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Ecofriendly synthesis of benzoxazines and benzothiazines at ambient temperature without catalyst and their anti-bacterial and anti-fungal activity

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ABSTRACT

Benzoxazine, Benzothiazines have been synthesized in excellent yields at ambient temperature by PEG -400 mediated protocol under solvent free conditions. The enviourment freely protocol with excellent yields are the interesting features of the present method. All the synthesized compounds have been tested for their anti-bacterial and anti-fungal activity.

Keywords: PEG-400, Benzoxazine, Benzothiazine, antibacterial, anti-fungal activity.

INTRODUCTION

The applications of most of the today's antimicrobial agents are boundless, not only by the rapidly developing drug resistance, but also by the inadequate status of present treatments of bacterial and fungal infections and drug side effects[1a-c]. Heterocycles are an important class and highly applicable compounds found in pharmaceuticals and natural product[2]. Benzoheterocycles particularly benzoxazine and benzthiazine are an important class of N-containing heterocycles as they exhibit interesting biological activities and are used as key structural motifs for the synthesis of various pharmaceutical agents and natural products. Benzoxazines show diverse biological activities including plant resistance factor against microbial disease and insects, potassium channel modulators, antirheumatic and antihypertensive activity[3a-c]. Benzothiazines have received increasing attention as useful intermediate in organic synthesis. They have found applications in peptide synthesis and as precursors to thioesters for natural chemical ligation[4a-d.]

Various annulation methods for the synthesis of benzoxazine from 2-aminophenols and benzthiazines from 2-aminothiophenol are reported in the literature. These methods make use of

various Pd-catalyst [5 a-b] base catalysts [6] or microwave conditions [7]. Hence a method which is simple and environmental friendly is highly desirable.

With the increasing awareness of environmentally benign chemical processes both in academia and industry; volatile, toxic and hazardous organic solvents are continuously replaced either by use of solvent free techniques or by using ionic liquid, water or PEG. Use of PEG as a reaction medium offers many advantages such as good solvating ability, aptitude to act as a phase transfer catalyst, negligible vapor pressure, easy recyclability, reusability, ease of work up, ecofriendly nature and economical cost [8a-c]. In this context we not only report an efficient and facile method for the synthesis of benzoxazines and benzothiazines but the synthesized compounds were also tested for their anti-bacterial and anti-fungal activity.

MATERIALS AND METHODS

Melting points (uncorrected) were determined in open capillary tube IR analyses were performed on Perkin Elmer FT IR-783 spectrometer using KBr pellets. ^{1}H NMR and C^{13} NMR spectra in $CDCl_3$ on Bruker (300 MHz) using TMS as an internal standard. The GCMS analyses data was collected on Schimadzu GCMS-2010.The biological activity tested as mentioned above.

2.1 General procedure for the synthesis of benzoxazine / benzothiazine derivatives:

Aromatic amine (2mmol) and dialkyl acetylenedicarboxylate (2mmol) were dissolved in PEG (1ml) and the reaction mixture was stirred at Room Temperature and monitored by TLC. Upon completion of the reaction, reaction was poured on crushed ice the solid formed was filtered, dried and recrystallised from ethanol.

2.2 Anti-bacterial Activity

S.aureus is bacterium with thick cell wall, which offen clump together like small clusters of grapes. *S.aureus* most commonly found on human body, especially nose. They are associated with wounds, cuts, needle pricks, surgery, catheters, etc. *S. aureus* infections can cause Septic arthritis and staphylococcal endocarditis. *E. Coli* is short form of *Escherichia coli*. They are a large and diverse group of bacteria. Some of them can cause sickness, urinary tract infections, respiratory illness, pneumonia and some other illnesses. Some, such as *E. coli* O157:H7 can cause severe illness. Infection often causes severe bloody diarrhea, abdominal cramps, and possibly fever (these symptoms are common to a variety of diseases, and may be caused by sources other than contaminated drinking water).

Trichothecium is a filamentous metaphoric fungus found in decaying vegetation and the soil. Therefore these species have been selected for the testing. *Rhizopus* is a cosmopolitan filamentous fungus frequently isolated from soil, decaying fruit and vegetables, animal feces, and old bread. Aside from being known as common contaminants, *Rhizopus* species are also occasional causes of serious, and often fatal, infections in humans.

Nutrient agar have been prepare prepared by dissolving bacteriological peptone and 0.82% sodium chloride in double distilled water. Then 2 % agar was added. The resulting mixture was sterilized for 30 minutes at 15 Ibs pressure. The anti-bacterial and anti-fungal activity was tested by Disc Diffusion Method.

2.3 *Disc diffusion method*

In this method, first 15-20 ml of molten agar was poured into to each sterile plate. The plates are inoculated with the help of sterile loop by using spread plate technique. The inocubated plates were allowed to stand for 3 to 15 min before applying paper disc. Using sterile forceps the paper absorbent disc of 6mm diameter coated with respective chemical was placed on the surface of plate. The plates were inocubated at 37°C temperature.

For comparison, the DMF solvent control was run under similar condition to know activity of blank. The zone of inhibition if any developed, was measured for tested organism for particular compound. The results are introduced in table II

RESULTS AND DISCUSSION

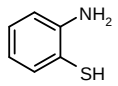
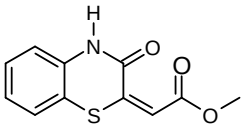
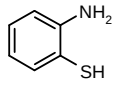
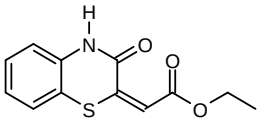
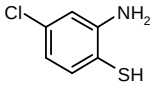
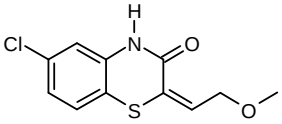
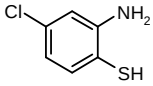
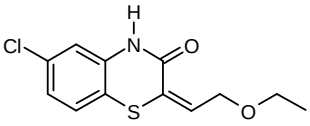
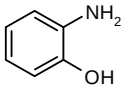
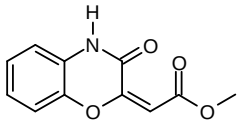
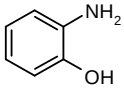
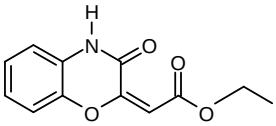
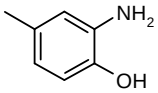
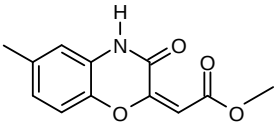
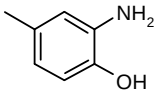
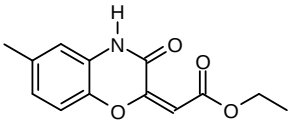
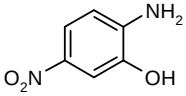
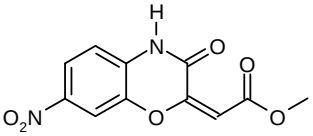
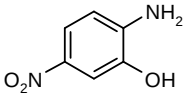
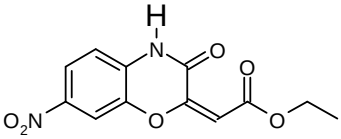
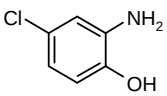
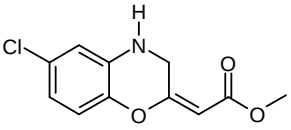
In order to standardize the reaction, 2-aminophenol (2mmol) and dimethyl acetylene dicarboxylate were mixed together and reaction mixture was stirred at R.T. without any catalyst but with PEG 400(1ml). After the completion of reaction, the reaction mixture was poured on crushed ice the separated solid was filtered and washed with water. The product was recrystallised from ethanol without any need of further purification. The product was confirmed on the basis of IR, ^{1}H NMR and C^{13} spectral data. The success of the above results and our previous success in the synthesis of nitrogen heterocycles inspired us [9a-c] to check the generality of the present protocol. In this 2-aminothiophenol, 2-amino-4-chlorothiophenol, 2-aminophenol, 2-amino-4-chlorophenol, 2-amino-4-methylphenol and 2-amino-5-nitrophenol gives the corresponding benzothiazines and benzoxazines in quantitative yield. The results are given in table-I.

The reactions proceed through the binucleophilic addition from aminophenol to the DMAD and DEAD. The intermediates formed might be stabilized through the hydrogen bonding. This support the mechanism proposed by Xian Fu Lin et al.[10] .Both the groups either electron donating or electron withdrawing favors the formation of benzothiazines and benzoxazines in shorter times.

The entry 8, 10 and 12 have been reported as new compounds and spectroscopic data found were in consistent with the proposed structure. The reported compounds confirmed with their authentic value. The details are given in the spectroscopic data.

All the synthesized compounds were screened for their antimicrobial activity against gram positive and gram-negative organisms by disc diffusion method.

Table 1 The reactions of 2-aminothiophenol, 2-aminophenol with DMAD or DEAD in PEG

Entry	Substrate	Time (min)	Product	M.P. (°C)	Yield (%)
1		45		263(264-266°c)	90
2		45		220(220-222°C)	92
3		45		234(236°C)	91
4		45		200(201°C)	91
5		45		149(150°C)	95
6		45		96(97°C)	94
7		60		147(148-149°C)	90
8		90		104°C	91
9		60		240(241-242°C)	95
10		120		192°C	93
11		90		158 (159°C)	91

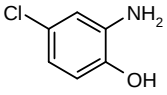
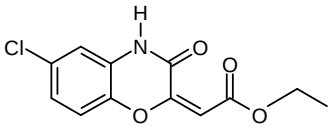
12		120		110°C	90

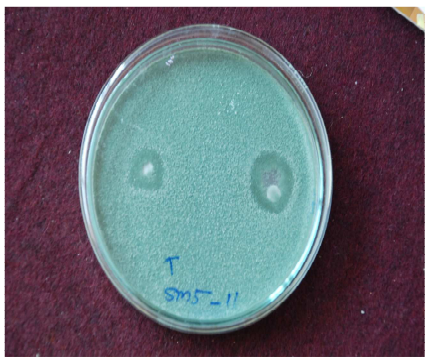
Table-2 The results of the Zone of inhibition against tested bacteria

Entry	*E.coli*		*S.aureus*	
	300	**500**	**300**	**500**
1.	17	21	18	22
2.	15	19	05	10
3.	05	11	04	08
4.	06	10	-----	-----
5.	14	19	08	12
6.	04	05	00	00
7.	18	24	10	18
8.	16	17	00	04
9.	07	09	09	14
10.	09	10	08	11
11.	10	13	----	----
12.	12	14	19	22

Table 3 The results of the Zone of inhibition against tested fungia

Entry	*Trichothecium*		*Rhizopus*	
	300	**500**	**300**	**500**
1.	17	25	15	19
2.	7	11	10	14
3.	---	----	12	17
4.	--	--	07	13
5.	--	--	09	14
6.	--	--	11	16
7.	12	16	18	23
8.	10	15	13	17
9.	--	--	02	06
10.	--	--	05	09
11.	14	18	16	19
12.	--	--	17	26

Figure 1 The photographic results of the biological activity against the tested bacteria and fungi



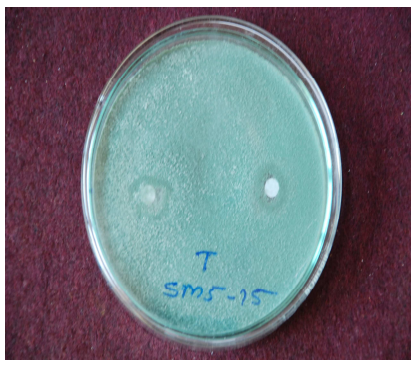
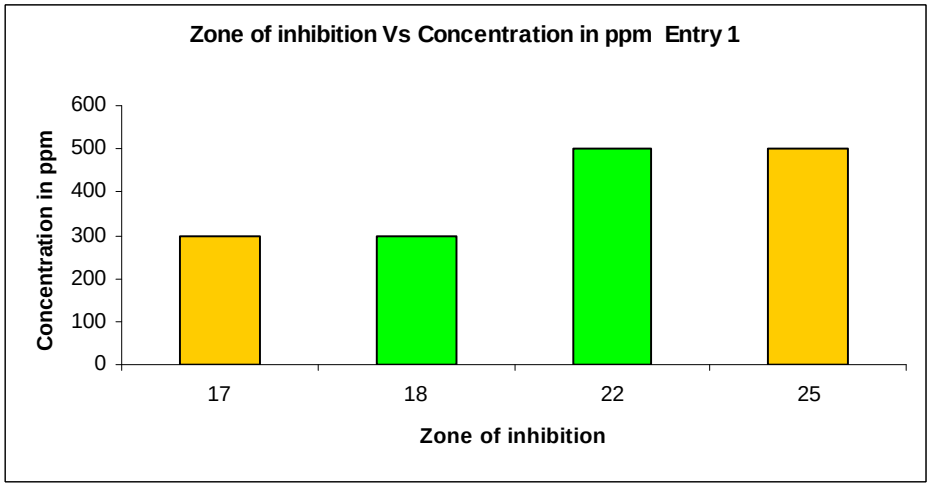


Figure 2 The graphical representation (Concentration vs. zone of inhibition) of the anti-bacterial and anti-fungal activity of 2-methoxycarbomethylene-3,4-dihydro-2*H*-1,4-benzothiazin-3-one



3.1 SAR study and significance of the biological activity:

The structure activity relationship deals with the activity of the compound under study with respect to the structure. This has great importance in medicinal chemistry as it helps to develop new bioactive compound on the basis of the reported literature. As far as our study is concerned, we have reported different benzothiazine and benzoxazine molecules and further tested their anti-bacterial and anti-fungal activitities against the *S.aureus, E.coli, Rhizopus, and Trichothecium.*

The results are shown in the **Table-II** and **III**, shown that some of the tested compounds showed prominent activity. But no compound is found to be selective against all of the tested bacteria and fungi. On careful analysis, it has been observed that benzothiazines have shown excellent zones of inhibitions as compared benzoxazines. This showed the importance of the sulfur atom in exhibiting the anti-bacterial and anti-fungal activities.

The presence of the groups likes nitro, chloro have decreases the anti-bacterial activity of the benzothiazines and benzoxazines the activity. However, the presence of these groups enhances anti-fungal activity of these molecules.

In conclusion, the benzoxazines and benzothiazines reported in the present study are of medicinal importance and their pharmacological evaluation is under investigation.

3.2 Spectroscopic data of the representative and new compounds:

2-Methoxycarbomethylene-3,4-dihydro-2H-1,4-benzoxazin-3-one(Table-I, entry 5)[12] M. P. 149°C

IR (KBr): 3114,1769,1673,1650,1624,1510,1480 cm^{-1}; H^1 NMR ($CDCl_3$ 300MHz): δ 3.79 (s, 3H, -**CH_3**), 5.94 (s, 1H, -**CH**), 6.96-6.98(m, 2H, **Ar-H**), 7.02–7.17(m, 2H, **Ar-H**), 10.67 (S,1H,-**NH**); ^{13}C NMR ($CDCl_3$): δ 170.30, 140.01, 138.15, 125.71, 122.83, 117.06, 114.84, 90.71 and 51.50; M^+ 219

6-Methyl-2-ethoxycarbomethylene-3,4-dihydro-2H-1,4-benzoxazin-3-one(Table-I, entry 8)M. P. 104°C

IR (KBr): 2985,2914,1769,1673,1650,1624,1510,1480,1362 cm^{-1}; H^1NMR ($CDCl_3$ 300MHz): 1.30-1.34 (t, 3H, -CH_2**CH_3** J = 7.5Hz), 3.78 (s, 3H, **-CH_3**) 4.22-4.24 (q, 2H, **-CH_2**CH_3, J=6.9Hz), 5.90 (s, 1H, **-CH**), 6.76-6.81 (m, 2H, **Ar-H**), 7.01-7.03 (d, 2H, **Ar-H**) 10.63 (s, 1H, **-NH**); ^{13}C NMR ($CDCl_3$): δ 169.97, 156.16, 138.13, 138.01, 135.71, 123.40, 116.68, 115.07, 90.91, 60.32, 20.95 and 14.20; M^+247

7-Nitro-2-ethoxycarbomethylene-3,4-dihydro-2H-1,4-benzoxazin-3-one(Table-I, entry 10)M. P. 192°C

IR (KBr): 3215, 3085,1782, 1676, 1638, 1606, 1522, 1479 cm^{-1}; H^1NMR ($CDCl_3$ 300MHz):1.31-1.36 (t, 3H, -CH_2-**CH_3**, J = 7.2Hz), 4.23-4.30 (q, 2H, **-CH_2**-CH_3, J = 7.2Hz), 6.09(s,1H,**-CH),** 7.04-7.07 (d, 1H, **Ar-H**), 8.05-8.09 (m, 2H, **Ar-H**), 10.98 (s,1H,**-NH**), ^{13}C NMR ($CDCl_3$): δ 169.36, 159.48, 142.11, 139.21, 136.28, 129.91, 121.75, 114.54, 113.45, 95.68, 61.10 and 14.46; M^+278

6-Chloro-2-ethoxycarbomethylene-3,4-dihydro-2H-1,4-benzoxazin-3-one(Table-I, entry 12)M. P. 110°C

IR (KBr): 2911,1761,1656,1630,1497,1364 cm^{-1}; H^1NMR ($CDCl_3$ 300MHz): δ 1.30 –1.35 (t, 3H, -CH_2-**CH3** J=7.2Hz), 4.20-4.27 (q, 2H, **-CH_2**CH_3, J=7.2Hz), 5.96(s, 1H,**-CH**), 6.95-7.26 (m, 3H, **Ar-H**,), 10.67 (s, 1H, **-NH**); ^{13}CNMR ($CDCl_3$75MHz): δ169.69, 155.46, 137.29, 129.83, 125.17, 122.51, 118.10, 114.67, 92.68, 60.62 and 14.22; M^+267

CONCLUSION

In summary, not only the novel and practical protocol but also here we have described the applicability of the synthesized compounds through eco-friendly protocol for the synthesis and anti-bacterial and anti-fungal activity of benzoxazine and benzothiazines at ambient temperature as well. The shorter reaction time with excellent yield from readily available chemicals made it practical method for the synthesis of desired compounds. Some of these compounds have excellent zone of inhibition against the tested species.

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