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Original Article

Synthesis and Characterization of Schiff and Mannich bases of Isatin and Evaluation of their Pharmacological Activity

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Abstract

Objective: The aim of present work is to synthesize a novel series of (Isatin) 1H-indole-2, 3-diones derivatives and evaluate its antioxidant activity. The structure of these compounds was established on basis of NMR data and IR data. **Introduction**: Isatin (1H-indole-2, 3-dione) is a synthetically versatile substrate, where it can be used for the synthesis of large variety of heterocyclic compounds, such as Indoles and quinolones. Isatin modify can considered as an important pharmacophore in the field of medicinal chemistry which can be used for conjugating it with other bioactive molecules such as antibacterial, antifungals, anticonvulsants and antiviral agents due to its potent pharmacological activity. Looking at the pharmacological potential of isatin we thought it worthwhile to synthesize and characterize some Schiff's and Mannich bases of isatin. Isatin and its derivatives undergo nucleophilic attack at position C-3. The chemo selectivity of these reactions depends on the nature of the substituents attached to the isatin nucleus, and especially of those bonded to the nitrogen atom, as well upon solvent and temperature employed. **Chemistry:** Isonitrosoacetanilide was prepared from aniline by treating aniline with chloral hydrate and hydroxylamine hydrochloride and from Isonitrosoacetanilide, indole-2, 3-dione was prepared in presence of conc. Sulphuric acid. Then various derivatives of indole-2, 3-diones were prepared by treating indole-2, 3-dione with various amines in presence of glacial acetic acid and formaldehyde to yield Schiff's and Mannich bases. The structure of synthesized compounds were confirmed by chromatographic and spectral analysis **Antioxidant activity:** The synthesized compounds were then evaluated for their Free radical scavenging activity by the DPPH assay method at 10, 20, 30, 40, 50 µg/ml concentrations of ligands & standard. Ascorbic acid was used as the standard. The data obtained were analyzed and results were expressed as mean absorbance ± standard error mean for each compound. The results of the pharmacological screening indicated that Compound ISS-2 shows more significant Free radical scavenging activity in comparison to other ligands and compounds ISS-1, ISS-5 shows moderate antioxidant activity and compounds ISS-4 and ISS-3 shows less antioxidant activity. **Conclusion:** The compounds synthesized were then characterized using various spectroscopic techniques i.e.IR, 1H-NMR. The spectroscopic studies showed spectral data confirmed the formation of new compounds. The synthesized compound possesses significant antioxidant activity.

Keywords: Isatin, antioxidant, Manichh and Schiff base, DDPH assay, ascorbic acid.

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Introduction

The term antioxidant originally was used to refer specifically to a chemical that prevented the

consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines .An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful.

Kinds of Antioxidants

Natural antioxidants:

- Tocopherols (delta>gamma>beta>alpha)
- Nordihydroguaretic Acid (NDGA)
- Sesamol
- Gossypol

Synthetic antioxidants:

- Butylated Hydroxy Anisole (BHA)
- Butylated Hydroxy Toluene (BHT)
- Propyl Gallate (PG)
- Tertiary Butyl Hydroquinone (TBHQ)

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid per oxidation. These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level.

The role of antioxidants in health

The process of aging and degenerative diseases such as cancer, cardiovascular disease, blood vessel blockage that includes hiperlipidemik, atherosclerosis, stroke and high blood pressure and disrupted his body's immune system can be caused by oxidative stress.

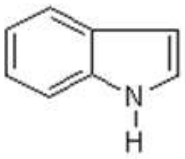
Oxidative stress is a state of balance and its total oxidant in the body. In this condition, the molecular activity of free radicals or reactive oxygen species (ROS) can cause cellular and genetic damage. Nutrient deficiencies and the existence of Xenobiotic compounds from food or too polluted environment will worsen the situation.

When the Japanese public generally or some Asian communities rarely have problems with a variety of degenerative diseases, this is due to its healthy menu of traditional rich nutrients and bioactive components. These substances have the ability as an antioxidant, which plays an important role in inhibiting the oxidation of chemical reactions, which can damage macro-molecules and can cause various health problems.

Indole and its Derivatives

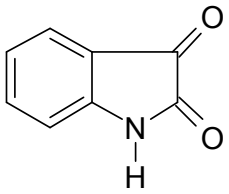
The word Indole (I) is coined from the word India, a blue dye imported from India known as Indigo. Bayer first prepared it in 1866 by zinc distillation of ox-indole. The I.U.P.A.C name of indole is 1H-benzo[b] pyrrole. Indole is a planar molecule with a conjugated system of 10 electrons. It exists in resonance form with resonance energy of 47-49 K cal/mole. It is a very weak base with pKa value 3.63. In structure a, b, and d the benzenoid 6-p system is preserved. The electrophilic attack results at 3rd position. Presence of high electron density at 3rd position has been also supported by the calculation of p electron density and by molecular orbital method.

Isatin (1H-indole-2,3-dione) (VIII) is considered as synthetically versatile molecule due to its indole-2,3-dione moiety. It was first obtained in 1841 by **Erdman**



Indole/1-H Benzopyrrole (I)

and **Laurent** as a product from the oxidation of indigo by nitric acid or chromic acid.



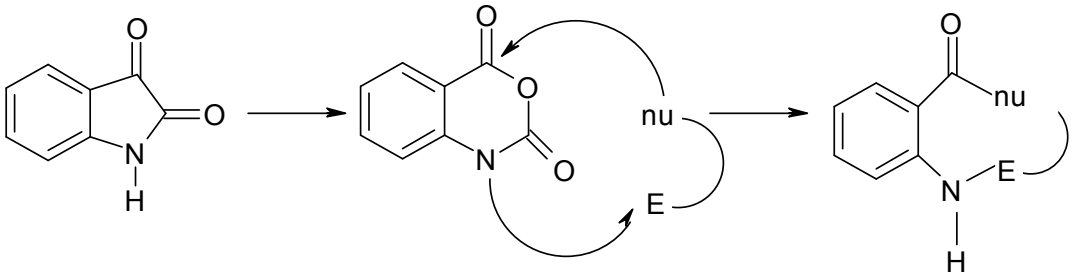
(1H-indole-2, 3-Dione) (VIII)

It forms red needles melting at 200-201°C and readily undergoes clean aromatic substitution reaction at C-5, N-alkylation via anions, and ketonic reaction at the C-3 carbonyl groups. If the 5th position is already occupied then electrophile takes the 7th position. The carbonyl group at position 2 is adjacent to the hetero atom and is stabilized by resonance. Thus it behaves as typical amide in its properties. It can be recrystallized from the hot water or ethanol. Although isatin with substituent attached to the aromatic ring are usually obtained from the corresponding functionalized anilines, they can be synthesized by electrophilic aromatic substitution too. Many synthetic methodologies have been described for the conversion of isatin to other heterocyclic systems. The chemistry of isatin can be generalized as one of the following strategies:

a) Partial or total reduction of the heterocyclic ring, leading to indoles and its derivatives.

b) Oxidation of the heterocyclic ring. For example, conversion of isatin to isatoic anhydride with subsequent conversion to other heterocyclic systems.

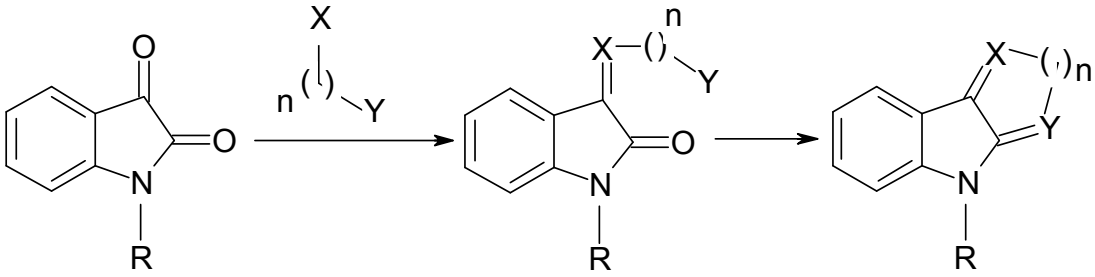
c) Nucleophilic addition at position C-3, which may be further followed by a cyclization process,



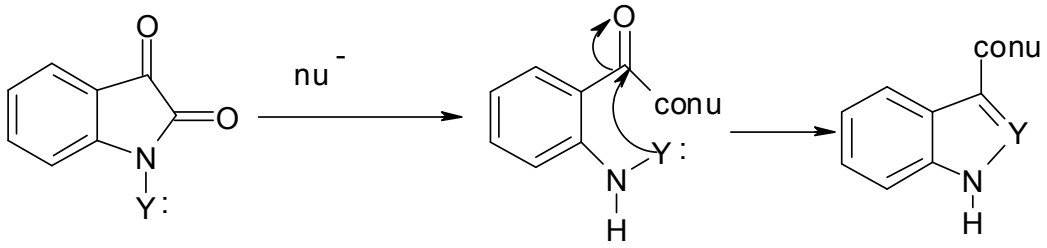
Isatoic anhydride

with or without N1-C2 bond cleavage, or by a spiro-annelation at position C-3.

d) Nucleophilic substitution at position C-2, leading to the opening of the heterocyclic ring. This



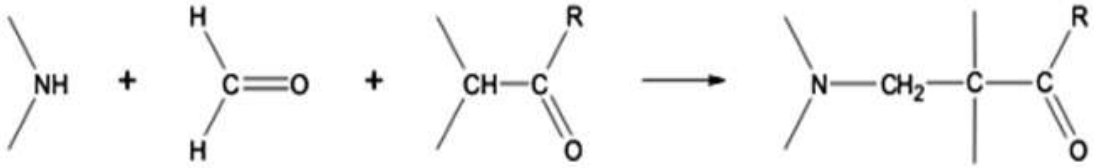
process may be followed by an intramolecular or by an intermolecular exotrig cyclization.



selectivity of these reactions depends on the nature of the nucleophile, on the nature of the substituents attached to the isatin nucleus, and especially of those bonded to the nitrogen atom, as well as upon the solvent and temperature employed. The initial products obtained can suffer further reaction in the presence of a second nucleophilic group to give cyclization products. For didactic reasons, these reactions have been sorted by the nature of the nucleophile.

Schiff Reaction

A **Schiff base** (or **Azomethine**), named after Hugo Schiff, is a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, but not hydrogen.



an amine to a carbonyl group followed by elimination of a hydroxyl anion to the Schiff base. The Schiff base is an electrophile which reacts in step two in a second nucleophilic addition with a carbanion generated from a compound containing an acidic proton.

Material and Methods

All of the chemicals were procured from CDH, Acros and Himedia. Melting point (m.p) was recorded on Veego melting point apparatus and is uncorrected. Infra red (IR) spectra were taken using FTIR thermo Scientific; NICOLET Is10, KBR disk spectrophotometer. The 1HNMR spectra were recorded on sophisticated multinuclear FT-NMR spectrometer model Avance-II (Bruker) 400 NMR Spectrometer, using DMSO-d6 solvent. Chemical

Isatin and its derivatives can suffer nucleophilic attack at positions C-2 and/or C-3. The chemo

Schiff bases are of the general formula R1, R2, C=N-R3, where R3 is an aryl or alkyl group that makes the Schiff base a stable imine. A Schiff base derived from an aniline, where R3 is a phenyl or substituted phenyl.

Mannich Reaction

The Mannich reaction is an organic reaction and consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. The final product is a â-amino-carbonyl compound. Reactions between aldimines and á-methylene carbonyls are also considered Mannich reactions because these imines form between amines and aldehydes.The reaction is named after Chemist Carl Mannich. The Mannich reaction is an example of Nucleophilic addition of

shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Rankem) and activated at 110°C for 30 min. The plates were developed by exposing to iodine vapours. All reagent and solvents were purified and dried by standard techniques.

Preparation of Indole -2,3-dione (Isatin).

Method of preparation of Isonitrosoacetanilide from aniline:-

In a 250 ml round bottom flask placed 9 gm (0.05M) of chloral hydrate and 85 ml of water. To this solution added, in order 13 gm (0.18M) of crystallized

anhydrous sodium sulfate (dried in oven), a solution of aniline (0.05 M) in 30 ml of water to which 4.3 ml (0.052M) of concentrated hydrochloric acid was added to dissolve the amine and finally, a solution of 11gram (0.158M) of hydroxylamine hydrochloride in 50 ml of water. The reaction mixture was heated to 80-900C. Vigorous boiling started in about 30-35 minutes. The reaction completed after 1-2 minutes of vigorous boiling. During the heating period, some crystal of isonitrosoacetanilide separates. On cooling the solution in running water the remainder crystallizes, was filtered under suction, and air dried.

Method of preparation of isatin from isonitroso acetanilide

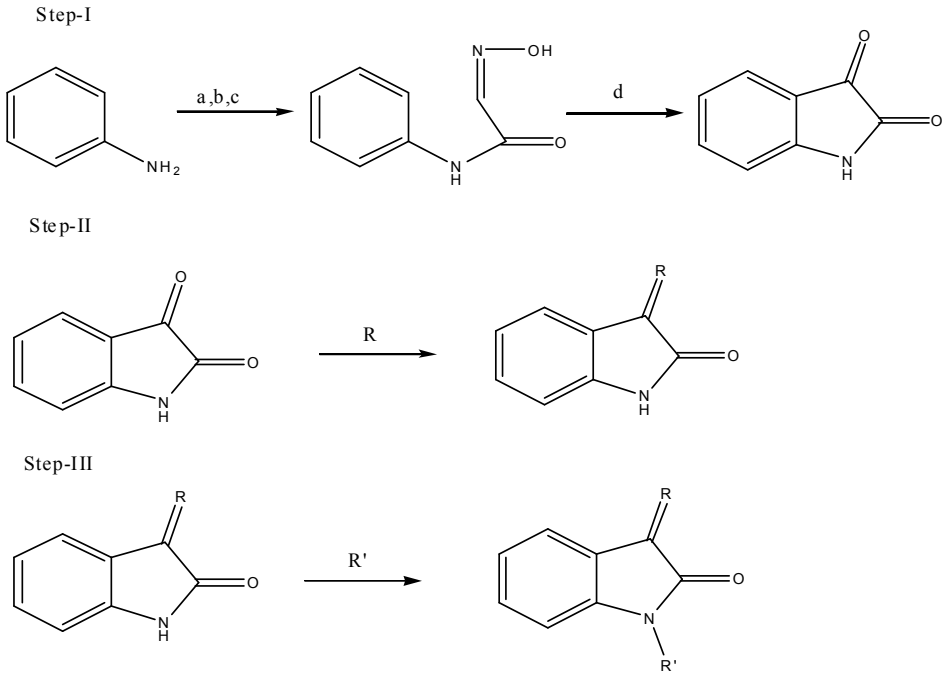
32.6 ml of concentrated sulfuric acid was warmed to 50°C in a 100 ml round bottom flask fitted with and efficient mechanical stirrer, add 7.5 gram of (0.04 M) of dry isonitroso acetanilide to such a rate that to keep the temperature 60-700C but not higher. External cooling should be applied at this stage so that the reaction can be carried out more rapidly. After the addition of the isonitrosoacetanilide compound was finished, the solution was heated to 80°C and kept at this temperature for about 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured in to 10-12 times of its volume of ice. After standing for about one and half hour the isatin was filtered under suction, washed several times with cold water to remove the sulfuric acid, and then dried in the air. It was reprecipitated by dissolving in 10 % sodium hydroxide solution followed by acidification with dil. HCl to get dark red colored product which on cooling was filtered, washed with water and dried to get pure crystals melting at 209-212 0 C.

General procedure for synthesis of novel Schiff bases of Isatin

0.01mole of Isatin was dissolved in 5 ml of ethanol. To this, was added 0.01 mole of Primary amine (4-fluoroaniline). Catalytic amount of Glacial acetic acid (1 ml) was added to the above mixture and the contents were refluxed for 10 h. The resulted solution was allowed to stand overnight and the precipitated solid was filtered, washed, dried and recrysallized from ethanol to yield the Schiff bases.

General method for the preparation of Mannich bases

0.01 mole of Schiff base was dissolved in minimum amount of hot ethanol (4ml). To this, 1 ml of 40% formaldehyde was added. To the above solution, 0.01 mole of secondary amine dissolved in minimum ethanol (1ml) was introduced. The mixture was stirred for 24 h at room temperature. The solid separated was filtered, dried and recrysallized from ethanol, to yield the Mannich bases.



Antioxidant Activity

Free radical scavenging activity by DPPH assays method.

DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron or hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug .The change in absorbance produced at 517 nm has been used as measure of its antioxidant activity.

Chemicals used

1, 1-diphenyl-2-picryl-hydrazil (DPPH)-Sigma Ltd., Ascorbic Acid-Qualigens, Methanol-Qualigens.

Preparation of DPPH solution

It was prepared by dissolving 33 mg of DPPH in 1 lit. Of methanol just before use and kept in dark amber colored bottle to protect from sunlight.

Sample preparation

Preparation of stock solution of Isatin derivatives

It was prepared by dissolving 50 mg of **Isatin** derivatives in 100 ml of methanol.

Standard preparation

Preparation of Ascorbic Acid solution

It was prepared by dissolving 50 mg of ascorbic acid in 100 ml of methanol.

DPPH Radical scavenging assay of Compd ISS-1 and Ascorbic acid.

Compd ISS-1			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.0116±0.0014	39.6	10	0.8240±0.0015	49.3
20	0.9033±0.0004	41.2	20	0.7620±0.0022	53.2
30	0.8300±0.0005	47.8	30	0.6830±0.0002	57.9
40	0.7320±0.0005	57.2	40	0.5540±0.005	65.8
50	0.5410±0.0014	64.5	50	0.4810±0.0002	70.2

DPPH Radical scavenging assay of Compd ISS-2 and Ascorbic acid

Compd ISS- 2			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.0200±0.0006	40.7	10	0.8240±0.0015	49.3
20	0.9230±0.0005	43	20	0.7620±0.0022	53.2
30	0.8900±0.0002	47.8	30	0.6830±0.0002	57.9
40	0.7270±0.0014	50.8	40	0.5540±0.005	65.8
50	0.5133±0.0006	58.9	50	0.4810±0.0002	70.2

DPPH Radical scavenging assay of Compd ISS-3 and Ascorbic acid

Compd ISS- 3			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.1233±0.0004	31	10	0.8240±0.0015	49.3
20	1.0063±0.0008	37.2	20	0.7620±0.0022	53.2
30	0.8413±0.0004	47.4	30	0.6830±0.0002	57.9
40	0.7110±0.0014	55.2	40	0.5540±0.005	65.8
50	0.5310±0.0002	65.2	50	0.4810±0.0002	70.2

DPPH Radical scavenging assay of Compd ISS-4 and Ascorbic acid

Compd ISS- 4			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	0.9850±0.0005	36.2	10	0.8240±0.0015	49.3
20	0.9116±0.0014	43.4	20	0.7620±0.0022	53.2
30	0.8336±0.0014	48.7	30	0.6830±0.0002	57.9
40	0.7813±0.0002	55.2	40	0.5540±0.005	65.8
50	0.6516±0.0014	65.2	50	0.4810±0.0002	70.2

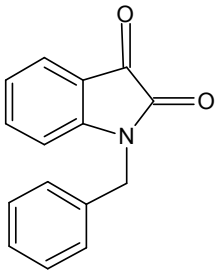
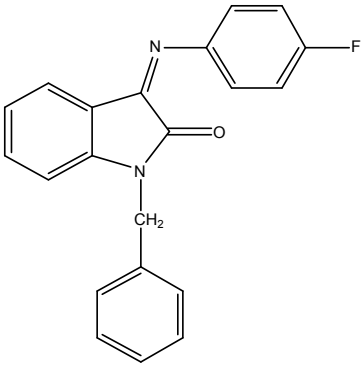
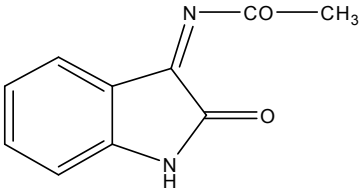
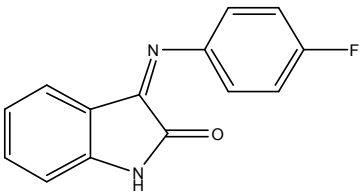
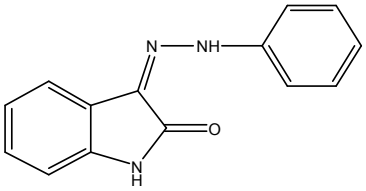
DPPH Radical scavenging assay of Compd ISS-5 and Ascorbic acid

Compd ISS-5			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	0.9813±0.0014	37.8	10	0.8240±0.0015	49.3
20	0.9510±0.0002	43.4	20	0.7620±0.0022	53.2
30	0.8316±0.0014	48.7	30	0.6830±0.0002	57.9
40	0.6810±0.0002	55.2	40	0.5540±0.005	65.8
50	0.5616±0.0014	65.2	50	0.4810±0.0002	70.2

Procedure

A 10, 20,30,40,50 µg/ml concentrations of ligands, and ascorbic acid were prepared. From this stock solution 1ml has been pipette out and 5ml methanol solution of DPPH was added, shaken well and the mixture was incubated at 370C for 30 min. The absorbance of all samples were measured against blank at 517 nm. The absorbance of DPPH reagent alone was taken as control (see in table 1,2,3,4,5,6). The % radical scavenging activity can be calculated following formula:

Physicochemical data for compounds

Code	Compound	M. F.	M.wt.	% Yield	M.P °C.
ISS-1		$C_{15}H_{11}NO_2$	237.25	82	156-158
1SS-2		$C_{21}H_{15}FN_2O$	330.36	84	178-180
ISS-3		$C_{10}H_6N_2O_2$	188.18	86	190-192
ISS-4		$C_{14}H_9FN_2O$	240.23	77	189-190
ISS-5		$C_{14}H_{11}N_3O$	237.26	80	198-200

Spectral Analysis Data of Synthesized Derivatives

Compound	Spectral Peaks (cm^{-1}) and Peak Characteristics
ISS-1	2922.15Ar C-H Stretching, 2851.26 Alkane C-H Stretching, 1594.94-1432.42, Ar C=C Stretching, 1739.60-C=O Stretching, 1677.27-C=O Stretching , 1349.48C-N Bending, 731.19 Oop(out of plane bending) H NMR 4.9 (2 H, N-CH_2), 6.7-7.6 (9 H, Ar-H)
ISS-2	3087.87 Ar C-H Stretching, 2839.49 Alkane C-H Stretching, 1590.98-1499.18 Ar C=C Stretching, 1612.78 -C=N Stretching, 1336.06 C-N Bending, 1728.78 -C=O Stretching, 1213.98 C-F, 754.81 Oop H NMR 4.9 (2 H, N-CH_2), 6.9-7.5 (13H, Ar-H)
ISS 3	3441.71 -N-H Stretching, 3177.70 Ar C-H Stretching, 3057.55 Alkane C-H Stretching, 1555.92-1462.74 Ar C=C Stretching, 1728.24 -C=O Stretching, 1685.47 -C=O Stretching, 1616.03 C=N Stretching 1331.92 C-N Bending, 746.79 Oop H NMR 3.3(1H,CH_3), 6.8-7.4 (4H, Ar-H)
ISS 4	3446.67 -N-H Stretching, 3063.03 Ar C-H Stretching, 2922.26 Alkane C-H Stretching, 1592.89-1467.15 Ar C=C Stretching, 1735.91 -C=O Stretching, 1611.28 -C=N Stretching, 1241.46 C-F, 1331.92 C-N Bending, 746.79 Oop H NMR 3.83(8 H, Ar-H), 8.29(1H,NH)
ISS 5	3432.16 -N-H Stretching, 3030.11 Ar C-H Stretching, 2783.50 Alkane C-H Stretching, 1494.39-1470.64 Ar C=C Stretching, 1732.10 -C=O Stretching, 1612.67 -C=N Stretching, 1348.97 C-N Bending, 753.96 Oop H NMR 6.9-7.5(9H, Ar-H), 10.9 (1H,NH), 12.8 (1H,NH)

%free radical = Absorbance of control - Absorbance of sample X 100 Scavenging activity Absorbance of control And calculated IC50 value

Results and Discussion

The synthesized compounds of Isatin (ISS-1 to ISS-5) showed diversified antioxidant activity. A series of mannish base and Schiff base of derivatives of Isatin were synthesized by mannich reaction and Schiff reaction with different secondary amines and primary amines and formaldehyde. The antioxidant activity was evaluated by the free radical scavenging activity by DPPH assay method. Comp ISS-2 showed more significant free radical scavenging activity when compared with that of standard drug i.e.

Ascorbic acid. Comp ISS-1 and Comp ISS-5 showed significant free radical scavenging activity when compared with that of standard drug i.e. Ascorbic acid. Comp ISS-3 and Comp ISS-4 showed less free radical scavenging activity when compared with that of standard drug i.e. Ascorbic acid. DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron on hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug.

Conclusion

The five compounds were synthesized with the standard chemicals and procedure. The compounds were characterized through their respective IR, ^1H NMR, UV and TLC. The compound member ISS-1 and ISS-3 show promising antioxidant activity

Acknowledgement

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Conflict of Interest

The author does not have any conflict of interest.

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