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A Research on Synthesis of Oxazine Derivatives & Screening of Oxazine Derivatives for Certain Pharmacological Activities

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HUMAN

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ABSTRACT

Oxazine derivatives are an important class of heterocycles, which has attracted much synthetic interest due to their wide range of biological activities. Oxazine is a heterocyclic compound can be formally derived from benzene, and its reduction products, by suitable substitution of carbon (and hydrogen) atoms by nitrogen and oxygen. In the last few years oxazine derivatives have proved to be valuable synthetic intermediates and also possess important biological activities like sedative, analgesic, antipyretic, anticonvulsant, antitubercular, antitumour, antimalarial and antimicrobial. In these days, development of drug resistance is a major problem and to overcome this situation, it is necessary to synthesize new classes of compounds.

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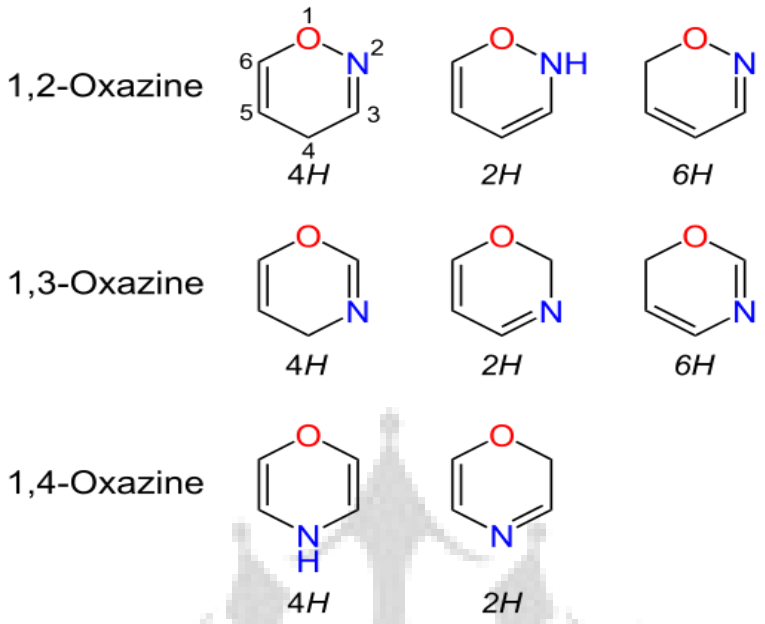
1. INTRODUCTION

Many heterocyclic analogous of chalcones have been synthesized and subsequently demonstrated to possess biological and pharmacological activities, which may possibly result in chemotherapeutic agents. Because of great potentiality, the heterocyclic analogous of chalcones are most helpful synthons. In the view of the varied biological and pharmacological application, we synthesized some heterocyclic derivatives of chalcones. Chalcones found to possess various activities like antimicrobial, anti-inflammatory, analgesic, anticancer, antimalarial, antiviral, antileishmanial, antioxidant, antitubercular, antiulcer, antihyperglycemic. In recent years, attention has increasingly been given to the synthesis of oxazine derivatives as a source of new antimicrobials. The synthesis of novel oxazine derivatives remains the main focus of medicinal research. Oxazine derivatives have been reported to possess antifungal, antibacterial, cytotoxic, antiviral and analgesic activity. Oxazine derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis. Due to the rapid development of bacterial resistant to antibacterial agents, it is vital to discover novel scaffold for the design and synthesis of the new antibacterial agents to help in the battle against pathogenic microorganisms. Much research has been carried out with the aim to discover the therapeutic value of chalcones.[3]

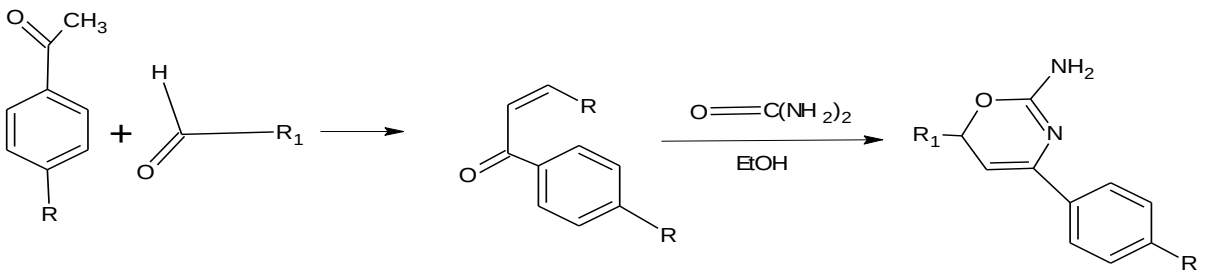
Oxazines are heterocyclic compounds containing one oxygen and one nitrogen. Many isomers exist depending on the relative position of the heteroatoms and relative position of the double bonds. 1,3-Oxazines attract more attention as they constitute an important class of both natural and non-natural products. Heterocycles containing the oxazine nucleus were found to possess a wide range of valuable biological properties like analgesic, anti-inflammatory, anti-leukemic, antimalarial1-3, antipyretic, anticonvulsant and antimicrobial activities4-8. Benzo-1,3-oxazines are also known to be biologically active, demonstrating anti-rheumatic, antianginal, antihypertensive effects, cytotoxic, and anti-osteoclastic bone resorption activity. Efavirenz, a trifluoromethyl-1, 3oxazin-2-one, is a non-nucleoside reverse transcriptase inhibitor which displays significant activity against HIV-1 mutant strains. 1, 3-Oxazine derivatives are also known to function as progesterone receptor agonists. Naphthoxazines are found to possess psycho-stimulating and antidepressant activity and are used in the treatment of Parkinson's disease. Only a few reports are available regarding the antimicrobial activity of 1, 3-oxazines.

Hence, there is enough scope to explore new oxazine derivatives for their antibacterial & antifungal activity.

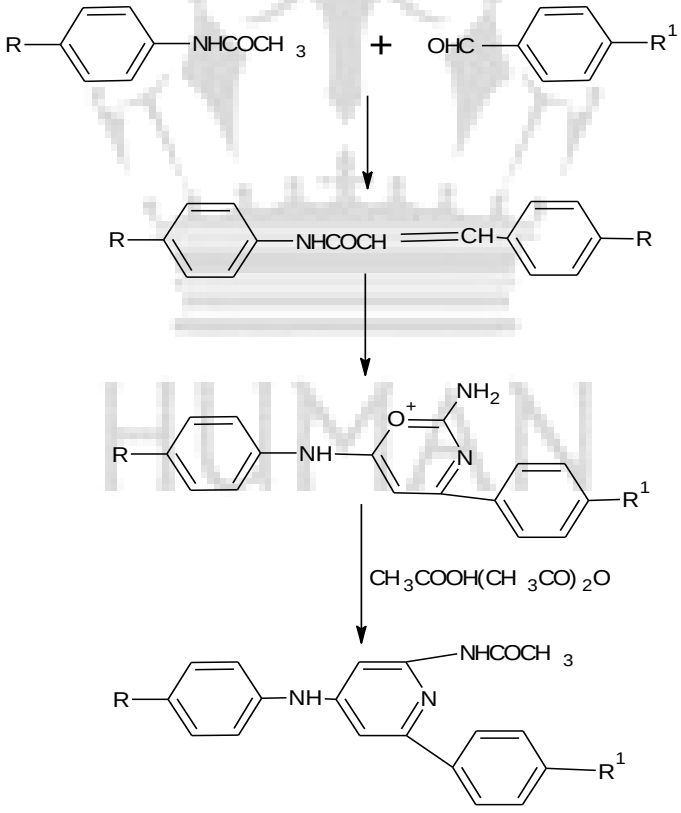


2. LITERATURE REVIEW

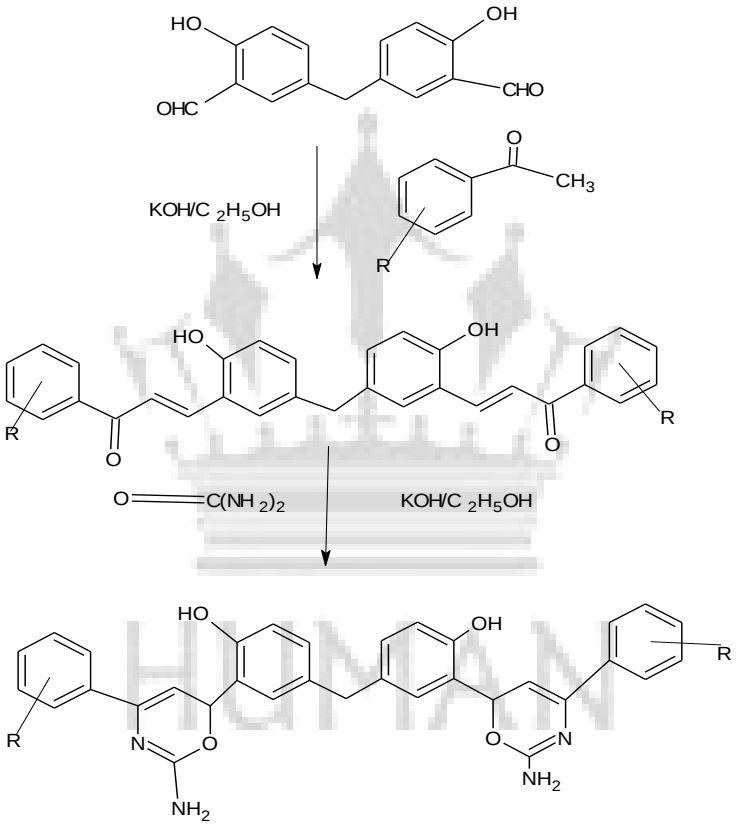
1.**Sunil Dhanya**, *et al* in 2013,a new series of 4-(4-substituitedphenyl)-6-substituited-6H-1,3-oxazines 2a-f have been synthesized from acid-catalyzed reaction between chalcones 1a-f and urea. The structures of all compounds were confirmed by advanced spectral techniques like IR, 1HNMR, and mass spectroscopy. The purity of the compounds was checked by thin layer chromatography and elemental analysis. Excellent antibacterial activity was exhibited by 2f against gram +ve bacteria. 2c and 2e were found to be highly sensitive against gram –ve bacteria. 2b and 2f displayed excellent antifungal activity. The quantitative structure-activity relationships (QSAR) studies of these compounds were performed using Easy QSAR 1.0 by simple linear regression analysis. The logarithm of the zone of inhibition of microorganisms was used as key properties to evaluate the QSAR models. The best-correlated QSAR model depicted that the autocorrelation charge 1 (ATSc1) and Crippen's molar refractivity (Crippen MR) from PaDEL-Descriptor 2.13 were significant for the antibacterial activity of oxazines against *S. aureus* and *E.coli* respectively. A close correlation between the observed and the predicted antibacterial activities (Log ZOI values) for the compounds indicated the development of the best QSAR model.[5]



2.**Beena K. P.** *et al* 2013,a series of [6-(p-substituted aminophenyl)-4-(p-substituted phenyl)—6H-1,3oxazin-yl]-acetamides were synthesized via Claisen-Schmidt condensation. The title compounds were characterized by IR, NMR analysis. The synthesized compounds were screened for their antibacterial and antifungal activity disc diffusion method. Among the synthesized compounds, A-2 was found to have a strong antibacterial and antifungal activity. Compounds A-1, A-3, A-4, and A-5 were found to have promising antimicrobial.activity.[6]



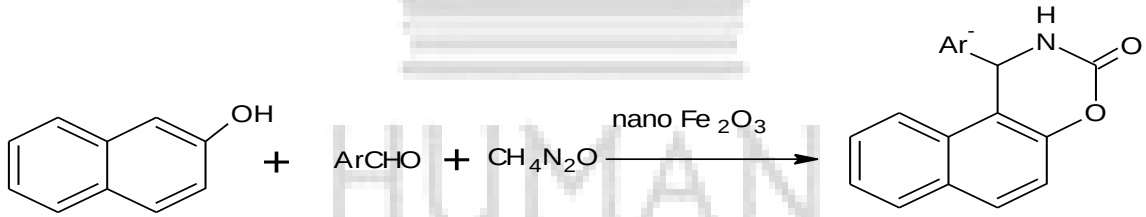
3.Sayaji.S *et al* in 2013, A series of novel 2-[2-Amino-4(4-bromophenyl)-6H-1,3-oxazine-6-yl]-4-{3-[2-amino-4(4-bromophenyl)-6H-1,3oxazine-6-yl]-4-hydroxybenzyl}phenol derivatives [3a-3i] were prepared from Bis[3-[(E)-3(4-bromophenyl)-3-oxo1-propenyl]-4-hydroxyphenyl]methane [2a-2i] with urea and potassium hydroxide in ethanol. All synthesized compounds were characterized on the basis of IR,NMR spectroscopic data, and Elemental Analysis. Antimicrobial activity was evaluated and compared with the standard drugs, some compounds of the series exhibited promising anti- bacterial and anti- fungal activity compared to standard drugs.[7]



4.Farhad Hatamjafari *et al* A Facile One-Pot Solvent-Free Synthesis of 1,2-Dihydro-1 arylnaphtho [1,2-e] [1,3] oxazine-3-ones Received: 29 March 2014; Accepted: 2 June 2014 His study aimed to synthesis of some 1,2-Dihydro-1-arylnaphtho [1,2-e] [1,3] oxazine-3-ones. The question this study tried to answer was this reaction can be performed in the presence of nano-Fe2O3 as an acid catalyst and solvent-free conditions or not. Therefore, to find the answer to the question, some of the1,2-Dihydro-1-arylnaphtho [1,2-e] [1,3] oxazine-3-one derivatives with medicinal properties were synthesized with rapid, high yield, novel, facile, and one-pot

condensation of β-naphthol, aromatic aldehydes, and urea using by nano-Fe2O3 under solvent-free conditions. The one-pot synthesis on solid inorganic support provides the products in good yields. The synthesized some of oxazine-3-one derivatives have been reported.Nano-Fe2O3 was reused for four runs without significant loss of activity and the effect of the solvents on the model reaction was carried out in a various solvent.[8]

5.**Ramesh L. Sawant** *et al* in 2012 A new series of Schiff bases of 1, 3-oxazines were synthesized in three steps. In the first step, 4-bromoacetophenone and substituted aromatic aldehyde reacted in the presence of sodium hydroxide to give substituted chalcones (Claisen-Schmidt condensation). In the second step, substituted chalcones reacted with urea to produce 4-(4-bromophenyl)-6-(substituted phenyl)-6H-1,3-oxazin-2-amine analogs. In third step, these compounds were reacted with substituted aromatic aldehydes to produce 4-(4-bromophenyl)-6(substituted phenyl)- 2-{[(1E) (substituted phenyl) methylidenene]}-6H-1,3-oxazin-amine. The newly synthesized compounds were characterized with IR, NMR and screened for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and antifungal activity against *Candida albicans*. The study revealed that compounds exhibited excellent antibacterial as well as antifungal activity.[9]



3. OBJECTIVES

❑ To synthesize oxazine derivatives by the cyclization of unstable chalcone with a mixture of urea, sodium acetate, ethanol.

❑ Determination of physicochemical properties of the synthesized compounds by

i) Melting point determination

ii) Solubility profile

iii) Thin layer chromatography

❑ Structure elucidation of the synthesized compounds by

i) I.R.: For functional group determination.

❑ Study of biological activities of the synthesized compounds

i) Anti-microbial activity

ii) Skeletal muscle relaxant property

iii) Anti-inflammatory activity

iv) Analgesic activity

v) Anticonvulsant activity

vi) Locomotor activity

4. MATERIALS AND METHODS

- **List of chemicals**

Name of chemical	**Company name**
Benzaldehyde	Spectrum
Alcohol	Spectrum
4-chlorobenzaldehyde	Ozone international
4-nitrobenzaldehyde	Otto
3-nitrobenzaldehyde	Otto
4-benzyloxybenzaldehyde	Chemco
3,4,5-trimethoxybenzaldehyde	Chemco
Ferric chloride	Nice chemicals Pvt. Ltd.
Bovine Serum Albumin	Otto
acetophenone	Ozone international

Urea.	Spectrum
Tris-HCl buffer	Otto
Starch powder	Spectrum
Diclofenac sodium	Spectrum
Diazepam	Neon laboratories Ltd
Carboxymethyl cellulose	Spectrum

- **All the glassware used is of borosilicate**
- **Instrument used**

Name of instrument	**Company name**	**Model no.**
UV	JASCO	V-630
IR	Shimadzu	IR Affinity 1
Electronic balance	Citizon	CY 220
Melting point apparatus	Veego	VMP-D
Actimeter cum hole board apparatus	Orchid Scientifics	Act -01
B.O.D Incubator	Rotex	0601
Laminar air flow unit	Rotex	0603
Actimeter cum hole board apparatus	Orchid scientifics	Act-01

- **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^{0}C. The experiments we carried out as per the guidelines of CPCSEA

5. METHODS

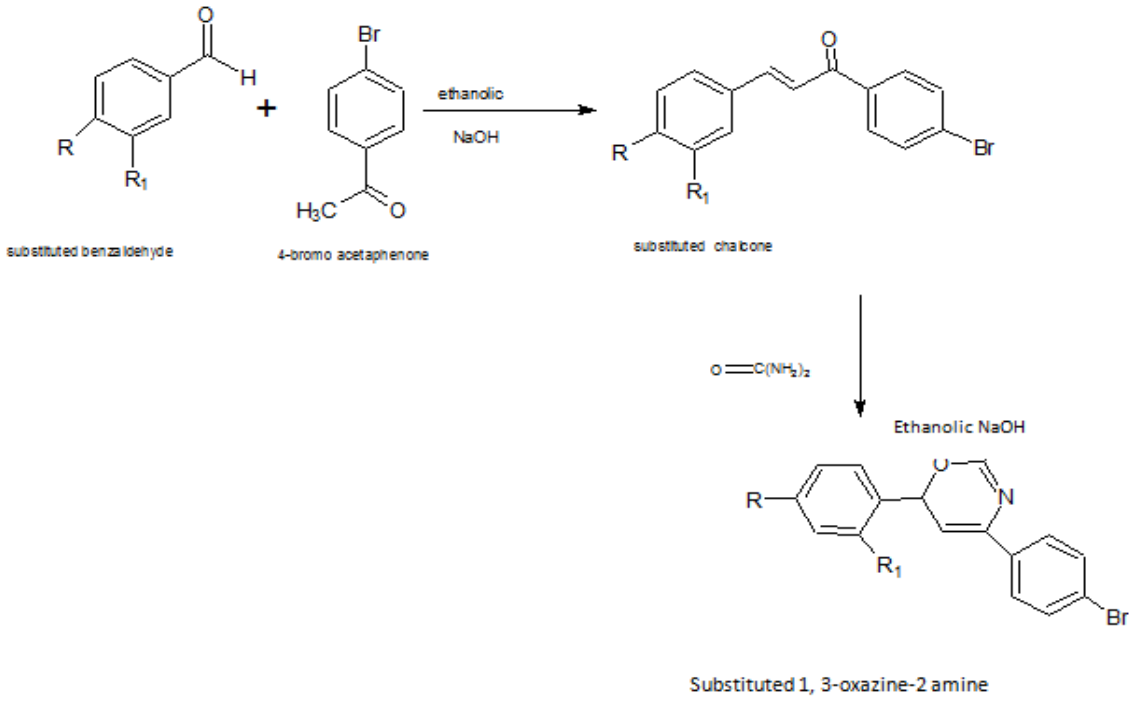
5.1 METHODOLOGY FOR SYNTHESIS

1. Synthesis of Chalcones[1]

An equimolar mixture of Benzaldehyde (1 mol) and Acetophenone (1 mol) was dissolved in minimum amount of alcohol. Sodium hydroxide (0.02 mol) was added slowly and the mixture stirred with a magnetic stirrer. Then the mixture was poured slowly into 4 ml of water with constant stirring the precipitate obtained was filtered.

2. Synthesis of oxazine derivative

Cyclization step: Formed unstable chalcones were further cyclized with 0.015 M Urea, 0.05M anhydrous sodium acetate in 20ml ethanol ,reflux for 6 hours .mixture poured into crushed ice precipitate was recrystallized from acetone.



5.2 CHARACTERIZATION

The product obtained is characterized by

- Checking the melting point,
- Performing TLC, and from
- IR spectra – CIF Pushpagiri

5.2.a. TLC

TLC plate was used as solid phase

Uv detection chamber was used for the spot detection

Mobile phase n-hexane: ethanol: water in the ratio 2:1:1

R_f value calculated using the equation

R_f = distance traveled by solute / distance traveled by the solvent.

6. ACUTE TOXICITY TEST

Acute toxicity studies of the synthesized compounds were carried out using OECD/OCED guideline 423. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Procedure

Healthy young adult *albino rats* of either sex (normally females) were used for this study. Females should be nulliparous and non-pregnant. Each animal was 8 to 12 weeks old at the commencement of dosing. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of animals dosed at one step will determine the next step. Animals 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. After the substance has been administered, food was withheld for further 3-4 hours. The dose level used as the starting dose is selected from one of

four fixed levels, 5, 50,300 and 2000 mg/kg body weight. As there is no information on the substance to be tested, the starting dose is 300mg/kg. The animals are observed individually after dosing, at least once during the first 30 minutes, periodically during the first four hours and thereafter for a total of 14 days. Body weight of the rat before and after treatment will be noted. Any change in skin colour, fur, eyes, locomotor activity, and behavioral pattern will be observed and also signs of tremors, convulsions, diarrhea, lethargy and sleep were noted.

7. SCREENING FOR CERTAIN BIOLOGICAL ACTIVITIES

7.1. SCREENING FOR ANTIBACTERIAL ACTIVITY

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

The antibacterial screening was carried out in a laminar air flow unit and all types of precautions were strictly maintained to avoid any type of contamination during the test. Ultraviolet light was switched on for half an hour before working in the laminar hood to avoid any accidental contamination. 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. Micropipette tips, culture media, cotton, forceps, blank disks, and so forth, were also sterilized. In disc diffusion method bacterial inoculum is 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 (500µg/ml in ethanol) was placed on the surface of each of agar plates using a sterile pair of forceps. The 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.

7.2. MUSCLE RELAXANT PROPERTY

1. **BY USING MUSCLE GRIP STRENGTH APPARATUS**

Force transducer meters should be set to zero or reset before each measurement is made so all

low proper values to be detected . At least 2 per group are generally needed if statistical significance is to be reached for this parameter.

Forelimb grip strength measurement: The most frequently used configuration is to measure forelimb grip strength.

1. Reset the meter.
2. Visually check that the grip is good i.e. a symmetric ,tight grip with both paws and exerting a detectable resistance against the investigator's pull.
3. Lift the mouse by the tail to the height where the front paws are at the same height as the bar.
4. Move the mouse horizontally towards the bar.
5. Gently pull the mouse away until its grasp is broken. The pulling should be at a constant speed and sufficiently slow to permit the mouse to build up a resistance against it. The transducer saves the value at this point.
6. Repeat the test a set number of times (but no more than 5 times) to obtain the best performance.

Group1: control (distilled water, p.o)
Group2: standard (diazepam 5 mg/kg ,i.p)
Group3: test (product)

2. BY USING ROTAROD APPARATUS

Turn on the apparatus, select an apparatus speed (20-25rpm), and place the animal one by one on the rotating rod. Inject diazepam (standard drug) to the group of standard animals. And administer the test drug to the test group. Note down the reading (fall off time) before after drug administration.

7.3. SCREENING FOR ANTI-INFLAMMATORY ACTIVITY

***In vitro* protein denaturation method**

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 a standard. The test tubes were heated at 72^0C for 5 minutes and then cooled for 10 minutes. The absorbances 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

***In vivo* studies; Carrageenan-induced paw edema in rats**

The animals were divided into three group of two animals in each group(one control, one standard ,one test group).Acute inflammation was induced by sub-planar injection of o.1% freshly prepared carrageenan suspension into the right hind paw of the rats. The product was suspended in distilled water and administered orally (400mg/kg).1 hour before carrageenan injection. Diclofenac sodium (10mg/kg) was given to standard group. The control group animals received vehicle.

Control group: carrageenan + water

Standard group: carrageenan+ Diclofenac sodium (10mg/kg)

Test group: carrageenan+ product

The paw volume was measured with mercury plethysmometer at 0,1,2,3 and 4 hours after carrageen injection .the percentage inhibition of edema was calculated for each group with respect to control group .The percentage inhibition of edema was calculated using the formula;

% inhibition of edema = $(V_c - V_t)/ V_c \times 100$

Where V_c = paw volume in test group

V_t = paw volume in control group animal

7.4. ANALGESICS PROPERTY

1. Weigh and number the animals. Divide the animals into control, test and standard. Take the basal reaction time by observing hind paw licking or jump response in animals when placed on

the hot plate maintained at constant temperature (55^0c).normally animal shows such response in 6-8 s .a cut of period is maintained to avoid damage to the paw. Inject standard to the animal & note down the basal reaction time of animal on hot plate at 15m, 30m, and 60m after the drug administration. Calculate the increase in the reaction time (as an index of analgesia).

7.5. ANTICONVULSANT PROPERTY

Weigh and number the animals. Divide the animals into three groups. One group for studying the protective effects of diazepam and other to study whether the test compound has an anticonvulsive effect. Inject pentylenetetrazole to control animals and note the onset of action. Inject product to second group. After 30 min inject pentylenetetrazole to these animals which have received diazepam. Note onset and severity of convulsions. Note either delay or complete abolition of convulsions in rat treated with diazepam. Inject test compound to third group .After 30min inject pentylenetetrazole to these animals which have received test. Note the onset and severity of convulsions.

7.6. LOCOMOTOR ACTIVITY

The locomotor activity can be easily studied with the help of IR actimeter. The actimeter consists of a square frame, frame stand, and hole board plate. The frame is equipped with 32 IR cells, out of which 16 cells are on X-axis and 16 cells are on Y- axis. The instrument control panel will display number of beam brakes by animal on all axis and total of all in hole board mode and actimeter mode.

Swiss albino rat weighing between 150-250g were divided into 3 groups, each group comprising of two animals. Each animal was placed individually and the basal activity score of all the animals was recorded after 30 and 60 minutes of drug treatment. The Dose of drug given was 60mg/kg orally. Diazepam at a dose of 2mg/kg was given as standard intraperitoneally. The activity on each rat was tested for 10 min. Finally, percentage decrease in locomotor activity was calculated.

6 RESULTS AND DISCUSSION

6.1 PHYSICOCHEMICAL PROPERTIES

Oxazine derivatives were synthesized by cyclization of unstable chalcone with 0.015 M Urea, 0.05M anhydrous sodium acetate in 20ml ethanol,which in turn was synthesized from 3-aminoacetophenone and different benzaldehyde. Physico-chemical properties of the synthesized compounds are given below.

Table 4. PHYSICOCHEMICAL PROPERTIES

Sample Code	State	Colour	Molecular Formula	Molecular Weight	M.P (^{0}C)	Yield %w/w	R_F value
HS 1	Solid powder	Dark yellow	$C_{16}H_{14}ClN_3O$	299.75486	173	48.8	0.78
HS 2	Solid powder	Orange yellow	$C_{16}H_{14}N_4O_3$	310.30736	160	65.4	0.87
HS 3	Solid powder	Dark red	$C_{16}H_{14}N_4O_3$	310.30736	99	57.2	0.89
HS 4	Solid powder	Dark yellow	$C_{17}H_{19}N_3O_2$	297.35166	138	61.3	0.40
HS 5	Solid powder	yellow	$C_{19}H_{21}N_3O_4$	355.38774	80	64	0.13

Table 5. SOLUBILITY

Sl.No	SAMPLE CODE	SOLVENT		
		n-hexane	ethanol	water
1	HS1	insoluble	soluble	soluble
2	HS2	insoluble	soluble	soluble
3	HS3	insoluble	soluble	soluble
4	HS4	insoluble	soluble	soluble
5	HS5	insoluble	soluble	soluble

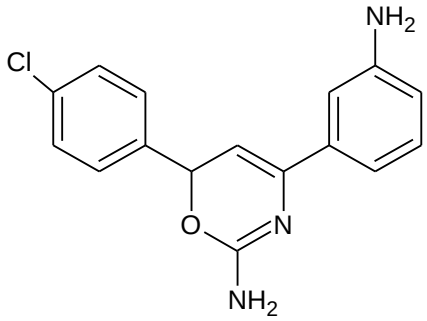
6.2 SPECTRAL CHARACTERIZATION

IR SPECTRAL ANALYSIS OF HS 1

Table 6, IR spectra of HS 1

Frequencyobtained (cm^{-1})	Frequency Range	Functional group
2921.32	3000-2850	CHstretching
825.57	600-800	C-Cl bond in ring
1487.18	1400-1600	Aromatic c=c stretch

By the above spectral features, the structure of HS1 is as follows



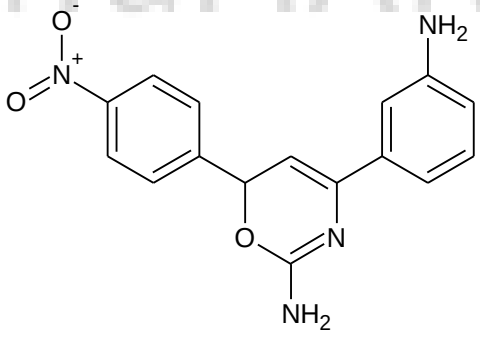
4-(3-aminophenyl)-6-(4-chlorophenyl)-6*H*-1,3-oxazin-2-amine

IR SPECTRAL ANALYSIS OF HS 2

Table 7, spectra of HS 2

Frequency obtained (cm^{-1})	Frequency Range	Functional group
3356.28	3400-3250	N-H Stretching
2919.38	3000-2850	CH stretching
1519.07	1600-1500	C=N Stretching
1107.19	1320-1000	C-O Stretching in ring

By the above spectral features the, the structure of HS2 is as follows



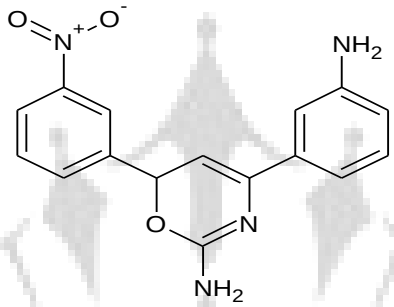
4-(3-aminophenyl)-6-(4-nitrophenyl)-6*H*-1,3-oxazin-2-amine

IR SPECTRAL ANALYSIS OF HS 3

Table 8, IR spectra of HS3

Frequency obtained (cm^{-1})	Frequency Range	Functional group
3373.64	3400-3250	N-H Stretching
2918.48	3000-2850	CH stretching

By the above spectral features, the structure of HS 3 is as follows



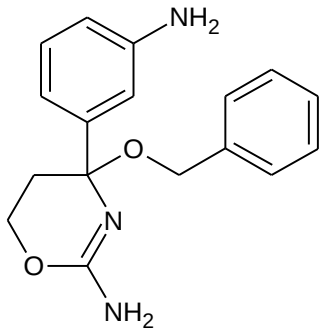
4-(3-aminophenyl)-6-(3-nitrophenyl)-6*H*-1,3-oxazin-2-amine

IR SPECTRAL ANALYSIS OF HS 4

Table 9, IR Spectra of HS4

Frequency obtained (cm^{-1})	Frequency Range	Functional group
3362.07	3400-3250	N-H Stretching
2925.17	3000-2850	CH Stretching
1657.89	1680-1640	C=C Stretching
1169.8	1320-1000	C-O Stretching in ring

From the above spectral features the structure of HS4 is as follows



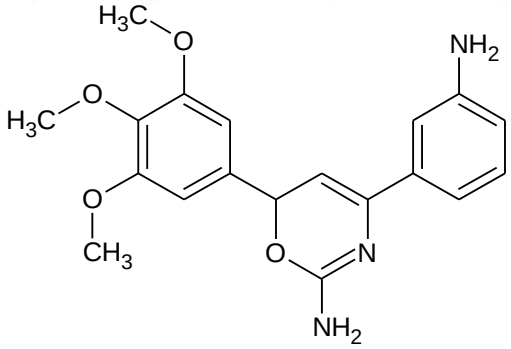
4-(3-aminophenyl)-4-(benzyloxy)-5,6-dihydro-4*H*-1,3-oxazin-2-amine

IR SPECTRAL ANALYSIS OF HS5

Table 10, IR Spectrum HS 5

Frequency obtained (cm^{-1})	Frequency Range	Functional group
2938.68	3000-2850	CH Stretching
1656.92	1680-1640	C=C Stretching
1579.	1600-1500	C=N Stretching
1418	1500-1400	C-C Stretching

From the above spectral features, the structure of HS 5 is as follows



4-(3-aminophenyl)-6-(3,4,5-trimethoxyphenyl)-6*H*-1,3-oxazin-2-amine

6.3 ACUTE TOXICITY STUDY

Acute toxicity study of the synthesized compounds was conducted as per OECD guidelines 423 in Swiss albino rat. The compounds showed toxic effects at a dose of 2000mg/kg so the safe dose of the drug is 300mg/kg. So 1/5th dose i.e., 60mg/kg were selected for *in vivo* screening studies.

6.4 SCREENING FOR BIOLOGICAL ACTIVITIES

6.4a ANTIBACTERIAL ACTIVITY

Table 11, Antibacterial activity of HS1-HS 5

Sl.no	Sample	Zone of inhibition in cm	
		Bacillus subtilis	*Pseudomonas aerogenosa*
1	Standard 1 (chloramphenicol, 30mcg)	-	1
2	Standard 2 (ciprofloxacin, 10mcg)	3.7	-
3	HS 1(1)	1	-
4	HS 2(3)	O.7	-
5	HS 3(4)	0.2	-
6	HS 4(2)	-	-
7	HS 5(5)	0.3	-

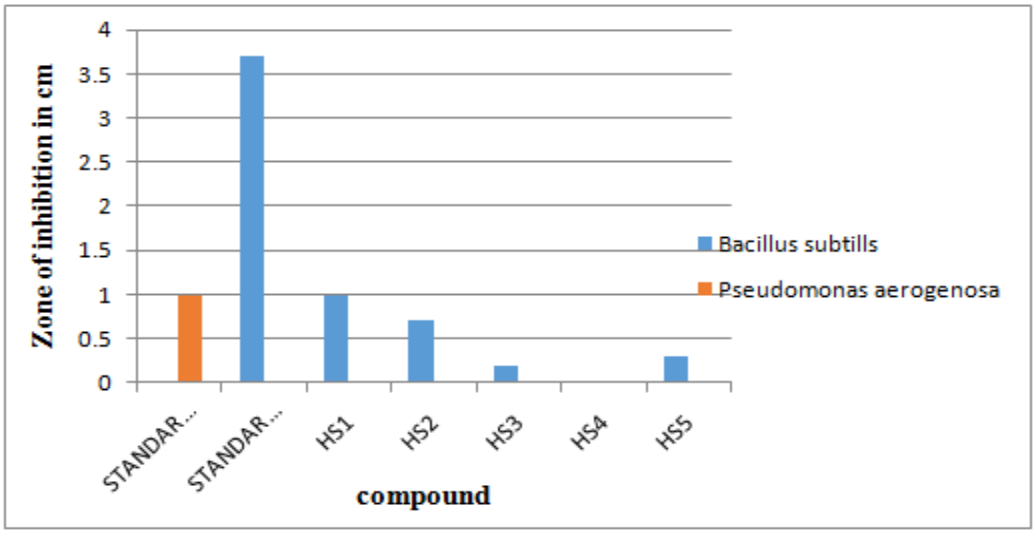


Figure 1, Antibacterial activity of HS1-HS 5 by measuring the zone of inhibition in cm.

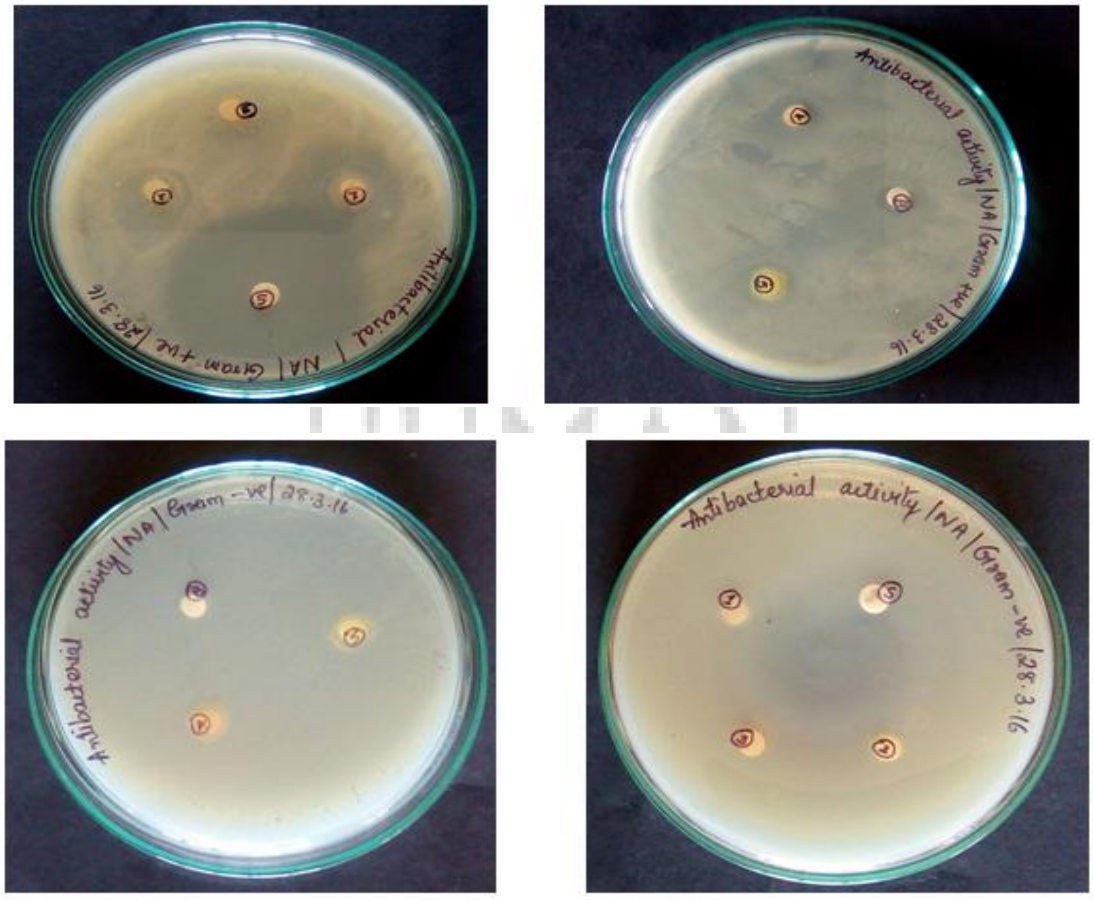


Figure 2, Antibacterial activity of HS1-HS 5 against *Bacillus subtilis* & *Pseudomonas aerogenosa.*

All the synthesized compounds have been screened for their antibacterial activity by disc diffusion method by measuring zone of inhibition in mm. 10µg Ciprofloxacin &30µg chloramphenicol were used as a standard. The compounds were screened for their antibacterial activity against Gram-positive bacteria*[Bacillus subtilis (*NCIM No. 2063), *Staphylococcus &*Gram-negative bacteria [*Pseudomonas aerogenosa*(NCIM No. 5029).

Compounds HS1, HS2, HS3, and HS5 showed activity against gram-positive bacteria *Bacillus subtilis.* Compounds HS1, showed moderate antibacterial activity against *Bacillussubtilis*, compounds, HS2, HS3, HS5 showed mild activity against *Bacillus subtilis.* The HS4 compound did not show any antibacterial activity. The synthesized oxazine derivatives did not show any antibacterial activity against gram negative bacteria, *Pseudomonas aerogenosa*.

6.4b MUSCLE RELAXANT PROPERTY

1**. BY USING MUSCLE GRIP STRENGTH APPARATUS**

Sl.no	sample	color	Before.drug administration	After.drug administration
1	HS1	black	160	150
2	HS2	Rose	260	140
3	HS3	colorless	140	98
4	HS4	Brown	110	87
5	HS5	Green	210	105
6	Standard	Blue	426	120
7	Control	yellow	125	110

All the synthesized compounds have been screened for their muscle relaxant property by using muscle grip strength apparatus. All the compound like HS1, HS2, HS3, HS4, HS 5 having

activity high compared to standard the compound show less activity. CompoundHS2, HS5 having high activity compared to other compound & HS4 having less activity .

2. BY USING ROTAROD APPARATUS

Sl.no	sample	color	Fall of time in minute	
			Before.drug administration	After.drug administration
1	HS1	black	3.52	3.2
2	HS2	Rose	4.12	1.32
3	HS3	colorless	3.71	1.6
4	HS4	Brown	4.55	2.25
5	HS5	Green	3.8	1.3
6	Standard	Blue	4.36	1.3
7	Control	yellow	4.3	2.8

All the synthesized compounds have been screened for their muscle relaxant property by using rota rod apparatus. All the compounds like HS1, HS2, HS3, HS4, HS 5 having activity high compared to the standard the compound show less activity. Compound HS2, HS5 having high activity compared to other compound& HS4 having less activity .

6.4c *In vitro* ANTI-INFLAMMATORY ACTIVITY

Table 13, Percentage inhibition of protein denaturation by HS1-HS 5

Sl.no.	SAMPLE	Absorbance at 660nm	Percentage of inhibition
1	HS1	0.2132	46%
2	HS2	0.2457	38%
3	HS3	0.2287	42%
4	HS4	0.2812	25.4%
5	HS5	0.2786	30.15%
6	Control	0.3988	-
7	standard	0.2014	49.51%

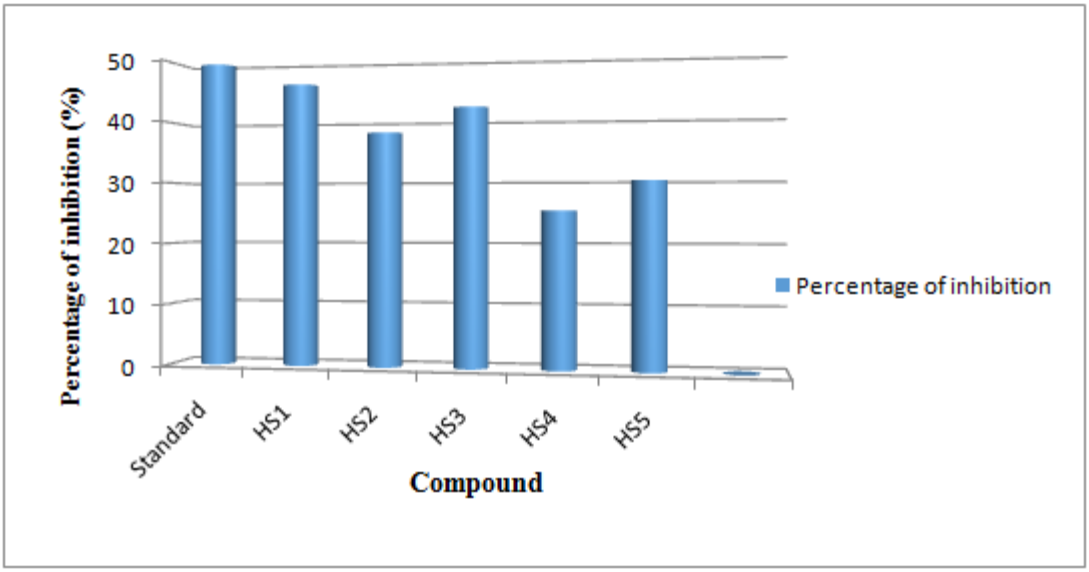


Figure 3, Percentage of Inhibition of Protein Denaturation by HS1-HS 5

The synthesized oxazine derivatives were tested for anti-inflammatory activity at a concentration of 100µg/ml by *in vitro* protein denaturation method using bovine serum albumin. The result obtained is shown in **Table 13**.

All the compounds HS1, HS 2, HS3, HS4 showed significant anti-inflammatory activity compared to standard drug diclofenac (100µg/ml). phenyl .4-chlorobenzaldehyde derivative showed maximum inhibition of heat-induced protein denaturation of 46% which is comparable to standard drug diclofenac which showed 49.51% of inhibition of heat-induced protein denaturation.

Inflammation is the response of living tissues to injury. It involves a complex mechanism of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown, and repair. Denaturation of protein is one of the main causes of inflammation. Several anti-inflammatory drugs have shown the ability to inhibit thermally-induced protein denaturation in dose-dependent manner. The ability of oxazine derivative to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity.

8.4. ANALGESICS PROPERTY

Sl.no	sample	color	Basal.reaction before..drug admn.**(**sec**)**	After drug administration (sec)
1	HS1	Red	4	15m=4
				30m=4
				60m=5
2	HS2	Green	3	15m=5
				30m=6
				60m=3
3	HS3	Brown	6	15m=6

				30m=9
				60m=7
4	HS4	Blue	4	15m=5
				30m=7
				60m=4
5	HS5	violet	3	15m=4
				30m=7
				60m=5
6	Control	Colorless	4.	15m=4
				30m=5
				60m=4
7	standard	rose	5	15m=7
				30m=11
				60m=9

All the synthesized compounds have been screened for their analgesic property. All the compound shows analgesic property. Compared to standard all compounds shows less activity. The compound HS5, HS2 having high activity compared to other compound.

8.5. ANTICONVULSANT PROPERTY

Sl no.	sample	color	convulsion		Observation
			onset of action (OOA) (sec)	Death/recovery	
1	HS1	Black	65	No death	Less convulsion
2	HS2	Rose	60	No death	Less convulsion
3	HS3	Colorless	50	death	Less convulsion
4	HS4	Brown	65	No death	Less convulsion
5	HS5	Green	78	No death	Less convulsion
6	CONTROL	yellow	50	No death	Less convulsion
7	STANDARD	orange	95	No death	No Convulsion

All the synthesized compounds have been screened for their anticonvulsant property. All the compounds show anticonvulsant property. Compared to standard all compounds show less activity. The compound HS3, HS2 having high activity compared to other compound.

8.6 LOCOMOTOR ACTIVITY

SL.no	sample	colour	Before drug admn.	Locomotor activity		
				15 min	30min	60min
1	HS 1	blue	743	529	334	115
2	HS 2	orange	175	195	213	85
3	HS 3	colorless	795	603	689	332
4	HS 4	rose	221	434	521	348
5	HS 5	green	182	262	306	116
6	standard	black	846	166	136	156
7	control	violet	162	158	169	166

CONCLUSION

Oxazine and related heterocyclic compounds were reported to have antimycobacterial, antibacterial, antifungal, anticoagulant, anticancer, antioxidant, and cytotoxic activities. It has been found that oxazine derivative can be synthesized in a number of ways. So this review article can extend the synthetic utility of new heterocyclic oxazine derivatives. Therefore, the biological significance of oxazine compounds could be utilized for the development of new chemical entities to various diseases.

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