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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL SCREENING OF SOME NOVEL SUBSTITUTED 2-AMINO [1, 3] OXAZINE DERIVATIVES

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ABSTRACT

The present study deals with synthesis of some novel substituted 2-amino [1, 3] oxazine derivatives and their spectral characterization by means of UV, IR and 1H NMR. The compounds were screened for antibacterial activity against standard strains of both Gram positive and Gram negative bacteria. Results obtained establish compounds S3 and S4 to be significantly responsive against different bacterial strains and as such these compounds can pave the way for development of potent antibacterial agents.

KEYWORDS: Chalcone, Urea, Antibacterial, Cup-plate method

INTRODUCTION

Oxazines which have been the object of the interest for the past three decades, still remain little studied compounds. Oxazines are heterocyclic compounds containing one nitrogen and one oxygen[1-2]. Aromatic oxazines were first synthesized in 1944 by Holly and Cope through Mannich reactions. Comparatively little work has been done on simple derivatives of these ring system and most of these concerns the

reduced 1, 3 and 1, 4 compounds. The most important simple1, 4- oxazine is morpholine or tetrahydro-1, 4 oxazine, which is a colour less liquid, which is miscible with water[3]. Oxazine heterocycles have special interest because they constitute an important class of natural and non natural products and show useful biological activities[4]. Its increasing importance in pharmaceutical and biological field, through this review article, we are planned to collect synthesis of oxazine derivatives for their biological activities.

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. 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 remain a main focus of medicinal research.

Oxazine derivatives have been reported to posses' antifungal[5-9], antibacterial[10-12], anticoagulant[13], antimicrobial activity[14], anticoagulant activity[15]. 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. The present methodology bears the merits of reduced worthwhile to synthesis the titled compounds, as they appeared to be highly promising.

MATERIALS AND METHODS

All the reagents used for synthesis were of analytical grade commercial products and used without further purification. The melting points of the synthesized compounds were determined using an electric melting point apparatus by open capillary method. (Expressed in degree Celsius) and are uncorrected. The progress of reactions and purity of synthesized compounds were checked on silica gel-G TLC plates using various solvent combinations of different polarity. The spots were detected with iodine vapors as visualizing agent. The λmax (in nm) of the synthesized compounds was recorded on *Elico SL 164* UV-visible spectrophotometer using alcohol as solvent. The FT-IR spectra of the synthesized compounds were recorded on a FT-IR *Perkin Elmer Spectrum RX-I* spectrometer using KBr disc in the range of 4000-400 cm−1. The Proton NMR (1H NMR) spectra were recorded in *Bruker AC-F 400* FT-NMR spectrometer at a frequency of 400 MHz. Spectra were obtained in deuterated acetone (acetone-d6) using TMS (δ 0.00 ppm) as an internal standard at room temperature. Chemical shift (δ) values are expressed in ppm relative to internal standard.

Synthetic Scheme:

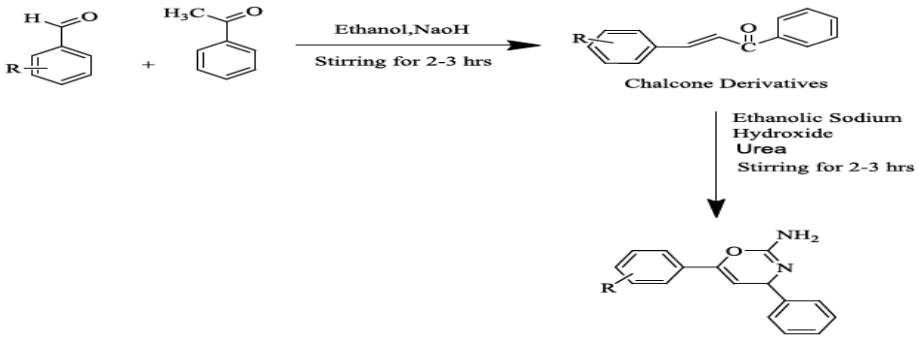
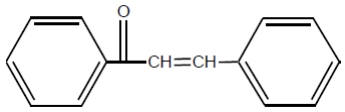
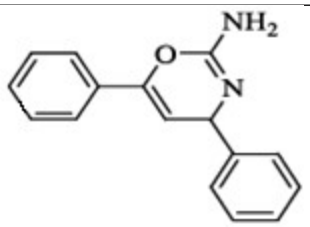
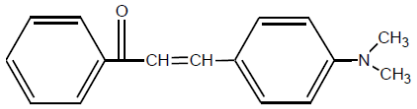
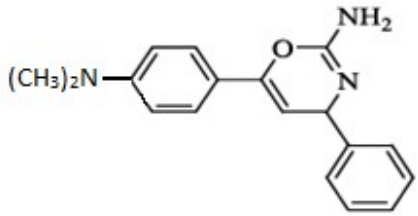
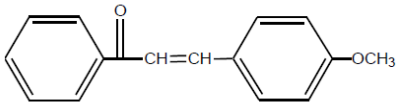
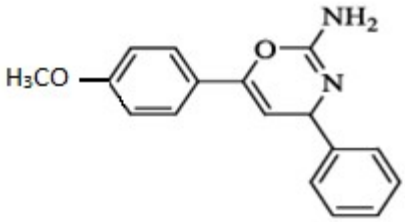
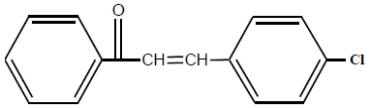
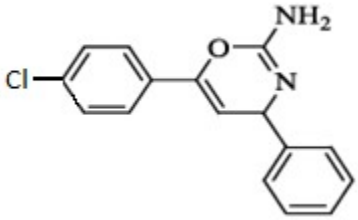
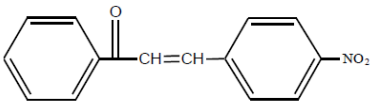
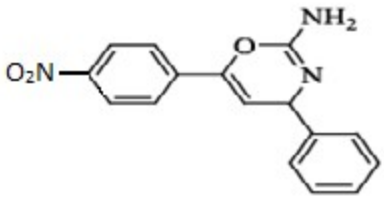


Fig.1: Synthetic Scheme for Substituted 2-amino [1, 3] oxazine derivatives Substituted Chalcones used for synthesis

Table.1: Substituted Chalcones used for reaction process to form target compounds

Sample Code	Substrate	Compound
S1		
S2		
S3		
S4		
S5		

General method for synthesis of Substituted 2-amino [1, 3]-oxazine derivatives:

Chalcone and Chalcone derivatives were prepared in Step I, treated with Urea in presence of Ethanolic NaOH to get various substituted 2-amino [1, 3]-oxazine derivatives. This reaction process involves two steps reaction is given below:

STEP I:

A solution of 5.5 gm Sodium Hydroxide and 30 ml alcohol is cooled (Crushed Ice) and the solution stirred. Freshly distilled 0.01mol acetophenone is added followed by addition of 0.01mol of different aromatic aldehydes. The reaction mixture is kept at 25°C and stirring continued until stirring is no longer possible (2-3 hr). The reaction mixture is left overnight in a refrigerator. The separated product is filtered on buchner funnel and washed with cold water and then with ice cold alcohol. It is recrystallized with alcohol.

STEP II:

Above obtained compounds [0.02mol] was taken in beaker and add 0.02 mol of urea dissolved in 10ml of 10% ethanolic NaOH solution. It is stirring for 3 hrs. Pour to 200 ml of cold water with constant stirring for 1 hr. Keep for overnight. Filter and collect the precipitate product and recrystallized with ethanol. The products were confirmed by IR, $_1$H NMR analysis and melting point determination[16].

Antibacterial Evaluation

The antibacterial activity of the synthesized compounds was evaluated systematically against different strains of Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria like *E.Coli* and *Pseudomonas aeruginosa*. The inhibition zones (in mm) of synthesized compounds were determined by cup-plate method[17]. The sterilized medium (autoclaved at 121°C for 20min) was inoculated using 18 hr slant cultures of the test organisms and transferred into sterile Petri dishes and allowed to the media to solidify. Cups of 8mm diameters were made on solidified media. Solutions of the synthesized compounds at a concentration of 50µg/ml and 100µg/ml were prepared in

acetone. 50µl of each solution was placed in cups by means of sterile pipette. In each plate one cup was used for standard and other two for test solutions. The plates thus prepared were left for 90 min in a refrigerator for diffusion. The plates were incubated at 37°C for 24 hrs and examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition was recorded Amikacin (50µg/ml) was used as standard.

RESULTS AND DISCUSSON

Physico-Chemical Properties and Spectral data of the Synthesized Compounds:

The yields of all the synthesized compounds were found to be satisfactory within the range of 75 to 80%. The spectral data generated upon analysis were found in accordance with the anticipated structure of the synthesized compounds.

S1: 4, 6-diphenyl-6H-1, 3-oxazin-2-amine:

Yield: 78%; **Melting point**: 88-90ºC; **R_f value**: 0.81; **λ_{max}**: 420; **IR (KBR) [cm^{-1}]**:3384(N-H str.), 2930(Ar C-H str.), 1640(N-H str.), 1627(C=N), 1475, 1559(Ar ring stretching); **$_1$HNMR (DMSO-d6) [ppm]**: 7.7 (m, 4H, Ar), 7.5 (m, 4H, Ar), 7.35 (m, 5H, Ar), 5.3 (d, 1H, CH, Oxazine), 1.7(s, 1H, CH).

S2: 6-[4-(dimethylamino) phenyl]-4-phenyl-6H-1, 3-oxazin-2-amine:

Yield: 80%; **Melting point**: 90-95ºC; **R_f value**: 0.785; **λ_{max}**: 410; **IR** (KBr cm^{-1}): 3400 (N-H str.), 3090 (Ar C-H str.), 2922 (CH_3 asym. str.), 2840 (CH_3 sym. str.), 1585 (Ar C-C str.), 1230 (C-O str). **^{1}H NMR**: 2.01 (2H, Ar- NH_2) δ 2.82 (6H, N $(CH_3)2$), δ 4.9 (1H, CH=C of oxazine ring), δ 7.28 (1H of oxazine ring), δ 7-7.8 (8H, Ar.H).

S3: 6-[4-(methoxy) phenyl]-4-phenyl-6H-1, 3-oxazin-2-amine:

Yield: 82%; **Melting point**: 105ºC- 110ºC; **R_f value**: 0.90; **λ_{max}**: 430; **IR** (KBr cm^{-1}): 3134.72 (NH stretch), 1012.36 (C-O-C stretch), 1665.23 (Ar C=C stretch); **^{1}H NMR** 2.13 (s, 3H, OCH3), 2.12 (d, 3H, CH3), 9.36 (s, 1H, NH), 9.54 (s, 1H, NH),7.09-7.54 (m, 1H, Aromatic).

S4: 6-[4-(chloro) phenyl]-4-phenyl-6H-1, 3-oxazin-2-amine:

Yield: 79%; **Melting point**: 115-120ºC; **R_f value**: 0.915; **λ_{max}**: 415; **IR** (KBr cm^{-1}): 2220.66 (Ar-H stretch), 1616.06 (C=O stretch), 1048.35 (C-O-C stretch), 1492.63 (Ar C=C stretch), 755.95 (C-Cl stretch); **^{1}H NMR**: 2.32 (d, 3H, CH3), 9.52 (s, 1H, NH), 9.76 (s, 1H, NH), 7.01-7.59 (m, 10 H, Aromatic).

S5: 6-[4-(nitro) phenyl]-4-phenyl-6H-1, 3-oxazin-2-amine:

Yield: 81%; **Melting point**: 110-120ºC; **R_f value**: 0.82; **λ_{max}**: 440; **IR** (KBr cm^{-1}): 3107 (Aromatic C-H str), 2854 (C-H), 1744 (C=O), 605 (C=C str), 1524 (NO2 str.), 855 (C-H out plane bending), 1431(C-N str.); **^{1}H NMR** (400 MHz, acetone-d_6), δ (ppm): 6.25 - 6.29 (2d, 2H, -CH=CH-), 6.9-8.01 (m, 8H, Ar-H).

Antibacterial activity data of the synthesized compounds

Antibacterial screening of the synthesized compounds against different strains of Gram positive and Gram negative bacteria show compounds S3 and S4 exhibiting marked inhibition of both Gram positive and negative strains.

Table.2 Antibacterial Activity Data:

Compound	Zone of Inhibition							
	Gram Positive bacteria				Gram Negative bacteria			
	S.Aureus		B.Subtilis		E.Coli		P.Aeruginosa	
	50	100	50	100	50	100	50	100
S1	12	14	14	20	15	18	14	17
S2	16	19	12	19	14	18	15	17
S3	16	18	20	25	16	20	16	20
S4	18	19	21	22	17	21	18	23
S5	15	17	19	24	15	19	15	19
Control	-	-	-	-	-	-	-	-
Amikacin	16	-	22	-	17	-	19	-

Amikacin (50 µg/ml) was used as positive control; Acetone was used as negative control

CONCLUSION

In this study, we have synthesized five derivatives of substituted 2- amino [1, 3] oxazine derivatives by the scheme depicted in Figure 1. The test compounds were synthesized in good percentage of yield their physical and analytical determination was done by using melting point apparatus, purification of compounds by TLC, and the structural assignments of new compounds were made on the basis of IR and $_1$HNMR data. This scheme of reaction went to completion within 3 hr. After completion of reaction and work up the products were identified and characterized by using IR and $_1$HNMR techniques and their structures were elucidated as *4,6-diphenyl-6H-1,3-oxazin-2-amine, 6-[4-(dimethylamino)phenyl]-4-phenyl-6H-1,3-oxazin-2-amine,6-[4-(methoxy)phenyl]-4-phenyl-6H-1,3-oxazin-2-amine,6-[4 (chloro)phenyl]-4-phenyl-6H-1,3-oxazin-2-amine, 6-[4-(notro)phenyl]-4-phenyl-6H-1,3-oxazin-2-amine*. The isolated yield was *78%, 80%, 82%, 79%, 81%.* From our present investigation, it can be concluded that we synthesized five derivatives of substituted 2- amino [1,3] oxazines by treated chalcone derivatives with urea in presence of ethanolic NaOH and the structural assignments of these compounds are made on the basis of IR and $_1$HNMR data.

Stressing on the structural influence on the activity of the synthesized novel analogues, it can be observed that the para methoxy group (-OCH_3) present in S3 and para chloro S4 may have a vital role in the activity of the compounds. On the other hand S2 and S1 too showed notable inhibitory activity to a considerable extent. It is evident from the research work that this series of synthesized and screened compounds along with further explored ones following the above mentioned convenient synthetic procedure may pave the way for development of some very potent antibacterial agents.

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REFERENCES

1. Raj K. Bansal, Heterocyclic Chemistry, Fourth edition; New Age International Publishers; 501-502.
2. Alan R. Katritzky, the Principles of Heterocyclic Chemistry; Pharma Med Press Publishers; 80-81.
3. Morrin Acheson R, An Introduction to the Chemistry of Heterocyclic Compounds, Third edition; A Wiely -Inter Science Publication; 410-414.
4. Zuhal T, Emel P, Adem K. Synthesis of New 1, 3-Disubstituted-2,3-dihydro-1H-naphth- [1,2e] [1,3]oxazines. Molecules 2007, 12: 345-352.
5. Bhat AR, Pawar PD. synthesis and biological evaluation of some [1, 4]-thiazine-2-one and [1-4]-oxazin-2-one derivatives. Indian drugs 2008, 45(12):962-965.
6. Beena KP, Akelesh T. Design, synthesis, characterization and evaluation of some 1,3-oxazine derivatives as potent antimicrobial agents. Scholars Research Library, 2013, 5(4): 257-260.
7. Sayaji SD, Pravina BP. Novel synthesis and antimicrobial activity of bis-oxazine derivatives, JCPR, 2013, 5(5): 271-274.
8. Kategaonkar AH, Sonar SS, Pokalwar RU, Kategaonkar AH, Shingate BB, and Shingare MS: An efficient and novel one- pot synthesis of new 3,4-dihydro-3-substituted-2H-naphtho [2,1-e]

[1,3]oxazine derivatives and reported their antimicrobial activities. Bull. Korean Chem. Soc 2010; 31(6): 1657 -1660.

9. Anil NM. Synthesis and antimicrobial study of new 8-bromo-1, 3-diaryl-2,3-dihydro-1H-naphthol[1,2e][1,3] oxazines. Int. J. Chem. 2011, 3: 74-86.
10. Zanatta N, Borchhardt DM, Alves SH, Coelho H S, Squizani MC, Marchi TM, Bonacorso HG, Martins MP: Synthesis of oxazines and They exhibited significant activity against tested microorganism strains. Bioorg. Med. Chem 2006; 14: 3174.
11. Elarfi MJ, AL-Difar HA: Synthesis of oxazine, thiazine and isoxazole. These compounds were also screened for their antibacterial activities. Sci. Revs. Chem. Commun 2012; 2(2):103-107.
12. Sunil D, Upadhya S, Rama M. Synthesis, characterization and QSAR studies of some New 1, 3-oxazines as patent antimicrobial agents. Res.J. Pharma. Sci. 2013, 2(2): 15-19.
13. Ramesh LS, Mahesh SM, Jyoti BW. Anticoagulant potential of Schiff Bases of 1, 3-oxazines, Internet J Pharm Sci, 2012, 4:320-323.
14. Sayaji SD, Piste BP. Novel one-pot synthesis and anti-microbial activity of 6-chloro-2, 4-diphenyl3, 4-dihydro-2H-1, 3-benzoxazines derivatives. Internet J chem. Tech Research, 2013, 5: 2199-2203.
15. Sawant RL, Mhaske MS, Wadekar JB: A series of Schiff bases of 1, 3-oxazines were synthesized via reaction of 1, 3- oxazine-2-amine with substituted benzaldehyde and they exhibited significant anticoagulant activity. Int J Pharm Pharm Sci 2012; 4(4):320-323.
16. Elarfi MJ, AL-Difar HA: Synthesis of oxazine, thiazine and isoxazole. These compounds were also screened for their antibacterial activities. Sci. Revs. Chem. Commun 2012; 2(2):103-107.
17. Hawkey PM, Lewis DA. Medical bacteriology-A practical approach, 2nd ed., United Kingdom: Oxford University press; 1994.