Simple isatin derivatives as free radical scavengers: Synthesis, biological evaluation and structure-activity relationship

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Abstract
To develop more potent small molecules with enhanced free radical scavenger properties, a series of N-substituted isatin derivatives was synthesized, and the cytoprotective effect on the apoptosis of PC12 cells induced by H₂O₂ was screened. All these compounds were found to be active, and N-ethyl isatin was found with the most potent activity of 69.7% protective effect on PC12 cells. Structure-activity relationship analyses showed the bioactivity of N-alkyl isatins decline as the increasing of the chain of the alkyl group, furthermore odd-even effect was found in the activity, which is interesting for further investigation.

Background
Oxidative stress has been implicated as a major role in the onset and progression of a vast variety of clinical abnormalities including neurodegenerative disorders. Free radicals play important roles in many physiological and pathological conditions [1]. In general, the generation and scavenging of oxygen free radicals is balanced and any imbalance or excessive amounts of active radicals may contribute to disease development. It has been found that free radical reactions can produce deleterious modifications in membranes, proteins, enzymes, and DNA [2], increasing the risk of diseases such as cancer [3], Alzheimer’s [4], Parkinson’s [5], angiocardiopathy [6], arthritis [7], asthma [8], diabetes [9], and degenerative eye disease [10]. Therefore, it is important to find effective scavengers of free radicals for prevention and treatment of such disorders.

Isatin is an endogenous indole present in mammalian tissues and fluids [11]. The substance was initially discovered as a component of endogenous monoamine oxidase (MAO) inhibitory activity, tribulin, and subsequently identified as a selective inhibitor of MAO B [12]. Further investigations have shown that isatin acts as an antagonist of both atrial natriuretic peptide-stimulated and nitric oxide-stimulated guanylate cyclase activity [13-15]. Isatin has a distinct and discontinuous distribution in rat brain and other tissues; the highest concentrations in the brain are found in the hippocampus and cerebellum [10]. Many Isatin derivatives, such as isatin hydrazono, isatin Mannich bases, isatin based spiroazetidinones and 3-(methylene)indolin-2-ones, have also been reported to possess neuroprotection activity [16-19].

To develop more potent small molecules with enhanced free radical scavenger properties, a series of N-substituted isatin derivatives was synthesized by substitution reactions (as shown in Scheme 1), and the cytoprotective effect on the apoptosis of PC12 cells induced by H₂O₂ was screened.

Results and Discussion

Chemistry
The N-substituted isatin derivatives were synthesized by reactions of substitution reaction. The reaction between isatin and halohydrocarbon has been reported being carried out in the presence of NaOEt using EtOH as solvent or in the presence of NaH using DMF as solvent [16]. The reactants and the solvents involved in the reactions must be anhydrous. To develop a simple method to synthesize N-substituted isatin derivatives, we firstly screened the effect of the base and solvent on the yield of the reaction of isatin and bromoethane (C₂H₅Br), and the results was shown in Table 1.
In this reaction, the protons transfers from N-H (a Brøsted acid) to a Brøsted or Lewis base via the hydrogen-bonded covalent and ionic complexes [20], producing the isatin anion which is the nucleophilic reactant to the halohydrocarbon. Higher solvent polarity can promote the proton-transfer equilibrium and leads to the higher yield [20]. From this table, it can be found that K₂CO₃-DMF system was an effective promotion for this reaction and other base-solvent systems were not effective with the yield no more than 60%. The possible reason might be that weak base can not help the proton transfer at the beginning effectively, but the too strong bases will lead to the substitution reaction between bromoethane and OH⁻. DMF exhibits the highest yield of 89% with K₂CO₃ for its highest solvent polarity, so the K₂CO₃-DMF was selected as the reactant reaction system in the following synthesis, and the results were shown in Table 2.

**Bioactivity**

The chemical modification of lead compound 1, focusing on the N-substituent, was carried out to further improve the free scavenging ability. A series of new N-substituted isatin derivatives (compounds 2-12) was synthesized. The free radical scavenging properties of these derivatives were evaluated to elucidate structure-activity relationships. The protective effect on the apoptosis of PC12 cells induced by H₂O₂ by free radical scavenging of these compounds against H₂O₂ were evaluated by cell survival assay in PC12 cells using a reported method [21]. The results were given in Table 3.

From the table, we can find almost all of the compounds showed potent activity at the condensation of 2 μg/ml, which were more effective than VE ((±) α-Tocopherol with the percentage of 22.5%). There is a noteworthy phenomenon that the activities of all compounds at the condensation of 2 μg/ml are more potent than that at the condensation of 20 μg/ml, and the mechanism will be interesting for the further investigation. Compound 3 and 8 exhibited the most potent activity with the protective effect of 69.8% and 69.5% at the condensation of 2 μg/ml respectively, which are more potent that that at the condensation of 20 μg/ml.

Almost all of these compounds were weakly cytotoxic to PC12 cells at the concentrations of 2-20 μg/ml except compound 11 and 12. Almost all compounds are cytotoxic to PC12 cells at the concentrations of 200 μg/ml, the PC12 cells inhibitory effects are more than 40%. Based on the factors, we can conclude the addition of halogenous atom in the substituents (compound 11 and

### Table 1 The substitution reaction between isatin and bromoethane

| No. | Solvent | Base   | Time (h) | Yield (%) |
|-----|---------|--------|----------|-----------|
| 1   | DMF     | Na₂CO₃ | 24       | 33        |
| 2   | DMF     | K₂CO₃  | 12       | 89        |
| 3   | DMF     | NaOH   | 12       | 40        |
| 4   | DMF     | KOH    | 12       | 41        |
| 5   | DMF     | TEA    | 24       | 60        |
| 6   | MeOH    | K₂CO₃  | 24       | 12        |
| 7   | THF     | K₂CO₃  | 24       | 25        |
| 8   | DCM     | K₂CO₃  | 24       | 19        |
| 9   | Acetonitrile | K₂CO₃ | 24       | 15        |

### Table 2 Synthesis of N-substituted isatin derivatives

| Compound | R          | Time (h) | Yield (%) |
|----------|------------|----------|-----------|
| 1        | H          | 24       | 33        |
| 2        | CH₃        | 4        | 87        |
| 3        | C₂H₅       | 12       | 89        |
| 4        | (CH₂)₂CH₂  | 12       | 93        |
| 5        | (CH₂)₃CH₃  | 24       | 90        |
| 6        | (CH₂)₄CH₄  | 24       | 95        |
| 7        | CH₂CH = CH₂| 12       | 93        |
| 8        | CH₂C₂H₅    | 12       | 90        |
| 9        | CH₂COOC₂H₅| 12       | 93        |
| 10       | C₂H₄Cl     | 24       | 79        |
| 11       | C₃H₄Br     | 24       | 83        |

### Table 3 Inhibitory and protective effects of N-substituted isatin derivatives

| Compound | Inhibitory effect/%<sup>a</sup> | Protective effect/%<sup>b</sup> |
|----------|---------------------------------|---------------------------------|
|          | 200 μg/ml | 20 μg/ml | 2 μg/ml | 200 μg/ml | 20 μg/ml | 2 μg/ml |
| 1        | 92.0   | 0.0   | 2.6   | -        | 20.1    | 40.4    |
| 2        | 60.6   | 2.7   | 5.0   | -        | 31.4    | 50.9    |
| 3        | 45.6   | 0.4   | 7.0   | -        | 38.2    | 69.8    |
| 4        | 43.0   | 3.3   | 0.0   | -        | 10.1    | 39.7    |
| 5        | 53.3   | 0.0   | 0.0   | -        | 30.0    | 60.8    |
| 6        | 51.9   | 0.0   | 0.0   | -        | 8.2     | 24.1    |
| 7        | 50.4   | 0.0   | 0.0   | 0        | 15.5    | 20.9    |
| 8        | 54.0   | 2.1   | 0.0   | -        | 14.8    | 69.5    |
| 9        | 63.1   | 0.0   | 0.0   | -        | 9.3     | 51.3    |
| 10       | 67.9   | 0.0   | 5.3   | -        | 46.6    | 54.5    |
| 11       | 63.5   | 20.5  | 5.6   | -        | 0       | 25.1    |
| 12       | 61.6   | 24.1  | 12.3  | -        | 0       | 62.1    |
| VE       | 0.0    | 22.5  |       |          |         |         |

<sup>a</sup> Inhibition of PC12 cell growth; <sup>b</sup> protective effect on the apoptosis of PC12 cells induced by H₂O₂.
enhance the cytotoxicity at the concentrations of 2-
20 μg/ml.

The substitution reaction between isatin and halohy-
drocarbon (C1 to C6) gave compounds 2-7, which pro-
vided the appropriate material for the structure-activity
relationship analyses. The cytoprotective activities of N-
substituted isatin derivatives with the alkyl group con-
taining one to six carbon atoms were shown in Figure 1.
The activity approximately declines as the increase of
the chain of the alkyl group. With a further analysis, it
was found that there was a clear odd-even effect in
these activities. The activities of N-substituted isatin
derivatives with odd carbon atoms alkyl group (one,
three and five carbon atoms, corresponding compound
2, 4 and 6, marked with solid pillars in Figure 1) decline
as the chain of the alkyl group increases, and the same
regulation can be found in the activities of the N-sub-
stituted isatin derivatives with even carbon atoms alkyl
group (two, four and six carbon atoms, corresponding
compound 3, 5 and 7, marked with virtual pillars in Fig-
ure 1). This regulation exhibits both under the conden-
sation of 2 μg/ml and 20 μg/ml, and the activities of N-
substituted isatin derivatives with even carbon atoms
alkyl group are more potent than the that of N-substi-
tuted isatin derivatives with parallel odd carbon atoms
alkyl group. Besides, by the structure-activity relation-
ship analyses, it was found that the unsaturated bond of
the substituent (compound 8-10) can improve the activ-
ity compared with the other substituents with similar
carbon atoms.

Experimental
All starting materials and solvents (A.R. grade) were
commercially available and were used without further
purification. NMR spectra were recorded using a Bruker
Drx-400 spectrometer operating at 400 MHz for 1H.
Mass spectra were recorded on a Micromass Platform
spectrometer using a direct-inlet system operating in the
electron impact (EI) mode at 75 eV. Elemental analyses
were obtained using a Carlo Erba 1106 elemental
analyzer.

General synthesis of N-alkyl substituted isatin derivatives
Isatin (1 mmol) and halohydrocarbon (1.2 mmol) were
dissolved in DMF (20 ml), and 3 mmol anhydrous
K2CO3 was added. The mixture was stirred under room
temperature until the disappearance of isatin, as evi-
denced by thin-layer chromatography. The solvent was
removed in vacuo and the residue was separated by col-
umn chromatography (silica gel, petroleum ether/ethyl
acetate = 20:1), giving N-alkyl substituted isatin com-
 pound (compound 2-12).

1-Methylindoline-2,3-dione (Compound 2) 1H-NMR
(D6-DMSO, 400 MHz): 7.66 (1 H, td, J = 1.2, 7.6 Hz),
7.52 (1 H, d, J = 7.6 Hz), 7.12 (2 H, t, J =7.6 Hz), 3.12
(3 H, s); MS (EI) m/z: 161 (M+); Anal. Found: C, 67.01;
H, 4.40; N, 8.66 (%). Calc. for (C9H7NO2): C, 67.07; H,
4.38; N, 8.69 (%).

1-Ethylindoline-2,3-dione (Compound 3) 1H-NMR
(CDCl3, 400 MHz): 7.57 (2 H, m), 7.09 (1 H, t, J = 7.6
Hz), 6.89 (1 H, d, J = 7.6 Hz), 3.76 (2 H, q, J = 7.6 Hz),
1.29 (3 H, t, J = 7.6 Hz); MS (EI) m/z: 175 (M+); Anal.

Figure 1 The cytoprotective activities of N-substituted isatin derivatives with the alkyl group containing 1-6 carbon atoms (The corresponding compounds are compounds 2-7.)
Found: C, 68.59; H, 5.22; N, 8.01 (%). Calc. for (C10H8NO2): C, 70.92; H, 6.54; N, 5.90 (%). Calc. for (C11H11NO2): C, 70.58; H, 4.67; N, 5.90 (%). Calc. for (C15H11NO2): C, 75.94; H, 4.65; N, 5.90 (%).

1-Benzoylindoline-2,3-dione (Compound 9) 1H-NMR (D6-DMSO, 400 MHz): 7.56 (2 H, m), 7.42 (2 H, d, J = 7.6 Hz), 7.30 (2 H, t, J = 7.6 Hz), 7.27 (1 H, m), 7.10 (1 H, t, J = 7.6 Hz), 6.96 (1 H, m), 4.90 (2 H, s); MS (EI) m/z 233 (M+); Anal. Found: C, 75.99; H, 4.65; N, 5.90 (%). Calc. for (C14H13NO2): C, 75.94; H, 4.67; N, 5.90 (%).

Ethyl 2-(2,3-dioxindolin-1-yl)acetate (Compound 10) 1H-NMR (CDCl3, 400 MHz): 7.62 (1 H, d, J = 7.6 Hz), 7.57 (1 H, t, J = 7.6 Hz), 7.14 (1 H, t, J = 7.6 Hz), 6.77 (1 H, d, J = 7.6 Hz), 4.47 (2 H, s), 4.22 (2 H, q, J = 7.2 Hz), 1.26 (3 H, t, J = 7.2 Hz); MS (EI) m/z 233 (M+); Anal. Found: C, 61.84; H, 4.72; N, 6.00 (%). Calc. for (C12H13NO2): C, 61.80; H, 4.75; N, 6.01 (%).

1-2-Chloroethylindoline-2,3-dione (Compound 11) 1H-NMR (D6-DMSO, 400 MHz): 7.67 (1 H, td, J = 9.12 Hz), 7.56 (1 H, dd, J = 7.6, 1.2 Hz), 7.29 (1 H, d, J = 8.0 Hz), 7.14 (1 H, dd, J = 7.6, 0.8 Hz), 4.10 (2 H, t, J = 6.4 Hz), 3.70 (2 H, t, J = 6.4 Hz); MS (EI) m/z 211 (M+);

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Authors’ contributions
GC has formulated the research idea and prepared the manuscript draft version,YW prepared the manuscript for submission and coordinated further formalities, SM and QS carried out the chemical and biological studies, XH conceived of the study, participated in its design and coordination. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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