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Autocatalytic synthesis of 3-ethyl-3-hydroxy-indole-2-ones and their neuroprotection and antitumor activities' evaluation

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ABSTRACT

In this study, a series of 3-ethyl-3-hydroxy-indole-2-ones were synthesized through the addition reaction of Et_2Zn and N-substituted isatin by an autocatalytic process. The synthesized compounds were characterized by NMR spectroscopy and mass spectrometry, and the reaction mechanism was discussed. The antitumor and neuroprotection activities of these compounds were evaluated. The results showed that several compounds display protection activity on H_2O_2-induced apoptosis of PC12 cells, which are more effective than that of (±)-α-tocopherol Vitamin E (VE). Moreover, these compounds also show antitumor activity against A549 and P388 cell lines.

1. Introduction

Alkaloids are versatile natural products with various bioactivities, among which isatin (indole-2,3-dione, a kind of endogenous indole alkaloid) present in mammalian tissues and fluids has drawn much attention from the fields of chemistry and biology [1]. It has been identified as a selective inhibitor of monoamine oxidase B [2] and is an interesting compound involved in nerve protection. Besides, isatin derivatives have been reported with various activities [3–5]. The 3-hydroxyindole moiety has been found in many alkaloid natural products, such as welwitindolinone C, 3-hydroxyglucoisatisin, dioxibrassinine, and donaxaridine [6], and it has also been found in many biologically active molecules such as convolutamydines [7] and convolutamydines A and B, which show potent antitumor activity against HL-60 cell line. It is an important structural motif in proteasome inhibitors [8]. With an active carboxyl group, isatin can easily transform to 3-hydroxyindole by an addition reaction [5,9]. We have reported the synthesis and bioactivity of several kinds of isatin derivatives [5,10,11]; to explore the bioactivity of the derivatives, a series of new compounds, 3-ethyl-3-hydroxy-indole-2-ones, were synthesized by an addition reaction, and then we studied the cytoprotectivity of H_2O_2-induced apoptosis of PC12 cells and the antitumor activity against A549 and P388 cell lines.

2. Experimental section

2.1. Materials

The agents and solvents of analytical reagent grade were purchased from Xi'an Chemical Co, Ltd and were used without any further purification. NMR characterization was

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performed using a Bruker DRX-400 spectrometer, 400 MHz for ^{1}H and 100 MHz for ^{13}C. Mass spectra were recorded using a Micromass Platform spectrometer with electron impact mode at 75 eV.

2.2. Synthesis of 3-ethyl-3-hydroxy-indole-2-ones

The addition reaction of Et_2Zn and common aldehyde ketone has been used for long time, but it was used for the first time in the addition of N-substituted isatin and Et_2Zn. In this study, 1 mmol Et_2Zn solution and 10 mL toluene were added in a dried flask protected under nitrogen atmosphere. The flask was placed into an ice-water bath cooled to 0 °C. Then 1 mmol N-substituted isatin was added to the flask, and the resulting solution was stirred at 0 °C for 4 h. After the mixture was warmed to room temperature, the reaction was quenched by adding a 10 mL saturated NH_4Cl aqueous solution. The aqueous layer was extracted with AcOEt (3 × 10 mL). The AcOEt solution was dried over anhydrous Na_2SO_4, and concentrated by vacuum. The target product was obtained by purification with silica gel flash column chromatography (hexane:AcOEt = 10:1 to 4:1). The reaction is summarized in Scheme 1.

2.3. Biological activity screening

The synthesized compounds were screened for their biological activities on protective activity of H_2O_2-induced apoptosis of PC12 cells and antitumor activity against A549 and P388 cell lines by the reported methods [5,12].

3. Results and discussion

3.1. Chemistry

The structure, yield, NMR, and MS data of the synthesized 3-ethyl-3-hydroxy-indole-2-ones compounds in this study are summarized in Table 1. From the results, it was found that all the reactions provide high yields except the reaction of Et_2Zn and *N*-2-oxo-2-ethoxy-ethyl isatin, which gave a yield of 65%. The reason may be due to the carboxyl group on the N-substitutent, which can react with Et_2Zn.

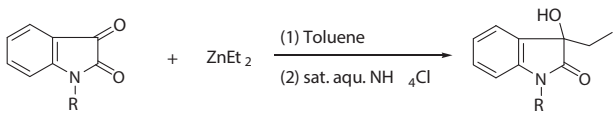
Nakazaki et al. [13] have reported the reaction between alkyllithium and *N*-phenyl isatin catalyzed by LiBr with a yield of 68–98%. It should be noted that the reaction was carried out at −78 °C, and use of cosolvents was required. Kumar et al. [14] investigated the reaction between trialkylaluminum without any catalyst at 70 °C and found the reaction is very rapid and yields are high. But more trialkylaluminum was needed than the reaction molar ratio, isatin:organoaluminum = 1:2.

The mechanism is described in Scheme 2. In this reaction, the Zn^{2+} coordinates with the O atom of the carboxyl group in isatin, by which the carboxyl group is activated. Then the $CH_3CH_2^-$ attacks the C atom of the activated carboxyl group, by a classical addition reaction process, and a zinc salt intermediate is formed accordingly. Through a similar process, the zinc salt activates another molecule of isatin and initiates another addition reaction, which results in a bi-oxindole zinc salt. Finally, the target product, 3-ethyl-3-hydroxy-indole-2-one, was produced by the hydroxylation process in a saturated aqueous solution of NH_4Cl.

3.2. Biological activity

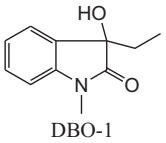
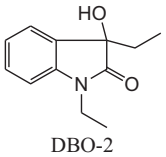
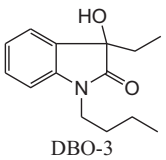
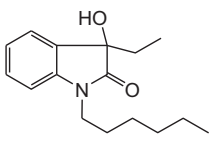
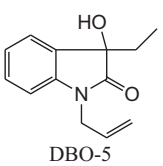
PC12 cell line derives from rat adrenal medulla, which has been used to get detailed information about diseases related to brain, such as hypoxia and Parkinson's disease. In our study, PC12 cell has been used to evaluate the neuroprotection activity of oxindole compounds [11]. In this article, the neuroprotection of the 3-ethyl-3-hydroxy-indole-2-ones in vitro screening results was evaluated, and the results are shown in Table 2. From the result, it was found that compounds DNO-2, DNO-3, and DNO-5 show some protection activity on the H_2O_2-induced apoptosis of PC12 cells with the higher activity than that of VE ((±)-α-tocopherol), with the percentage of 34.3%, 32.2%, and 28.8% at 2 μM, respectively. Also they are almost noncytotoxic against the PC12 cell at the concentration less than 2–20 μM, whereas DNO-1, DNO-2, DNO-6, and DNO-7 show high cytotoxicity at 200 μM. Concerning the structure of these compounds, the 3-ethyl-3-hydroxy-indole-2-ones with *N*-alkyl groups show high neuroprotection activity, and they are not cytotoxic against PC12 cell at the concentration less than 2–20 μM. The unsaturated C=C bond in the N-substituent group can enhance the protection activity, whereas the ester group and phenyl group can decrease the activity and lead to high cytotoxicity against PC12 cell.

Many isatin derivatives have been reported displaying high antitumor activity [15,16]. In this article, the antitumor activity of these compounds against A549 and P388 cell lines was screened, and the results are summarized in Table 3. From this table, we can find that almost all compounds show inhibition against both cancer cells at the concentration of 100 μM. DBO-3, DBO-4, DBO-5, DBO-6, and DBO-7 show high activity against A459 cell line, with the inhibition percentage more than 50% at 100 μM. Concerning the structures of these compounds, the long chains contribute to the high activity against the A459 cell line, and the highest activity of DNO-6 may be due to the ester group in the N-substituent group.



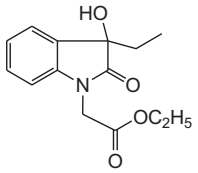
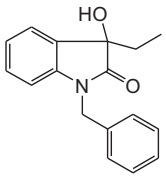
Scheme 1. The synthesis of 3-ethyl-3-hydroxy-indole-2-ones.

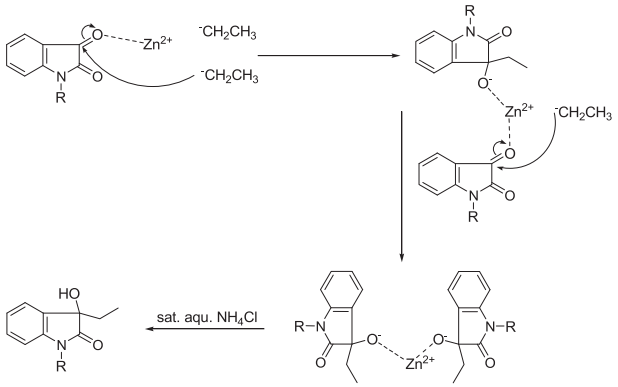
Table 1
The structure, yield, NMR, and MS of the synthesized 3-ethyl-3-hydroxy-indole-2-ones.

Compound structure and the number	Yield (%)	NMR and MS
 DBO-1	89	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.38 (1H, d, J = 7.6 Hz), 7.34 (1H, t, J = 7.6 Hz), 7.11 (1H, t, J = 7.6 Hz), 6.85 (1H, d, J = 7.6 Hz), 3.20 (3H, s), 3.04 (1H, s), 1.99–2.07 (1H, m), 0.75 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.3, 143.5, 129.7, 129.5, 128.7, 123.8, 123.1, 108.3, 31.4, 26.1, 7.5; MS (ESI) m/z: 214 (M^+)
 DBO-2	92	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.39 (1H, d, J = 7.2 Hz), 7.32 (1H, t, J = 7.6 Hz), 7.10 (1H, t, J = 7.6 Hz), 6.86 (1H, d, J = 7.6 Hz), 3.75–3.85 (1H, m), 3.63–3.71 (1H, m), 3.45 (1H, br), 2.00–2.03 (2H, m), 1.26 (3H, t, J = 7.2 Hz) 0.72 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.2, 143.6, 129.8, 129.5, 128.4, 124.0, 122.9, 108.4, 34.6, 31.7, 12.6, 7.6; MS (ESI) m/z: 228 (M^+)
 DBO-3	93	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.44 (1H, d, J = 7.2 Hz), 7.32 (1H, t, J = 7.6 Hz), 7.10 (1H, t, J = 7.6 Hz), 6.85 (1H, d, J = 7.6 Hz), 3.76–3.80 (1H, m), 3.58–3.62 (1H, m), 3.21 (1H, br), 2.00–2.04 (2H, m), 1.70 −1.73 (2H, m), 1.32–1.38 (2H, m), 0.91 (3H, t, J = 7.2 Hz), 0.73 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.1, 143.3, 129.7, 129.0, 123.9, 122.8, 108.5, 39.8, 31.6, 26.9, 22.4, 13.1, 7.6; MS (ESI) m/z: 256 (M^+)
 DBO-4	88	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.38 (1H, d, J = 7.6 Hz), 7.32 (1H, t, J = 7.6 Hz), 7.10 (1H, t, J = 7.6 Hz), 6.86 (1H, d, J = 8.0 Hz), 3.72–3.80 (1H, m), 3.57–3.65 (1H, m), 3.13 (1H, s), 1.90–2.18 (2H, m), 1.60 −1.66 (2H, m), 1.34–1.45 (4H, m), 0.95 (3H, t, J = 7.6 Hz), 0.73 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.1, 143.1, 129.9, 129.5, 123.9, 122.8, 108.6, 40.0, 31.7, 31.4, 27.3, 26.6, 22.5, 13.9, 7.6; MS (ESI) m/z: 284 (M^+)
 DBO-5	85	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.40 (1H, d, J = 7.6 Hz), 7.31 (1H, td, J = 7.6, 1.2 Hz), 7.10 (1H, t, J = 7.6 Hz), 6.84 (1H, d, J = 8.0 Hz), 5.79–5.85 (1H, m), 5.20–5.26 (2H, m), 4.25 (1H, dd, J = 15.6, 7.6 Hz), 4.20 (1H, dd, J = 15.6, 7.6 Hz), 3.11 (1H, s), 2.03 (2H, q, J = 7.6 Hz), 0.76 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.1, 142.8, 131.2, 129.7, 129.5, 123.9, 123.1, 117.7, 109.3, 42.3, 31.7, 7.7; MS (ESI) m/z: 240 ($M + Na^+$)

(*continued on next page*)

Table 1 (*continued*)

Compound structure and the number	Yield (%)	NMR and MS
 DBO-6	65	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.39 (1H, d, J = 7.6 Hz), 7.31 (1H, t, J = 7.16 Hz), 7.13 (1H, t, J = 7.6 Hz), 6.72 (1H, d, J = 7.6 Hz), 4.62 (1H, d, J = 17.6 Hz), 4.18–4.27 (3H, m), 3.0 (1H, s), 2.04 (2H, q, J = 7.2 Hz), 1.25 (3H, t, J = 7.2 Hz), 0.77 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.1, 167.5, 142.2, 129.6, 129.5, 124.0, 123.4, 108.3, 61.8, 41.3, 31.7, 14.1, 7.5; MS (ESI) m/z: 286 ($M + Na^+$)
 DBO-7	87	^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.39 (1H, d, J = 7.6 Hz), 7.19–7.30 (6H, m), 7.07 (1H, d, J = 8.0 Hz), 5.02 (1H, d, J = 15.6 Hz), 4.76 (1H, d, J = 15.6 Hz), 3.06 (1H, s), 2.03–3.01 (2H, m), 0.79 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 178.4, 142.7, 129.7, 129.5, 128.8, 128.6, 127.7, 127.6, 126.7, 123.9, 123.1, 109.4, 43.8, 31.7, 7.7; MS (ESI) m/z: 290 (M^+)



Scheme 2. The reaction between Et_2Zn and N-substituted isatin.

Table 2
Inhibitory and protective effects of 3-ethyl-3-hydroxy-indole-2-ones on PC12 cell.

Compound	Inhibitory effect[a] (%)			Protective effect[b] (%)		
	200 μM	20 μM	2 μM	200 μM	20 μM	2 μM
DBO-1	23.6	0	0	–	25.1	15.4
DBO-2	33.9	0	0	–	36.5	34.3
DBO-3	0	0	0	0	37.7	32.2
DBO-4	0	0	0	49.0	8.4	2.7
DBO-5	0	0	5.7	0	53.9	28.8
DBO-6	37.6	16.5	9.3	–	0	1.8
DBO-7	84.4	11.9	5.9	–	0	11.9
VE			0.0			22.5

[a] Inhibition against PC12 cell growth.
[b] Protective effect on the H_2O_2-induced apoptosis of PC12 cells.

Table 3
In vitro cytotoxicity against A549 and P388 cell lines.

Compound	P388 (%)		A459 (%)	
	100 μM	10 μM	100 μM	10 μM
DBO-1	38.9	20.5	40.5	8.2
DBO-2	36.8	19.0	47.3	9.8
DBO-3	32.6	16.7	55.8	13.9
DBO-4	20.0	6.7	64.1	14.1
DBO-5	13.6	13.7	62.0	14.4
DBO-6	35.4	17.1	70.2	12.6
DBO-7	22.8	21.3	54.8	9.5

4. Conclusions

A series of 3-ethyl-3-hydroxy-indole-2-ones were synthesized through the autocatalytic addition reaction of Et_2Zn and N-substituted isatin and characterized by NMR and MS. The antitumor and neuroprotection activities of these compounds were evaluated and showed protection activity on the apoptosis of PC12 cells induced by H_2O_2, which are more effective than that of (±)-α-tocopherol Vitamin E (VE) at 2 and 20 μM. Moreover, these compounds also showed antitumor activity against A549 and P388 cell lines.

Acknowledgments

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