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Synthesis, characterization and antimicrobial studies of (E)-N-((2-chloro-6-substituted quinolin-3-yl)methylene)-4-(substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines

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Research work is planned to synthesize novel (E)-N-((2-chloro-6-substituted quinolin-3-yl)methylene)-4-(substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines by the reaction of 6-(3-substituted phenyl)-4-(4-substituted phenyl)-5,6-dihydro-4*H*-1,3-oxazin-2-amines with 6-substituted-2-chloro-quinoline-3-carbaldehydes in alcoholic medium and in the presence of acetic acid. The structures of synthesized compounds are assigned on the basis of FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data. The new compounds are also screened for their antibacterial and antifungal activities. These compounds are showing potent antimicrobial activities due to their chemical structure.

Keywords: Quinoline, Oxazine, Spectroscopy, Antibacterial, Antifungal

Oxazines are heterocyclic compounds containing one nitrogen and one oxygen with three isomeric forms such as 1,2-oxazines, 1,3-oxaqzines and 1,4-oxazines. 1,3-oxazine derivatives have gained much attention due to varied biological properties like analgesic[1], anticonvulsant[2], antitubular[3], antibacterial[4] and anticancer[5] activities. The quinoline derivatives has been found to possess antimalarial[6], antibacterial[7,8], antifungal[9], antiviral[10], receptoragonists[11], antineoplastic agents[12] and antituberculotic[13] etc. In an earlier communication[14] we have reported the synthesis of quinolino-thiazines by the reaction of 4-substituted phenyl-6-phenyl-6H-1,3-thiazin-2-amines with 6-substituted-2-chloro-quinoline-3-carbaldehydes. Keeping in view of these observations, we have studied the mode of reaction between 6-substituted-2-chloro-quinoline-3-carbaldehydes and substituted oxazines to evaluate their biological activity.

Experimental Details

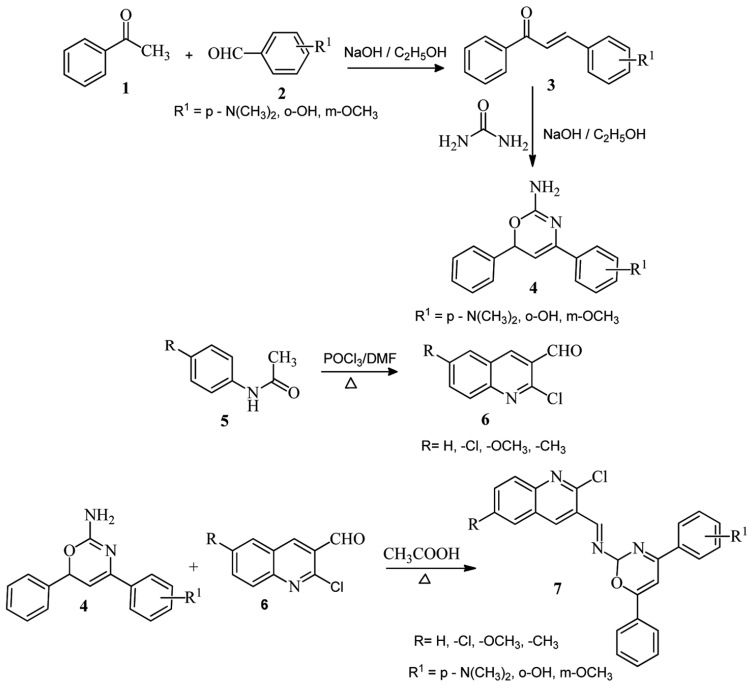
All the resources used from Sigma Aldrich, Alfa, and Spectrochem Chemicals Pvt. Ltd. Melting points of all the synthesized compounds were recorded by Bio Techniques India BTI-39 melting point instrument and are uncorrected. The completion of reaction was monitored by thin layer chromatography (TLC) on silica gel plates using ethyl acetate-hexane solvent mixture. IR spectra were found on a NICOLET AVATAR 330 FT-IR spectrophotometer, ^{1}H-NMR and ^{13}C-NMR were recorded on BRUKER 400 MHz NMR spectrometer and chemical shift valves are given on delta scale relative to TMS as internal reference. Mass spectra of some selected compounds were recorded on a WATERS SYNAPT G2 high resolution mass spectrometer.

General procedure for synthesis of Chalcones, 3

A mixture of acetophenones **1** (0.01 mol) and substituted aldehydes **2** (0.01 mol) was stirred in 90% ethanol (30 mL) and then an aqueous solution of potassium hydroxide (15 mL) was added to it. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with dilute hydrochloric acid. The chalcones derivative precipitates out as solid. Then it was filtered and recrystallized from ethanol.

General procedure for the synthesis of 4-substituted phenyl-6-phenyl-6H-1,3-oxazin-2-amines, 4

A mixture of substituted chalcones **3** (0.01 mol) and urea (0.01 mol) were dissolved in ethanol and sodium hydroxide (0.01 mol) solution (10 mL) was added. The reaction mixture was stirred for 3 h. Then it was poured



Scheme 1 — Synthesis of (E)-N-[(2-chloro-6-substituted quinolin-3-yl-methylene]-4-(substituted phenyl)-6- phenyl-2H-1,3-oxazin-2-amines

into 300 mL of cold water with continuous stirring for 1 h then left overnight. The precipitate formed was filtered, washed and recrystallized from ethanol.

General procedure for the synthesis of 6-substituted-2chloro-quinoline-3-carbaldehydes, 6

Dimethyl formamide (9.13 g, 9.9 mL, 0.125 mol) was cooled to 0°C in a flask equipped with a drying tube and phosphoryl chloride (53.7g, 32.2 mL, 0.35 mol) was added dropwise with stirring. To the solution was added substituted acetanilide **5** (6.55 g, 0.05 mol) and the solution was heated under reflux for 16 h. The reaction mixture was poured into ice water and stirred for 30 min at 0-10°C when 2-chloro-6-substituted quinoline-3-carbaldehyde **6** separated as yellow precipitate. It was filtered, washed with water and recrystallized from ethyl acetate.

General procedure for the synthesis of (E)-N-((2-chloro-6-substituted quinolin-3-yl)methylene)-4-(substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines, 7a-l

To an equimolar mixture of 4-substituted phenyl-6-phenyl-6H-1,3-oxazin-2-amines **4** (0.01 mol) and 6-substituted-2-chloro-quinoline-3-carbaldehydes **6** (0.01 mol) in ethanol (30 mL), two drops glacial acetic acid was added and refluxed on a water bath for two hours. The reaction mixture was cooled to room temperature and filtered. The precipitated products were recrystallized with ethanol.

Results and Discussion

The reaction was started by the preparation of 4-substituted phenyl-6-phenyl-6H-1,3-oxazine-2-amines **4** from substituted chalcones **3**, urea in ethanolic sodium hydroxide solution as per the literature procedure[15]. The substituted chalcones **3** were prepared by the reaction between the substituted aldehydes **2** and ketones **1** in alcoholic sodium hydroxide solution as per the literature procedure[16].

6-Substituted-2-chloro-quinoline-3-carbaldehydes **6** were obtained by Vilsmeier-Haack formylation of substituted acetanilides **5** using dimethyl formamide and phosphoryl chloride as per procedure reported by Otto-Meth-Cohn[17].

The novel compounds (E)-N-((2-Chloro-6-substituted quinolin-3-yl)methylene)-4-((substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines **7** were synthesized by the condensation reaction of 4-substituted phenyl-6-phenyl-6H-1,3-oxazin-2-amines **4** with2-chloro-6-substituted quinoline-3-carbaldehydes **5** in alcoholic medium and in the presence of acetic acid (Scheme 1).The characterization data of the newly synthesized compounds **7a-l** was presented in Table 1.

The spectroscopic data of (E)-N-((2-chloro-6-substituted quinolin-3-yl)methylene)-4-(substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines **7a-l** recorded are explained as follows:

Table 1 — Characterization data of (E)-N-((2-chloro-6-substituted quinolin-3-yl)methylene)-4-(substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines, **7a-l**

Compound	R	R^1	Product description	Mol. formula	M.P (°C)	Yield (%)
7a	H	p-$N(CH_3)_2$	Yellow crystals	$C_{28}H_{23}ClN_4O$	151-13	63
7b	H	o-OH	Pale yellow crystals	$C_{26}H_{18}ClN_3O_2$	134-136	65
7c	H	m-OCH_3	White crystals	$C_{27}H_{20}ClN_3O_2$	153-155	65
7d	Cl	p-$N(CH_3)_2$	White crystals	$C_{28}H_{23}Cl_2N_4O$	144-146	66
7e	Cl	o-OH	Yellow crystals	$C_{26}H_{17}Cl_2N_3O_2$	144-146	66
7f	Cl	m-OCH_3	Yellow crystals	$C_{27}H_{19}Cl_2N_3O_2$	144-146	63
7g	OCH_3	p-$N(CH_3)_2$	Yellow crystals	$C_{29}H_{25}ClN_4O_2$	174-176	75
7h	OCH_3	o-OH	White crystals	$C_{27}H_{20}ClN_3O_3$	122-124	77
7i	OCH_3	m-OCH_3	Pale yellow crystals	$C_{28}H_{22}ClN_3O_3$	142-144	67
7j	CH_3	p-$N(CH_3)_2$	Yellow crystals	$C_{29}H_{25}ClN_4O$	163-165	66
7k	CH_3	o-OH	White crystals	$C_{27}H_{20}ClN_3O_2$	165-164	75
7l	CH_3	m-OCH_3	White crystals	$C_{28}H_{22}ClN_3O_2$	174-176	60

(E)-N-((2-Chloroquinolin-3-yl)methylene)-4-(4-(dimethyl-amino)phenyl)-6-phenyl-2H-1,3-oxazin-2-amine, 7a

FT-IR (KBr, cm^{-1}) spectrum of the compound **7a** showed absorption band at 1645.95 due to the(CH=N) stretching and other bands at 1568.32is dueto (C=N) stretching, 1168.60 is due to C-N [-$N(CH_3)_2$] stretching, 1020.49 is due to (C-O-C) stretching, and 814.12 due to (C-Cl) stretching. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm) spectrum of the compound **7a** showed signal at 10.55 (s) belongs to CH=N protons, 8.75 (s) belongs to quinoline H-4 proton, 7.76-8.05 (m) belongs to quinoline H-5, H-6, H-7 and H-8 protons, 6.67-7.64 (m) belongs to phenyl and oxazine protons, and 3.03 (s) belongs to $N(CH_3)_3$ protons. ^{13}C-NMR ($CDCl_3$, 400 MHz, δ ppm) spectrum of the compound**, 7a** showed signals at 40.10 (-$N(CH_3)_3$C-atoms), 77.07, 111.86, 116.91, 122.67, 123.35, 124.24, 128.09, 128.29, 128.44, 130.07, 130.27, 130.40, 132.14, 134.33, 139.04, 139.18, 145.87, 152.01, 156.40, 160.10(oxazine, quinoline, and phenyl C-atoms), 161.8 (CH=NC-atom). Mass spectrum (HRMS) of the compound **7a** showed the molecular ion peak at m/z, 468.0143 (M^++1) in agreement with molecular formula $C_{28}H_{23}ClN_4O$.

(E)-N-((2-Chloro-6-methoxyquinolin-3-yl)methylene)-4-(4-(di-methylamino)phenyl)-6-phenyl-2H-1,3-oxazin-2-amine, 7g

FT-IR (KBr, cm^{-1}) (7g): 1686.97 (CH=N stretching), 1570.95(C=N stretching), 1170.20 (C-N stretching, -$N(CH_3)_3$), 1075.08 (C-O-C stretching), 812.06 (C-Cl stretching). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm), 7g: 10.52 (s, 1H, CH=N proton), 8.61 (s, 1H, quinoline H-4 proton), 7.74-7.99 (m, 3H, quinoline H-5, H-7 and H-8 protons), δ 6.66-7.53 (m, 11H, phenyl and thiazine protons), 3.93 (s, 3H, -OCH_3 protons) and 3.02 (s, 6H, -$N(CH_3)_2$ protons). ^{13}C-NMR ($CDCl_3$, 400 MHz, δ ppm), 7g: 40.10 (-$N(CH_3)_3$C-atoms), 55.52 (-OCH_3C-atoms), 77.07, 104.98, 111.86, 116.91,123.35, 127.80, 128.09, 128.29, 128.32, 128.44, 130.07, 130.27, 130.90, 134.33, 145.11, 152.01, 153.40, 157.40, 159.90 (oxazine, quinoline, and phenyl C-atoms), 161.8 (CH=N, C-atom). Mass spectrum (HRMS), 7g: Molecular ion peak, m/z 498.0019 (M^++1) (M.F: $C_{29}H_{25}ClN_4O_2$).

(E)-N-((2-chloro-6-methylquinolin-3-yl)methylene)-4-(4-(di-methylamino)phenyl)-6- phenyl-2H-1,3-thiazin-2-amine, 7j

FT-IR (KBr, cm^{-1}), 7j: 1649.11 (CH=N stretching), 1544.07 (C=N stretching), 1169.37 (C-N stretching, -$N(CH_3)_3$), 1016.78 (C-O-C stretching), 815.74 (C-Cl stretching). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm), 7j: 10.51 (s, 1H, CH=N proton), 8.62 (s, 1H, quinoline H-4 proton), 7.92-7.99 (m, 3H, quinoline H-5, H-7 and H-8 protons), 6.69-7.78 (m, 11H, phenyl and oxazine protons), 3.02 (s, 6H, -$N(CH_3)_2$ protons) and 2.53 (s, 3H, -CH_3 protons). ^{13}C-NMR ($CDCl_3$, 400 MHz, δ ppm), 7j: 21.6 (-CH_3, C-atoms), 40.10 (-$N(CH_3)_3$,C-atoms), 77.07, 111.86, 116.91, 124.36, 124.66, 127.80, 128.09, 128.29, 128.44, 130.07, 130.20, 130.27, 132.40, 136.43, 137.30, 148.64, 151.64, 153.40, 157.40, 159.90 (oxazine, quinoline, and phenyl C-atoms), 161.8 (CH=N, C-atom). Mass spectrum (HRMS), 7j: Molecular ion peak, m/z 482.0242 (M^++1) (M.F: $C_{29}H_{25}ClN_4O$).

Antibacterial and antifungal activity studies

All the synthesized compounds, **7a-l** were tested for their antibacterial and antifungal activity by employing cup-plate method[18]. In this technique pores were made using a sterile cork borer in the solidified agar medium and an aliquot of 0.05 mL of 1000 μg/mL of the tested substance is placed in each pore in the nutrient agar medium on which a culture of the tested bacteria has been spread to produce uniform growth. After 24 h incubation at 37°C, the diameter of inhibition zone is measured in mm. The antibacterial activity against Gram positive and Gram negative bacterial strains namely

Table 2 — Antimicrobial studies of (E)-N-((2-chloro-6-substituted quinolin-3-yl)methylene)-4-(substituted phenyl)-6-phenyl-2H-1,3-oxazin-2-amines, 7**a-l**

Compound	Antibacterial activity (Diameter of zone of inhibition in mm)		Antifungal activity (Diameter of zone of inhibition in mm)	
	S. aureus	*K. pneumoniae*	*A. niger*	*C. albicans*
7a	16	18	18	18
7b	16	18	18	18
7c	16	18	18	18
7d	**26**	**25**	22	24
7e	**23**	**24**	20	24
7f	**22**	**21**	24	20
7g	16	18	18	18
7h	20	19	25	18
7i	18	18	18	18
7j	16	18	18	18
7k	20	22	18	18
7l	16	18	18	18
Ciprofloxacin	**20**	**22**		
Ciclopiroxolamine			**24**	**26**
DMF(control)	-	-	-	-

Staphylococcus aureus and *Klebsiella pneumoniaie* respectively. The standard antibacterial drug Ciprofloxacin was screened under similar conditions at a concentration of 100 μg/mL. Nutrient agar was used as culture medium and DMF was used as solvent control. Out of twelve compounds, most of them showed moderate antibacterial activity comparable with that of the standard. The compounds **7d, 7e,** and **7f** showed maximum activity against *S. aureus* and *K. pneumoniae* due to the presence of –Cl substituent at the quinoline moiety.

The in vitro antifungal activities of the synthesized compounds **7a-l** were evaluated against *Aspergillus niger* and *Candida albicans*. The screening data revealed that compounds showed good antifungal activity comparable with the standard drug ciclopyraoxolamine. The results of antibacterial and antifungal activity studies are given in Table 2.

Conclusion

A novel series of quinolino-oxazines were synthesized and characterized by spectroscopic techniques such as FT-IR, ^{1}H-NMR,^{13}C-NMR and HRMS. These newly synthesized compounds were evaluated for antibacterial and antifungal agents. These compounds showed potent antimicrobial activities due to their chemical structure which contains chlorine and dimethylamino substituents.

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