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### RESEARCH ARTICLE

#### MICROWAVE IRRADIATION SYNTHESIS AND ANTIOXIDANT ACTIVITY OF ISATIN- OXADIAZOLE DERIVATIVES.

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Isatin, Oxadiazole, Antioxidant activity.

#### Abstract

Based on the literature review, we have found that Isatin- Oxadiazole derivatives are exhibiting different activities like anti-oxidant, anti-viral, anti-microbial and anti-convulsant and we synthesized Isatin-Oxadiazole hybrids. Among the series of compounds (*E*)-5-chloro-3,(2,(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one(IVb), (*E*)-5-bromo-3,(2,(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one(IVe) showed most significant antioxidant activity.

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#### Introduction:-

Heterocyclics form the largest of classical divisions of organic chemistry and are of immense importance both biologically and industrially. The majority of pharmaceuticals and agrochemicals are heterocyclics while countless additives and modifiers used in industrial applications ranging from cosmetics, reprography, information storage and plastics. One of the striking structural features inherent to heterocyclics, which continue to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations. For more than a century, heterocyclics have constituted one the largest areas of research in organic chemistry. They have contributed to the development of society through health care and industrial point of view as well as in understanding life processes and thus improving the quality of life. Various compounds such as nucleic acids, alkaloids, antibiotics, essential amino acids, vitamins, hemoglobin, hormones and a large number of synthetic drugs contain heterocyclic ring systems. Among the wide variety of heterocyclic compounds that have been explored for developing pharmaceutically important molecules, Isatin have played an important role in the field of research.

#### Chemistry Of Isatin:-

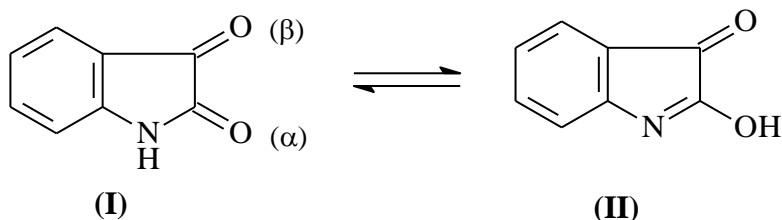
Isatin (1H-indole-2,3-dione) (I) was first discovered by Erdmann and Laurent in 1841, independently as a product from oxidation of indigo by nitric and chromic acids.

It is a unique molecule possessing both amide and ketocarbonyl groups. Apart from this, it has an active hydrogen atom attached to nitrogen (or oxygen) and an aromatic ring which should substitute at 5- and 7-positions. It exists in

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a tautomeric form (II) and these functional characteristics play an important role in governing the various reactions of the molecule.



The C-3 carbonyl group of isatin is strongly electrophilic. As a result, isatins are readily involved in condensation and addition reactions with carbanion type nucleophiles into 3-substituted oxindoles. In general, there are three possibilities during condensation reactions.

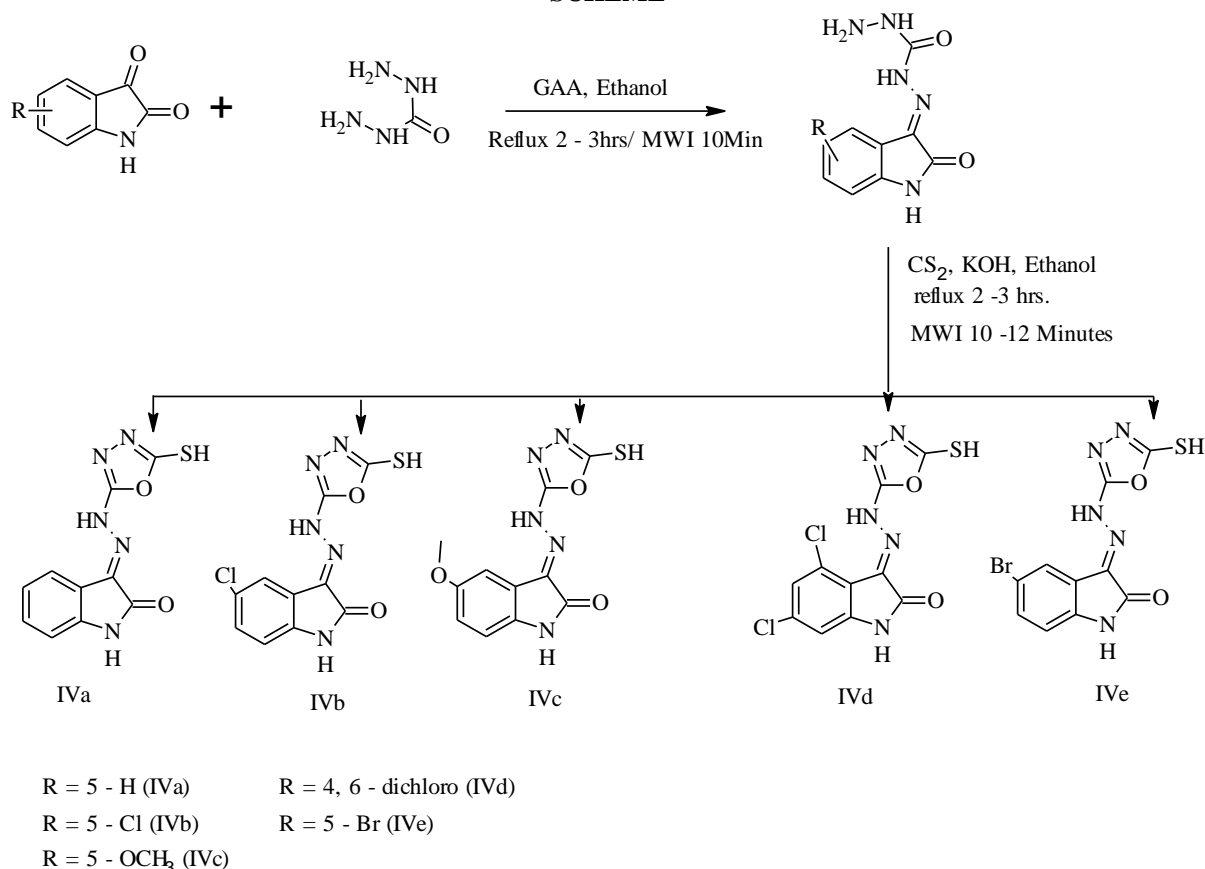
1. Both the  $\alpha$ ,  $\beta$ -carbonyl groups, having varying reactivity are involved,
2. Ring cleavage takes place and
3. Ring expansion occurs

A general observation has been that the nature of final product always depends on the experimental conditions and substituents at nitrogen atom, which may affect the electron density at,  $\alpha$  and  $\beta$  carbonyl carbon atoms respectively.

#### Oxadiazoles:-

Oxadiazole is a heterocyclic aromatic chemical compound containing an oxygen and two nitrogen atoms in a five membered ring. There are 4 isomers 1,2,4-oxadiazole; 1,2,3-oxadiazole; 1,2,5-oxadiazole and 1,3,4-oxadiazole. 1,3,4-oxadiazole is better known and more widely studied because of its many important chemical and biological properties. Among heterocyclic compounds 1,3,4-oxadiazole has become an important construction for the development of new drugs.

#### SCHEME



**Experimental Procedure:-****Synthesis Of Isatincarbohydrazone:-**

A mixture of Isatin(0.1M), Carbohydrazide(0.1M) are dissolved in Ethanol, then add 2-3 drops of Glacial acetic acid. Refluxed for 90 minutes /MWI10 Minutes, the progress of the reaction was monitored by TLC. The reaction mixture was filtered by using Whitman filter paper and funnel. And dried product was purified by using ethyl acetate.

**SYNTHESIS OF (E)-3-(2-(5-MERCAPTO-1,3,4-OXADIAZOL-2-YL)HYDRAZONO)INDOLIN-2-ONE:**

Weigh accurately 0.028M of Isatin carbohydrazone and 0.028M KOH and dissolved in Ethanol, when it is in heating conditions, add 35mmol(0.035M) of carbon disulphide and put it reflux for 2-3 hrs/MWI 10-12 Min. until the evaluation of hydrogen sulphide ceased. The reaction mixture was cooled to room temperature and poured into ice cold water (100ml). It was neutralized with dilute hydrochloric acid. The precipitated solid was filtered, washed with water and dried product was recrystallized from ethanol.

**Derivatives:-**

5-Chloro Isatin, 5-Methoxy Isatin, 4,6-Dichloro isatin and 5-Br isatin are the starting materials which are used for the respective (E)-5-chloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one; (E)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-5-methoxy indolin-2-one; (E)-4,6-dichloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one; (E)-5-bromo-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one.

**Table 1:-**

S.No		Derivatives	Molecular Formula	Molecular Weight	Melting Point
1	IV a	R-5H	C <sub>10</sub> H <sub>7</sub> N <sub>5</sub> O <sub>2</sub> S <sub>1</sub>	261	205-208
2	IV b	R-5Cl	C <sub>10</sub> H <sub>6</sub> N <sub>5</sub> O <sub>2</sub> S <sub>1</sub> Cl <sub>1</sub>	295	206-208
3	IV c	R-5-OCH <sub>3</sub>	C <sub>10</sub> H <sub>9</sub> N <sub>5</sub> O <sub>3</sub> S <sub>1</sub>	291	204-207
4	IV d	R-4-Cl,R1-6-Cl	C <sub>10</sub> H <sub>5</sub> N <sub>5</sub> O <sub>2</sub> S <sub>1</sub> Cl <sub>2</sub>	330	207-209
5	IV e	R-5Br	C <sub>10</sub> H <sub>6</sub> N <sub>5</sub> O <sub>2</sub> S <sub>1</sub> Br <sub>1</sub>	340	205-207

**Pharmacological Evaluation:-****Anti-oxidant Activity:-**

In view of varied biological and pharmacological importance of different series of pyrimidine derivatives, the synthesized compounds were screened for anti-oxidant activity. Antioxidants are intimately involved in the prevention of free radicals, which cause DNA damage. By studying various methods, the following methods describes anti-oxidant activity.

1. Hydrogen peroxide scavenging method
2. Nitric oxide scavenging method

**Hydrogen peroxide scavenging method:-****Materials:-**

Hydrogen Peroxide, Phosphate Buffer, Ascorbic Acid

**Method:-**

The synthesized compound scavenged to hydrogen peroxide was determined according to the method of Ruch et al (1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both C and standard compounds were calculated:

$$\% \text{ Scavenged } [H_2O_2] = [(A_c - A_s/A_c) \times 100]$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the test compounds or standards.

**Preparation of phosphate buffer (pH7.4):-**

6.8gms of  $\text{KH}_2\text{PO}_4$  (potassium dihydrogen phosphate) +1.5gms of NaOH (sodium hydroxide) were dissolved in 1000ml of Millipore water.

**Preparation of 40mM Hydrogen Peroxide:-**

0.228ml of  $\text{H}_2\text{O}_2$  is dissolved in 50ml of phosphate buffer (pH7.4)

**Preparation of standard solution (Ascorbic Acid):-**

10mg of ascorbic acid is dissolved in 10ml of phosphate buffer (pH7.4)

**Preparation of test solution (synthesized compound):-**

10mg of sample is dissolved in 10ml of phosphate buffer (pH7.4)

**Nitric oxide scavenging Assay:-****Materials:-**

Sodiumnitro prusside, phosphate buffered saline, Griess reagent, methanol.

**Method:-**

Nitric oxide scavenging activity was measured spectrophotometrically. Sodiumnitroprusside (3.0ml of 5mM) in phosphate buffered saline pH 7.4, was mixed with different concentrations of the compound prepared in methanol and incubated at 25 °C for 30 min. A control without the test compound, with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1- naphthylethylenediamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

**Results and Discussion:-**

The preliminary studies on possible pharmacological activities of isatin-oxadiazole derivatives have generated some useful data.

**Hydrogen peroxide scavenging Method.****Table 2:-**

S.NO.	COMPOUNDS	IC <sub>50</sub> (µg/ml)
IV(a)	5 -H	3.68±0.0307
IV(b)	5 -Cl	3.74±0.303
IV(c)	5-OCH <sub>3</sub>	3.55±0.026
IV(d)	4 ,6-DiChloro	3.65±0.0204
IV( e)	5-Br	3.72±0.043
standard	Ascorbic acid	3.66±0.033

Antioxidant activity of (E)-3-(2-(5- mercapto-1,3,4-oxadiazol-2-yl)hydrazono)indolin-2-one derivatives by Hydrogen peroxide scavenging activity.

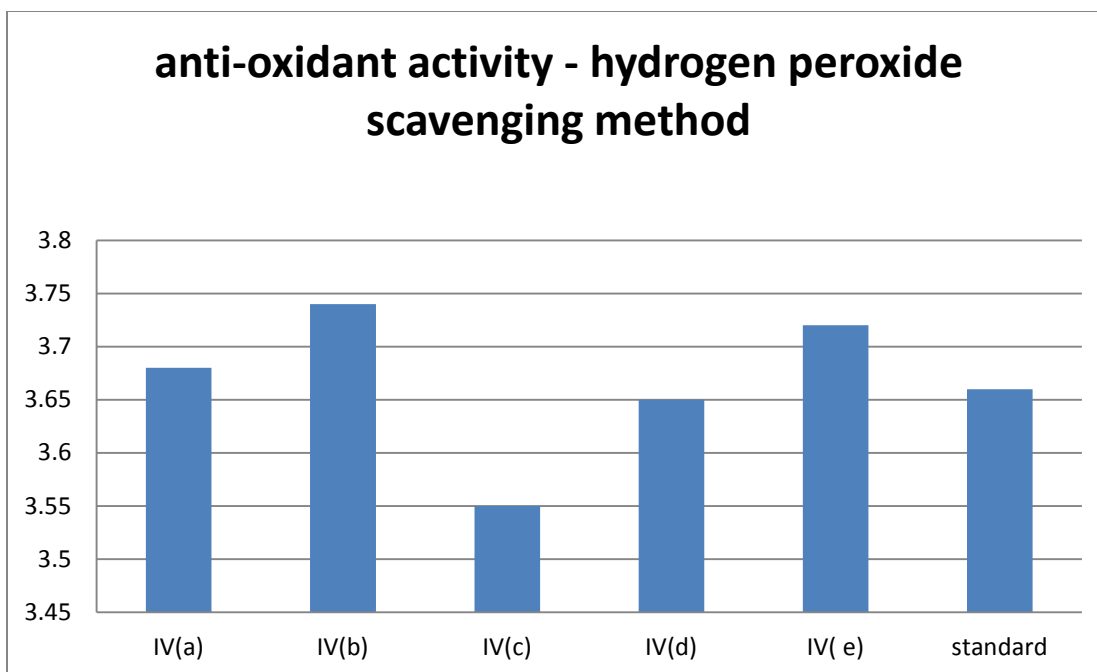


Fig 1:-

The anti-oxidant activity of the synthesized compounds IVa-e was evaluated by in vitro hydrogen scavenging assay method. The results are recorded in Table 2.

- Among the series (IVa-e) (*E*)-5-chloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one; (*E*)-5-bromo-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one showed most significant activity with IC<sub>50</sub> values of 3.74±0.303, 3.72±0.043.
- Compounds (*E*)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one, (*E*)-4,6-dichloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one showed good antioxidant activity with IC<sub>50</sub> values of 3.68±0.0307, 3.65±0.0204.
- Compound (*E*)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-5-methoxy indolin-2-one, showed less antioxidant activity with IC<sub>50</sub> value of 3.55±0.026.

#### Nitric oxide scavenging method

Table 3:-

S.NO.	COMPOUNDS	IC <sub>50</sub> (μg/ml)
IVa	5-H	3.77±0.030
IVb	5-Cl	3.56±0.023
IVc	5-OCH <sub>3</sub>	3.68±0.038
IVd	R-4Cl, R <sup>1</sup> -6Cl	3.50±0.026
IVe	R 5-Br	3.59±0.029
Standard	Ascorbic acid	3.66±0.033

Antioxidant activity of (*E*)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one derivatives by Nitric oxide scavenging activity.

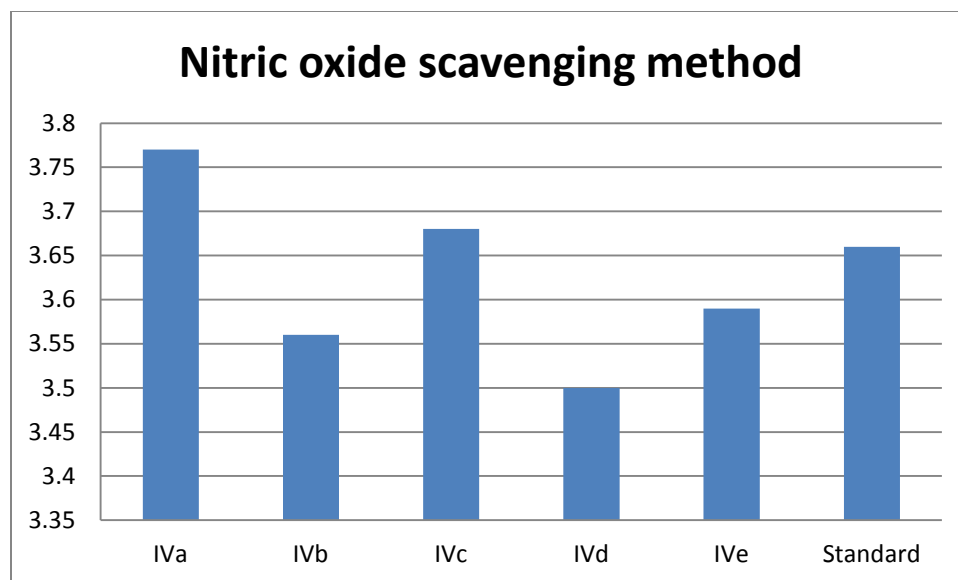


Fig 2:-

- Among the series compound of (*E*)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one showed significant antioxidant activity by IC<sub>50</sub> value of 3.77±0.030.
- Compound (*E*)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-5-methoxy indolin-2-one, shows good antioxidant activity by IC<sub>50</sub> value of 3.68±0.038.
- Compounds (IVb),(IVd),(IVe), showed less antioxidant activity

Five new Isatin- oxadiazole derivatives have been synthesized by using appropriate synthetic route.

Final compounds are characterized by physical & spectral data (FT-IR, NMR, Mass spectroscopy)

From the FT-IR spectrum, we have found that the peak at 3419.39cm<sup>-1</sup> is due to the presence of N-H stretching and the peak at 1661.12cm<sup>-1</sup> is due to the presence of C=O stretching.

From the <sup>1</sup>HNMR spectrum, we confirmed the presence of number of protons the peaks absorbed between 6.8-7.6 indicate the presence of aromatic protons.

From the Mass spectrum, we have found that the molecular ion peak appears at 261(M+1) there confirms the most abundant peak confirms the formation of final compound.

#### ➤ Activity:

All the synthesized compounds are evaluated for antioxidant activity by Hydrogen peroxide scavenging assay & Nitric oxide scavenging assay, the results are clearly reveals that most of the compounds showing good antioxidant activity as compared with the standard drug( Ascorbic acid).

#### Conclusion:-

Isatin-Oxadiazole derivatives have been synthesized & their structures are elucidated by physical& spectral data.

#### Spectral Data:-

##### (*E*)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVa):-

- ❖ From the FT-IR spectrum, we have found that the peak at 3419.39cm<sup>-1</sup> is due to the presence of N-H stretching, and the peak at 1661.12cm<sup>-1</sup> is due to the presence of C=O stretching.
- ❖ From the <sup>1</sup>HNMR spectrum :SH Proton peak absorbed at 13, NH amide proton at 8, NH hydrazide peak at 7, CH protons in benzylidenimin as 7.86,7.81,7.50 and 7.26 ppm.
- ❖ From the Mass spectrum, we have found that the molecular ion peak appears at 261(M+1) there confirms the most abundant peak confirms the formation of final compound.

**(E)-5-chloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVb):-**

- ❖ FTIR: From the FT-IR spectrum, we have found that the peak at  $3329.6\text{cm}^{-1}$  is due to the presence of N-H stretching, and the peak at  $1625.7\text{cm}^{-1}$  is due to the presence of C=O stretching.
- ❖ From the  $^1\text{H}$ NMR spectrum :SH Proton peak absorbed at 13.05, NH amide proton at 8, NH hydrazide peak at 7, CH protons in benzylidenimin as 7.80, 7.50, 7.89 ppm.
- ❖ From the Mass spectrum, we have found that the molecular ion peak appears at 296(M+1) there confirms the most abundant peak confirms the formation of final compound.

**(E)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-5-methoxy indolin-2-one (IVc) :-**

- ❖ From the FT-IR spectrum, we have found that the peak at  $3298.7\text{cm}^{-1}$  is due to the presence of N-H stretching, and the peak at  $1623.9\text{cm}^{-1}$  is due to the presence of C=O stretching
- ❖ From the  $^1\text{H}$ NMR spectrum :SH Proton peak absorbed at 13.05, NH amide proton at 8, NH hydrazide peak at 7, CH protons in benzylidenimin as 7.75, 7.48, and 7.04 ppm.
- ❖ From the Mass spectrum, we have found that the molecular ion peak appears at 292(M+1) there confirms the most abundant peak confirms the formation of final compound.

**(E)-4,6-dichloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVd) :-**

- ❖ From the FT-IR spectrum, we have found that the peak at  $3395.4\text{cm}^{-1}$  is due to the presence of N-H stretching, and the peak at  $1619.2\text{cm}^{-1}$  is due to the presence of C=O stretching
- ❖ SH 13.05 aromatic C-SH NH 7.00 hydrazid, CH 9.02, 7.29 benzylidenimin, CH 7.44, 7.29 benzylidenimin.
- ❖ From the Mass spectrum, we have found that the molecular ion peak appears at 330(M+1) there confirms the most abundant peak confirms the formation of final compound.

**(E)-5-bromo-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVe) :-**

- ❖ From the FT-IR spectrum, we have found that the peak at  $3213.4\text{cm}^{-1}$  is due to the presence of N-H stretching, and the peak at  $1616.4\text{cm}^{-1}$  is due to the presence of C=O stretching
- ❖ SH 13.05 aromatic C-SH NH 7.00 hydrazid, CH 8.05, 7.70 benzylidenimin, CH 7.32, benzylidenimin
- ❖ All the synthesized compounds are evaluated for antioxidant activity by Hydrogen peroxide scavenging assay & Nitric oxide scavenging assay, the results are clearly reveals that most of the compounds showing good antioxidant activity as compared with the standard drug ( Ascorbic acid).
- Among the series compound (E)-5-chloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVb), have shown good yield.
- All the compounds are evaluated for antioxidant activity by using standard protocols.
- Among the series compounds (E)-5-chloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVb), (E)-5-bromo-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVe) showed most significant antioxidant activity by Hydrogen peroxide scavenging assay.
- Compounds (E)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVa), (E)-4,6-dichloro-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVd) showed good antioxidant activity in the series by Hydrogen peroxide scavenging assay.
- Among the series compound of 3(E)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-indolin-2-one (IVa) showed significant antioxidant activity by Nitric oxide scavenging assay.
- Compound (E)-3-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)hydrazono)-5-methoxy indolin-2-one (IVc) showed good antioxidant activity by Nitric oxide scavenging method.
- The compound (R=Cl) and (R=H) have shown most significant antioxidant activity by Nitric oxide scavenging method and Hydrogen peroxide scavenging method and owing to its Strong activating electron donating nature.

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