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Synthesis, Characterization and QSAR studies of some New 1, 3-oxazines as Potent Antimicrobial agents

Sunil Dhanya, Upadhya Sadhana H., Savitha and Rama M.
Department of Chemistry, Manipal Institute of Technology, Manipal University, 576104, INDIA

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Abstract

A new series of 4-(4-substituitedphenyl)-6-substituited-6H-1,3-oxazines 2a-f have been synthesized from acid catalysed reaction between chalcones 1a-f and urea. The structures of all compounds were confirmed by advanced spectral techniques like IR, ^{1}HNMR 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 was 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 zone of inhibition of micro-organisms was used as key properties to evaluate the QSAR models. The best correlated QSAR model depicted that the autocorrelationcharge 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.

Keywords: Chalcones, oxazines, antimicrobial activity, QSAR.

Introduction

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, anti-malarial[1-3], antipyretic, anticonvulsant and antimicrobial activities[4-8]. Benzo-1,3-oxazines are also known to be biologically active, demonstrating anti-rheumatic, antianginal, antihypertensive effects, cytotoxic[9,10] and anti-osteoclastic bone resorption activity[11]. Efavirenz, a trifluoromethyl-1,3-oxazin-2-one, is a non-nucleoside reverse transcriptase inhibitor which displays significant activity against HIV-1 mutant strains[12]. 1,3-Oxazine derivatives are also known to function as progesterone receptor agonists[13]. Naphthoxazines are found to possess psycho stimulating and antidepressant activity and are used in the treatment of Parkinson's disease[14,15].

Only 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 and antifungal activities. In this connection, the present paper describes the synthesis and antimicrobial studies of six new 1,3-oxazine derivatives.

Material and methods

Chemistry: Claisen-Schmidt condensation of substituted aromatic aldehydes with 4-substituted acetophenones yielded six (2*E*)-3-[(substituted) phenyl)]-1- [(4-substituted) phenyl] prop-2-en-1-ones (chalcones) 1a-b[16,17] (scheme-1). Six new oxazines were synthesized by the reaction between chalcones and urea (0.001 mole) in ethanol medium in presence of concentrated hydrochloric acid[18]. The synthetic pathway is given in scheme-1.

Experimental: General method for the *synthesis of 4-(4-substituitedphenyl)-6-substituited-6H-1,3-oxazine 2a-c*.
Chalcone (0.001 mole) in alcohol was added to a solution of 12.5 mM of urea in alcohol. 5 mL of conc. HCl was added, refluxed for 9 h and concentrated to half its volume. The reaction mixture was poured into crushed ice water and kept overnight. The oxazine separated was filtered, dried and recrystallized using ethanol.

6-[4-(dimethylamino)phenyl]-4-(4-fluorophenyl)-6*H*-1,3-oxazin-2-amine (2a): Yellow solid (73 %) mp 98-100 °C; 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.*), 1010 (C-F *str.*); ^{1}HNMR (DMSO-d_6) [ppm]: 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); MS (m/z): 311 (M^+); Anal. calcd. for $C_{18}H_{18}N_3OF$: C, 69.45; H, 5.79; N, 13.50. Found: C, 69.56; H, 5.81; N, 13.54.

6-[4-(dimethylamino)phenyl]-4-(4-chlorophenyl)-6*H*-1,3-oxazin-2-amine (2b): Yellow solid (70 %) mp 102-104°C, IR (KBr) [cm^{-1}]: 3400 (N-H *str.*), 3100 (Ar C-H *str.*), 2910 (CH_3 *asym. str.*), 2820 (CH_3 *sym. str.*), 1580 (Ar C-C *str.*), 1240 (C-O *str.*), 790 (C-Cl *str.*); ^{1}HNMR (DMSO-d_6) [ppm]: 2.03 (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); MS (m/z): 327 (M^+); Anal. calcd. for $C_{18}H_{18}N_3OCl$; C, 66.06; H, 5.50; N, 12.84. Found: C, 66.19; H, 5.53; N, 12.89.

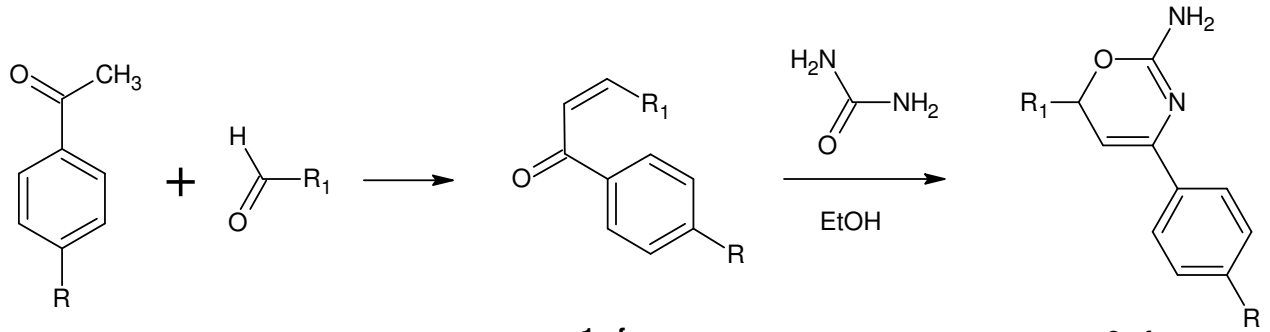
6-[4-(dimethylamino)phenyl]-4-(4-methoxyphenyl)-6*H*-1,3-oxazin-2-amine (2c): Orange solid (70 %) mp 118-122°C, IR (KBr) [cm^{-1}]: 3400 (N-H *str.*), 3100 (Ar C-H *str.*), 2910 (CH_3 *asym. str.*), 2850 (CH_3 *sym. str.*), 1600 (Ar C-C *str.*), 1250 (C-O *str.*); ^{1}HNMR (DMSO-d_6) [ppm]: 2.03 (2H, Ar-NH_2) δ 2.82 (6H, N(CH_3)$_2$), δ 3.43 (3H, OCH_3), δ 4.9 (1H, CH=C of oxazine ring), δ 7.28 (1H of oxazine ring), δ 7-7.8 (8H, Ar.H); MS (m/z): 323 (M^+); Anal. calcd. for $C_{19}H_{21}N_3O_2$; C, 70.59; H, 6.50; N, 13.00. Found: C, 70.69; H, 6.53; N, 12.99.

6-[2,4-dimethoxyphenyl]-4-(4-fluorophenyl)-6*H*-1,3-oxazin-2-amine (**2d**): Brown solid (82 %) mp 82-84°C, IR (KBr) [cm^{-1}]: 3450 (N-H *str.*), 3095 (Ar C-H *str.*), 2940 (CH_3 *asym. str.*), 2860 (CH_3 *sym. str.*), 1600 (Ar C-C *str.*), 1245 (C-O *str.*), 1010 (C-F *str.*); ^{1}HNMR (DMSO-d_6) [ppm]: 2.03 (2H, Ar-NH_2), δ 3.44 (6H, OCH_3), δ 4.84 (1H, CH=C of oxazine ring), δ 7.23 (1H of oxazine ring), δ 7-7.8 (7H, Ar.H); MS (m/z): 328 (M^+); Anal. calcd. for $C_{18}H_{17}N_2O_3F$; C, 65.85; H, 5.18; N, 8.54. Found: C, 66.02; H, 5.20; N, 8.59.

6-[2,4-dimethoxyphenyl]-4-(4-chlorophenyl)-6*H*-1,3-oxazin-2-amine (**2e**): Brown solid (79 %) mp 112-115°C, IR (KBr) [cm^{-1}]: 3400 (N-H *str.*), 3100 (Ar C-H *str.*), 2950 (CH_3 *asym. str.*), 2840 (CH_3 *sym. str.*), 1605 (Ar C-C *str.*), 1240 (C-O *str.*), 750 (C-Cl *str.*); ^{1}HNMR (DMSO-d_6) [ppm]: 2.03 (2H, Ar-NH_2) δ 3.43 (6H, OCH_3), δ 4.84 (1H, CH=C of oxazine ring), δ 7.23 (1H of oxazine ring), δ 7-7.8 (7H, Ar.H); MS (m/z): 344 (M^+); Anal. calcd. for $C_{18}H_{17}N_2O_3Cl$; C, 62.79; H, 4.94; N, 8.14. Found: C, 62.92; H, 4.95; N, 8.18.

6-[2,4-dimethoxyphenyl]-4-(4-methoxyphenyl)-6*H*-1,3-oxazin-2-amine (**2f**): Brown solid (75 %) mp 86-88°C, IR (KBr) [cm^{-1}]: 3450 (N-H *str.*), 3095 (Ar C-H *str.*), 2930 (CH_3 *asym. str.*), 2840 (CH_3 *sym. str.*), 1605 (Ar C-C *str.*), 1250 (C-O *str.*); ^{1}HNMR (DMSO-d_6) [ppm]: 2.03 (2H, Ar-NH_2) δ 3.43 (9H, OCH_3), δ 4.84 (1H, CH=C of oxazine ring), δ 7.23 (1H of oxazine ring), δ 7-7.8 (7H, Ar.H); MS (m/z): 340 (M^+); Anal. calcd. for $C_{19}H_{20}N_2O_4$; C, 67.06; H, 5.88; N, 8.24. Found: C, 67.22; H, 5.88; N, 8.27.

All the chemicals and solvents used for this work were obtained from Merck and Aldrich Chemicals. The structures of the newly synthesized compounds were confirmed by spectral data and elemental analysis. Thin layer chromatography (TLC) was conducted on 0.25 × 10^{-3} m silica gel plates to follow the progress of the reactions and to check the purity of the compounds. A 1:1 mixture of ethyl acetate and hexane solution was used as the eluent for chalcones. A 4:1:5 mixture of n-butanol, acetic acid and water was used as developer for oxazines. Visualization was made by using UV light. The IR spectra were recorded in a Schimadzu FTIR 8400S spectrophotometer in the range of 4000-400 cm^{-1} using KBr pellets. ^{1}H-NMR spectra were recorded in a AV500 NMR spectrometer in deuterated dimethyl sulphoxide and are reported as parts per million (ppm) downfield from tetramethyl silane used as an internal standard. The mass spectra were taken in a Schimadzu GCMS-QP5050 mass spectrometer. The IR, ^{1}HNMR and MS were consistent with the assigned structures.



1a-f **2a-f**

Comp. no.	R_1	R
1a, 2a	4-dimethylaminophenyl	F
1b, 2b	4-dimethylaminophenyl	Cl
1c, 2c	4-dimethylaminophenyl	OCH_3
1d, 2d	2,4-dimethoxyphenyl	F
1e, 2e	2,4-dimethoxyphenyl	Cl
1f, 2f	2,4-dimethoxyphenyl	OCH_3

Scheme-1
Synthetic route for the preparation of oxazines

The mass spectrum showed molecular ion peaks which were in accordance with their respective molecular mass. The elemental analysis was done in Flash thermo 1112 series CHN analyser. All the compounds gave C, H and N analysis within the permissible limit of 0.4 %. Melting points were determined by open capillary method and are uncorrected.

Pharmacology: Antimicrobial activity of all the newly synthesized oxazines was studied by Disc Diffusion Method (Kirby-Bauer Method)[19].

Antibacterial activity: The antibacterial activity of oxazines was evaluated against *Staphylococus aureus* representing Gram-positive bacteria and *Escherichia coli* representing Gram-negative bacteria. The compounds were dissolved in DMSO at a concentration of 100 µg/mL. Antibacterial activity of solvent DMSO against the test organisms was investigated and was found to be nil. Nutrient agar media was prepared and plated on petri-plates. Plates were inoculated by swab culturing using stock culture. Different discs were dipped in dissolved solution of oxazines and placed in inoculated plates using sterile forceps and gently pressed. Plates were incubated for 24 h at 37°C. Tetracycline was taken as the reference drug against Gram-positive and Gram-negative bacteria. The results were recorded for each tested compound as the average diameter of inhibition zones (ZOI) of bacterial growth around the discs in mm.

Antifungal activity: The oxazines were screened for their *in vitro* antifungal activity at 100 µg/mL against *Aspergillus niger*. The discs after treatment with oxazines were incubated for a week at room temperature and ZOI was measured in mm. The antifungal activities of test compounds were compared to the standard drug, Ketaconazole.

QSAR studies: In order to carry out Quantitative Structure Activity Relationship (QSAR) studies, the 2D structures of the molecules were converted to 3D and the descriptors for those molecules were predicted using PaDEL software[20]. To determine the correlation between the physicochemical descriptors and the Log ZOI values of oxazines against the microbes, simple linear regression analysis was performed using Easy QSAR 1.0[21]. The best QSAR model was chosen based on the statistical parameters like the square of the correlation coefficient (r^2), the Fischer's value of significance (F) and the standard error of estimate (s). The experimentally obtained antimicrobial activity of oxazines was then correlated with their predicted antimicrobial activity using the tool Easy QSAR.

Results and Discussion

Compounds 2c and 2e containing methoxy and chloro substituent respectively showed excellent antibacterial activity against *E. coli*. Compounds 2d and 2e showed moderate antibacterial activity, whereas 2f containing methoxy substituent displayed high sensitivity against *S. aureus*. Compound 2b and 2f demonstrated excellent antifungal activity by inhibiting spore germination of *A. niger*. The results of the present study are in agreement with the earlier literature which had shown the efficacy of a methoxy group in enhancing the antimicrobial properties of a molecule[22]. The structure-antimicrobial activity relationship of the synthesized compounds revealed that the compounds with methoxy and chloro substituents in the phenyl ring exhibited maximum antimicrobial activity. This can be attributed to the increased dipole moment in C-X bond which might have enhanced the intermolecular interactions and might have augmented the antimicrobial property of the molecule.

Various QSAR models were developed by the simple linear regression analysis. The highest value of the square of the correlation coefficient (r^2 = 0.93), and the satisfactory values of F and s (27.64 and 0.03 respectively), were obtained when the antibacterial activity (Log ZOI) of four oxazines, against *E.coli* at 100 µg/mL, was correlated with the Crippen MR. This correlation is represented by equation 1.

Y=4.900683986699E + 000 + -2.589732673611E-002*(X1) (1)
n = 4, r^2 = 0.93, F = 27.64, s = 0.03

To identify the contribution of the descriptor to the antibacterial activity of oxazines, this correlation was done. The negative sign associated with the parameter Crippen MR, indicated that the lower the value of Crippen MR, the higher would be the activity of oxazines against *E. coli*. The observed and the predicted antibacterial activities of the compounds are listed in table-1 and the correlation between them is represented graphically in figure 1. The highest value of the square of the correlation coefficient (r^2 = 1), and the satisfactory values of F and s (955014.92 and 0.00 respectively), were obtained when the antibacterial activity (Log ZOI) of three oxazines, against *S.aureus* at 100 µg/mL, was correlated with ATSc1. This correlation is represented by equation 2.

Y= 1.350553374177E+000 + 1.731905179576E-002*(x1) (2)
n = 3, r^2 = 0.998, F = 955014.92, s = 0.00

The positive sign associated with the parameter, ATSc1, indicated that the higher the value of ATSc1, the higher would be the activity of oxazines against *S. aureus*. The observed and the predicted antibacterial activities of the compounds are listed in table 1 and the correlation between them is represented graphically in figure 2. No statistically significant results were obtained for inhibitory activity against Fungi using PaDEL descriptors with the synthesized compounds.

Table-1
Observed and the predicted activities (Log ZOI) of the compounds using the best QSAR model

Comp. No.	S. aureus			E. coli			A. niger		
	ZOI	Observed Log ZOI	Predicted Log ZOI	ZOI	Observed Log ZOI	Predicted Log ZOI	ZOI	Observed Log ZOI	Predicted Log ZOI
2a	0	0	-	11	2.4	2.53	14	2.639	2.72
2b	0	0	-	13	2.56	2.45	20	2.996	2.81
2c	0	0	-	22	3.09	3.1	0	0	-
2d	12	2.48	2.49	0	0	-	0	0	-
2e	12	2.48	2.48	24	3.18	3.16	16	2.773	2.90
2f	21	3.04	3.04	0	0	-	19	2.944	2.88

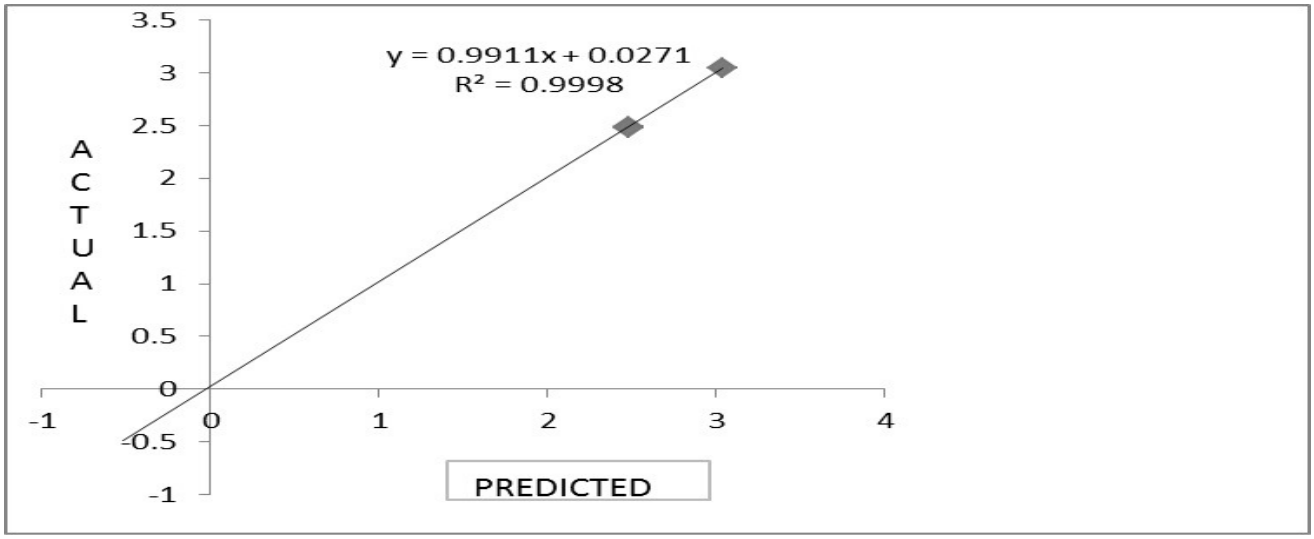


Figure-1
Correlation between the observed and the predicted activities using the best QSAR model for the compounds against *S. aureus*

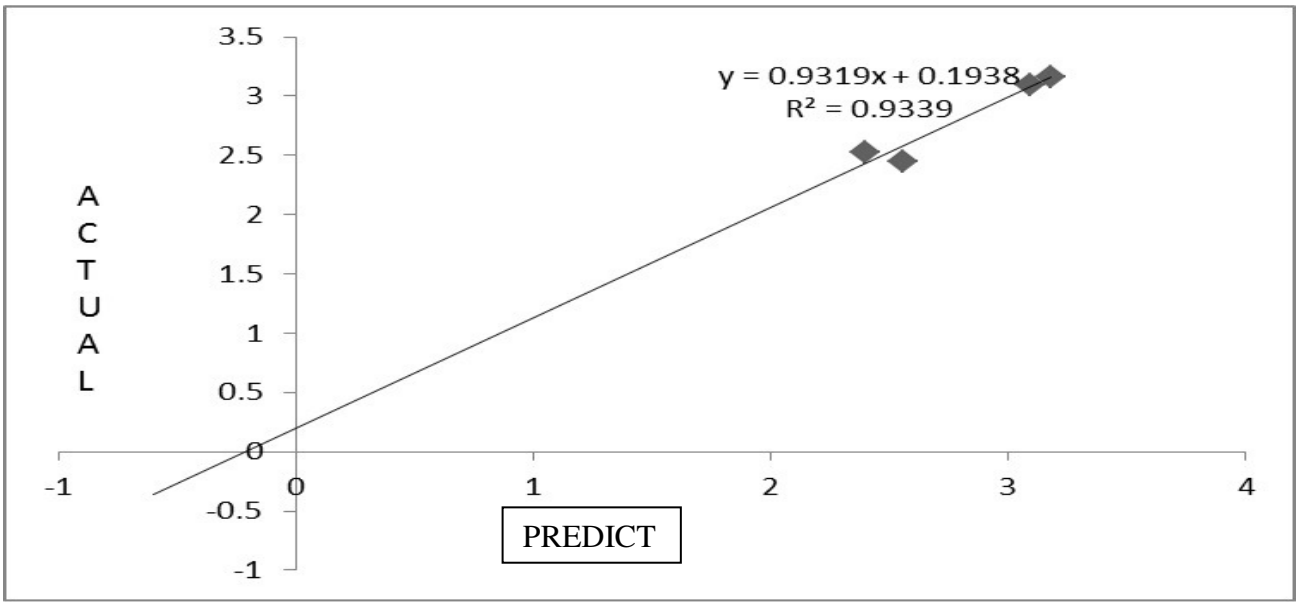


Figure-2
Correlation between the observed and the predicted activities using the best QSAR model for the compounds against *E. coli*

Conclusion

The objective of the present study was to synthesize a series of six new oxazines by the reaction between chalcones and urea in presence of concentrated hydrochloric acid. The different spectral techniques and the elemental analysis established the structure of the compounds. The parameter, Crippen MR, was significantly correlated to the antibacterial activity of chalcones against Gram - ve bacteria at 100 µg/mL. A close correlation between the observed and the predicted Log ZOI values for oxazines indicated the development of the best QSAR model. Moreover this class of compounds needs further investigation and this model can be used for the design of oxazines as novel antibacterial agents.

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