-phenyl-propenal might be the key functional group.

Experimental

General Remarks: All starting materials and solvents (A.R. grade) are commercially available and were used without further purification. NMR spectrum were recorded in the stated solutions, on a Bruker Drx-400 or 500 spectrometer, operating at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C; δ values are reported in ppm and *J* values in hertz. Mass spectrum were recorded on a Micromass Platformllspectrometer, using the direct-inlet system operating in the electron impact (EI) mode at 75 eV.

General procedure for the 1,3-dipolar cycloaddition reaction of isatin, *α*-amino acid and (*E*)-β-substituted-styrene

A solution of isatin (1 mmol), α-amino acid (1 mmol) and (*E*)-β-substituted-styrene (1.2 mmol) was heated under reflux in a mixture of methanol (9 mL) and water (3 mL). Completion of the reaction was monitored by TLC check, the solvent was distilled off under reduced pressure and the residue was separated by column chromatography (silica gel, petroleum ether/ethyl acetate = 10: 1-4: 1) to give the target products.

Biological activity screening

The compounds were screened for their biological activities on protective effect on cytotoxicity against A549 and P388 cell line at various concentrations by the reported methods.[17]

4a,^{1}H-NMR (D_6-Acetone, 500 MHz),δ: 9.47 (1H, s), 7.44 (1H, d, *J* = 7.5 Hz), 7.35 (3H, m), 7.25 (3H, m), 7.0 (1H, m), 6.95 (1H, d, *J* = 7.5 Hz), 3.96 (1H, d, *J* = 12.0 Hz), 3.92 (1H, m), 3.65 (1H, m), 3.05 (3H, s), 2.63 (1H, m), 2.48 (1H, m), 1.87 (2H, m), 1.76 (2H, m); ^{13}C-NMR (D_6-Acetone, 125 MHz),δ: 179.2, 169.7, 142.6, 139.9, 129.7, 128.8, 127.8, 127.0, 126.4, 125.4, 121.3, 110.0, 72.6, 61.1, 51.7, 51.3, 47.6, 30.1, 27.3; EI-MS, *m/z* (%): 362 (M^+)

4b, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.45 (1H, s), 7.44 (2H, d, *J* = 7.2 Hz), 7.36 (3H, m), 7.25 (2H, m), 6.99 (1H, m), 6.94 (2H, d, *J* = 7.6 Hz), 4.39 (1H, m), 3.93 (2H, d, *J* = 8.0 Hz), 3.86 (1H, m), 3.63 (1H, dd, *J* = 9.2, 12.0 Hz), 2.55 (1H, m), 2.46 (1H, m), 1.87 (2H, m), 1.73 (2H, m), 0.86 (3H, d, *J* = 6.4 Hz), 0.48 (3H, d, *J* = 6.4 Hz); ^{13}C-NMR (D_6-Acetone, 100 MHz),δ: 181.1, 168.6, 141.8, 139.6, 129.6, 128.6, 128.0, 127.0, 126.3, 125.8, 122.2, 110.6, 73.7, 73.0, 68.2, 63.7, 61.5, 52.8, 47.9, 31.4, 27.8, 21.4, 20.4; EI-MS, *m/z* (%): 390 (M^+).

4c, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.48 (1H, s), 7.36 (3H, m), 7.26 (1H, dt, *J* = 7.6, 0.8 Hz), 7.0 (1H, t, *J* = 7.6 Hz), 6.96 (1H, d, *J* = 7.6 Hz), 6.91 (1H, d, *J* = 8.8 Hz), 3.91 (1H, d, *J* = 6.0 Hz), 3.77 (3H, s), 3.61 (1H, dd, *J* = 12.0, 1.2 Hz), 3.06 (3H, s), 2.64 (1H, m), 2.48 (1H, m), 2.08 (1H, m), 1.91 (1H, m), 1.75 (1H, m); ^{13}C-NMR (D_6-Acetone, 100 MHz),δ: 179.9, 170.7, 159.6, 143.6, 130.2, 129.6, 127.3, 126.8, 121.9, 114.8, 110.6, 110.5, 73.4, 73.3, 62.9, 55.4, 52.8, 51.4, 48.4, 31.1, 30.5, 28.1; EI-MS, *m/z* (%): 392 (M^+).

4d, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.45 (1H, s), 7.49 (2H, d, *J* = 7.6 Hz), 7.38 (1H, d, *J* = 7.6 Hz), 7.29 (3H, m), 7.21 (1H, d, *J* = 7.6 Hz), 7.01 (2H, m), 4.18 (1H, m), 3.98 (1H, m), 3.92 (1H, d, *J* = 9.6 Hz), 3.25-3.31 (3H, m), 2.90-2.97 (4H, m), 2.85 (1H, m), 2.55 (1H, m), 2.46 (1H, m), 1.95 (1H, m), 1.87 (1H, m), 1.73 (2H, m); ^{13}C-NMR (D_6-Acetone, 100 MHz),δ: 180.1, 169.4, 143.6, 142.1, 130.4, 129.4, 129.1, 128.9, 127.4, 127.1, 122.1, 110.6, 72.7, 71.2, 66.7, 66.5, 59.2, 55.0, 49.7, 46.1, 42.9, 28.8, 26.0; EI-MS, *m/z* (%): 417 (M^+).

4e, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.22 (1H, s), 7.52 (2H, d, *J* = 7.2 Hz), 7.43 (2H, m), 7.39 (1H, d, *J* = 7.2 Hz), 7.31 (2H, d, *J* = 7.2 Hz), 7.22 (2H, d, *J* = 8.0 Hz), 7.18 (2H, m), 7.06 (1H, td, *J* = 7.6, 1.2 Hz), 6.90 (1H, td, *J* = 7.6, 0.8 Hz), 6.58 (1H, d, *J* = 7.6 Hz), 4.94 (1H, d, *J* = 11.2 Hz), 4.11 (1H, m), 3.94 (1H, t, *J* = 10.8 Hz), 2.75 (1H, m), 2.43 (1H, m), 1.92 (2H, m), 1.79 (2H, m); ^{13}C-NMR (D_6-Acetone, 100 MHz),δ: 180.9, 158.4, 143.0, 141.4, 138.2, 133.6, 130.0, 129.4, 129.0, 128.8, 128.6, 128.4, 127.6, 126.2, 122.0, 110.4, 110.3, 73.7, 72.1, 64.8, 52.1, 48.5, 30.8, 27.2; EI-MS, *m/z* (%): 408 (M^+).

4f, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.29 (1H, s), 7.58 (2H, d, *J* = 7.2 Hz), 7.35-7.42 (5H, m), 7.13-7.25 (8H, m), 6.95 (2H,m), 6.81 (1H, t, *J* = 7.2 Hz), 6.48 (2H, d, *J* = 7.6 Hz), 4.63 (1H, d, *J* = 11.2 Hz), 4.21 (1H, m), 3.92 (1H, t, *J* = 10.8 Hz), 2.81-2.94 (2H, m), 2.74 (1H, m); ^{13}C-NMR (D_6-Acetone, 100 MHz),δ: 197.7, 182.1, 142.5, 141.3, 140.2, 138.2, 133.5, 130.5, 130.0, 129.6, 129.5, 129.3, 129.0, 128.9, 128.2, 127.7, 126.8, 126.6, 122.5, 109.8, 69.5, 66.4, 64.1, 54.8, 39.8; EI-MS, *m/z* (%): 458 (M^+).

4g, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.34 (1H, s), 7.53 (1H, d, J = 7.6 Hz), 7.44 (1H, d, J = 8.0 Hz), 7.39 (1H, m), 7.34 (1H, t, J = 7.6 Hz), 7.22 (3H, m), 7.12 (1H, dd, J = 7.6, 0.8 Hz), 7.0 (1H, d, J = 7.6 Hz), 6.88 (1H, t, J = 7.2 Hz), 6.53 (1H, d, J = 8.0 Hz), 4.72 (1H, d, J = 10.8 Hz), 4.22 (1H, t, J = 10.4 Hz), 3.94 (1H, t, J = 5.6 Hz), 3.89 (1H, m), 3,71 (1H, m), 3.53 (1H, m), 3.01 (1H, s, b); ^{13}C-NMR (D_6-DMSO, 100 MHz),δ: 197.2, 181.0, 141.6, 140.2, 136.7, 133.1, 129.3, 129.0, 128.7, 128.4, 128.0, 127.1, 126.8, 125.3, 121.6, 109.1, 68.7, 66.4, 62.4, 59.8, 49.2; EI-MS, *m/z* (%): 398 (M^+).

4h, ^{1}H-NMR ($CDCl_3$, 400 MHz),δ: 7.87 (1H, s), 7.73 (1H, d, J = 7.6 Hz), 7.41 (2H, d, J = 8.0 Hz), 7.37 (1H, dd, J = 8.0, 1.2 Hz), 7.28 (2H, m), 7.13 (4H, m), 7.0 (1H, td, J = 7.6, 1.2 Hz), 6.93 (1H, t, J = 7.6 Hz), 6.49 (1H, d, J = 7.6 Hz), 4.74 (1H, d, J = 10.4 Hz), 4.52 (1H, t, J = 10.4 Hz), 3.93 (1H, m), 1.60 (1H, m), 1.50 (1H, m), 1.43 (1H, m), 0.81 (6H, dd, J = 6.4, 0.8 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz),δ: 197.3, 181.6, 140.1, 137.5, 137.1, 135.1, 132.7, 129.9, 129.3, 129.0, 128.8, 128.7, 128.1, 127.8, 127.6, 127.2, 125.5, 123.1, 109.4, 69.4, 64.4, 63.1, 51.1, 43.3, 30.9, 25.9, 23.5, 21.9; EI-MS, *m/z* (%): 460 (M^+).

4i, ^{1}H-NMR (D_6-Acetone, 400 MHz),δ: 9.52 (1H, s), 7.48 (1H, d, J = 7.2 Hz), 7.41 (2H, m), 7.28-7.35 (7H, m), 7.14-7.22 (3H, m), 6.95 (1H, m), 6.79 (1H, d, J = 7.2 Hz), 6.36 (1H, d, J = 16.0 Hz), 4.51 (1H, d, J = 11.6 Hz), 3.99 (1H, m), 3.87 (1H, m), 2.63 (1H, m), 2.46 (1H, m), 1.91 (2H, m), 1.76 (2H, m); ^{13}C-NMR (D_6-Acetone, 100 MHz),δ: 195.6, 181.0, 143.6, 141.0, 139.4, 133.8, 130.4, 129.3, 128.5, 128.4, 128.0, 127.6, 126.9, 126.7, 124.9, 122.0, 110.2, 97.3, 73.7, 72.1, 66.2, 52.0, 47.7, 30.5, 27.1; EI-MS, *m/z* (%): 434 (M^+).

4j, ^{1}H-NMR (CCl_3D, 400 MHz),δ: 8.77 (1H, s), 8.12 (1H, s), 7.13 (3H, m), 6.95 (1H, t, J = 7.6 Hz), 6.88 (2H, t, J = 7.2 Hz), 6.74 (1H, d, J = 7.6 Hz), 6.69 (2H, m), 6.60 (1H, d, J = 7.6 Hz), 6.50 (2H, m), 5.89 (1H, d, J = 9.6 Hz), 4.26 (1H, m), 2.67-2.75 (2H, m), 2.19 (2H, m), 2.01 (1H, m), 1.79 (1H, m); ^{13}C-NMR (CCl_3D, 100 MHz),δ: 179.2, 174.4, 141.5, 141.2, 135.7, 133.3, 129.3, 129.2, 128.9, 128.8, 128.7, 127.7, 126.6, 126.3, 126.2, 125.4, 121.1, 121.0, 109.8, 109.2, 70.7, 70.4, 49.3, 47.2, 47.2, 30.3, 30.2; EI-MS, *m/z* (%): 455 (M^+).

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