



IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF SOME β -LACTAMS AND PHENOLICS

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ABSTRACT

Nitrogen containing heterocyclic compounds such as oxindoles especially isatins (β -lactams) and their derivatives have excellent biological properties such as anti-inflammatory, antimicrobial, anticancer activity. In the present study the antioxidant activity of the said class of compounds was investigated by DPPH method with respect to ascorbic acid. To determine the antioxidant activity, a number of methyl/chlorinated isatins (**2a-f**), their Schiff-bases (**2a'**, **2d'**, **2e'** and **2f'**), *spiro*-thiadiazolines (**1g-i**), and optically active phenolics (**1a-f**) of different isatins were synthesized by both microwave and conventional heating methods. The present findings revealed that some of the synthesized compounds (**1b**, **1c**, **1e**, **1f**, **2a-b**, **2d**, **2a'** and **2b'**) exhibited a marked scavenging effect on DPPH radical.

Keywords: Isatins, Thiadiazolines, Phenolics, Antioxidant activity, DPPH

INTRODUCTION

Isatins, their Schiff-bases and *spiro*-thiadiazolines are important class of compounds due to their diverse types of biological activities, including antifungal, antiviral, antiproliferative, CNS-depressant, anticonvulsant and anxiogenic actions.¹⁻² In addition, isatins being synthetically versatile substrates are very useful for the synthesis of a large variety of heterocyclic compounds, as raw material for drug synthesis and can also function as suitable building blocks to synthesize some bioactive natural products. Hence a significant rising research interest in the design of oxindoles as drugs is currently observed in the field of medicinal chemistry. The Schiff-bases and *spiro*-thiadiazoline derivatives of isatins have shown remarkable cytotoxic activities in earlier reports³⁻⁵ but literature search reveals no previous report on the investigation of isatins for their ability of scavenging free radicals to prevent oxidation. Oxidation is a very crucial process of the aerobic life system. Thus radicals are generated either naturally or due to some biological dysfunction. Unpaired electrons which are centered in atoms of nitrogen or oxygen are called reactive nitrogen species (RNS) or reactive oxygen species (ROS) and its excess has a harmful effect, such as the peroxidation of the membrane lipids, aggression to tissue proteins and membranes, on damage to DNA and enzymes.^{6,7} Consumption of citrus fruits, vegetables, red wines, juices, etc. provides protection against such diseases.^{8,9} A variety of natural substances such as flavonoids^{10,11} from many other plants are used by humans as "antioxidants" to trap and deactivate damaging ROS and RNS. This protection can be explained by the capacity of these antioxidants to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids.^{12,13} It is worth to be noted here that many antioxidant-enriched sources contain mainly *vitamin C*, which is chemically a cyclic ester (lactone). Since isatins and related compounds have very close structural similarity, cyclic amide (lactam) with *vitamin C*, it would not be surprising if isatins, their thiadiazoline derivatives and optically active phenolics show antioxidant properties. Accordingly numerous isatin-based Schiff-bases, their *spiro*-thiadiazolines, and the optically active isomeric phenolics like *m*-cresol derivatives were synthesized (scheme 1) in fairly good yields. In this paper, we have reported for the first time the *in vitro* free radical scavenging ability of the synthesized compounds using DPPH assay.

MATERIALS AND METHODS

Synthesis

A number of methyl/chlorinated isatins (**2a-f**), their Schiff-bases (**2a'**, **2d'**, **2e'** and **2f'**), *spiro*-thiadiazolines (**1g-i**), and optically active

phenolics (**1a-f**) of different isatins were synthesized according to scheme 1.

The ring closure reaction of oximinoacetanilides (**3a-d**) with conc. H₂SO₄ to give **2a-f** was carried out by both conventional heating and microwave (MW) method in high yields (>80%). IR (KBr, disc) spectrum of these compounds such as **2a** showed absorption at ν 1742 (s, C=O, lactam) and 1735 (s, C=O) cm⁻¹ for carbonyl groups respectively. Compounds **2a-f** such as **2a** showed clear peaks in the ¹H-NMR spectrum (CD₃OD) at δ 11.13 (s, 1H, NH) as singlet for lactam proton. Aromatic protons appeared at δ 7.53-6.99 (m, 4H, ArH) as a multiplet. Synthesis of *spiro*-thiadiazoline (**1g-j**) from the respective isatins via their Schiff-bases (**2a'-f**) was carried out under MW and conventional heating methods in a nice manner. For example compound **2a** (2.0 mmol) and freshly distilled acetic anhydride (15.0mL) was refluxed for 6h. The progress of the reaction was monitored by TLC (EtOAc:CHCl₃, 1:3). The reaction mixture was cooled at room temperature. A yellow solid was obtained, which was filtered off and crude solid was recrystallized from MeOH to give **1g** (78%). IR (KBr, disc) spectrum of thiadiazoline molecules such as **1g** showed absorption at ν 1812 (s, C=O, lactam) and 1694 (s, C=O) cm⁻¹ for carbonyl groups respectively. ¹H-NMR (DMSO) spectra of **1g-j** are substantially explicable. If we consider compound **1g**, two distinct singlet appeared at δ 11.96 (s, 1H, lactam NH) and 10.69 (s, 1H, amide NH) for lactam and amide protons respectively. Aromatic protons appeared at δ 7.30-6.83 (m, 4H, ArH) as a multiplet. Two other peaks were seen at δ 2.08 (s, 3H, COCH₃) and 1.98 (s, 3H, COCH₃) for two methyl protons. ¹³C-NMR (DMSO) showed peaks at δ 173.26, 169.94, 166.85 (3 \times C=O, lactam and amide) for carbonyl carbons. Aromatic protons appeared at δ 142.68 (N=C, C-9), 141.0, 130.04, 128.55, 123.81, 122.52 and 116.24. Methyl carbons appeared at δ 74.58 (C-5), 22.29, 22.10 (2 \times CH₃) respectively. The treatment of isatins (**2a-f**) with *m*-cresol varying temperature gave different optically active phenolics of isatins (**1a-f**). The spectral and other physical constants were consistent with their structures.

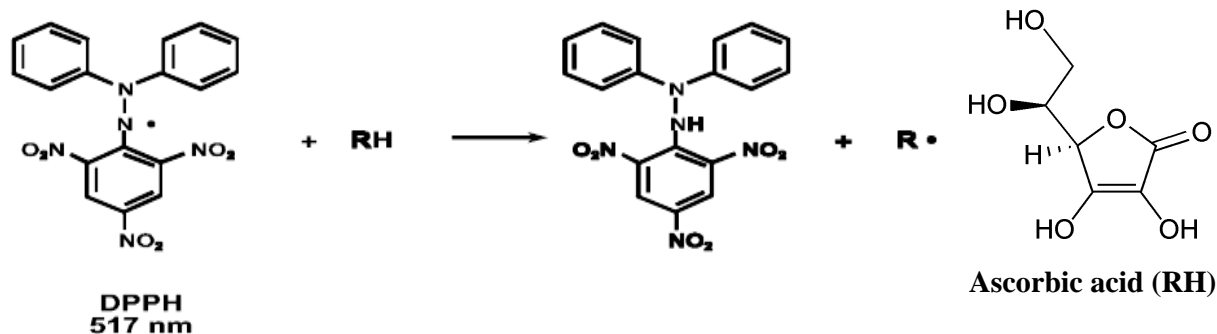
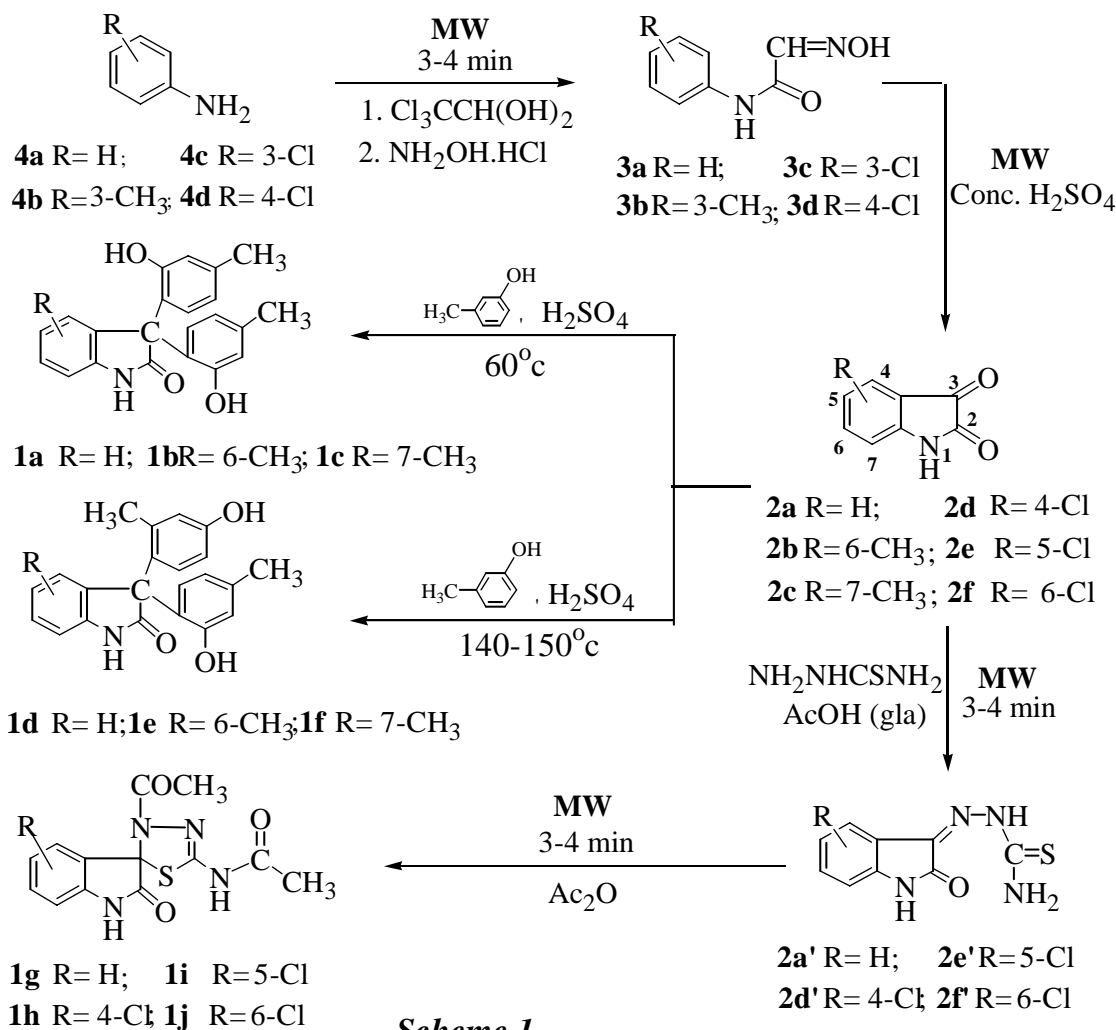
DPPH Assay

The anti-oxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as described by Sadhu *et al.*¹⁴ DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquot of the different concentrations (5-500 μ g/mL) of the test sample is added to 3 mL of a 0.004% ethanolic solution of DPPH. Absorbance at 517 nm is determined after 30 min, and IC₅₀ (Inhibitory concentration to scavenge 50% free radicals) is

also determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

The equation used to measure free radical scavenging is: % Inhibition = $100 \times (A_0 - A_s)/A_0$, where A_0 and A_s are the values for the absorbance of the positive control and the absorbance of the

sample, respectively. The experiment was performed in triplicate and average absorption was noted for each concentration. Ascorbic acid was used as a positive control. Results are expressed as mean inhibitory concentration (IC_{50}). A lower value of IC_{50} indicates a higher free radical scavenging activity.



RESULTS AND DISCUSSION

The present investigation emphasized mainly on two important things. One of these is to the synthesis of molecules having *N*-heterocycles and phenolic moieties and the other one is to examine their free radical scavenging activity. Nitrogen containing heterocyclic organic compounds having extra keto group show interesting chemical property as well as biological activity.¹⁵ The synthetic route of the compounds (**1a-f**, **1g-i** and **2a-f**, **2a'-f'**) was mainly based on the nucleophilic addition and consequent ring extension reactions through cyclization. Hence the synthetic pathway facilitated to introduce further hetero atoms (*N*, *S*) with C=O moieties. Furthermore, each of the compounds contains a high percentage of nitrogen. Therefore, it might have enhanced the reducing power to scavenge free radicals, especially reactive ROS and RNS. This finding is also concomitant with the earlier work.¹⁶ The screening of the synthesized compounds through Structure Activity Relationship (SAR) showed that isatins and other series of compounds were found to be anomalous in their free radical

scavenging activity. The 1,1-diphenyl-2-picrylhydrazyl radical is widely used to evaluate the free radical scavenging capacity of different antioxidants.^{17,18} With this method it is possible to determine the antiradical power of an antioxidant by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule shows an absorbance at 517 nm, which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic spin paired molecule.¹⁹ The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity (ϵ , optical density) of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen radical from a free radical scavenging antioxidant compound to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to the number of electrons captured as shown in scheme 2.

Table 1: shows relative antioxidant potential of the synthesized compounds

Compound no.	IC ₅₀ value (µg/ml)	Remarks	Compound no.	IC ₅₀ value (µg/ml)	Remarks
1a	1833.66	Less Active	2a'	553.71	Moderately Active
1b	395.58	Highly Active	2d'	2220.23	Less Active
1c	1009.81	Moderately Active	2e'	1998.55	Less Active
1d	4405.25	Less Active	2f'	2405.25	Less Active
1e	449.43	Highly Active	2a	1166.86	Moderately active
1f	865.33	Moderately Active	2b	281.16	Highly Active
1g	7880.51	Less Active	2c	1466.76	Less Active
1h	1168.55	Less Active	2d	398.270	Highly Active
1i	6675.46	Less Active	2e	2975.00	Less Active
1j	1865.66	Less Active	2f	1897.21	Less Active

* Ascorbic acid as a positive control showed an IC₅₀ value of 49 (µg/mL)

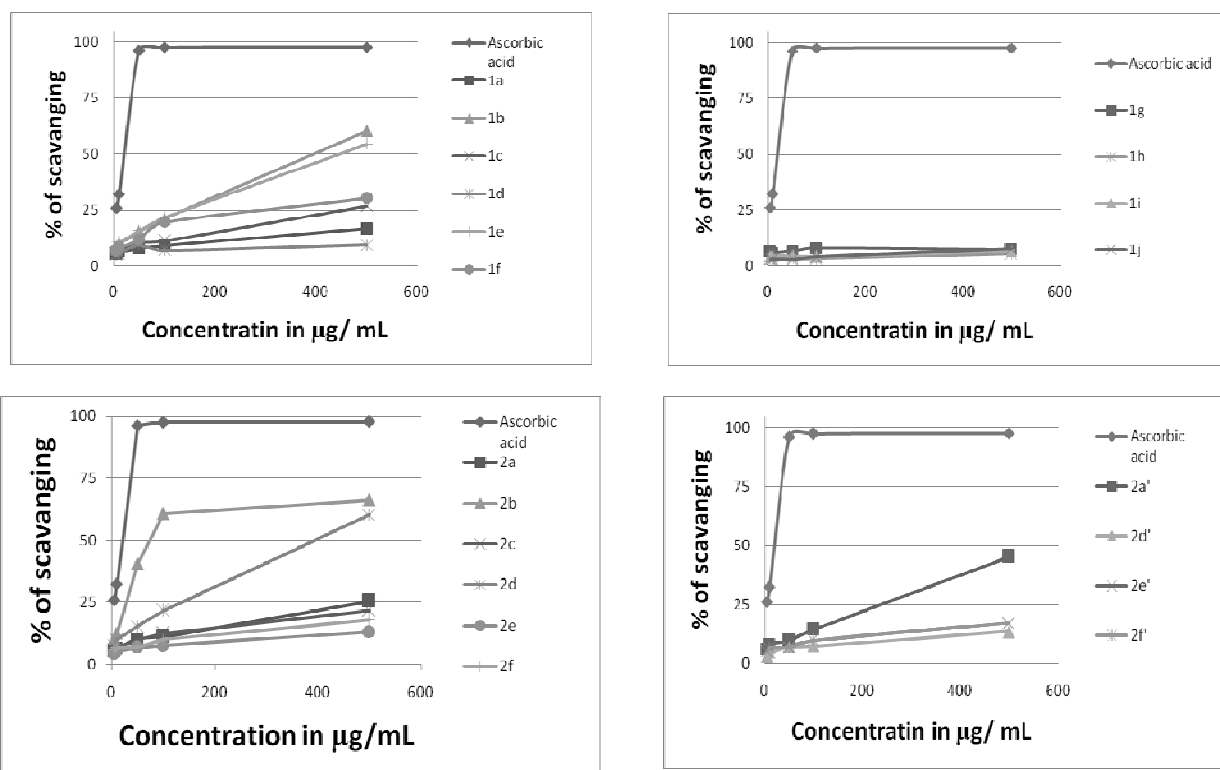


Fig. 1: It shows comparative free radical scavenging capacity of tested samples against ascorbic acid

Antioxidant results of different series of synthesized compounds (**1a-f**, **1g-i**, **2a-f** and **2a'-f'**) with respect to ascorbic acid were reported in Table I and Figure 1. As per chemical structural features there were three different types of compounds synthesized under the study area. It is obvious that structural variation brings about the bioactivity and, of course, structural modification on molecules alters the biological activity in a regular trend. Without exception this has been reflected in the case of the Schiff-bases (**2a'** and **2d'**, precursors of thiadiazolines), which showed upward tendency of antioxidant activity. But, thiadiazoline derivatives (**1g-i**) showed downward tendency of antioxidant potency. It is presumably due to the higher reducing potential of open chain thioamides (Schiff-bases) enhanced the radical trapping capacity. On the other hand, isatins (**2a-f**) showed irregular trend in their antioxidant activity. Compound **2a-b** and **2d** were attuned to show greater antioxidant activity although we have no clarification why the other isomers **2c**, **2e** and **2f** show poor activity. As it mentioned earlier that the antioxidant efficacy of any compound depends strongly on its reducing property and compound **2a-b** and **2d** may have the higher reducing potential than the other isomers. In the case of phenolics, compounds **1a-f** have shown greater propensity in showing antioxidant potency. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant activity of phenolics plays an important role in the adsorption or neutralization of free radicals. It was also found that the methyl substituted phenolics (**1b-c**, **1e-f**) are stronger in scavenging free radicals than the un-substituted ones.

It is clear from our present findings that heterocyclic systems with substituted pattern (*CH₃*, *Cl*-) show greater antioxidant property. Almost every case it was observed that molecules with methyl or halogen (*CH₃/Cl*) substitution such as compound **1b-c**, **1e-f**, **2b**, **2d**, **2d'** etc. were found to be more active than their un-substituted counterpart. Compound **2b** was found to be the most efficacious among all synthesized compounds. Although we don't have the exact clarification with regard to its excellent antioxidant potency, we have some logical arguments in favor of the fact. Actually ketolactam ring is responsible to initiate the free radical scavenging activity due to its N-H and C=O moieties. It is thought that the methyl group at 6-position has electron releasing affinity, which enhanced the reducing potential of the β -lactam ring through intervening aromatic system in case of compound **2b** than its other isomers and analogues. Due to extra stabilization compound **2b** would have higher aptitude to trap radical in a faster rate than the other similar type of molecules.

CONCLUSION

This study reports the antioxidant activity of *m*-cresol derivatives of isatins (phenolics) and other series of synthesized heterocyclic compounds for the first time. Some of the phenolics, Schiff-bases and β -lactams exhibited a marked scavenging effect on DPPH radical among all synthesized compounds. Therefore, the strategies in designing and synthesis of phenol-based lactams and other related compounds and the consequent *in vivo* screening of those should be the main objective of further investigations. This would lead to the synthesis of short listed target molecules of the active metabolites with potent antioxidant activity.

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