

Free Radical Scavenging Activity of Some Isatin-5-Sulphonamide Derivatives

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Summary

The present study was aimed at evaluating the *In vitro* free radical scavenging activity of some new isatin-5-sulphonamide derivatives using DPPH, Hydrogen peroxide, and Nitric oxide methods. All the five test compounds showed dose dependent free radical scavenging activity. Among the tested compounds I_e and I_c exhibited most promising anti-oxidant activity than other compounds and which are compared with standard.drug.

Key words: Antioxidant activity, Isatin-5-Sulphonamide derivatives, free radical scavenging activity

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INTRODUCTION

The imbalance between reactive oxygen species (ROS) and antioxidant defense mechanism leads to oxidative modifications in cellular membrane or intracellular molecules (1). ROS are continuously produced during normal physiological events and are removed by antioxidant defense mechanisms (2). O_2^- is, an oxygen-centered radical with selective reactivity, produced by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. H_2O_2 is non free radical species and can be formed *in vivo* by many oxidize enzymes such as superoxide dismutase (3). Under pathological conditions, ROS are overproduced and results in lipid peroxidation and oxidative stress (1). Antioxidant based drugs or formulations are used for the treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (4) to prevent damage to cellular component arising as a consequence of chemical reactions involving free radicals (5).

Isatin (1H-indole-2, 3- dione) was first discovered by Erdmann and Laurent in 1841, independently as a product from oxidation of indigo by nitric and chromic acids. Isatin (1H-indole-2, 3- dione) is a versatile molecule and possesses wide range of biological activities. In the present study, we selected some previously synthesized isatin-5-sulphonamide derivatives (prepared by chlorosulphonation of isatin to prepare isatin-5-sulphonic acid chloride and it is subjected to reaction with different amines or anilines to form respective sulphonamide derivatives) and screened for the *In vitro* free radical scavenging activity.

MATERIALS AND METHODS

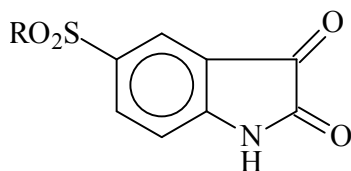
Materials: 1,1-Diphenyl-2 picryl hydrazyl (DPPH), Curcumin were obtained from Sigma Aldrich Co., St.Louis, USA. Phosphate buffer Saline (PBS) was obtained from Himedia, Mumbai, India and Ascorbic acid and other chemicals were purchased from SD fine chemicals Ltd., Mumbai, India. All chemicals used were of analytical grade.

Test compounds: In the present study, we screened five (Ia to Ie) isatin-5-sulphonamide derivatives (prepared by chlorosulphonation of isatin to prepare isatin-5-sulphonic acid chloride and it is subjected to reaction with different amines or anilines to form respective sulphonamide derivatives) for the *in vitro* free radical scavenging activity. The physical data of test compounds were shown in table-1.

Scavenging of Diphenyl Picryl Hydrazyl (DPPH) radicals

The free radical scavenging activity of compounds was measured by DPPH using the method of Blios (6). To the 0.1 ml of different concentrations (0.01 to 10 mg/ml) of test compounds, 2.5 ml of methanol and 0.5 ml of 0.2mM DPPH solutions were added and mixed thoroughly and the absorbance was read at 517 nm against blank. Ascorbic acid was used as a reference standard. The IC_{50} (Inhibitory concentration) is the concentration of sample required to scavenge 50% of DPPH free radicals.

Table I: Physical data of Isatin-5-Sulphonamide derivatives



S.No.	Compound	R	Mol. Formula	Mol. Wt	m.p. (°C)	% yield
1	Ia		C ₁₀ H ₁₀ N ₂ O ₄ S	254.2	170-72	82
2	Ib	-NHC ₂ H ₅	C ₁₀ H ₁₀ N ₂ O ₄ S	254.2	165-67	81
3	Ic		C ₁₅ H ₁₂ N ₂ O ₄ S	316.3	173-75	75
4	Id		C ₁₄ H ₁₀ N ₂ O ₄ S	302.3	175-77	85
5	Ie		C ₁₅ H ₁₂ N ₂ O ₄ S	316.3	175-78	68

Scavenging of Hydrogen Peroxide (H₂ O₂) radicals

The ability of test compounds to scavenge hydrogen peroxide was determined according to the method of Sanchez (7) and Famey (8). The solution of hydrogen peroxide (20mM) was prepared in PBS (pH 7.4). Various concentrations (0.01 to 10 mg/ml) of 1 ml of test compounds and standard were added to 2 ml of H₂ O₂. Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the PBS without H₂ O₂. The % of H₂ O₂ scavenging of test and standard compounds was calculated by;

$$\% \text{ scavenged } [H_2 O_2] = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] * 100$$

Nitric Oxide Radical Scavenging Activity:

Nitric Oxide generated from Sodium nitroprusside in aqueous solution at physiological P^H interacts with oxygen to produce nitrite ions which were measured by Griess reaction (9,10). The reaction mixture (3ml) containing sodium nitroprusside (10mM) in phosphate buffered saline (PBS) and test compounds in different concentrations was incubated at 25⁰C for 150 min. At intervals, samples (0.5 ml) of incubation solution were removed and 0.5ml of Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm. The percentage

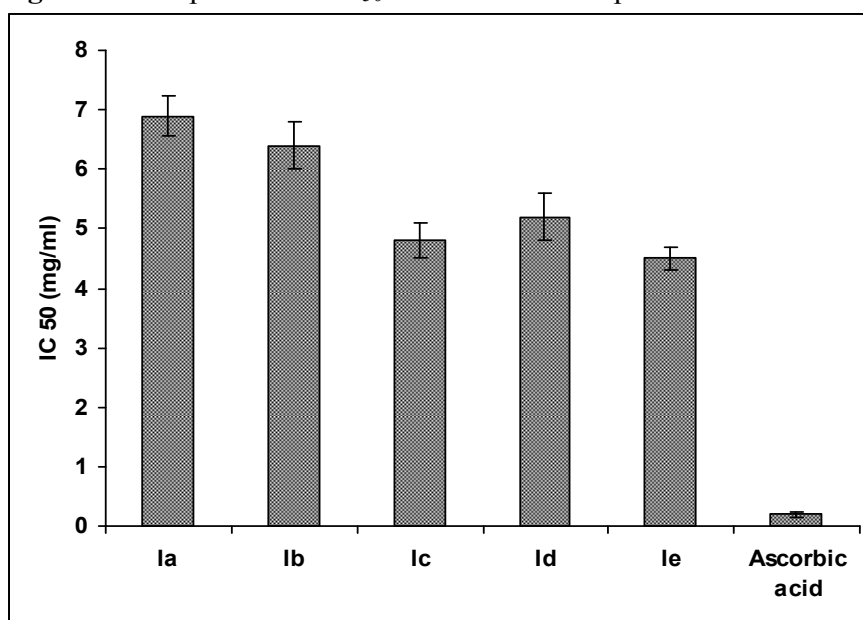
inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test compounds.

RESULTS AND DISCUSSION

DPPH free radicals scavenging activity

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidant activity of test compounds (11). The IC₅₀ values (mean \pm SD) of all the test compounds were shown in figure 1. Among the compounds tested for DPPH radical scavenging activity, compounds Ic and Ie exhibited more promising activity than other compounds.

Figure 1: Comparison of IC₅₀ values of test compounds for DPPH scavenging activity



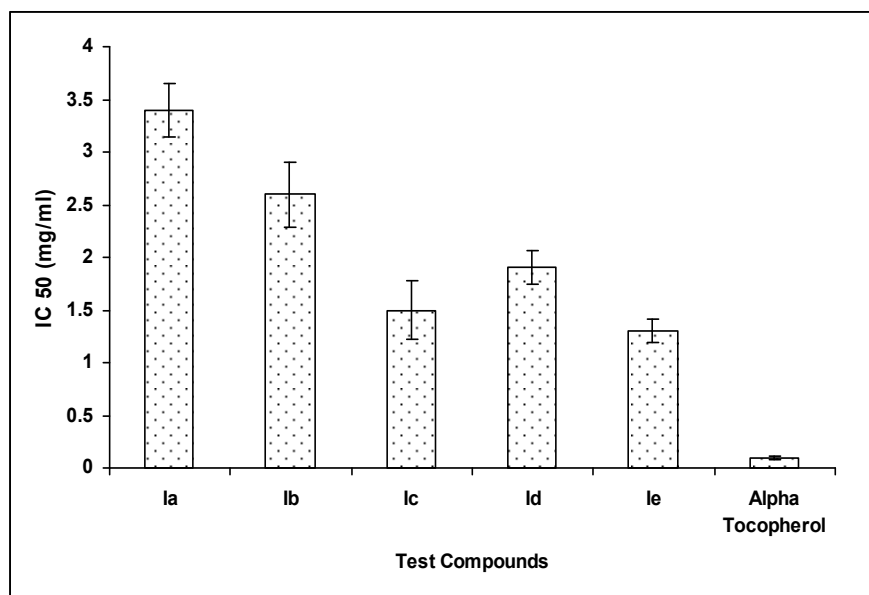
Data was mean \pm SD (n=5)

Hydrogen Peroxide (H₂O₂) radicals scavenging activity

All the test compounds scavenged the hydrogen peroxide radicals in a concentration dependant manner. The IC₅₀ values of test compounds and on hydrogen peroxide scavenging ability was shown in figure 2.

From above observations, compound Ie and Ic showed the strong free radical scavenging activity than others. The order of free radical scavenging activity of test compounds was Ie, Ic, Id, Ib and Ia respectively.

Figure 2: Comparison of IC₅₀ values of test compounds for H₂O₂ scavenging activity

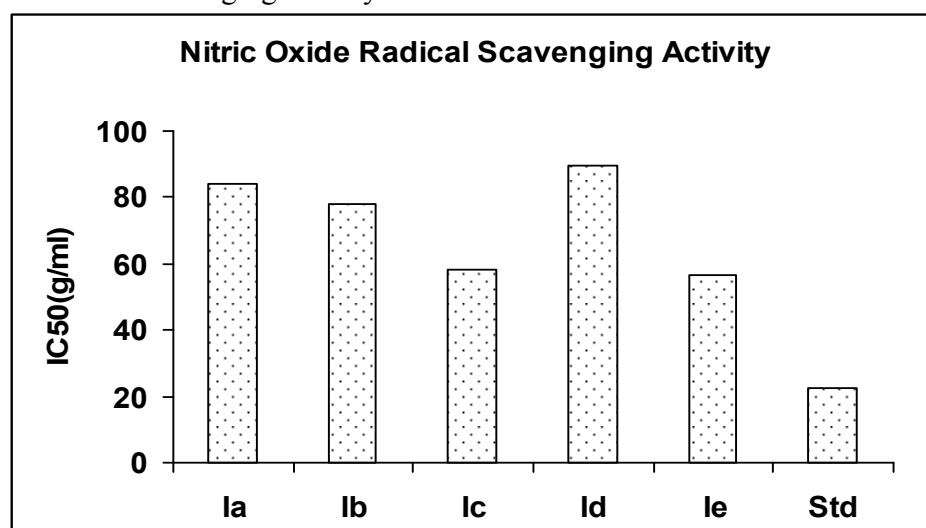


Data was mean ± SD (n=5)

Nitric Oxide Radical Scavenging Activity

All the test compounds scavenged the nitric oxide radicals in a concentration dependant manner. The IC₅₀ values of test compounds and on nitric oxide scavenging ability was shown in figure 3. Nitric oxide radical generated from sodium nitropruside at physiological pH was found to be inhibited by all the test compounds. Curcumin was used as a reference compounds.

Figure 3: Comparison of IC₅₀ values of test compounds for nitric oxide Scavenging activity



CONCLUSION

The present study results suggest the free radical scavenging activity of all the five isatin 5-sulphonamide derivatives. Among the compound tested, Ie and Ic showed potent antioxidant activity under DPPH, nitric oxide and hydrogen peroxide methods.

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