SYNTHESIS OF 2- ((4-(BENZO[D]OXAZOL-2-YL) PHENYL) AMINO)-N'-(2-OXOINDOLIN-3-YLIDENE) ACETOHYDRAZIDES FOR ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

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ABSTRACT

To find novel substances with anti-inflammatory properties, a series of new 2- ((4-(benzo[d]oxazol-2-yl) phenyl) amino)-N'- (2-oxoindolin-3-ylidene) acetohydrazides (**8a-o)** were synthesized and their structures were confirmed by spectroscopic methods. In vivo anti-inflammatory activity of the synthesized compounds was determined using the carrageenan induced rat paw edema method. Compound **8m**(R=5-F,6-Cl) and **8d**(R=5-F) demonstrated potent anti-inflammatory activity with IC50 **50.63±0.23** and **52.65±0.32** respectively.Structure-activity relationship studies revealed that the substitution of chloro, bromo, and fluoro at 5 and 7 positions of indole moiety significantly increased the anti-inflammatory potency. Substitution of 5flouro 6-Chloro groups on indole increased anti-oxidant activity. Introduction of methyl group, nitro group on the indole moiety resulted in decreasing the anti-inflammatory activity.

KEYWORDS: Anti-inflammatory activity, Indole, Carrageenan, Indomethacin.

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1. INTRODUCTION

Nitrogen and oxygen based heterocyclic compounds are prominent and distinct domain of organic chemistry, with extensive research invested for the synthesis of new compounds. Over the lasttwo decades, these compounds have gained growing interest. They found a wide range of uses in the chemical sciences and contributed to the development of several organic synthetic techniques [1-4].

Numerous naturally occurring N-heterocyclic molecules, such as vitamins, nucleic acids, antibiotics, dyes and agrochemicals, among many others, have physiological and pharmacological effects [5-6].

Moreover, they also play an important role in the formation of numerous compounds that have pharmacological activity. Purines, pyrimidines, and other N-heterocyclic chemicals are also present in the base pairs of DNA and RNA. The rapidly developing fields of organic and medicinal chemistry, as well as the pharmaceutical industry, have given significance to these nitrogen-containing heterocyclic compounds with distinctive properties and applications [7-8]. Furthermore, the electron-rich nitrogen heterocycle can easily generate varied weak connections as well as accept or donate a proton.

In biochemical reactions, heterocyclic compounds play an important role due to the presence of aromatic heterocycles in the side chain of most the component of all living cells (Al-jubouri et al. 2015). These heterocyclic compounds are used as pharmaceuticals in veterinary and human medicine as well as herbicides and insecticides in agriculture, together with organic compounds with biological activity. Most of formulations which are available in the market show the presence of these chemical rings. The presence of these rings exhibited that these substances had pharmacological properties and can simultaneously provide a base for a variety of pharmacophoric groups that cancombine with receptors [8-9].

2. EXPERIMENTAL SECTION:

2.1.Materials and Methods:

In this present work chemicals were obtained from local dealer with SD Fine chem, Himedia and Sigma-Aldrich. All chemicals were 98-99% pure; purity of the synthesized compounds has been checked by TLC and melting point was carried out by using Thieles tube apparatus. The structure was established by spectral data (IR, ^{1}HNMR, ^{13}CNMR and Mass).

2.2. General procedure.

Step-I: Synthesis of 4-(benzo[d]oxazol-2-yl) aniline (3): Equimolar quantities of orthoaminophenol (1; 0.109 mol) and paraaminobenzoic acid (2; 0.137 mol) were mixed with 4N HCl until they dissolve in RBF. The reaction mixture was refluxed for 12-18hrs.The completion of reaction was indicated by TLC. The reaction mixture was poured on crushed ice with constant stirring to get precipitate and later it was allowed to stand aside. Compound 4- (benzo[d]oxazol-2-yl) aniline (3) obtained was filtered, purified, dried and recrystallized from ethanol [10-11].

Step-II: Synthesis of ethyl 2-((4-(benzo[d]oxazol-2-yl) phenyl) amino) acetate (5): A mixture of 4-(benzo[d]oxazol-2-yl) aniline (3; 0.250 mol) and Ethyl chloro acetate (4; 0.122 mol) were refluxed in 20 ml of acetone in the presence of a catalytic amount of Potassium Carbonate (K_2CO_3). TLC was performed to check the completion of reaction. The compound was filtered, evaporated and washed with petroleum ether. The solid separated was filtered, washed with cold

alcohol and the product ethyl 2-((4-(benzo[d]oxazol-2-yl) phenyl)amino)acetate (5).The product obtained was purified by the column chromatography using hexane:ethyl acetate mixture (9:1) as mobile phase.

Step-III: Synthesis of 2-((4-(benzo[d]oxazol-2-yl) phenyl)amino) acetohydrazide (6): A mixture of ethyl 2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)acetate (5, 0.282 mol) and hydrazine hydrate (0.032 mol) in 100 ml methanol was refluxed for 8 hours, the completion of the reaction was monitored by TLC. The mixture was poured into ice cold water and filtered. The compound 2-((4-(benzo[d]oxazol-2-yl) phenyl) amino) acetohydrazide (6) was collected after recrystallizing with methanol.

Step-IV: Synthesis of 2- ((4-(benzo[d]oxazol-2-yl) phenyl) amino)-N'-(2-oxoindolin-3-ylidene) acetohydrazides (8): A mixture of 2-((4-(benzo[d]oxazol-2-yl)phenyl)amino) aceto hydrazide (6; 1.93g, 0.01 mol) and an appropriate isatin (7; 1.47g, 0.01 mol) in methanol was refluxed for 10 hours. The reaction mixture after cooling was poured in to the crushed ice and kept aside for 3-4hrs. The solid separated was filtered and washed with cold alcohol. Adopting this procedure fifteen 2-((4-(benzo[d]oxazol-2-yl) phenyl) amino)-N'-(2-oxoindolin-3-ylidene) acetohydrazides (8a-o) were prepared. These compounds were purified by the column chromatography using hexane: ethyl acetate mixture (9:1) as mobile phase in pure form.

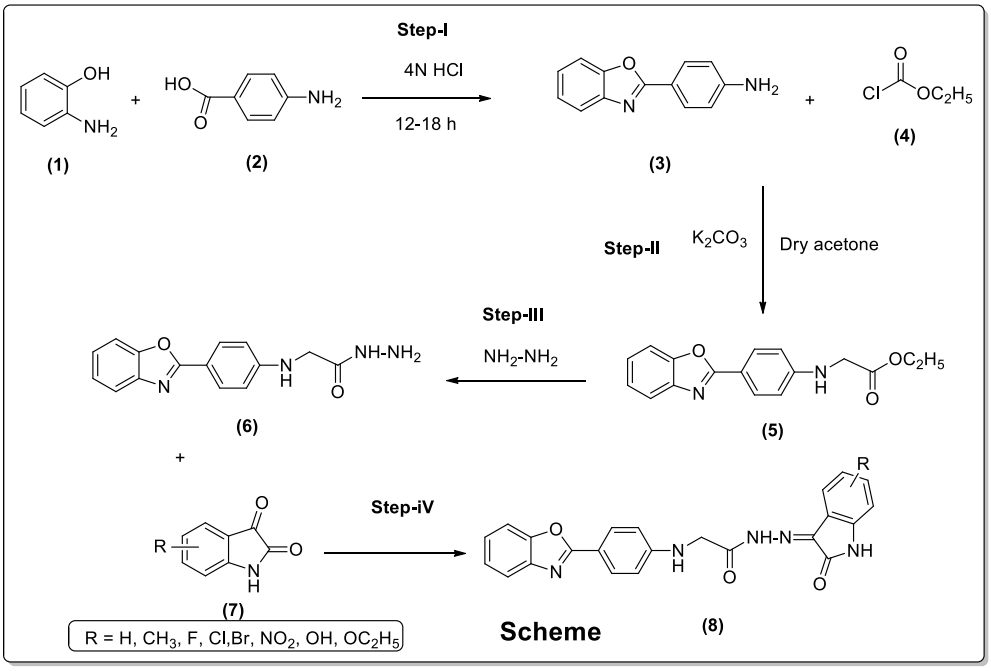

Fig.no:1. Scheme-1: Synthesis of 2-((4-(Benzo[d]oxazol-2-yl) phenyl) amino)-N'-(2-oxoindolin-3-ylidene) acetohydrazides (8a-o).

2.3. Biological Activity: In the present study, the newly synthesized compounds

(8a-o) were evaluated for anti-inflammatory and antioxidant properties as for IAEC protocol.
(IAEC/45/SURA/HYD/2021).

In-vitro Anti-inflammatory Activity [13]

Using a chromogenic assay based on the oxidation of N,N,N′,N′-tetramethyl-p-phenylene diamine during the COX-2 enzyme's conversion of prostaglandin G2 to prostaglandin H2, the enzyme activity was determined. The peroxidase component of cyclooxygenases is measured by the colorimetric COX Inhibitor Screening Assay. By examining the colorimetric appearance of oxidised N,N,N',N'-tetramethyl-p-phenylenediamine at 590 nm, the peroxidase activity was determined.

Reagent Preparation: Assay buffer: 3 ml of Assay Buffer concentration were diluted with 27 ml of HPLC-grade water. Heme and the COX-2 enzyme were diluted in this final assay buffer (0.1 M Tris-HCl, pH 8) before being assayed. It was stored at a temperature of 4 °C.

Heme: Heme in dimethyl sulfoxide is dissolved in the contents of this vial. Heme was diluted before use by adding 1.912 ml of Assay Buffer to 88 µl of heme. This diluted heme is stable at room temperature for 12 hours. Ovine COX-2: When thawed, the ovine COX-2 solution in this vial needs to be maintained on ice. 400 µl of Assay Buffer were used to dilute 200 µl of enzyme, and the mixture was then stored on ice. This can complete 60 wells. When analysing more wells, scale the amount up. The enzyme remains stable for an hour after diluting it.

Arachidonic Acid (substrate): Arachidonic acid in ethanol is dissolved in this vial. A final concentration of 1.1 mM is obtained by transferring 100 µl of the given substrate to a different vial, adding 100 µl of KOH, vortexing the mixture, and diluting it with 1.8 ml of HPLC-grade water. Use the arachidonic acid solution within 30 minutes of preparation. A 20 µl aliquot produced a final concentration in the wells of 100 µM.
Potassium Hydroxide: 0.1 M KOH is present in this vial. The given reagent is prepared for usage.

Colorimetric Substrate: A TMPD solution is contained in this vial. As given, the reagent is ready to use.

Calculations:

To determine the average absorbance of all the samples. Subtract the absorbance of the background wells from the absorbances of the 100% Initial Activity and the Inhibitor wells. To get the per cent inhibition, subtract each inhibitor sample from the 100% initial activity sample, divide the result by 100% initial activity sample, and multiply the result by 100.

Procedure: The final volume of the assay was 220 µl in all the wells.

Background Wells - added 160 µl of Assay Buffer, and 10 µl of heme to three wells and 100% Initial Activity Wells - added 150 µl of Assay Buffer, 10 µl of heme, and 10 µl of Enzyme (COX-2) to three wells.

Inhibitor Wells - added 150 µl of Assay Buffer, 10 µl of heme, and 10 µl of Enzyme (COX-2) to three wells. Added 10 µl of inhibitor* to the Inhibitor wells and 10 µl of solvent (which ever solvent you dissolved your inhibitor in) to the 100% Initial Activity wells and background wells. Carefully shaken the plate for a few seconds and incubated for five minutes at 25°C.

Added 20 µl of the colorimetric substrate solution to all the wells that were used. Added 20 µl of arachidonic acid to all the wells that were used. Carefully shaken the plate for a few seconds and incubated for five minutes at 25°C. Read the absorbance at 590 nm using a plate reader.

In Vivo Acute Anti-inflammatory Activity: [14-16]

Carrageenan induced paw edema in rats: Wistar strain Albino rats of either sex, weighing between 200 and 250 g were placed into fourteen groups of six animals each. A plethismometer was used to calculate the right hind paw's volume. This constituted the initial reading. The test compounds were used a dose i.e., 100mg/kg body weight. Indomethacin10mg (31.4 µM)/kg was used as standard. All of them were given as suspensions with sodium CMC (0.1% w/v) serving as the suspending agent. Only sodium CMC suspension was given to the animals in the control group. One hour before to the carrageenan injection, all of them were given orally. To treat the plantar area of the right hind paw, 0.1 ml of 1% w/v carrageenan suspension in normal saline was injected. Every hour for four hours, the swelling that was caused by the phlogistic agent injection was measured. Using the formula shown below, the percentage inhibition of edema was calculated:

$$\%\text{Inhibition of edema} = \frac{\text{mean edema of control group - mean edema of treated group}}{\text{mean edema of control group}} * 100$$

Anti-oxidant Activity:

DPPH radical scavenging assay[17]

2.5 ml of the compound (10- 100 g/ml) were combined with 1 ml of a 0.1 mM solution of DPPH in methanol. After that, the reaction mixture was let to stand at room temperature in a dim environment for 30 minutes. After 30 minutes, a UV-visible spectrophotometer was used to measure the absorbance at 517 nm. (Blios et al., 1958). Ascorbic acid was used as a standard.

The scavenging activity of DPPH radical (%) was calculated from the following equation:

$$\%\text{ scavenging activity} = \frac{\text{Absorbance of control/Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

3. RESULTS AND DISCUSSION

3.1. Chemistry: A mixture of orthoaminophenol(1) and paraaminobenzoic acid (2) were mixed in acetic anhydride was refluxed for 12-18hrs at 200ºC. The completion of reaction was monitored by TLC. The reaction mixture was poured on to crushed ice with constant stirring to get 4-(benzo[d]oxazol-2-yl)aniline **(3)**. A mixture of compoundn(3**;**0.250 mol) and Ethyl chloro acetate (4; 0.122 mol) were

refluxed in 20 ml of acetone in the presence of a catalytic amount of Potassium Carbonate(K_2CO_3). After completion of the reaction monitored by TLC the reaction mixture was washed with petroleum ether then recrystallized to give the compound ethyl 2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)acetate **(5).** Compound 5 and hydrazine hydrate in methanol was stirred and refluxed for 8 hours.The reaction mixture was poured into ice cold water and filtered to give compound 2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)acetohydrazide **(6).** Compound 2-((4-(benzo[d]oxazol-2-yl)phenyl) amino)acetohydrazide (**6**; 1.93g, 0.01 mol) and respective isatin (**7a-o;** 1.47g, 0.01 mol) in methanol were refluxed for 10 hours. The reaction mixture was poured in to the crushed ice and kept aside for 3-4hrs. The solid separated was filtered, washed with cold methanol and the products 2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3-ylidene) aceto hydrazides(8a-o) obtained were purified by the column chromatography using hexane:ethyl acetate mixture (9:1) as mobile phase to get the target molecules **(8a-o)** in pure form. Compounds were characterized by spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass).

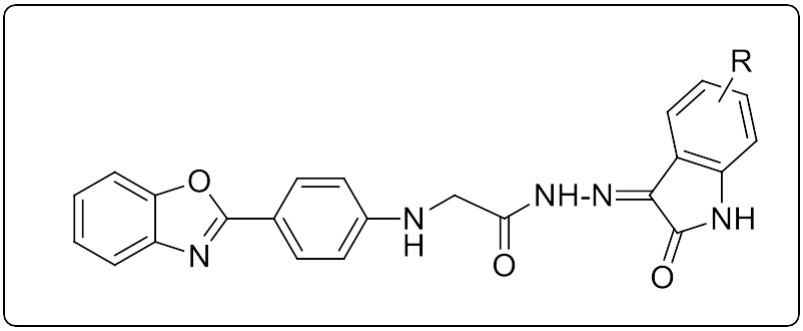

Fig.No.2. General Structure of compound-8(a-o)

Table:1: Physical data of synthesized 2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3-ylidene)acetohydrazides(8a-o)

S.No	Compound	Substituent (R)	Mol.Formula	Mol. Wt.	% Yield
1	8a	H	$C_{23}H_{17}N_5O_3$	411	78
2	8b	5-Cl	$C_{23}H_{16}ClN_5O_3$	445	84
3	8c	5-Br	$C_{23}H_{16}BrN_5O_3$	490	95
4	8d	5-F	$C_{23}H_{16}FN_5O_3$	429	85
5	8e	5-CH_3	$C_{24}H_{19}N_5O_3$	425	50
6	8f	5-NO_2	$C_{23}H_{16}N_6O_5$	456	70
7	8g	6-Br	$C_{23}H_{16}BrN_5O_3$	490	70

8	8h	7-F	$C_{23}H_{16}FN_5O_3$	429	80
9	8i	7-Cl	$C_{23}H_{16}ClN_5O_3$	445	65
10	8j	7-Br	$C_{23}H_{16}BrN_5O_3$	490	70
11	8k	7-CH_3	$C_{24}H_{19}N_5O_3$	425	50
12	8l	7-NO_2	$C_{23}H_{16}N_6O_5$	456	52
13	8m	5-Flouro-6-Chloro	$C_{23}H_{15}Cl_2FN_5O_3$	479	60
14	8n	5-COOH	$C_{24}H_{17}N_5O_5$	455	38
15	8o	5-$COOC_2H_5$	$C_{26}H_{21}N_5O_5$	483	60

3.2. Spectral data:

Compound.8a:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3ylidene)aceto hydrazide. IR spectrum (KBr, cm^{-1}): 3398.83(NH),3050.91(C-H Aromatic str), 2921.96(C-H Aliphatic str), 1749.43(C=O(str)), 1581.09(C=C (str)), 1318.44(C-O(str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.11 (s, 1H, Isatin NH), 8.79-8.83(d, 3H, aromatic CH, amide NH), 8.68-8.74(m, 2H, aromatic CH and aryl CONH), 8.12-8.14 (d, 3H, aromatic CH), 7.97-8.14 (m, 3H, aromatic CH), 7.83-7.88 (m, 2H, aromatic CH), 7.646-7.685 (m, 2H, aromatic CH), 4.02 (s, 1H, NH), 3.39(s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 165.53, 158.96, 152.72, 143.90, 137.00, 135.53, 134.92, 131.24, 128.89, 126.73, 124.41, 120.92, 119.82, 117.79. **MASS spectrum m/z:** 411$[M+H]^+$.

Compound.8b:2-((4-(Benzo[d]oxazol-2-yl)-phenyl)-amino)-N'-(5-chloro-2-oxoindolin-3-ylidene)-acetohydrazide. IR spectrum (KBr, cm^{-1}):3340.99(NH),3058.56(C-H Aromatic str), 2976.58 (C-H Aliphatic str), 1742.84 (C=O (str)), 1591.59 (C=C (str)), 1398.20 (C-O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.19 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.95-8.00(t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.80-6.82 (d, 1H, aromatic CH), 4.29 (s, 1H, NH), 2.40 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 164.13, 156.12, 154.18, 148.10, 138.10, 137.13, 134.92, 131.24, 128.89, 126.73, 124.41, 122.90, 119.82, 118.15.**MASS spectrum m/z:** 447.$[M+2]^+$.

Compound.8c:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(5-bromo-2-oxoindolin-3-ylidene)acetohydrazide. IR spectrum (KBr, cm^{-1}): 3348.83(NH),3071.10(C-H Aromatic str), 2985.15 (C-H Aliphatic str), 1745.85 (C=O (str)), 1575.64 (C=C Aromatic str), 1391.11(C-O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.09 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.91-8.00 (t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.81-6.83 (d, 1H, aromatic CH), 4.27 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2). **^{13}C NMR (100MHz, $CDCl_3$):** 161.12, 160.12, 158.18, 156.20, 148.18, 145.13, 143.01, 140.25, 138.84,

136.74, 129.45, 127.92, 127.90, 116.12. **MASS spectrum m/z:** 492.2 $[M+2]^+$.

Compound.8d:2-((4-(Benzo[d]oxazol-2-yl)phenyl)-amino)-N'-(5-fluoro-2-oxoindolin-3-ylidene) acetohydrazide. IR spectrum (KBr, cm^{-1}): 3359.84(NH), 3052.52(C-H Aromatic str), 2971.18 (C-H Aliphatic str), 1735.80 (C=O (str)), 1590.50 (C=C Aromatic str), 1390.25(C-O (str)). **^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.19 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.91-8.00(t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.80-6.82 (d, 1H, aromatic CH), 4.29 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2). **^{13}C NMR (100MHz, $CDCl_3$):** 161.15, 155.10, 154.12, 148.18, 138.18, 134.13, 132.92, 130.24, 126.80, 125.75, 124.46, 123.92, 120.80, 119.10. **MASS spectrum m/z:** 431.$[M+2]^+$.

Compound.8e:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(5-methyl-2-oxoindolin-3-ylidene)-acetohydrazide hydrate. IR spectrum (KBr, cm^{-1}): 3358.87(NH),3080.18 (C-H Aromatic str), 2985.90(C-H Aliphatic str), 1754.42(C=O (str)), 1580.07 (C=C Aromatic str), 1358.48 (C-O(str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.12 (s, 1H, Isatin NH), 8.79-8.83 (d, 3H, aromatic CH, amide NH), 8.68-8.74(m, 3H, aromatic CH), 8.12-8.15 (d, 1H, aromatic CH), 7.97-8.09 (m, 4H, aromatic CH), 7.83-7.88 (m, 2H, aromatic CH), 7.64-7.68 (d, 1H, aromatic CH), 4.03 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 162.52, 160.10, 158.78, 156.92, 150.15, 148.50, 137.94, 136.25, 135.80, 130.13, 128.48, 127.95, 124.80, 120.75. **MASS spectrum m/z:** 444.$[M+1]^+$.

Compound.8f:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(5-nitro-2-oxoindolin-3-ylidene)-acetohydrazide. IR spectrum (KBr, cm^{-1}): 3364.83(NH), 3068.60(C-H Aromatic str), 2989.12 (C-H Aliphatic str), 1738.70 (C=O (str)), 1595.40 (C=C Aromatic str), 1385.20(C-O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.00 (s, 1H, Isatin NH), 8.49-8.51 (d, 21H, aromatic CH), 7.91-8.01(t, 1H, aromatic CH), 7.89-7.91 (d, 2H, aromatic CH), 7.77-7.81 (t, 1H, aromatic CH), 7.62-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.81-6.82 (d, 1H, aromatic CH), 4.29 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2). **^{13}C NMR (100MHz, $CDCl_3$):** 168.15, 159.18, 157.18, 150.14, 148.12, 144.13, 132.92, 130.24, 128.80, 127.75, 124.46, 123.92, 122.80, 116.10. **MASS spectrum m/z:** 475 $[M+1]^+$.

Compound.8g:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(6-bromo-2-oxoindolin-3-ylidene)acetohydrazide. IR spectrum (KBr, cm^{-1}): 3348.84(NH),3075.17 (C-H Aromatic str), 2984.12 (C-H Aliphatic str), 1742.84 (C=O (str)), 1571.61 (C=C Aromatic str), 1392.12(C-O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.09 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.91-8.00 (t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.81-6.83 (d, 1H, aromatic CH), 4.27 (s, 1H, NH), 3.39 (s, 2H,

aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 164.12, 161.12, 157.18, 156.20, 148.18, 145.13, 143.01, 140.25, 138.84, 136.74, 129.45, 126.92, 125.90, 115.12.**MASS spectrum m/z:** 492.2 $[M+2]^+$.

Compound.8h:2-((4-(Benzo[d]oxazol-2-yl)phenyl)-amino)-N'-(7-fluoro-2-oxoindolin-3-ylidene)acetohydrazide. IR spectrum (KBr, cm^{-1}): 3348.89(NH), 3050.51(C-H Aromatic str), 2970.10 (C-H Aliphatic str), 1730.81 (C=O (str)), 1594.54 (C=C Aromatic str), 1394.24(C-O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.16 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.91-8.00(t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.80-6.82 (d, 1H, aromatic CH), 4.29 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 164.15, 155.10, 154.12, 148.18, 138.18, 134.13, 132.92, 130.24, 126.80, 125.75, 124.46, 123.92, 120.80, 114.10.**MASS spectrum m/z:** 431.$[M+2]^+$. **Comound.8i:2-((4-(Benzo[d]oxazol-2-yl)-phenyl)-amino)-N'-(7-chloro-2-oxoindolin-3-ylidene) – acetohydrazide. IR spectrum (KBr, cm^{-1}):** 3340.99(NH),3068.55(C-H Aromatic str), 2971.54 (C-H Aliphatic str), 1744.81 (C=O (str)), 1594.54(C=C Aromatic str), 1394.24 (C-O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.19 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.95-8.00 (t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.80-6.82 (d, 1H, aromatic CH), 4.29(s, 1H, NH), 3.39 (s, 2H, aliphatic CH). **^{13}C NMR (100MHz, $CDCl_3$):** 168.13, 156.12, 154.18, 148.10, 138.10, 137.13, 134.92, 131.24, 128.89, 126.73, 124.41, 122.90, 119.82, 115.15.**MASS spectrum m/z:** 447.$[M+2]^+$. **Compound.8j:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(7-bromo-2-oxoindolin-3-ylidene)acetohydrazide.IR spectrum (KBr, cm^{-1}):** 3397.83(NH), 3070.10 (C-H Aromatic str), 2980.11 (C-H Aliphatic str), 1744.85 (C=O (str)), 1577.66 (C=C Aromatic str), 1396.14(C-O (str)). **^{1}HNMR (400MHz $CDCl_3$, δ ppm):** 10.09 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.91-8.00 (t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.81-6.83 (d, 1H, aromatic CH), 4.27 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 162.11, 160.12, 157.18, 156.20, 148.18, 145.13, 143.01, 140.25, 137.84, 134.74, 127.45, 126.92, 125.90, 112.12.**MASS spectrum m/z:** 492.2 $[M+2]^+$.

Compound.8k:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(7-methyl-2-oxoindolin-3-ylidene)-acetohydrazide hydrate. IR spectrum (KBr, cm^{-1}): 3398.87(NH),3087.90 (C-H Aromatic str), 2980.95(C-H Aliphatic str), 1759.42(C=O (str)), 1585.09 (C=C Aromatic str), 1320.40 (C-O(str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.18 (s, 1H, Isatin NH), 8.79-8.83 (d, 2H, aromatic CH, amide NH), 8.68-8.74(m, 4H, aromatic CH), 8.12-8.14 (d, 1H, aromatic CH), 7.97-8.08 (m, 3H,

aromatic CH), 7.83-7.88 (m, 2H, aromatic CH), 7.64-7.68 (d, 2H, aromatic CH), 4.03 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 168.55, 156.10, 156.78, 145.92, 140.15, 138.50, 137.94, 136.25, 135.80, 130.13, 129.48, 127.95, 126.80, 119.75. **MASS spectrum m/z:** 444.$[M+1]^+$.

Compound.8l:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(7-nitro-2-oxoindolin-3-ylidene)-acetohydrazide hydrate. IR spectrum (KBr, cm^{-1}): 3398.88(NH),3074.15(C-H Aromatic str), 2980.18 (C-H Aliphatic str), 1742.81 (C=O (str)), 1570.60 (C=C Aromatic str), 1390.18(C-O (str)). **^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.09 (s, 1H, Isatin NH), 8.49-8.51 (d, 1H, aromatic CH), 7.91-8.00 (t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.63-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.81-6.83 (d, 1H, aromatic CH), 4.27 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 164.18, 160.17, 159.18, 156.20, 148.18, 145.13, 143.01, 140.25, 138.84, 136.74, 129.45, 128.92, 127.90, 118.12.**MASS spectrum m/z:** 475.2 $[M+1]^+$.

Compound.8m:2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(6-chloro-5-fluoro-2-oxoindolin-3-ylidene)acetohydrazide. IR spectrum (KBr, cm^{-1}): 3398.89(NH), 3046.89 (C-H Aromatic str), 2985.90(C-H Aliphatic str), 1752.40(C=O (str)), 1575.09(C=C Aromatic str), 1320.45(C-O(str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.12(s, 1H, Isatin NH), 8.79-8.83 (d, 4H, aromatic CH, amide NH), 8.68-8.74(m, 2H, aromatic CH), 8.12-8.14 (d, 1H, aromatic CH), 7.97-8.08 (m, 2H, aromatic CH), 7.83-7.88 (m, 2H, aromatic CH), 7.64-7.68 (d, 2H, aromatic CH), 4.03 (s, 1H, NH), 3.39(s, 2H, aliphaticCH2).**^{13}C NMR (100MHz, $CDCl_3$):** 161.51, 155.90, 154.70, 148.95, 139.09, 138.57, 135.94, 134.25, 130.80, 129.13, 128.48, 128.95, 120.80, 115.75.

MASS spectrum m/z: 467.$[M+4]^+$.

Compound.8n: Methyl3-(2-(2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)acetyl)hydrazono)-2-oxoindoline-7-carboxylate hydrate. IR spectrum (KBr, cm^{-1}): 3398.99(NH), 3075.40(C-H Aromatic str), 2980.18 (C-H Aliphatic str), 1740.21 (C=O (str)), 1574.50 (C=C Aromatic (str)), 1396.25 (C- O (str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.10 (s, 1H, Isatin NH), 8.48-8.51 (d, 1H, aromatic CH), 7.91-8.00 (t, 2H, aromatic CH), 7.89-7.91 (d, 1H, aromatic CH), 7.77-7.81 (t, 2H, aromatic CH), 7.66-7.67 (m, 2H, aromatic CH), 7.38-7.47 (m, 2H, aromatic CH), 7.19-7.23 (t, 1H, aromatic CH), 6.81-6.82 (d, 1H, aromatic CH), 4.29 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2). **^{13}C NMR (100MHz, $CDCl_3$):** 174.18, 164.5.17, 158.18, 155.20, 150.18, 145.13, 144.01, 139.25, 137.84, 130.74, 128.49, 123.92, 123.80, 119.12. **MASS spectrum m/z:** 488.$[M+1]^+$.

Compound.8o:Ethyl3-(2-(2-((4-(benzo[d]oxazol-2-yl)phenyl)amino)acetyl)hydrazono)-2-oxoindoline-5-carboxylate. IR spectrum (KBr, cm^{-1}): 3398.89(NH), 3080.18 (C-H Aromatic str), 2985.90(C-H Aliphatic (str)), 1754.42(C=O (str)), 1580.07 (C=C Aromatic str), 1358.48 (C-O(str)).**^{1}H NMR (400MHz $CDCl_3$, δ ppm):** 10.12 (s, 1H,

Isatin NH), 8.79-8.83 (d, 3H, aromatic CH, amide NH), 8.68-8.74(m, 3H, aromatic CH), 8.12-8.15 (d, 1H, aromatic CH), 7.97-8.09 (m, 4H, aromatic CH), 7.83-7.88 (m, 2H, aromatic CH), 7.64-7.68 (d, 1H, aromatic CH), 4.03 (s, 1H, NH), 3.39 (s, 2H, aliphatic CH2).**^{13}C NMR (100MHz, $CDCl_3$):** 162.52, 160.10, 158.78, 156.92, 150.15, 148.50, 137.94, 136.25, 135.80, 130.13, 128.48, 127.95, 124.80, 120.75. **MASS spectrum m/z:** 444.$[M+1]^+$.

In-vitro Anti-inflammatory Activity. All the fifteen compounds have been screened for *In-vitro* and *In-vivo* anti-inflammatory activity by COX inhibition method,Indomethacin was used as standard drug.Anti-inflammatory activity can be tangibly correlated with structure of all compounds depending on the substitutionon the isatin ring. Anti-inflammatoryactivity of all the compounds has been listedin **Table 2**.

3.3. Biological activity:

Table 2: COX-2 Inhibitory activity of 2-((4-(Benzo[d]oxazol-2-yl) phenyl)amino)-N'-(2-oxoindolin-3ylidene)acetohydrazides (8a-o)

S.No	Compound	Substituent (R)	Mol.Formula	COX-2 Inhibition IC50 (µM)
1	8a	H	$C_{23}H_{17}N_5O_3$	66.23±0.14
2	**8b**	**5-Cl**	**$C_{23}H_{16}ClN_5O_3$**	**56.35±0.54**
3	8c	5-Br	$C_{23}H_{16}BrN_5O_3$	61.590±0.11
4	**8d**	**5-F**	**$C_{23}H_{16}FN_5O_3$**	**52.65±0.32**
5	8e	5-CH_3	$C_{24}H_{19}N_5O_3$	68.34±0.45
6	8f	5-NO_2	$C_{23}H_{16}N_6O_5$	69.64±0.56
7	8g	6-Br	$C_{23}H_{16}BrN_5O_3$	59.21±0.33
8	**8h**	**7-F**	**$C_{23}H_{16}FN_5O_3$**	**54.71±0.67**
9	**8i**	**7-Cl**	**$C_{23}H_{16}ClN_5O_3$**	**58.56±0.40**
10	8j	7-Br	$C_{23}H_{16}BrN_5O_3$	60.63±0.23
11	8k	7-CH_3	$C_{24}H_{19}N_5O_3$	73.18±0.46
12	8l	7-NO_2	$C_{23}H_{16}N_6O_5$	69.42±0.19
13	**8m**	**5-F, 6-Cl**	**$C_{23}H_{15}Cl_2FN_5O_3$**	**50.63±0.23**
14	8n	5-COOH	$C_{24}H_{17}N_5O_5$	63.18±0.46
15	8o	5-$COOC_2H_5$	$C_{26}H_{21}N_5O_5$	69.42±0.19
16	Indomethacine	-	-	28.41±0.19

Data presented in the table 2 reveals that most active compound among the series was found to be **8m** (R=5-F, 6-Cl) with IC_{50} of **50.63±0.23**. Among the halo substitution compounds this is followed by compounds **8d (**R=5-F**)** with IC_{50} of , 52.65±0.32; **8h (**R=7-F**)** with IC_{50} of 54.71±0.67; **8b** (R=5-Cl) with IC50 of 56.35±0.54; **8i (**R=7-Cl**)** with IC_{50} of 58.56±0.40**;** showing COX-2 inhibitory activity Rest of the compounds showed moderate to mild COX- 2 inhibitory activity with IC50 values in the

range from 60.63±0.23 to 73..18±0.46.None of the compounds showed COX-2 inhibitory activity on a par with standard Indomethacin with IC 50 value of 28.41±0.19

***In-Vivo* Anti-inflammatory Activity:**

Six compounds were selected from those that shown the best invitro anti-inflammatory activity and testedfor in vivo anti-inflammatory activity using the carrageenan-induced rat paw edema method, at a dose of 100 mg/kg body weight. The data showed that all test compounds significantlyreduced carrageenan induced rat paw edema,and the results were shown in Table 3. Among the all,compound **8b (**R=5-Cl**), 8d (**R=5-F**),8h (**R=7-F**), 8i (**R=7-Cl**), 8m**(R=5F,6-Cl) are considered to possess potent anti-inflammatory activity with mean rat paw edema volume 0.49±0.039, 0.52±0.048, 0.42±0.028, 045±0.078 and 0.32±0.042 at 1st hour of experiment respectively . From the above data it clearly indicates that halo substituted derivatives found to be more potent among all the compounds. Anti-inflammatory activity of compound **8m**(R=5F,6-Cl) with mean paw volume of 0.32±0.042.was compared with the anti-inflammatory activity of standard indomethacin with mean paw volume 0.30±0.070 at first hour of experiment.

Table 3. ***In vivo*** **Anti-inflammatory activity of 2-((4-(Benzo[d]oxazol-2-yl) phenyl)amino)-N'-(2-oxoindolin-3ylidene)acetohydrazides (8a-o)**

S.No	Compound	R	Mean Paw Edema Volume in ml ± SD			
			1h	2h	3h	4h
1	8b	5-Cl	0.49±0.039	0.44±0.036	0.36±0.028	0.27±0.048
2	8d	5-F	0.52±0.048	0.50±0.018	0.39±0.068	0.36±0.29
3	8h	7- F	0.42±0.028	0.36±0.078	0.31±0.028	0.28±0.042
4	8i	7-Cl	0.45±0.078	0.40±0.042	0.35±0.039	0.29±0.018
5	8m	5-F, 6-Cl	0.32±0.042	0.29±0.052	0.25±0.084	0.21±0.031
6	8o	5-$COOC_2H_5$	0.49±0.076	0.43±0.031	0.41±0.056	0.39±0.054
7	**Control Group**		0.56±0.090	0.63±0.064	0.71±0.034	0.80±0.090
8	**Indomethacin**		**0.30± 0.070**	**0.24± 0.120**	**0.20 ± 0.080**	**0.16 ± 0.062**

The anti-inflammatory activity of standard indomethacin with mean paw volume at first hour of experiment was 0.30± 0.070

Anti-oxidant Activity: Antioxidant activity of the 2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3ylidene)acetohydrazides (8a-o) was

evaluated by 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity. Results, shown in Table 4, are expressed as µmol for DPPH test and are compared to the reference compound Ascorbic acid. For the best interpretation of the results of the DPPH test, each compound was tested at the concentration capable of inhibiting 50% of the radical scavenging activity. The radical scavenging activity of the 2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3ylidene)acetohydrazides (8a-o) that emerged from DPPH results indicates that compounds bearing a 5-F, 6-Cl on the indole group showed potent antioxidant activity with IC_{50} of 58.32 µM. By introducing Cl group in the 5- position of the indole group showed the antioxidant capacity increased of about 2-fold as compared to unsubstituted indole moiety. The shift of 5-Cl group into 7-position on indole moiety increased in activity. The introduction of a Methyl and Nitro group at 5-position of indole showed moderate anti-oxidant activity. the results are depicted in Table 4.

Table 4. Antioxidant activity of 2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3ylidene)acetohydrazides (8a-o)

S.No	Compound	Substituent (R)	Mol.Formula	IC50 (µM)
1	8a	H	$C_{23}H_{17}N_5O_3$	55.25
2	8b	5-Cl	$C_{23}H_{16}ClN_5O_3$	44.10
3	8c	5-Br	$C_{23}H_{16}BrN_5O_3$	46.87
4	8d	5-F	$C_{23}H_{16}FN_5O_3$	46.20
5	8e	5-CH_3	$C_{24}H_{19}N_5O_3$	54.42
6	8f	5-NO_2	$C_{23}H_{16}N_6O_5$	50.76
7	8g	6-Br	$C_{23}H_{16}BrN_5O_3$	48.11
8	8h	7-F	$C_{23}H_{16}FN_5O_3$	45.15
9	8i	7-Cl	$C_{23}H_{16}ClN_5O_3$	40.60
10	8j	7-Br	$C_{23}H_{16}BrN_5O_3$	49.33
11	8k	7-CH_3	$C_{24}H_{19}N_5O_3$	59.23
12	8l	7-NO_2	$C_{23}H_{16}N_6O_5$	56.87
13	8m	5-F, 6-Cl	$C_{23}H_{15}Cl_2FN_5O_3$	35.15
14	8n	5-COOH	$C_{24}H_{17}N_5O_5$	58.32
15	8o	5-$COOC_2H_5$	$C_{26}H_{21}N_5O_5$	52.47
16	Ascorbic acid	-	-	6.03

4. Conclusions: In the current study, 15 novel 2-((4-(Benzo[d]oxazol-2-yl)phenyl)amino)-N'-(2-oxoindolin-3ylidene)acetohydrazides (8a-o) have been synthesized and their anti-inflammatory activities have been investigated with respect to their inhibition of Carrageenan induced rat

paw edema. The synthesized compounds exhibited potent anti-inflammatory activity it is concluded that 15 compounds inhibited the cox-2 enzyme, All the compounds exhibited varied degrees of antioxidant activity.Structure-activity relationship has also been established with respect to the substituents present on the core indole moiety of the synthesized compounds. All these results yield valuable information for further optimization of structure-based drug design.

DECLARATION OF INTEREST.

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