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SYNTHESIS OF NOVEL 5, 6 – BENZ 1, 3 – OXAZEPINE 4, 7 – DIONE DERIVATIVES AND SCREENING FOR ANTIBACTERIAL, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT

The present study involves the synthesis of series of eight number of 5,6 – Benz 1,3 – Oxazepine 4, 7 – Dione (BO.1-BO.8) derivatives by cycloaddition reaction between schiff base (semicarbazone) and phthalic anhydride with dry benzene as the solvent. Schiff base is synthesized by the condensation reaction of semicarbazide hydrochloride with various aromatic aldehydes in the presence of sodium acetate. All the theoretically synthesised compounds were screened for *in vitro* antibacterial (Disc diffusion method), *in vitro* antioxidant (H_2O_2 scavenging method) and *in vitro* and *in vivo* anti-inflammatory activity (protein denaturation method and carrageenan induced inflammation respectively.

KEYWORDS: Semicarbazide Hydrochloride, Schiff bases, Cycloaddition, Phthalic anhydride, Oxazepinediones, antibacterial, antioxidant, anti-inflammatory activity.

INTRODUCTION

5, 6 – Benz 1, 3 – Oxazepine 4, 7 – Dione contains oxazepine[1] as the core nucleus, which is a seven membered heterocyclic compound which contain oxygen and nitrogen as the hetero atom in first and third position, were two ketone moiety attached to the fourth and seventh position of the ring, sixth and fifth carbon is fused with a benzene ring. The method used for the synthesis of 5, 6 – Benz 1, 3 – Oxazepine 4, 7 – Dione is pericyclic cycloaddition[2,3], which is classified as a 5+2 = 7, which implying five-atom component plus two-atom component leading to seven-membered heterocyclic ring. Here the five atom involved in the synthesis of oxazepinedione derivative component is the anhydride nucleus of phthalic anhydride and the two atom component is C=N of schiff base or imine. The mechanism involves the addition of one σ- carbonyl to π-bond (N=C) to give 4- membered cyclic and 5-membered cyclic ring of anhydride in the same transition state, which opens into various anhydride (E.g.: phthalic anhydride) to a given 7-membered cyclic ring 1, 3-oxazepine 4, 7 dione.

The intermediate (Schiff base) used in this reaction is semicarbazone[4] which is synthesized by the usual condensation reaction in which an aromatic aldehydes with a primary amine (semicarbazide) forms an imine in the presence of mild acid. Schiff-base compounds have been used as fine chemicals and medical substrates. Compared to other derivatives of oxazepine much less studies are so far conducted for oxazepinediones. Literature reviews shows that oxazepinedione nucleus have antimicrobial activity[1, 3], antitumor activity[5], anticorrosive activity[6], enzymatic activity[7] and anticonvulsant activity.[8] All the synthesised compounds (BO.1 – BO.8) were screened for antibacterial activity, *in vitro* antioxidant activity, *in vitro* and *in vivo* anti-inflammatory activity.

MATERIALS AND METHODS

(I) General Synthesis

(1) General procedure for synthesis of Schiff base {Semicarbazone}[9, 10]

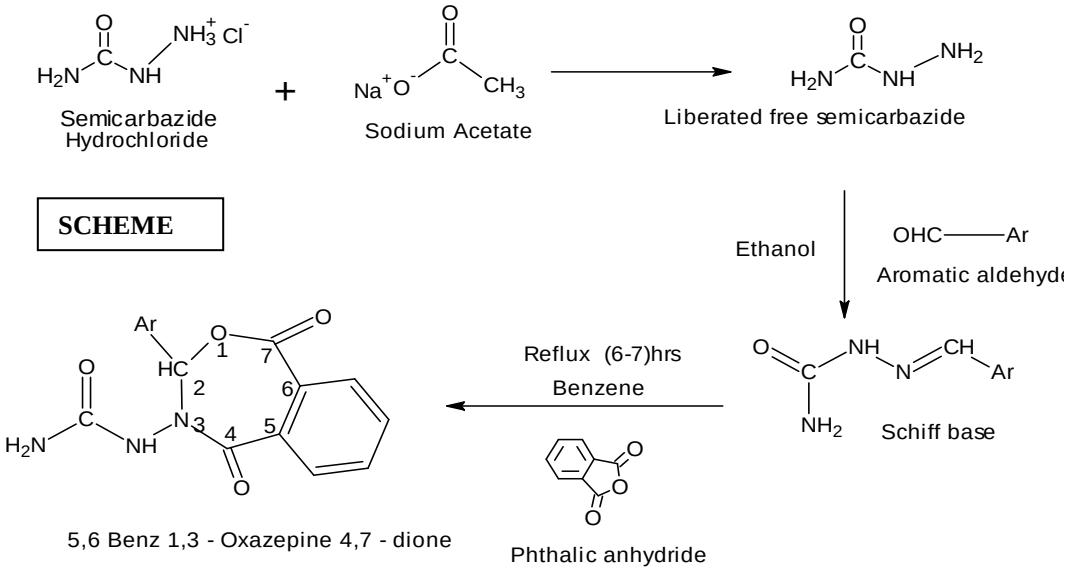
Dissolve 0.02M of semicarbazide hydrochloride and 2g of crystallized sodium acetate in 5ml of water in a conical flask, then add 0.02M of aromatic aldehyde (Ar–CHO) and shake well to obtain a turbid mixture. Add alcohol (acetone free) until a clear solution is obtained; shake the mixture for a few minutes and allow to stand. The semicarbazone crystallizes from the cold solution on stand. Filter off the crystals, wash with a little cold water and recrystallized from ethanol.

(2) General procedure for synthesis of 5, 6 – Benz 1, 3 – Oxazepine 4, 7 – Dione[11,12,13]

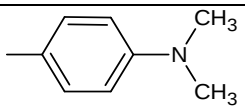
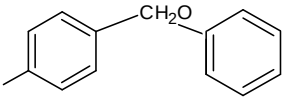
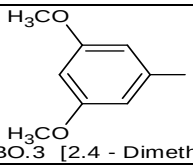
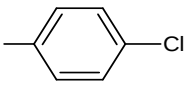
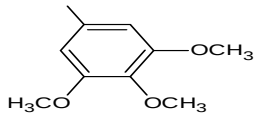
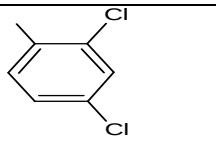
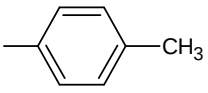
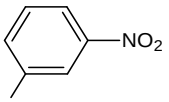
Accurately weighed about an equimolar quantity, i.e. 0.01M of synthesized semicarbazone (schiff base) in the above step and 0.01M of phthalic anhydride into a round

bottom flask. Add 25ml of benzene as solvent and then reflux the reaction mixture for 6 – 7hrs in a water bath. Cool the reaction mixture in an ice bath for several hours. Filter off the precipitated product and the recrystallized it from ethanol.

Scheme for the synthesis of Oxazepinedione from semicarbazide hydrochloride is as Follows.



Synthesis of Oxazepinedione from Schiff base {Semicarbazone}

SUBSTITUENTS USED			
 BO.1 [4 - Dimethyl amino	 BO.2 [4 - Benzyloxy]	 BO.3 [2.4 - Dimethoxy	 BO.4 [4 - Chloro]
 BO. 5 [3,4,5 - Trimethoxy	 BO.6 [2,4 - Dichloro]	 BO. 7 [4 - Methyl]	 BO. 8 [3 - Nitro]

B. Screening for Biological Activities

1. Antioxidant Activity (*In vitro* antioxidant activity by H_2O_2 scavenging method)[15,16]

A solution of H_2O_2 (20mM) was prepared in phosphate buffer saline (pH 7.4). 1ml of test sample and standard (ascorbic acid) in a concentration of 100µg/ml in ethanol were added to 2ml of H_2O_2 solution in phosphate buffer saline. The absorbance was measured at 230nm after 10minutes.

Percentage of H_2O_2 scavenged = $(A_c\text{-}A_t)/A_c*100$.

2. Antibacterial Activity (Disc diffusion method)[17]

The newly synthesized compounds were tested for their preliminary antibacterial activity against different microorganisms representing gram positive bacteria (*Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa, Escherichia. coli*) by disc diffusion method using ciprofloxacin as standard.

The antibacterial screening was carried out in a laminar air flow unit. Petri dishes and other glassware were sterilized in the autoclave at 121°C temperature and at a pressure of 15 lbs/sq inch for 15 minutes. In disc diffusion method bacterial inoculums were prepared and inoculated into the entire surface of solid agar plate with a sterile cotton- tipped swab to form an even lawn. The paper disc 6mm in diameter impregnated with diluted test drug solution (1mg/ml in ethanol) was placed on the surface of each of agar plates using a sterile pair of forceps. Standard used is ciprofloxacin 10The forceps were sterilized using flame. The plates were incubated for 2 - 3 days at 20 -25^{0}C and observed without opening them and the zone of inhibition was measured.

3. Anti Inflammatory Activity (*In vitro* protein denaturation method)[18, 19]

A solution of 0.2% w/v of Bovine Serum Albumin (BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Test drug of 100µg/ml concentration were prepared using ethanol as solvent. 50µl of each test drug was transferred to test tubes using micropipette. 5ml of 0.2% w/v BSA was added to the test tubes. The control consists of 5 ml of

0.2%w/v BSA solution and 50µl of alcohol. Diclofenac 100µg/ml is used as standard. The test tubes were heated at 72^0C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions were determined using UV-VIS spectrophotometer at a wavelength of 660nm.
Percentage inhibition = $(A_c - A_t)/A_c$ *100
A_c: absorbance of control, A_t: absorbance of test

4. Anti Inflammatory Activity[18]

Animals Used For the Study

Albino rats (wistar strain) were used to carry out the activities. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions i.e., 12:12 hour light or dark cycle at 25 ± 2^0C. The experiments were carried out as per the guidelines of CPCSEA, New Delhi, India.

Acute Toxicity Test[20]

Acute toxicity studies of the synthesized compounds were carried out using OECD/OCED guideline 423. Healthy young adult non pregnant female albino rats were used for this study. Animals were fasted prior to dosing (food but not water should be withheld overnight). Following the period of fasting, the animals were weighed and the test substance administered orally at different dose levels (5, 50,300 & 2000mg/kg) and the animals were tested for their mortality.

Carrageenan-induced oedema in rats[21]

For screening in vivo anti-inflammatory activity for each of the newly synthesized Benzoxazepinedione derivatives, 3 Groups were used.

Group I: Treatment with Vehicle/Control (0.1% Carboxy Methyl Cellulose); 10 ml/Kg
Group II: Treatment with Test (Synthesized Benzoxazepinedione Derivatives); 60mg/Kg
Group III: Treatment with Standard drug, Diclofenac Sodium (10mg/Kg).

Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The test dose of 60 mg/kg were administered orally 60 minutes before carrageenan injection. Diclofenac Sodium (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified bymeasuring the volume displaced by the paw, using a plethysmometer at time 0, 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of oedema was calculated in comparison to the control animals.

RESULTS AND DISCUSSION

A series of Eight number of 5, 6 – Benz 1, 3 – Oxazepine 4, 7 – Dione were synthesized by cyclo addition by refluxing semicarbazones and phthalic anhydride for 7hrs.

All the newly synthesized oxazepinedione derivatives (BO.1 – BO.8) were screened for *in vitro* antioxidant activity by hydrogen peroxide scavenging method. The results were graphically represented in figure no: 1. both the test drug (BO.1 – BO.8) and standard (Ascorbic acid) were used in the same concentration (100µg/mL). It was found that out of the eight newly synthesized compounds (BO.1 – BO.8), only three of the oxazepinedione derivatives shows hydrogen peroxide scavenging capacity. When compared to the standard ascorbic acid, 2-(p-Chloro phenyl)-3-(Semicarbazone)-2, 3-dihyro-5, 6 Benz [1, 3]-Oxazepine-4, 7-Dione (BO. 4) showed more ability to scavenging hydrogen peroxide. While 2-(p-toluyl)-3-(Semicarbazone)-2, 3-dihyro-5, 6 Benz [1, 3]-Oxazepine-4, 7-Dione (BO. 7) had shown less capacity. 2-(p-Benzoyloxyphenyl)-3-(Semicarbazone)-2, 3-dihyro-5, 6 Benz [1, 3] - Oxazepine-4, 7- Dione (BO.2) Showed activity in between BO. 4 and BO. 7.

Antibacterial screening of all the newly synthesized 5, 6 - Benz 1, 3 - Oxazepine 4, 7 – Dione derivatives were carried out on five micro-organism by disc diffusion method by measuring the zone of inhibition in millimetres .Both gram positive and gram negative bacteria were used. Gram positive bacteria include *Bacillus subtilis (*NCIM No. 2063), *Staphylococcus epidermidis* (NCIM No. 2493), *Staphylococcus aureus* (NCIM No.2079) and gram negative bacteria *Pseudomonas aeruginosa* (NCIM No. 5029), *Escherichia coli* (NCIM No. 2931). Ciprofloxacin (10µg) antibiotic is used as standard. Test drug include 1mg/ml of all the newly synthesized 5, 6 - Benz 1, 3 - Oxazepine 4, 7 – Dione derivatives. The result obtained is given as a graphical representation in the figure: 2 and the image for the petri plates are also given in figure: 3. It was found that most of the compounds are active against G (+) *Bacillus subtilis*. Highest activity was found out for BO. 4 having p- chloro phenyl substitution against *Pseudomonas aeruginosa.*

In vitro anti-inflammatory activity were carried out for all the newly synthesized , 6 - Benz 1, 3 - Oxazepine 4, 7 – Dione derivatives by the method of determining the percentage inhibition of protein denaturation using bovine serum albumin. Diclofenac (100 µg/ ml) is used as the standard drug. All test drug were used at a concentration of100 µg/ ml. The result obtained is shown in the figure 4. Durring analysing the result among the eight 3-(Semicarbazone)-2, 3-dihyro-5, 6 Benz [1, 3]-Oxazepine-4, 7-Dione derivatives only three were showing anti-inflammatory activity when compared with diclofenac standard. They are BO.2, BO.7 and BO.8 with 2-p-Benzoyloxyphenyl, 2-p-toluyl and 2-p-nitro phenyl substituent respectively. Among them BO.2, 2-(p-Benzoyloxyphenyl)-3-(Semicarbazone)-2, 3-dihyro-5, 6 Benz [1, 3]-Oxazepine-4, 7-Dione show highest (18.7%) anti-inflammatory activity and BO.7, 2-(p-toluyl)-3-(Semicarbazone)-2, 3-dihyro-5, 6 Benz [1, 3]-Oxazepine-4, 7-Dione showed the minimum inhibition of heat induced protein denaturation(2.9%).

In case of invivo antiinflammatory activity only benzoyl oxy derivative of 5, 6 benz 1, 3 oxazepine 4, 7 dione (BO.2) show slight reduction in the inflammation induced by carrageenan in albino rats.

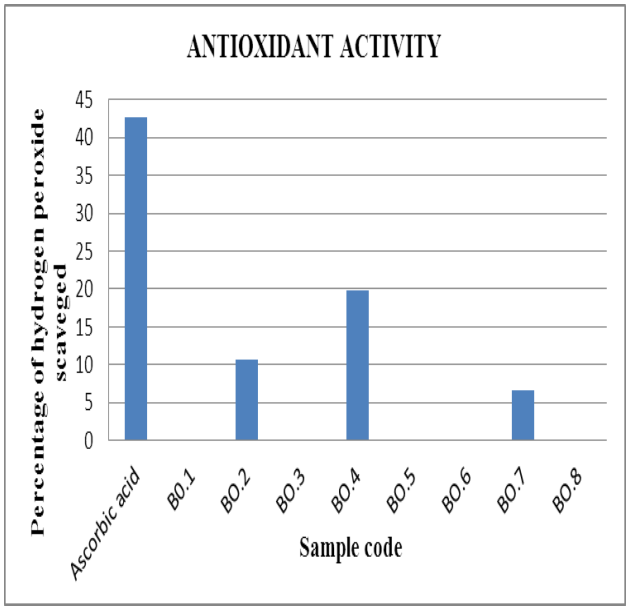


Figure 1 [Graphical representation of H_2O_2 scavenging activity of novel compounds (BO.1 – BO.8)]

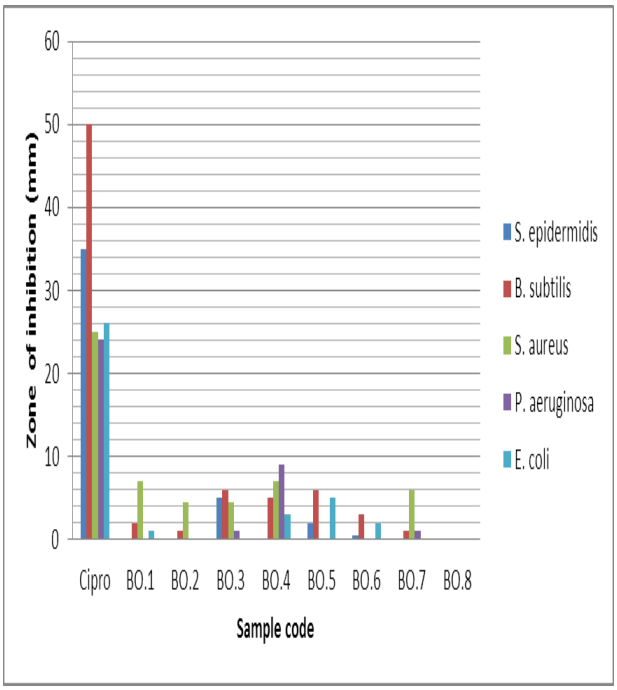
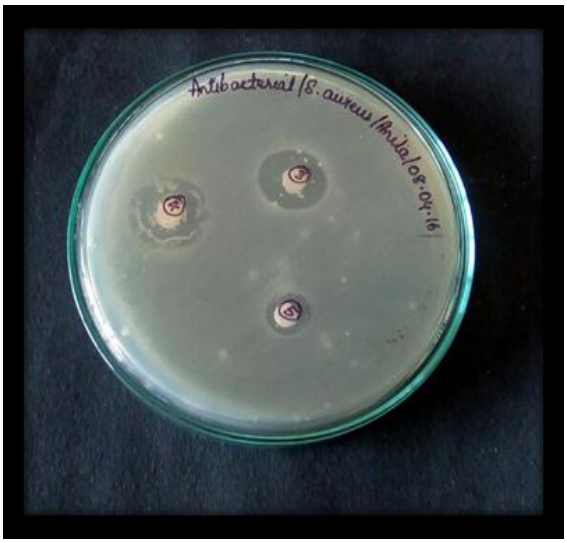


Figure 2(Graphical representation of Antibacterial activity of novel cmpounds (BO.1 –BO.8) by measuring the zone of inhibition in mm.)

Bacilus subtilis

Escherichia coli



Staphylococcus aureus

Pseudomonas aeruginosa

Staphylococcus epidermidis

Figure 3 Antibacterial activity of BO.1 – BO.8 against various microorganisms

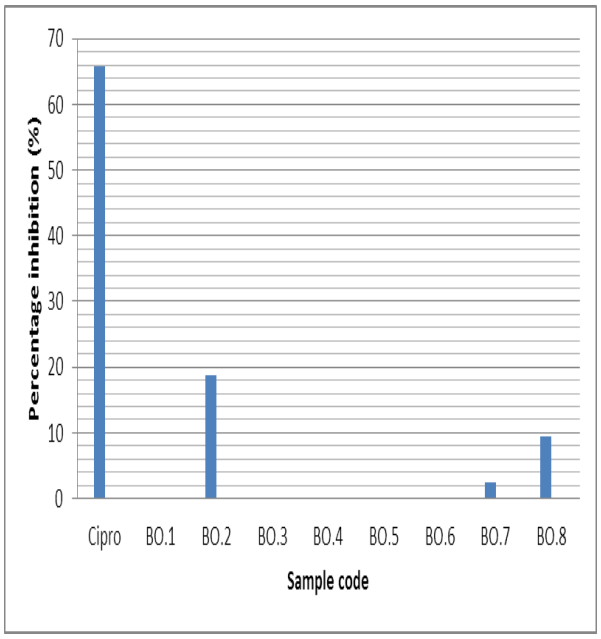


Figure 3 (Percentage of Inhibition of Protein Denaturation by BO.1 – BO.8).

CONCLUSION

The objective of the study was to synthesize 5, 6 – Benz 1, 3 – Oxazepine 4, 7 – Dione (B O.1- BO. 8). All the synthesized compounds were then biologically screened for *invitro* antibacterial, anti-oxidant, and anti-inflammatory activities. Among the three activities most of the synthesized 5, 6 – Benz 1, 3 – Oxazepine 4, 7 – Dione derivatives had antibacterial activity against both gram positive and gram negative organism.

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