Synthesis and Bioactivity Evaluation of 3-Hydroxy-3-(phenylethynyl)indol-2-one Analogues

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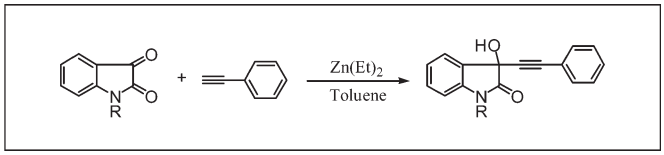

A series of propargylic alcohol was synthesized by the addition of phenylacetylene to isatin and its N-substituted derivatives for the first time. This reaction involves activation of zinc reagent *via* coordination with carbonyl substrates that behave ''ligand like.'' The bioactivities on protective effect on the apoptosis of PC12 cells induced by H_2O_2 and cytotoxicity against lung cancer A549 and P388 cell line of these compounds were investigated, and several compounds showed potent activities.

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INTRODUCTION

The reactions, which lead to carbon–carbon bond formation by addition of nucleophiles to carbonyl group electrophiles, are of great importance in the continuing development of efficient processes for synthesis. Addition of acetylides to carbonyl substrates has attracted the attention of organic chemists and is still a very active field of organic reactions. The reaction gives access to preparation of propargylic alcohols, which are well known as versatile building blocks in the synthesis of complex natural products [1,2] and can be transformed into other functional groups, such as chalcones [3], and oxasilacyclopentene [4,5]. The addition of alkynes to ketones is also a very useful practical strategy to create tertiary alcohols with a new stereogenic center [6–8].

Some natural products with the structure of propargylic alcohol showed potent bioactivities, such as panaxyno1 (PNN), which performs potent activities on neuro-protection [9] and antitumor [10]. Isatin has been found in many natural products, with the function of modulating biochemical processes [11–13]. The advances in the use of isatin for organic synthesis and a survey of its biological activities are continuously reported [14–16]. In 1963, Ried reported the synthesis of 3-hydroxy-3-(phenyl-ethynyl)indol-2-one for the first time, with a low yield of 28% [17]. But in the following years, there was no further attention paid to the synthesis and its bioactivity.

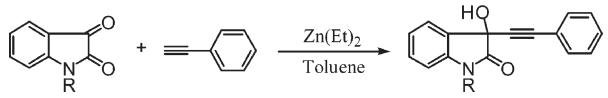
In this article, we describe the addition of phenyl-acetylene to isatin and (*N*-substituted) isatins promoted by Et_2Zn without employing specific ligands to form 3-hydroxy-3-(phenylethynyl)indol-2-one analogues (Scheme 1). Furthermore, the bioactivity on neuroprotection and antitumor of these isatin derivatives was evaluated for the first time.

RESULTS AND DISCUSSION

Chemistry. The *in situ* generated $ZnMe_2$-acetylides from Me_2Zn and acetylides have been used in a direct addition process with aldehydes and ketones without the employment of specific ligands [18]. In our preliminary studies, the reaction was carried out at room temperature. Unfortunately, the outcome was found very complex (by TLC), and the desired product was in a low yield after purification by flash chromatography. Higher yields were obtained at lower temperatures. Also, a variety of substrates were examined at 0°C, and the results are summarized in Table 1.

It can be found from Table 1 that the temperature deeply effects the reaction of the addition of phenyl-acetylene to (*N*-methyl) isatin. The reaction at relatively higher temperature (rt) gave poor yield, and the yields increased as the temperature is decreased, and the yield

Scheme 1



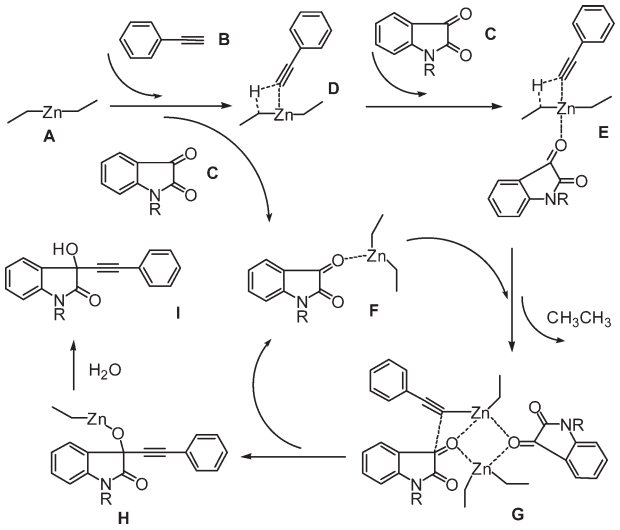
was highest at 0°C. Significantly, lower temperatures such as −10°C did not improve the yield.

In consideration of the accepted mechanism for ligand-assisted addition of Et_2Zn to aldehydes, Pier *et al*. [18] investigated the possibility that a zinc-ketone complex acts as a bifunctional catalyst, bringing together an additional 1 equiv of ketone and zinc reagent in a bicyclic transition state (TS). On the basis of the proposed mechanism, we assumed that this reaction involves activation of the zinc reagent *via* coordination with isatins. The activated TS are shown in Scheme 2. In the TS (G), two molecules of (*N*-substituted) isatin behaved in a ''ligand like'' fashion to activate a phenylacetylene by two oxygen atoms coordinating with two Zn as bridged-atoms. The addition of activated phenylacetylene to a neighbor carbonyl formed H, and F was released into another cycle. The desired product (I) is provided through hydrolysis in the final step of the reaction.

Because the substrates have more than one coordinative and reactive point, the TS might be more complex, which lead to several side reactions, the addition of ethyl to isatins can be considered as an example. We could find the yields were very low in entries 7 and 11 because of the more reactive substrates. (*N*-Acyl) isatins (entries 12 and 13) were also introduced into this reaction, but the product was too complex to determine whether there was a desired product.

Biological activity. Compounds 2–9 were screened for their biological activities on protective effect on the

Scheme 2



apoptosis of PC12 cells induced by H_2O_2 and cytotoxicity against A549 and P388 cell line at various concentrations by the reported methods [19,20]. The neuroprotection and cytotoxicity in *vitro* screening results are shown in Table 2.

From the table, we find that compounds **4**, **5**, and **6** showed potent activity, which were more effective than VE ((±) α-Tocophreol), with the percentage of 46.62, 65.93, and 35.89% at 2 μg/mL respectively, as compound **5** was not cytotoxic against PC12 cell under the concentration of 2–200 μg/mL, whereas compounds **4** and **6** showed some cytotoxicity. Other compounds were either inactive or cytotoxic against PC12 cell. The results of in *vitro* cytotoxicity of compounds 2–9 against A549 and P388 cell line were given in Table 3.

From the results, it was found that almost all compounds could inhibit both cancer cells effectively at the concentration of 100 μ*M*. Compounds **6** and **8** showed

Table 1

Alkynylation of (*N*-substituted)isatins with phenylacetylene using Et_2Zn as promoter.

Entry	R	Time (h)	Temp. (°C)	Yield (%)	Compound
1	H	12	0	38.7	1
2	CH_3	12	25	8.1	2
3	CH_3	12	10	31.3	2
4	CH_3	12	0	58.1	2
5	CH_3	24	−10	51.6	2
6	C_2H_5	12	0	66.7	3
7	$(CH_2)_2CH_3$	12	0	60	4
8	$(CH_2)_3CH_3$	12	0	70.2	5
9	$(CH_2)_5CH_3$	12	0	71.3	6
10	CH_2CHCH_2	12	0	40.5	7
11	Bn	12	0	67.9	8
12	$CH_2CO_2C_2H_5$	12	0	35.8	9
13	$COCH_3$	12	0	/	/
14	$COCH_3CH_3$	12	0	/	/

Table 2

Protective effect on the apoptosis of PC12 cells induced by H_2O_2.

	Inhibition of PC12 cell (%)			Inhibition of apoptosis of PC12 cells induced by H_2O_2 (%)		
Compound	200 μ*M*	20 μ*M*	2 μ*M*	200 μ*M*	20 μ*M*	2 μ*M*
2	55.56	18.43	26.61	–	–	–
3	54.36	3.59	15.02	–	0	0
4	18.77	0	3.53	–	43.70	46.62
5	0.00	0	0	44.20	62.88	65.93
6	90.06	14.67	5.63	–	0	35.89
7	89.33	5.66	2.99	–	0	0
8	89.51	7.41	2.36	–	0	0
9	29.56	14	9.07	–	0	0
VE						22.46

Table 3

In vitro cytotoxicity against A549 and P388 cell line.

	P388 (%)		A459 (%)	
Compound	100 μ*M*	10 μ*M*	100 μ*M*	10 μ*M*
2	48.7	9.4	75.6	12.2
3	54.6	4.5	58.5	0
4	54.0	10.1	50.7	0
5	17.8	8.1	55.8	0
6	97.2	0	95.3	3.7
7	61.4	2.5	76.5	9.8
8	68.7	5.6	76.0	14.2
9	97.9	14.4	85.9	3.2

potent activity, with the inhibition percentage of 97.2, 95.3 and 97.9, 85.9, only compound **5** was inactive against P388.

EXPERIMENTAL

In an oven-dried flask connected to a nitrogen/vacuum line was placed phenylacetylene (1.6 mmol) followed by the slow addition of Et_2Zn 1*M* solution in toluene (1.5 mmol, 1.5 mL) (2.5 mmol Et_2Zn for isatin). The resulting solution was stirred at room temperature for 1 h and then cooled to 0°C with an ice-water bath. The (*N*-substituted) isatin (1 mmol) was added to the reaction mixture. The resulting solution was stirred at 0°C for 12 h, and then warmed to room temperature. The reaction was quenched with water (10 mL). The mixture was stirred for 10 min and then filtered over Celite. The mixture evaporated under reduced pressure and was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate = 10: 1–4: 1).

3-Hydroxy-3-(phenylethynyl)indolin-2-one (1). ^{1}H NMR (CD_3OD, 400 MHz), δ: 7.50 (1H, d, J = 7.2 Hz), 7.43 (2H, m), 7.30–7.33 (4H, m), 7.10 (1H, m), 6.91 (1H, d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 177.6, 142.4, 132.8, 132.2, 131.3, 130.0, 129.5, 125.6, 124.2, 123.3, 111.5, 87.3, 86.3; MS (ESI): *m*/*z* 272 (M + Na^+). HR-ESI-MS: *m*/*z* 272.0684 (calcd. for $C_{16}H_{11}NNaO_2^+$ 272.0687); Anal. Calcd for $C_{16}H_{11}NO_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 77.33; H, 4.14, N, 5.52.

1-Methyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (2). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.62 (1H, d, J = 6.8 Hz), 7.43 (2H, d, J = 6.8 Hz), 7.38 (1H, t, J = 7.6 Hz), 7.24–7.30 (3H, m), 7.16 (1H, t, J = 7.6 Hz), 6.85 (1H, d, J = 8.0 Hz), 4.05 (1H, s), 3.23 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.9, 142.9, 132.0, 130.5, 128.9, 128.8, 128.2, 124.7, 123.7, 121.5, 108.8, 86.3, 85.4, 69.5, 26.6; MS (ESI): *m*/*z* 286 (M + Na^+). HR-ESI-MS: *m*/*z* 286.08849 (calcd. for $C_{17}H_{13}NNaO_2^+$ 286.0844); Anal. Calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.43; H, 4.77, N, 5.23.

1-Ethyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (3). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.62 (1H, d, J = 7.6 Hz), 7.27–7.45 (6H, m), 7.16 (1H, t, J = 7.6 Hz), 6.89 (1H, d, J = 7.6 Hz), 3.78 (2H, m), 3.75 (1H, s), 1.31 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.5, 142.2, 132.0, 130.4, 129.2, 128.9, 128.1, 124.9, 123.5, 121.7, 108.9, 86.2, 85.6, 35.2, 12.4; MS (ESI): *m*/*z* 300 (M + Na^+). HR-ESI-MS: *m*/*z* 300.1006 (calcd. for $C_{18}H_{15}NNaO_2^+$ 300.1000); Anal. Calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.78; H, 5.41, N, 5.11.

1-Propyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (4). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.62 (1H, d, J = 7.6 Hz), 7.27–7.45 (6H, m), 7.16 (1H, t, J = 7.6 Hz), 6.89 (1H, d, J = 7.6 Hz), 3.78 (2H, m), 3.75 (1H, s), 1.31 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.5, 142.2, 132.0, 130.4, 129.2, 128.9, 128.1, 124.9, 123.5, 121.7, 108.9, 86.2, 85.6, 35.2, 12.4; MS (ESI): *m*/*z* 314 (M + Na^+). HR-ESI-MS: *m*/*z* 314.1158 (calcd. for $C_{19}H_{17}NNaO_2^+$ 314.1157); Anal. Calcd for $C_{19}H_{17}NO_2$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.30; H, 5.76, N, 4.45.

1-Butyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (5). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.62 (1H, d, J = 7.2 Hz), 7.25–7.44 (6H, m), 7.14 (1H, t, J = 7.6 Hz), 6.87 (1H, d, J = 8.0 Hz), 3.83 (1H, s), 3.68 (2H, m), 7.74 (2H, m), 0.98 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.7, 142.3, 132.1, 130.3, 129.2, 128.9, 128.1, 124.9, 123.5, 121.7, 108.9, 86.2, 85.7, 33.2, 25.7, 12.8; MS (ESI) *m*/*z*: 328 (M + Na^+). HR-ESI-MS: *m*/*z* 328.1318 (calcd. for $C_{20}H_{19}NNaO_2^+$ 328.1313); Anal. Calcd for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27; N, 4.59. Found: C, 78.67; H, 6.33, N, 4.42.

1-Hexyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (6). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.61 (1H, d, J = 7.6 Hz), 7.43 (2H, m), 7.37 (1H, t, J = 8.0 Hz), 7.30 (2H, m), 7.15 (1H, t, J = 7.6 Hz), 6.88 (2H, d, J = 8.0 Hz), 3.71 (2H, t, J = 6.8 Hz), 3.64 (1H, s), 1.70 (3H, m), 1.25–1.38 (5H, m), 0.87 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.9, 142.4, 132.1, 130.1, 129.0, 128.9, 128.2, 124.9, 123.5, 121.8, 109.1, 86.1, 85.6, 69.5, 48.6, 40.0, 31.1, 28.9, 26.8, 22.3; MS (ESI): *m*/*z* 356 (M + Na^+). HR-ESI-MS: *m*/*z* 356.1625 (calcd. for $C_{22}H_{23}NNaO_2^+$ 356.1626); Anal. Calcd for $C_{22}H_{23}NO_2$: C, 79.25; H, 6.95; N, 4.20. Found: C, 79.31; H, 6.78, N, 4.41.

1-Allyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (7). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.62 (1H, d, J = 7.6 Hz), 7.44 (2H, d, J = 7.6 Hz), 7.26–7.36 (4H, m), 7.15 (1H, t, J = 7.6 Hz), 6.86 (1H, d, J = 7.6 Hz), 5.85 (1H, m), 5.26 (2H, m), 4.36 (2H, m), 3.80 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.8, 143.0, 130.6, 133.1, 131.3, 129.2, 128.4, 128.2, 124.9, 123.6, 121.4, 118.8, 109.4, 86.2, 85.5, 69.5, 43.3; MS (ESI): *m*/*z* 312 (M + Na^+). HR-ESI-MS: *m*/*z* 312.9997 (calcd. for $C_{19}H_{15}NNaO_2^+$ 312.1000); Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 78.66; H, 5.15, N, 4.77.

1-Benzyl-3-hydroxy-3-(phenylethynyl)indolin-2-one (8). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.62 (1H, d, J = 7.2 Hz), 7.45 (2H, d, J = 7.6 Hz), 7.23–7.33 (4H, m), 7.12 (1H, t, J = 7.6 Hz), 6.72 (1H, d, J = 7.6 Hz), 4.94 (2H, s), 3.80 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 174.1, 142.2, 135.0, 132.0, 130.3, 128.9, 128.8, 128.2, 127.7, 127.1, 124.8, 123.7, 121.6, 108.9, 86.5, 85.5, 69.6, 44.1. MS (ESI): *m*/*z* 362 (M + Na^+). HR-ESI-MS: *m*/*z* 362.1157 (calcd. for $C_{23}H_{17}NNaO_2^+$ 362.1157); Anal. Calcd for $C_{23}H_{17}NO_2$: C, 81.40; H, 5.05; N, 4.13. Found: C, 81.51; H, 5.13, N, 4.22.

Ethyl-2-(3-hydroxy-2-oxo-3-(phenylethynyl)indolin-1-yl)-acetate (9). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.64 (1H, d, J = 7.6 Hz), 7.43 (2H, d, J = 7.2 Hz), 7.25–7.37 (4H, m), 7.17 (1H, t, J = 7.6 Hz), 7.66 (1H, d, J = 8.0 Hz), 4.48 (2H, s), 4.21 (2H, q, J = 7.2 Hz), 3.76 (1H, s), 1.25 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.8, 167.1, 141.2,

132.1, 130.5, 129.0, 128.7, 128.2, 124.9, 124.1, 121.5, 108.9, 86.7, 85.1, 69.4, 61.9, 41.7, 14.1; MS (ESI): *m*/*z* 358 ($M + Na^+$). HR-ESI-MS: *m*/*z* 358.1054 (calcd. for $C_{20}H_{17}NNaO_4^+$ 358.1055); Anal. Calcd for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11; N, 4.18; O, 19.08. Found: C, 71.58; H, 5.20, N, 4.23.

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