Synthesis, characterization and evaluation of anti-microbial action of oxazolidine substituted chalcone derivatives

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Abstract

Five novel oxazolidine-chalcone compounds were synthesized and evaluated for antifungal action. The preliminary anti-fungal activity of the synthesized compounds was studied by zone of inhibition method and the IC_{50} was calculated by broth dilution method. The yield of the compounds was in the range of 61-38%. Moderate antifungal activity against both the tested fungal strains was exhibited by the synthesized oxazolidine derivatives. The zone of inhibition exhibited by RSO_2 was highest among all compound. RSO_1 exhibited slightly lower efficacy compared to RSO_2 but higher than other compounds. This suggests the presence of any electron donating group on the aromatic ring of side chain was beneficial for activity.

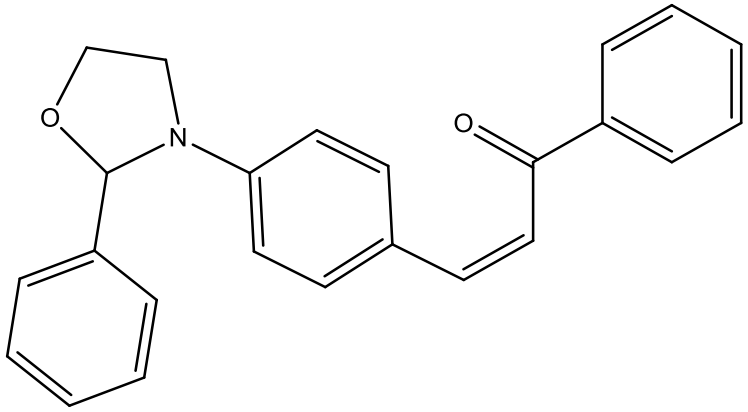
Keywords: Oxazolidine, chalcone, anti-fungal, zone of inhibition, inhibitory concentration

Introduction

Fungal pathogens are responsible for at least 13 million infections and 1.5 million deaths globally per year, primarily in those with some compromised immune function [1]. Combined with delays in diagnosis due to the nonspecific symptoms of severe disease, fungal infections are chronically underdiagnosed, with a high degree of variability in the prognosis of affected patients [2]. This is concerning as the number of immunocompromised patients, especially in those without human immunodeficiency virus (HIV), has risen in the past decade. Accumulative studies have indicated that Candida (albicans and non-albicans) and Aspergillus (Asp.) species account for most severe clinical fungal infections [3,4].

Thus, the development of novel antifungal agents with low resistance and high efficacy is a priority [5]. Azoles and chalcone based compounds have been able to make most of the clinically used antifungal agents. Literature also points that oxazolidine based compounds have been the first anti-fungal compounds and the nucleus is widely investigated and able to bring out some promising compounds with antifungal actions.

In the present investigation we planned to design and synthesize novel chalcone linked oxazolidine derivatives with an objective to obtain potent antifungal compounds.



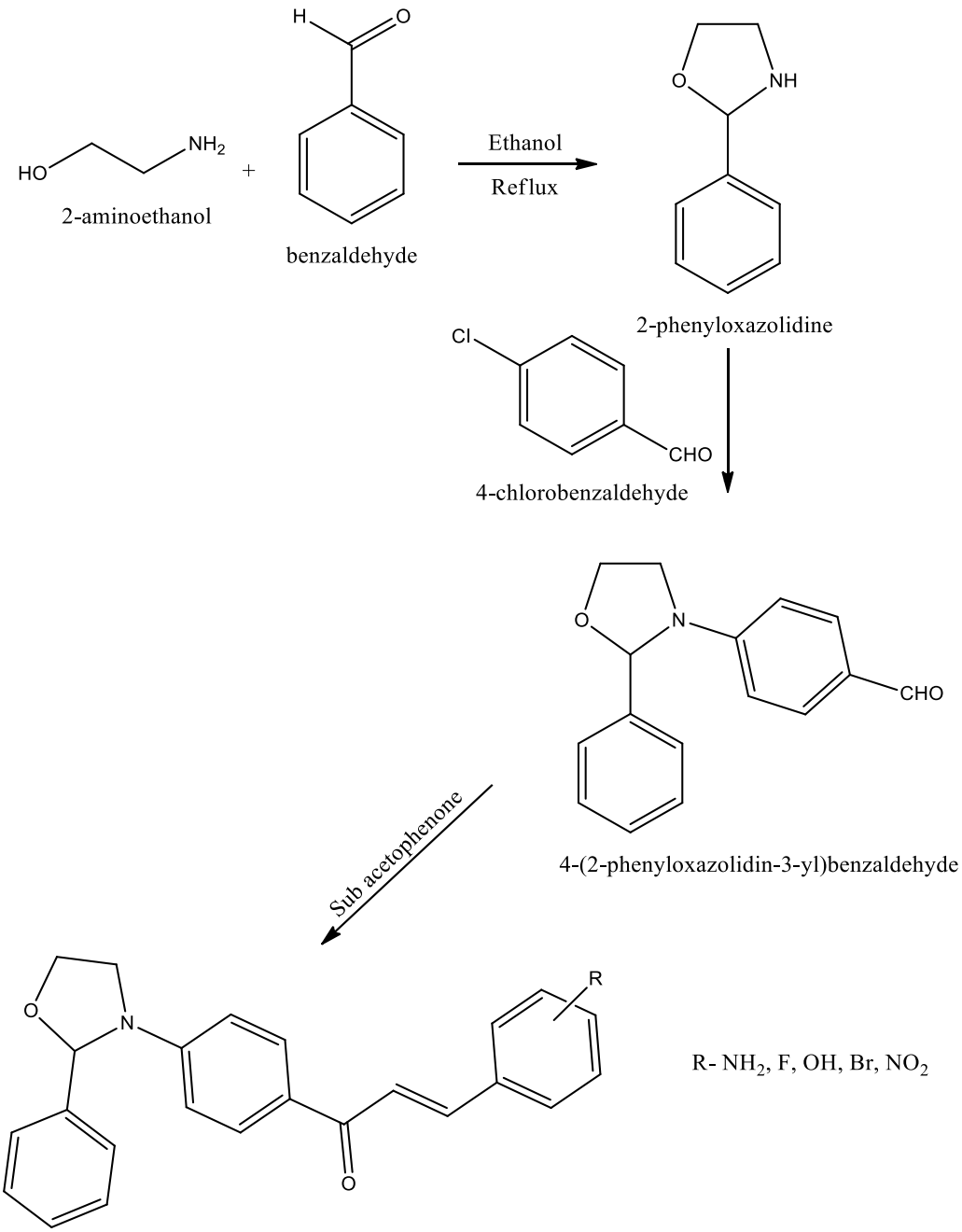
(*Z*)-1-phenyl-3-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one

Figure 1. Proposed structure

Material and Methods

Chemistry

The synthesis of target derivatives was prepared in accordance with the modification of scheme 1[6].



(*E*)-3-phenyl-1-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one, **RSO1-5**

Scheme 1. Synthesis strategy

The steps involved in the synthesis of target compounds involved

1. Synthesis of 2-phenyloxazolidine
2. Synthesis of 4-(2-phenyloxazolidin-3-yl)benzaldehyde
3. Synthesis of chalcone derivatives

Synthesis of 2-phenyloxazolidine

In a round bottom flask, 0.01 mol of ethanolamine was placed and to it was added 15 mL ethanol. To the solution was added 0.01 mol of benzaldehyde and 0.01 mol of potassium carbonate. The reaction mixture was refluxed on boiling water bath for 3.5 h and the solution was cooled to obtain the 2-phenyloxazolidine [3].

Synthesis of 4-(2-phenyloxazolidin-3-yl)benzaldehyde

A solution of 0.016 mol DIPEA and 0.01 mol 2-phenyloxazolidine was prepared in 20 mL acetone and stirred at 0 °C for 15 min. To this solution was added 0.02 mol of 4-chlorobenzaldehyde dropwise maintaining the temperature at 0 °C. On completion of addition, the reaction flask was attached to reflux condenser and heated at room temperature for 2h under constant stirring. 20 mL of cold water was added to the reaction mixture and stirring was continued for 1 h at room temperature. The solid that precipitated was filtered under vacuum and dried to obtain 4-(2-phenyloxazolidin-3-yl)benzaldehyde [7].

General method for the synthesis of chalcone derivatives

To a solution of 4-(2-phenyloxazolidin-3-yl)benzaldehyde (0.001 mol) and corresponding acetophenone (0.001 mol) in acetic acid (10 mL) was added conc. H_2SO_4 (0.3 mL) and stirred at 10-20°C until the completion of reaction (≈72 h). The precipitate was filtered off and recrystallized from acetic acid [4].

Chemical characterization of synthesized compounds

The various physical and chemical features of the synthesized compounds was studied using reported methods [8-11].

Antifungal evaluation

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Aspergillus niger* (MTCC 40) was used for the present investigation. A few drops of the bacterial suspension were injected onto the surface of pre-poured, 3 mm-thick nutritional agar plates. The disc diffusion method was used to screen for antifungal activity [12].

The synthesized oxazolidine-chalcone derivatives were dissolved in appropriate solvent to obtain the solutions of 25, 50, 75 & 100 µg/mL. These solutions were used as the test samples. Using a cork borer (10mm), wells were created in the agar plate at equal intervals, and 200 mL of the oxazolidine (25, 50, 75, and 100 µg/mL) were poured into each one. The plates were kept at 37 ± 0.1°C for 72 hours to promote microbial development. Each plate's zone of inhibition was measured in millimeters.

The broth dilution technique was used to determine the minimum inhibitory concentration of the synthesized compounds. The final inoculum size (of fungal culture) was maintained to 10^5 CFU/mL. A set of tubes containing only the inoculated broth was used as the growth control, and one containing only the broth was used to ensure the sterility of the medium. All the tubes were incubated at 37°C for 72 h to allow for growth of micro-organism. After incubation, the optical density of the content from each tube was observed at 600 nm using UV-Visible spectrophotometer. The concentration that led to half of the optical density (50%) of the growth control tube was observed for each sample [12].

Results and Discussion

Chemistry

The synthesized compounds were coded as **RSO_1-RSO_5** and these compounds were tested yield (%), solubility, retention factor (R_f, hexane-ethylacetate, 3:7) and melting point (°C).

(E)-3-(4-hydroxyphenyl)-1-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one, RSO_1

Yield: 65%; color: off-white; melting point: 221-223°C, R_f: 0.48; Mass: 371.4; 1H-NMR (δ, ppm): 3.59, 5.92, 6.87-7.83, 5.79; FT-IR(cm^{-1}): 3619, 1707, 1615, 815

(E)-3-(4-aminophenyl)-1-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one, RSO_2

Yield: 68%; color: pale-yellow; melting point: 204-206°C, R_f: 0.51; Mass: 370.45; 1H-NMR (δ, ppm): 3.91, 5.82, 6.69-7.83, 4.40; FT-IR(cm^{-1}): 3565, 1740, 1582, 852

(E)-3-(4-fluorophenyl)-1-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one, RSO_3

Yield: 61%; color: light brown; melting point: 206-208°C, R_f: 0.74; Mass: 373.43; 1H-NMR (δ, ppm): 3.59, 5.92, 6.99-7.83; FT-IR(cm^{-1}): 1708, 1628

(E)-3-(4-bromophenyl)-1-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one, RSO_4

Yield: 63%; color: pale-yellow; melting point: 219-221°C, R_f: 0.69; Mass: 434.4; 1H-NMR (δ, ppm): 3.91, 5.92, 6.99-7.83; FT-IR(cm^{-1}): 1708, 1582, 812

(E)-3-(4-nitrophenyl)-1-(4-(2-phenyloxazolidin-3-yl)phenyl)prop-2-en-1-one, RSO_5

Yield: 61%; color: dark yellow; melting point: 233-235°C, R_f: 0.61; Mass: 371.4; 1H-NMR (δ, ppm): 3.59, 5.92, 6.99-7.83; FT-IR(cm^{-1}): 1708, 1615

The synthesis of the oxazolidine in the first step was achieved by condensation of aminoethanol with carbonyl compound. The oxazolidine underwent substitution reaction with chlorobenzaldehyde to obtain the intermediate carbaldehyde. The carbaldehyde was subjected to claisen-schmidt condensation with acetophenones to obtain the desired chalcone.

The products were insoluble in water, methanol and DMSO while they were soluble in chloroform and were obtained in 61-68% yield. The structural identification of the compounds was done by FTIR spectroscopy to identify the functional groups present and ^{1}H-NMR spectroscopy to identify the protons in the molecules.

All the compounds exhibited the stretching and bending vibrations for aromatic C-C/C=C (1625-1575 cm^{-1}), C=O (1650-1750 cm^{-1}) and C-N (900-800 cm^{-1}), in the FTIR spectrum. The stretching vibrations corresponding to O-H (3500-3800 cm^{-1}) and N-H (3300-3600 cm^{-1}) and were also found in the corresponding compounds.

The proton NMR spectra presented the chemical shifts presence of protons of aromatic ring (6.8-8.3 ppm), oxazolidine hydrogen (3.59-3.91 ppm & 5.92 ppm) in all compounds.

Antifungal action

The zone of inhibition was measured to assess the preliminary antifungal activity of the synthesized oxazolidine-chalcone. Four concentrations of the oxazolidine were tested for antifungal action. Ketoconazole was used as the standard drug for antifungal action (Table 1).

Table 1. Zone of inhibition exhibited by compounds

Compound Code	Zone of Inhibition (mm)*			
	25µg	50µg	75µg	100µg
RSO_1	-	-	15	**21**
RSO_2	-	-	16	**22**
RSO_3	-	-	15	17
RSO_4	-	-	16	19
RSO_5	-	-	15	17
Ketoconazole	**23**	-	-	-

Minimum Inhibitory Concentration (MIC)

The MIC value of the test compounds was determined using broth dilution method by measuring the optical density of the broth solution incubated with diluted drug samples (Figure 2).

The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Table 2).

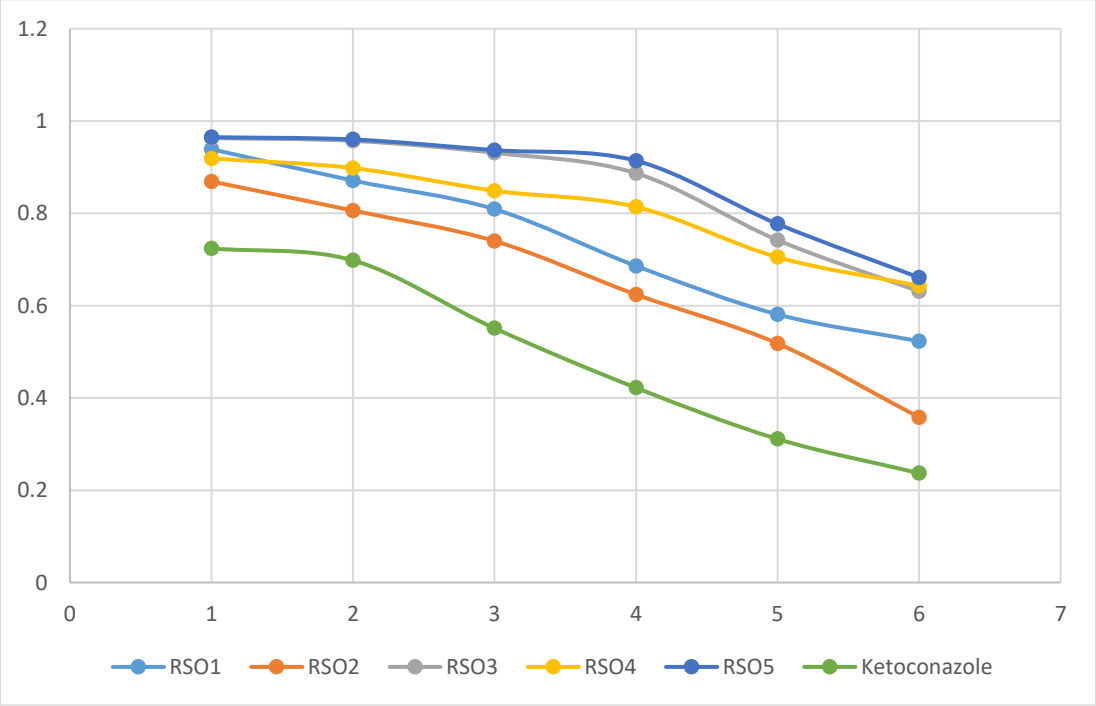


Figure 2. Plot of optical density vs. concentration

Table 2. Calculated IC_{50} values of the test samples

Test sample	IC_{50} (µg/mL)
RSO_1	107.17
RSO_2	62.51
RSO_3	168.97
RSO_4	144.76
RSO_5	161.43
Ketoconazole	18.12

The antifungal action was exhibited by the compounds against *A. niger* was exhibited by the compounds. The zone of inhibition exhibited by RSO_2 was highest among all compound. RSO_1 exhibited slightly lower efficacy compared to RSO_2 but higher than other compounds. This suggests the presence of any electron donating group on the aromatic ring of side chain was beneficial for activity.

Conclusion

In this work, oxazolidine-chalcone compounds were synthesized and evaluated for antifungal action. Compound RSO_2 was able to produce antifungal action against both the tested fungal strains in concentration less than 100 µg. The structure can hence be optimized to obtain novel anti-fungal compounds with good potency.

Acknowledgements

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