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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL OXAZINE AND THIAZINE DERIVATIVES AS POTENT ANTI-MICROBIAL AND ANTIOXIDANT AGENTS

Battala Swapna*, Poosala Rajkumar, Y. Vinay Sree and Ch. Harini

Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Secunderabad, Telangana, India.

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***Corresponding Author**
Battala Swapna
Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Secunderabad, Telangana, India.

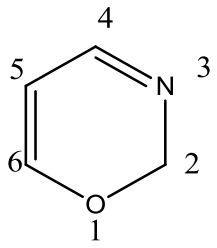
ABSTRACT

Oxazines and Thiazines are the heterocyclic compounds in which oxazine containing heteroatoms (N&O) and thiazine containing heteroatoms (N&S) in a doubly bond un-saturated six-membered ring. The present investigation is about synthesis and evaluation of oxazine and thiazine derivatives as potent antimicrobial and antioxidants. Anti-bacterial is done against the *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus acidophilus* by cup plate method and medium was nutrient agar medium and Antifungal is done against the *Aspergillus niger*, *Can- dida* albicans, *Malassezia fur fur* by cup plate method and medium was sabouraud dex trose agar medium and Antioxidants is done by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and Iron chelation method.

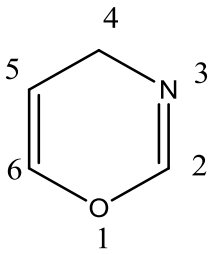
KEYWORDS: Oxazine, Thiazine, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

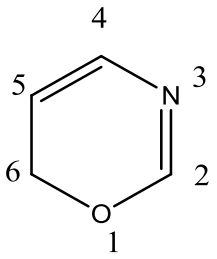
Chemistry of oxazine Oxazine is a Six-membered ring, which contains two heteroatoms (N&O) placed in the heterocyclic ring at 1,3 positions. These oxazines contain three isomers of 1,3-oxazine system which are named 2H,4H and 6H oxazines respectively.



2*H*-1,3-oxazine



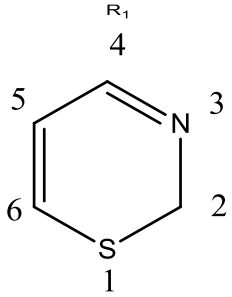
4*H*-1,3-oxazine



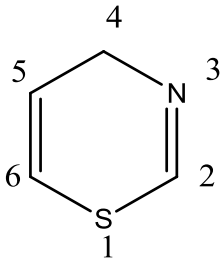
6*H*-1,3-oxazine

These compounds are aromatic compounds, in which all the carbon atoms are sp^2 hybridised. Oxazines play a vital role in medicinal chemistry. These oxazines are used as Antibiotics, Antitumor, Anticonvulsants, Antipsychotic, Antihistamine, Analgesics, Anti-inflammatory, Antihypertensive, Sedative, Amoebicidal, Fungicidal, Antiviral.

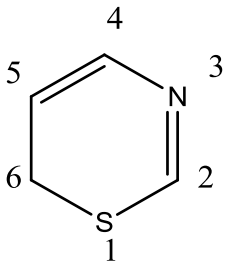
Chemistry of thiazine: Thiazine is a six-membered ring, which contains two heteroatoms (N&S) placed in the heterocyclic ring at 1,3 positions. These thiazines contain three isomers of 1,3-thiazine system which are named 2H, 4H and 6H Thiazine respectively.



2*H*-1,3-thiazine



4*H*-1,3-thiazine



6*H*-1,3-thiazine

These compounds are aromatic compounds, in which all the carbon atoms are sp^2 hybridised. These thiazine's play vital role in medicinal chemistry. So, these are used as Ant Antibiotics, Antitumor, Anticonvulsants, Antipsychotic, Antihistamine, Analgesics, Anti-inflammatory, Antihypertensive, Sedative, Amoebicidal, Fungicidal, Anti-viral.

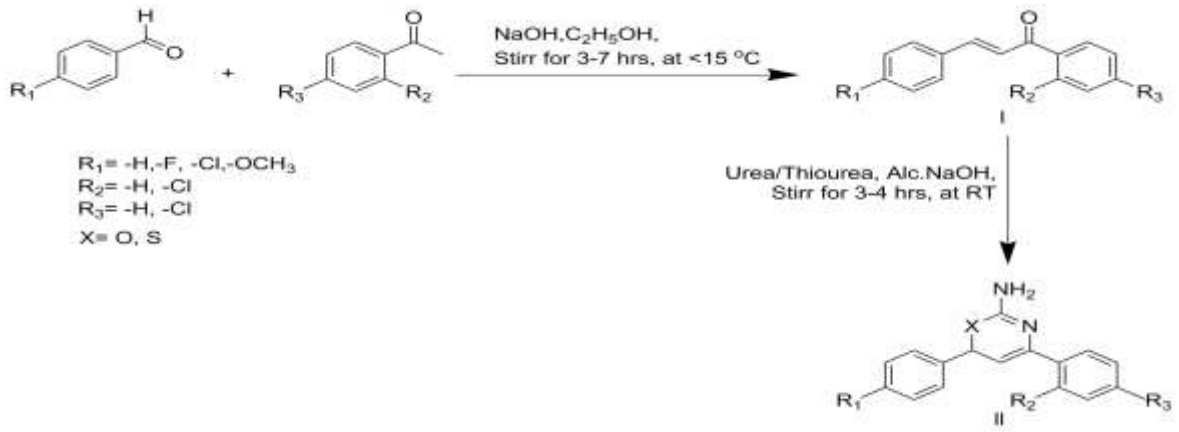
AIM AND OBJECTIVES

The oxazine and thiazine derivativespossess a wide variety of pharmacological activities such as antimicrobial, antifungal, antiviral, analgesic, anti-inflammatory and antimycobacterial activities. In the present work the effort is made to develop a convenient method for the synthesis of novel oxazine and thiazine derivatives by conventional method. Understanding the importance of oxazine and thiazine for antimicrobial and antioxidant activities, some

novel oxazine and thiazine derivatives were synthesized by structural modification on the oxazine and thiazine ring.

Therefore, the present work has been aimed to achieve the following objectives.

- To synthesize the novel oxazineand thiazine derivatives by using reported methodology. To purify the intermediates and final compounds by recrystallization/ chromatographic techniques using suitable solvents.
- To characterize the synthesized compounds by the help of physical (MP, Rf values), TLC and spectral data (FT-IR,1H-NMR, Mass spectroscopy).
- Finally, to evaluate the synthesized compounds for their possible antimicrobial and Anti-oxidant activity.
- To identify the potent compounds, if any for their specific activity & for future exploitation.



SYNTHETIC PROCEDURE

Step 1: Synthesis of chalcone by Claisen-Schmidt condensation reaction

A mixture of benzaldehyde derivatives (0.01 mol) and acetophenone derivati ves (0.01 mol) was dissolved in 10 ml rectified spirit in a 250 ml round bottomed flask equiped with a magnetic stirrer.

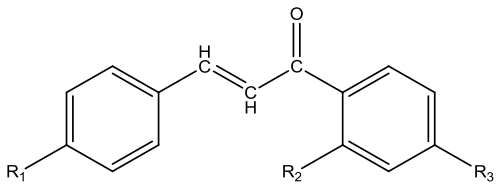
Then 10ml NaOH solution (1g in 10ml H2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution becomes turbid. The reaction temperature was maintained at 15˚C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hours the reaction mixture was left to stand overnight at refrigerator. The precipitate obtained was filtered, washed, dried in air and re-crystallized from ethanol.[19]

Step 2 Synthesis of Thiazine derivatives

Weigh Chalcone compound (0.2gm,o.oo6M) in 30ml of ethanol absolute is placed in a round bottom flask, after that (0.04gm,0.006M) of thiourea was added with 5ml of 10% sodium hydroxide, the mixture was stirred at room temperature for 3hrs , then 20 ml of cold water was added, the mixture was stirred for one hour and cooled in an ice-bath for two days. The precipitate obtained was filtered, washed and recrystallized. The completion of the reaction was monitored by TLC.[20]

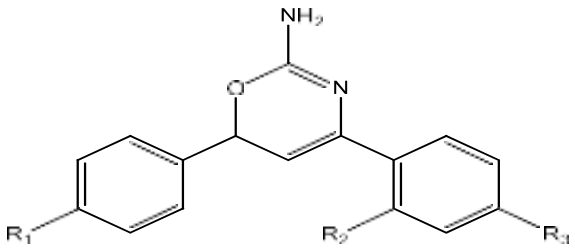
RESULT AND DISCUSSION

Table 1: Analytical data for chalcone derivatives.



Code	R_1	R_2	R_3	MF	M.W	% Yield	R_f*	M.P. °C
1	-H	-H	-H	$C_{15}H_{12}O$	208	88.6	0.68	76-78
2	-Cl	-H	-H	$C_{15}H_{11}ClO$	242.5	70.4	0.76	106-108
3	-Cl	-Cl	-Cl	$C_{15}H_9Cl_3O$	311.5	87	0.69	99-101
4	-F	-H	-H	$C_{15}H_{11}FO$	226	79.7	0.71	78-81
5	-F	-H	-Cl	$C_{15}H_{10}FClO$	260.5	83.8	0.52	114-117
6	-H	-H	-Cl	$C_{15}H_{11}ClO$	242.5	93.3	0.73	87-89
7	-F	-Cl	-Cl	$C_{15}H_9FCl_2O$	295	84.4	0.55	119-122
8	$-OCH_3$	-H	-H	$C_{16}H_{14}O_2$	238	89.5	0.61	74-76
9	$-OCH_3$	-H	-Cl	$C_{16}H_{13}ClO_2$	272.5	85.9	0.64	104-106

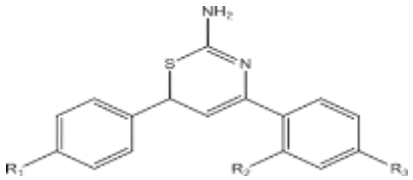
Table 2: Analytical data for oxazine derivatives.



Code	R_1	R_2	R_3	MF	M.W	% Yield	R_f^*	M.P. °C
1a	-H	-H	-H	$C_{16}H_{14}N_2O$	250	65.6	0.68	123-125
2a	-Cl	-H	-H	$C_{16}H_{13}ClN_2O$	284	69.6	0.45	116-118
3a	-Cl	-Cl	-Cl	$C_{16}H_{11}Cl_3N_2O$	353	73.5	0.45	141-144
4a	-F	-H	-H	$C_{16}H_{13}FN_2O$	268	70.8	0.45	100-102
5a	-F	-H	-Cl	$C_{16}H_{12}FClN_2O$	302	73.2	0.52	126-128
6a	-H	-H	-Cl	$C_{16}H_{13}ClN_2O$	284	63.2	0.51	116-118
7a	-F	-Cl	-Cl	$C_{16}H_{11}FCl_2N_2O$	337	56.7	0.53	128-130
8a	$-OCH_3$	-H	-H	$C_{17}H_{16}N_2O_2$	280	70.4	0.47	111-113
9a	$-OCH_3$	-H	-Cl	$C_{17}H_{15}ClN_2O_2$	314	75.8	0.57	110-112

R_f^* values are calculated by developing TLC in Hexane: Ethyl acetate: Methanol- 4:2:1 solvent system.

Table 3: Analytical data for thiazine derivatives.



Code	R_1	R_2	R_3	MF	M.W	% Yield	R_f^*	M.P. °C
1b	-H	-H	-H	$C_{16}H_{14}N_2S$	266.	78.8	0.77	128-131
2b	-Cl	-H	-H	$C_{16}H_{13}ClN_2S$	300	72.8	0.47	127-129
3b	-Cl	-Cl	-Cl	$C_{16}H_{11}Cl_3N_2S$	369	76.7	0.69	153-155
4b	-F	-H	-H	$C_{16}H_{13}FN_2S$	284	87.5	0.56	117-119
5b	-F	-H	-Cl	$C_{16}H_{12}FClN_2S$	318	73.4	0.75	154-156
6b	-H	-H	-Cl	$C_{16}H_{13}ClN_2S$	300	65	0.58	168-170
7b	-F	-Cl	-Cl	$C_{16}H_{11}FCl_2N_2S$	353	61.8	0.66	133-135
8b	$-OCH_3$	-H	-H	$C_{17}H_{16}N_2OS$	296	70.6	0.57	142-144
9b	$-OCH_3$	-H	-Cl	$C_{17}H_{15}ClN_2OS$	330	69.8	0.69	132-134

R_f^* values are calculated by developing TLC in Hexane: Ethyl acetate: Methanol- 4:2:1 solvent system.

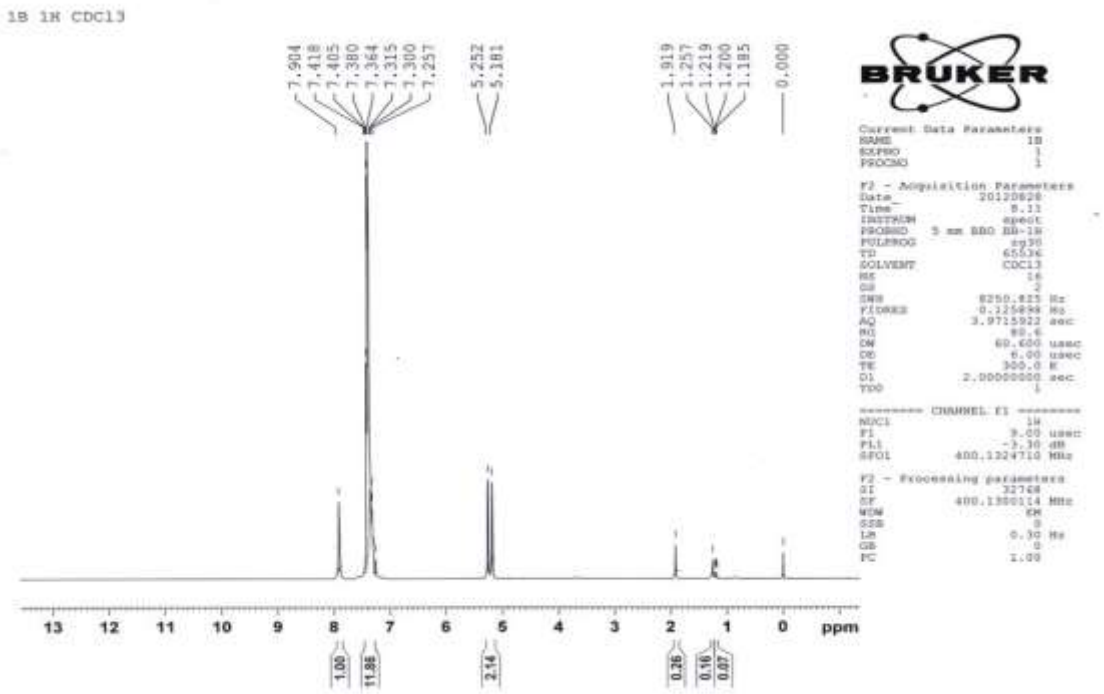


Figure 1: 4,6-diphenyl-6H-1,3-Thiazin-2-amine(1b).

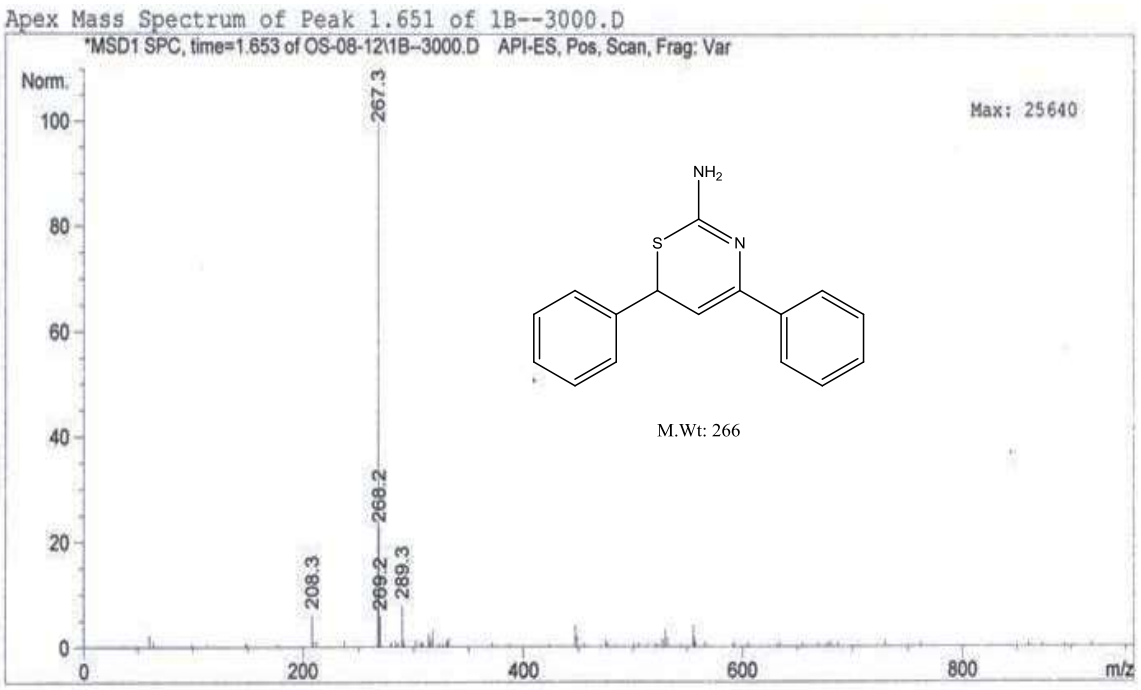


Figure 2: 4, 6-diphenyl-6H-1,3-Thiazin-2-amine(1b).

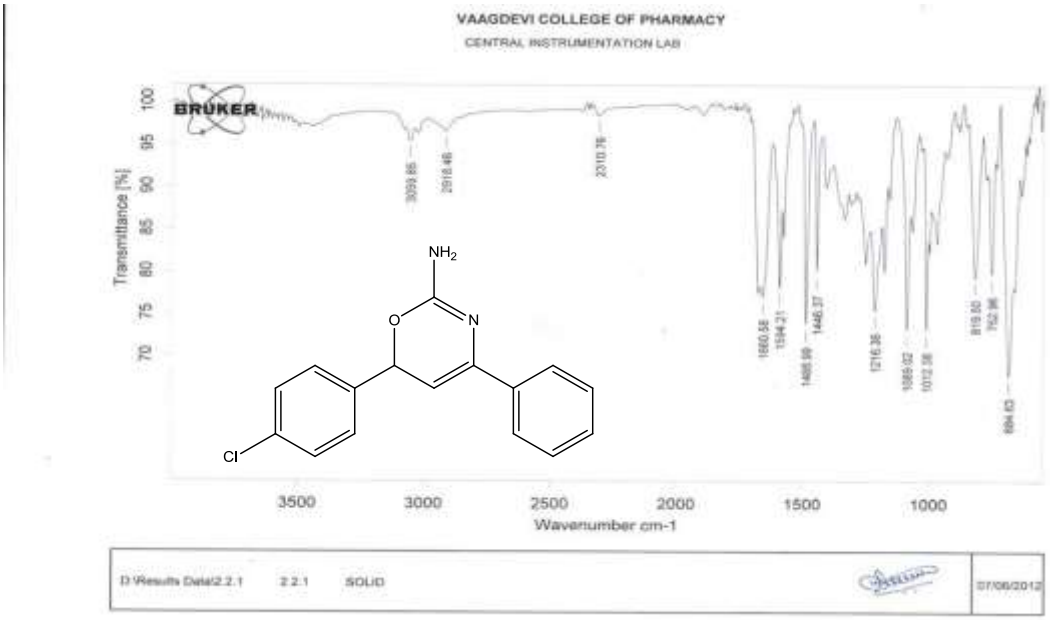


Figure 3: 6-(4-chlorophenyl)-4-phenyl-6H-1,3-oxazin-2-amine(2a).

819 cm-1 (C-Cl str.), 1012 cm-1 (C-O str.), 1089 cm-1 (C-C str.), 1216 cm-1 (C-N str. (Aromatic Amines)), 1488 cm-1 (C=C str.), 1660 cm-1 (C=N str.), 2918cm-1 (=C-H str.), 3459 cm-1 (NH str.).

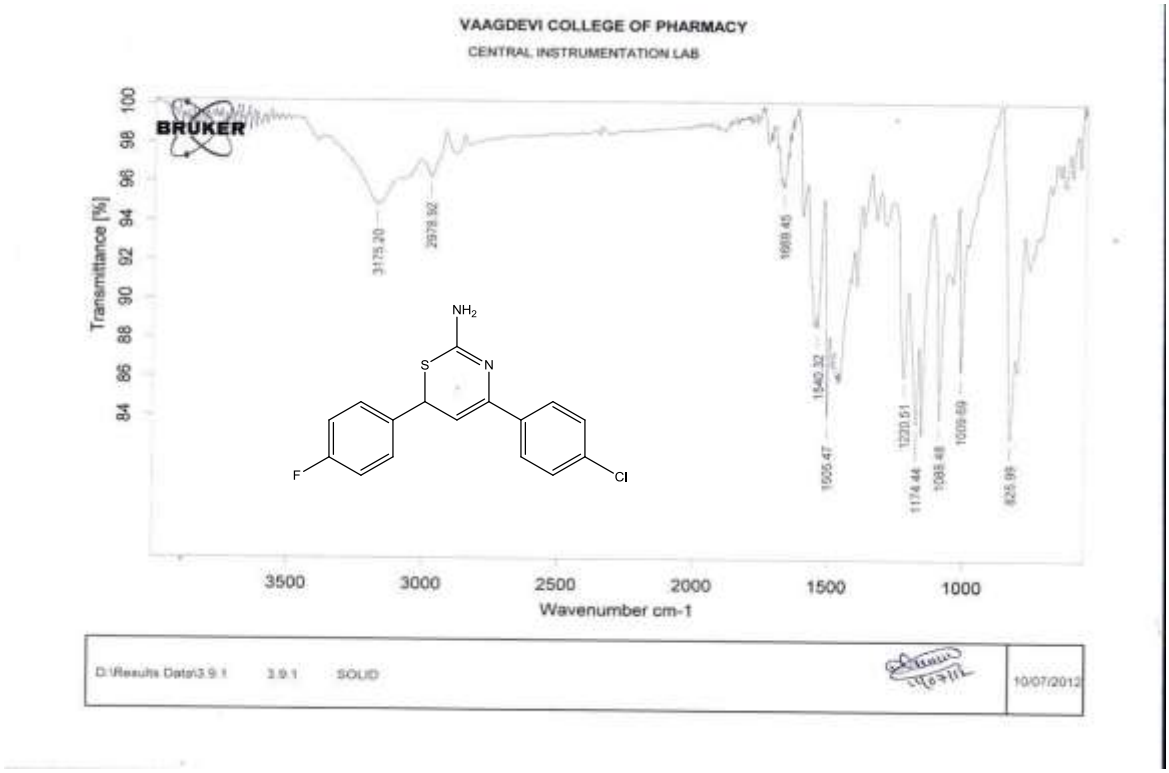


Figure 4: 4-(4-chlorophenyl)-6-(4-fluorophenyl)-6H-1,3-thiazin-2-amine(5b)

1. 825 cm-1 (C-Cl str.), 1009 cm-1 (C-F str.), 1220 cm-1 (C-N str. (Aromatic Amines)), 1540cm-1 (C-S str.), 1669 cm-1 (C=N str.), 3175 cm-1 (NH str.).

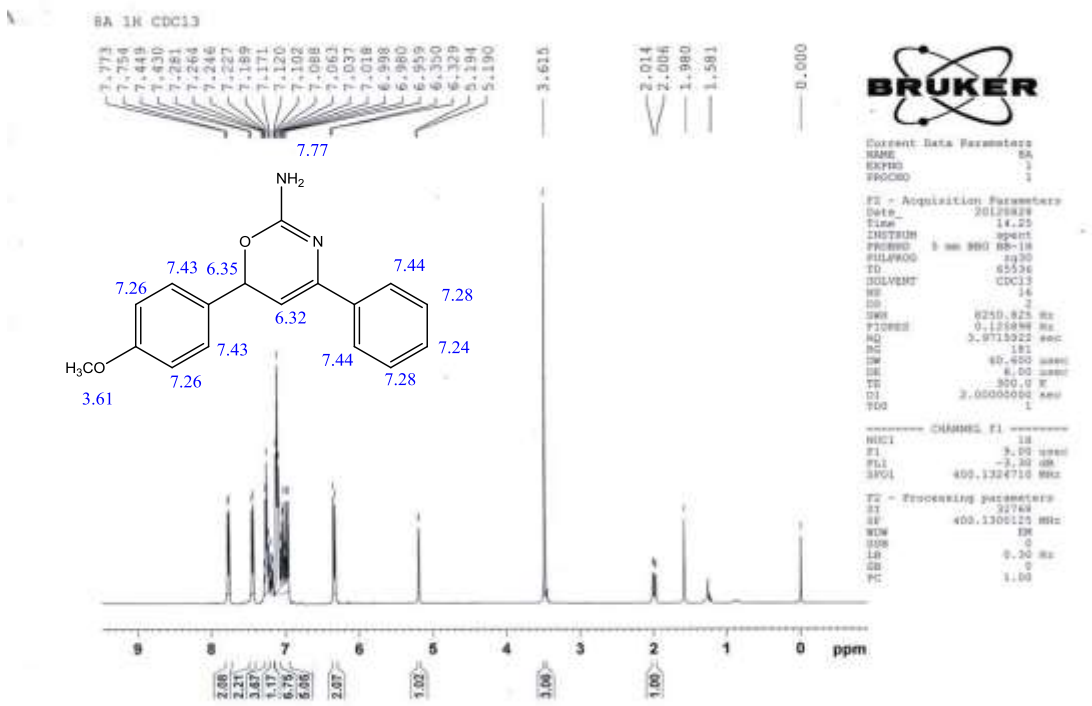


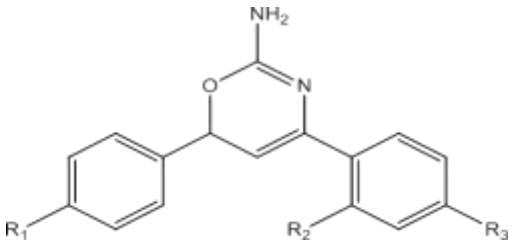
Figure 5: 6-(4-methoxyphenyl)-4-phenyl-6H-1,3-oxazin-2-amine(8a)

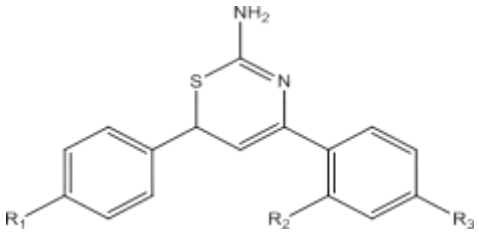
ANTIBACTERIAL ACTIVITY

The antibacterial activity of the test com- pounds was assayed against five strains of bacteria i.e. *Escherichia coli, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus, Lacto-bacillus acidophilus.*

The method adopted in the present investigation was cup plate method and medium isNutrient agar medium. The entire test com-pounds **1a-9a** and **1b-9b** equivalent to con-centration of 25, 50, ..., 200µg/ml were pre-pared by dissolving in DMSO. Weight equivalent to concentration of 100µg/ml was prepared by dissolving Ciprofloxacin in DMSO. Among eighteen derivatives all compounds showed mild antibacterial activity against *B. subtilis, S. aureus, L. acidophilus.* Compounds **3b, 7b, 3a** showed moderate activity against *E. coli, P.vulgaris.* When compared with standard (Ciprofloxacin) - low potent activity. Among oxazine derivatives **(1a-9a), 3a** compound is having potent antibacterial activity against *E. coli, P. vulgaris.* Due to thepresence electron withdrawing groups (-Cl)on R_1, R_2, R_3 positions of the phenyl ring. When compared to oxazine derivatives **(1a-9a),** all the thiazine derivatives **(1b-9b)** aremore potent. Among thiazine derivatives **(1b-9b), 3b** and **7b** compounds are having

potent antibacterial activity against *E. coli, P. vulgaris.* Due to the presence of electron withdrawing groups (-Cl) on R_1, R_2, R_3 positions of the phenyl ring. The un- substituted and substituted with electron donating groups like (8a,8b,9a,9b) on phenyl ring showed decreased in activity.





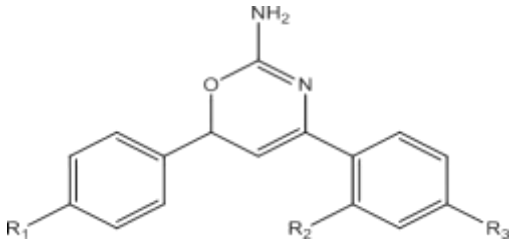
Code	R_1	R_2	R_3	MIC (µg/ml)		
				M. furfur	*A. niger*	*C.albicans*
1a	-H	-H	-H	200	175	>200
2a	-Cl	-H	-H	125	175	>200
3a	-Cl	-Cl	-Cl	125	100	200
4a	-F	-H	-H	175	195	180
5a	-F	-H	-Cl	175	150	>200
6a	-H	-H	-Cl	150	175	>200
7a	-F	-Cl	-Cl	125	110	>200
8a	$-OCH_3$	-H	-H	175	>200	>200
9a	$-OCH_3$	-H	-Cl	140	185	150
Std	Fluconazole			8	10	20

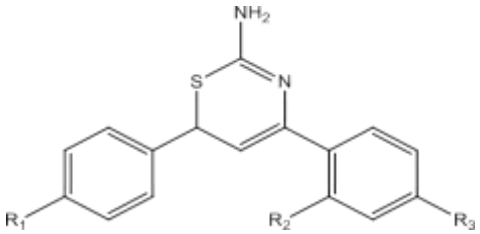
Code	MIC (µg/ml)		
	M. furfur	*A. niger*	*C.albicans*
1b	185	200	>200
2b	150	125	200
3b	110	110	200
4b	120	110	180
5b	120	150	200
6b	120	150	175
7b	110	90	175
8b	125	175	>200
9b	175	195	>200
Std	8	10	20

ANTIFUNGAL ACTIVITY

The antifungal activity of the test com-pounds was assayed against three strains of Fungi i.e., *Aspergillus niger, Candida al- bicans* and *Malassezia furfur.* The method adopted in the present investigation was cup plate method and medium is sabouraud dextrose agar medium. The entire test com- pounds **1a-9a** and **1b-9b** equivalent to con- centration of 25, 50, …, 200µg/ml were prepared by dissolving in DMSO. Weight equivalent to concentration of 100µg/mlwas prepared by dissolving fluconazole in DMSO. Among Eighteen derivatives all compounds showed low mild activity against *Candida albicans, Malassezia fur- fur.* Compounds **3a, 3b** and **7b** showed moderate activity against *Aspergillus niger.* When compared with standard (Fluconazole) - low potent activity. Among oxazine derivatives **(1a-9a),** compound **3a** showed potent antifungal activity against *Aspergillus niger.* Due to the presence of electron withdrawing groups(Cl)on R_1,R_2,R_3 positions of the phenyl ring The substitution of electron donating groups like (R =-OCH_3 on phenyl ring with showed de- crease in activity. When compared to oxazine derivatives **(1a-9a),** thiazine derivatives **(1b-**

9b) are more potent. Among thiazine derivatives **(1b-9b),** compounds **3b** and **7b** are having potent activity against *Aspergillus niger*. The un-substituted and substituted with electron donating groups like (8a,8b,9a,9b) on phenyl ring with showed decreased in activity.





Code	R_1	R_2	R_3	IC_{50} (nM)
1a	-H	-H	-H	670
2a	-Cl	-H	-H	353
3a	-Cl	-Cl	-Cl	249
4a	-F	-H	-H	238
5a	-F	-H	-Cl	211
6a	-H	-H	-Cl	246
7a	-F	-Cl	-Cl	228
8a	$-OCH_3$	-H	-H	342
9a	$-OCH_3$	-H	-Cl	213
Std		BHT		270

Code	IC_{50} (nM)
1b	440
2b	226
3b	151
4b	342
5b	184
6b	201
7b	150
8b	268
9b	187
Std	270

ANTIOXIDANT ACTIVITY

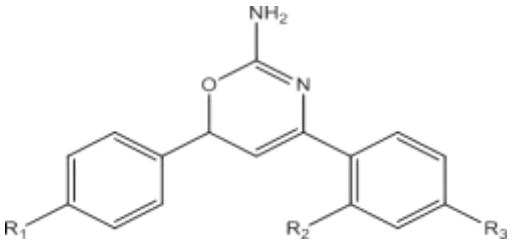
Method: 2,2-Diphenyl-1-picrylhydrazyl(DPPH) assay.[20]

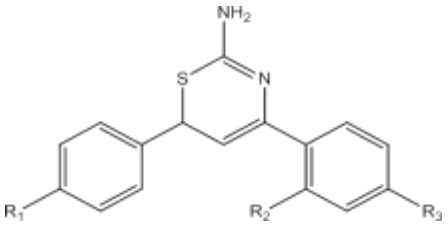
Principle: Determination of scavenging ac-tivity of DPPH free radical.

Procedure

1 ml of 0.135 mM DPPH in methanol + 3ml of aqueous solution of sample (20-100g/ml). Butylated hydroxyl toluene (BHT) as used as the standard material. The reaction mixture was kept at room temperaturefor 30 min. The absorbance was measuredspectrophotometrically at 517 nm.

% Inhibition of DPPH = $[A_C - A_S]/A_C \times 100$Where A_C is the absorbance of control and A_S is the absorbance of sample.





Code	R_1	R_2	R_3	IC_{50} (nM)
1a	-H	-H	-H	670
2a	-Cl	-H	-H	353
3a	-Cl	-Cl	-Cl	249
4a	-F	-H	-H	238
5a	-F	-H	-Cl	211
6a	-H	-H	-Cl	246
7a	-F	-Cl	-Cl	228
8a	$-OCH_3$	-H	-H	342
9a	$-OCH_3$	-H	-Cl	213
Std		BHT		270

Code	IC_{50} (nM)
1b	440
2b	226
3b	151
4b	342
5b	184
6b	201
7b	150
8b	268
9b	187
Std	270

All the synthesised compounds shown more potent than STD except **1a, 1b, 2a, 4b** and **8a.** The presenceof substituted aromatic ring in heterocyclic nucleus showed increase in activity when compared to the un-substituted aromatic ring. (-F> -Cl> -OCH > -H)

Iron chelation method

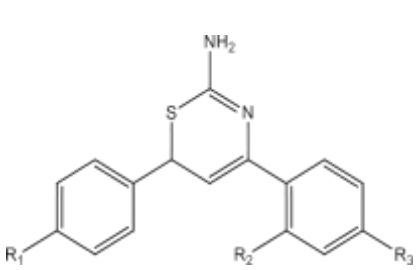
Procedure

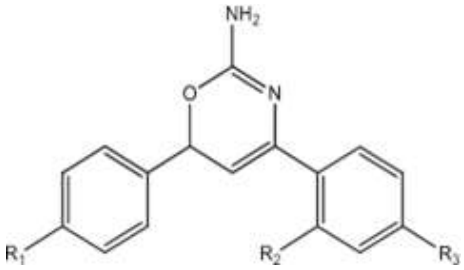
2ml of test solution of different concentrations (20-100 □g/ml) was mixed with 1ml of O-phenanthroline (prepared by dissolving 0.005 g of O-phenanthroline in 10 ml ofmethanol) and 2ml of $FeCl_3$ (prepared by dissolving 3.24 mg of $FeCl_3$ in 100 ml distilled water. The mixture is incubated 510nm. Ascorbic acid as used as the standard material.

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation.

% inhibition $= [A_c - A_s]/A_c \times 100$

Where A_c is the absorbance of control and A_s is the absorbance of sample.





Code	R_1	R_2	R_3	IC_{50} (nM)
1a	-H	-H	-H	561
2a	-Cl	-H	-H	384
3a	-Cl	-Cl	-Cl	199
4a	-F	-H	-H	273
5a	-F	-H	-Cl	170
6a	-H	-H	-Cl	281
7a	-F	-Cl	-Cl	137
8a	$-OCH_3$	-H	-H	245
9a	$-OCH_3$	-H	-Cl	179
Std	Ascorbic acid			241

Code	IC_{50} (nM)
1b	392
2b	276
3b	168
4b	186
5b	146
6b	209
7b	126
8b	203
9b	172
Std	241

All the synthesised compounds shown more potent than STD except **1a, 1b, 2a, 4b, 6a** and **8a.** The presence of substituted aromatic ring in heterocyclic nucleus showed increase in activity when compared to the unsubstituted ring.

CONCLUSION

The oxazine and thiazine derivatives are used as antiviral, analgesic, anti-inflammatory and antimyco bacterial activities, antitumor, antibiotics, anticonvulsants, anti-tubercular, antidepressant, antidiabetic. The synthesized oxazine and thiazine derivatives were characterized by physical and spectral data. Evaluated for their antimicrobial and antioxidant activities. All the thiazine derivatives **(1b-9b)** are showed more potent compared to oxazine derivatives **(1a-9a)**. Among all, the compounds **3b** and **7b** showed more potent activity. The unsubsti tuted and electron donating groups substituted phenyl ring showed less potent activity. The electron withdrawing groups substituted Phenyl (R_1, R_2, R_3) ring of hetero-cyclic oxazine and thiazine derivatives showed more potent activity(-F> -Cl> $-OCH_3$ > -H).

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