



# Synthesis, Characterization And Antibacterial Activity Of Aryl Sydnone Compounds

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## Abstract

The sydnone-morpholine derivatives (**VS<sub>1-5</sub>**) were synthesized in 6 steps involving synthesis of glycine derivative in the first step, followed by its nitroso salt formation in the second step; the third step involved the cyclization to sydnone nucleus followed by acylation of the sydnone ring in the fourth step, preparation of imine derivative in the fifth step and finally reaction of the carboxyl group with morpholine in the sixth step leading to the formation of target compounds (**VS<sub>1-5</sub>**). The synthesized compounds were tested yield (%), solubility, retention factor ( $R_f$ ) and melting point ( $^{\circ}\text{C}$ ). The completion of reaction was monitored by TLC and the dried products were again subjected to TLC using precoated TLC plates to calculate the  $R_f$  value of the compounds. The antibacterial action of the synthesized compounds was testing using two bacteria, *Escherichia coli* and *Staphylococcus aureus* by measuring the zone of inhibition as preliminary test and determining the minimum inhibitory concentration ( $\text{IC}_{50}$ ) of the compounds. The  $\text{IC}_{50}$  value for **1VS4** was the lowest against both the pathogen ( $10\ \mu\text{g/mL}$  for *E. coli* and  $<10\ \mu\text{g/mL}$  for *S. aureus*). Also from the results it was very evident that attachment of electron withdrawing groups in the molecule increased the  $\text{IC}_{50}$  (decreased antibacterial potency) as seen in compounds **VS<sub>2</sub>**, **VS<sub>3</sub>** and **VS<sub>5</sub>**.

**Keywords** : Aryl sydnone, mesoionic, antibacterial, morpholine, zone of inhibition, minimum inhibitory concentration

## Introduction

Bacteria are unique among the prokaryotes in that so many of them are normal flora that colonize the host without causing infection. New species and new variants of familiar species continue to be discovered, particularly as we intrude into new ecosystems. Among the top causes of mortality in the world, lower respiratory infection is the third most common and diarrhea is the sixth. Both are often caused by bacteria. Tuberculosis is the seventh most common cause of death [1].

An ideal antimicrobial agent acts at a target site that is present in the infecting organism but not the host cells. Four major sites in the bacterial cell can be targeted by antibiotics because they are sufficiently different from human cells. These are the cell wall, the cell membrane, the nucleic acid synthetic pathway, and the ribosome. Antibacterial agents, or antibiotics, are typically products of other microorganisms, elaborated by them in order to compete for space and resources.

Some bacteria are innately resistant to certain classes of antibiotics, either because they lack the target or are impermeable to the drug. Others are innately susceptible but develop resistance by one of a growing variety of mechanisms. Resistant strains of bacteria have a selective advantage, surviving in the presence of antibiotics, and can spread throughout the host and even be transferred to other hosts. This phenomenon is important where antibiotic use is common, such as in hospitals or in congregate housing such as nursing homes.

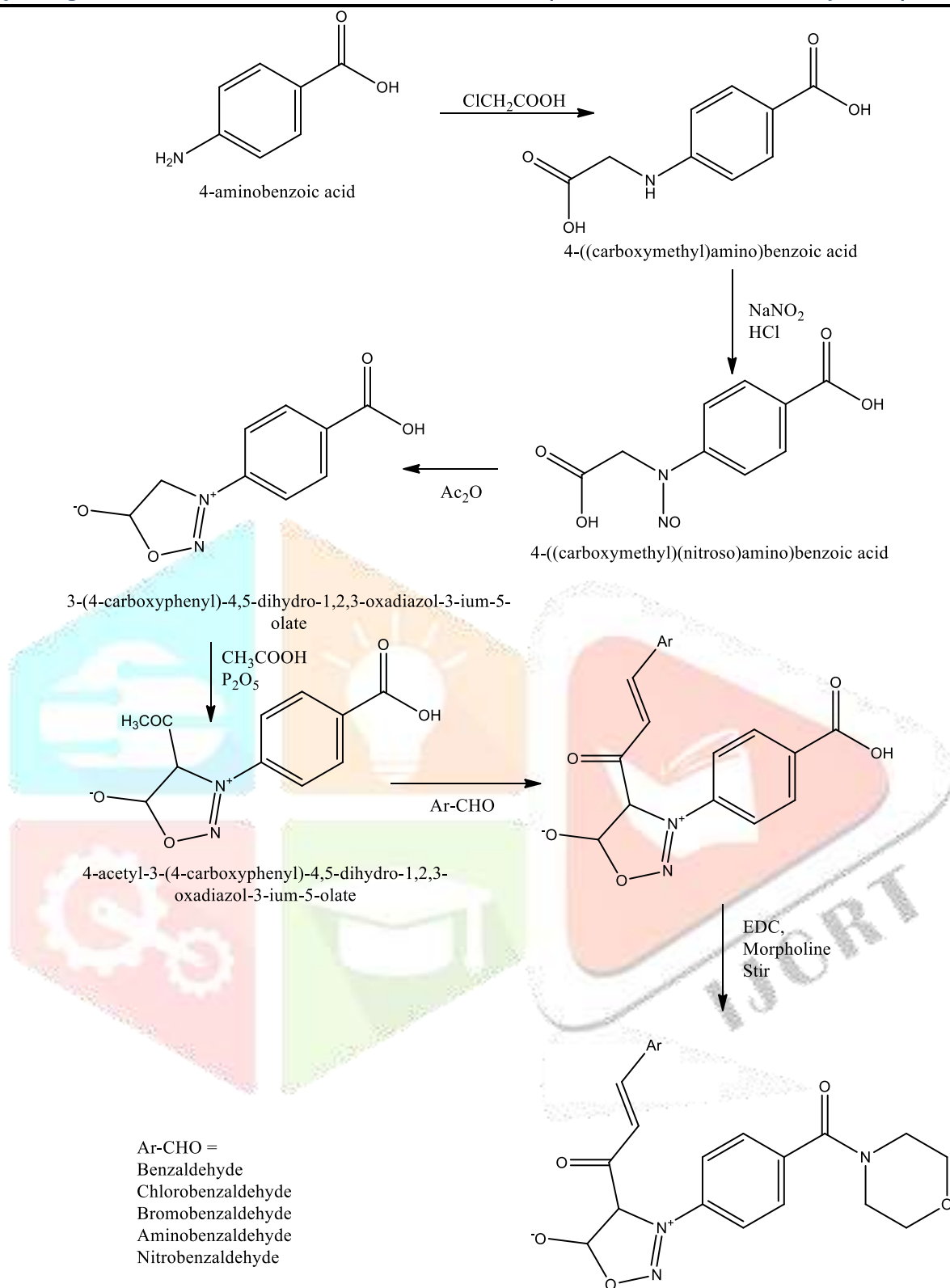
Sydnone is a mesoionic compound having the 1,2,3-oxadiazole skeleton bearing an oxygen atom attached to the fifth position [2,3]. Sydnone has portrayed several pharmacological actions including antimicrobial actions [4,8]. In an attempt to develop a new better molecule for treatment of bacterial infections, it was envisioned to synthesize and evaluate the antibacterial properties of some new sydnone based compounds.

### Material and methods

All chemicals used were of synthetic grade and used as obtained. The synthesized compounds were subjected to characterization for solubility, melting point, yield,  $^1\text{H}$  NMR study, and IR spectral study.

A few novel sydnone Schiff base derivatives were synthesized using the steps reported by Bhosale et al [9], Deshpande et al [10] and Butkovic et al [11] (Scheme 1).





### Scheme 1. Synthetic pathway

The synthesis has been achieved into 6 steps involving synthesis of glycine derivative in the first step, followed by its nitroso salt formation in the second step; the third step involved the cyclization to sydnone nucleus followed by acylation of the sydnone ring in the fourth step, preparation of imine derivative in the fifth step and finally reaction of the carboxyl group with morpholine in the sixth step leading to the formation of target compounds (VS1-5).

### **Synthesis of 4-((carboxymethyl)amino)benzoic acid, 1**

Chloroacetic acid (0.6 mol), and 4-aminobenzoic acid (0.5 mol) in 60 ml of ethanol were refluxed for 6 h. The reaction mixture was left overnight at room temperature and poured into ice-cold water; a precipitate of 4-((carboxymethyl)amino)benzoic acid. To the precipitate was added sodium hydroxide (0.5 mol) in 150 ml of water and the mixture was refluxed for 0.5 h. After cooling, the reaction mixture was acidified to pH 2 using hydrochloric acid in an ice bath. The precipitate of 4-((carboxymethyl)amino)benzoic acid was obtained, filtered and dried.

### **Synthesis of 4-((carboxymethyl)(nitroso)amino)benzoic acid, 2**

A suspension of 4-((carboxymethyl)amino)benzoic acid (0.05 mol) in water (20 ml) was cooled in an ice bath to 0°C. To the suspension was added dropwise concentrated hydrochloric acid (0.05 mol) over a duration of 0.5 h under stirring. The stirring was continued for 2.5 h and then a solution of sodium nitrite (0.05 mol) in water (20 ml) was added dropwise to it, maintaining the temperature to 0°C. The mixture was stirred for 1h and dichloromethane (20 mL) was added to it and stirred for 15 min. The organic layer was dried over sodium sulphate and evaporated to obtain 4-((carboxymethyl) (nitroso)amino)benzoic acid.

### **Synthesis of Sydnone, 3**

The 4-((carboxymethyl)(nitroso)amino)benzoic acid was dissolved in acetic anhydride (25 mL) and the reaction mixture was left in a dark overnight at room temperature. Then the reaction mixture was poured onto cold water (50 mL) with cooling and forceful stirring and was neutralized with sodium bicarbonate. The product was extracted thrice with dichloromethane (20 mL) and the organic layer was dried over sodium sulphate, filtered and evaporated to obtain the sydnone afford the crude sydnone

### **Synthesis of 4-acetyl-3-(4-carboxyphenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, 4**

To a suspension of (0.015 mol) of phosphorus pentoxide in 125 mL of benzene in a three-necked round bottom flask fitted with a reflux condenser equipped with a calcium chloride drying tube was added (0.005 mol) of sydnone. The magnetically stirred mixture was heated to reflux and glacial acetic acid (0.005 mol) was added dropwise, through a dropping funnel, over a 10 minute period. The stirred reaction mixture was refluxed for 5 hours during which the resultant clear solution turned brown-black. After cooling to room temperature, the benzene was decanted and the remaining black residue washed twice with 20 mL of benzene. The combined washings and decants were evaporated to dryness to yield a solid which was recrystallized from ethanol to obtain 4-acetyl-3-(4-carboxyphenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate [12].

### **Synthesis of 3-(4-carboxyphenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, 5**

To a suspension of 4-acetyl-3-(4-(methoxycarbonyl)phenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate (0.005 mol) in ethanol (20 mL) was added sodium hydroxide (10% w/v, 10 mL). The solution was cooled in an ice bath to 5-10°C and benzaldehyde (0.005 mol) was added to it with stirring. The stirring was continued for up to 4 h and the precipitate obtained was filtered off and recrystallized from ethanol-ethylacetate (1:1) to obtain 3-(4-carboxyphenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate.

### **General method for synthesis of target compounds, VS<sub>1-5</sub>**

The measured quantity of 3-(4-carboxyphenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate (0.001 mol) and morpholine (0.001 mol) were dissolved in dichloromethane. To the reaction mixture was added EDC (0.0012 mol) and the mixture was stirred vigorously at 40°C under reflux. The precipitate was filtered and the solvent was evaporated under reduced pressure to obtain the target compopunds [13].

## Antibacterial study

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investigation. The lyophilized cultures obtained from IMT, Chandigarh were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria. The synthesized Schiff's bases were dissolved in methanol to obtain the solutions of 25, 50, 75 & 100 µg/mL. These solutions were used as the test samples.

## Zone of inhibition

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method [14]. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the sydnone derivative (25, 50, 75 & 100 µg/mL) were placed in each hole. The plates were incubated for 24h at  $37 \pm 0.1^\circ\text{C}$  to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

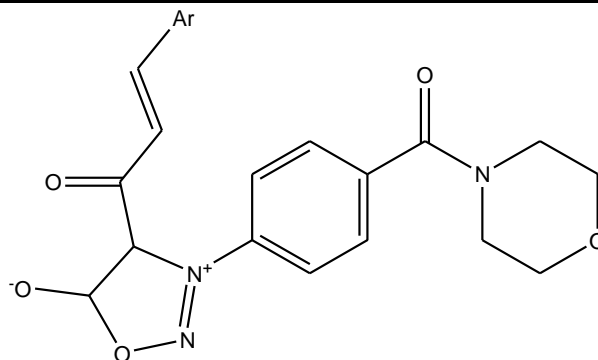
## Minimum Inhibitory Concentration

The broth dilution technique was used to determine the minimum inhibitory concentration of the synthesized compounds. The final inoculum size (of bacterial 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. A set of six tubes was labelled as 1 to 6 and to each tube was added 3 mL of nutrient broth. To the first tube was added 300µL of 1mg/mL of the test sample. The contents were mixed by swirling between hands and 300µL of content from the 1<sup>st</sup> tube was transferred to 2<sup>nd</sup> tube. The process was repeated from 2<sup>nd</sup> to 3<sup>rd</sup> tube up to the 6<sup>th</sup> tube. From the 6<sup>th</sup> tube, 300µL of content was withdrawn and discarded. To each of these tubes was added 200µL of the bacterial inoculum. All the tubes were incubated at  $37^\circ\text{C}$  for 24-48 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 [15].

## Results and Discussion

The sydnone-morpholine derivatives (**VS<sub>1-5</sub>**) were synthesized in 6 steps involving synthesis of glycine derivative in the first step, followed by its nitroso salt formation in the second step; the third step involved the cyclization to sydnone nucleus followed by acylation of the sydnone ring in the fourth step, preparation of imine derivative in the fifth step and finally reaction of the carboxyl group with morpholine in the sixth step leading to the formation of target compounds (**VS<sub>1-5</sub>**).

The synthesized conjugates were characterized by determining the practical yield, melting point, solubility (Table 1) and spectral studies (Table 2).



### General structure of the VS<sub>1-5</sub>

Table 1. Structure and molecular formula of VS<sub>1-5</sub>

| Compound code   | R' | Molecular Formula   | Yield (%) | Melting Point | Solubility           |
|-----------------|----|---|-----------|---------------|----------------------|
| VS <sub>1</sub> |    | C <sub>22</sub> H <sub>21</sub> N <sub>3</sub> O <sub>5</sub>   | 64        | 198-200       | Methanol, Chloroform |
| VS <sub>2</sub> |    | C <sub>22</sub> H <sub>20</sub> ClN <sub>3</sub> O <sub>5</sub> | 71        | 188-190       | Methanol, Chloroform |
| VS <sub>3</sub> |    | C <sub>22</sub> H <sub>20</sub> BrN <sub>3</sub> O <sub>5</sub> | 68        | 223-225       | Methanol, Chloroform |

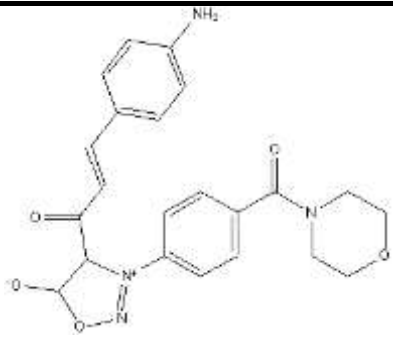
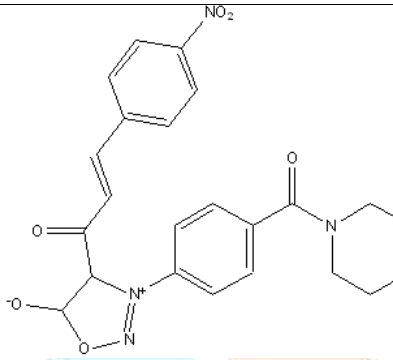
|                 |   |   |    |         |                         |
|-----------------|---|---|----|---------|-------------------------|
| VS <sub>4</sub> |   | C <sub>22</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub> | 60 | 222-224 | Methanol,<br>Chloroform |
| VS <sub>5</sub> |  | C <sub>22</sub> H <sub>20</sub> N <sub>4</sub> O <sub>7</sub> | 65 | 212-214 | Methanol,<br>Chloroform |

Table 2. Spectral data of VS<sub>1-5</sub>

| Compound Code   | <sup>1</sup> H-NMR (CDCl <sub>3</sub> , δ, ppm)   | FTIR (cm <sup>-1</sup> )  | Mass (m/e, calculated) |
|-----------------|---|---|------------------------|
| VS <sub>1</sub> | 5.48, 6.02 (C-H, sydnone), 7.38 to 7.97 (C-H, aromatic), 6.89, 7.59 (C-H, chalcone), 3.64, 3.52 (C-H, morpholine) | 1718.3 cm <sup>-1</sup> (C=O), 1448.1 cm <sup>-1</sup> (C=N), 1170.4 cm <sup>-1</sup> (C-N) and bending vibrations at around 743.6 cm <sup>-1</sup> (C-N)                 | 407.15                 |
| VS <sub>2</sub> | 5.48, 6.02 (C-H, sydnone), 7.38 to 7.97 (C-H, aromatic), 6.89, 7.63 (C-H, chalcone), 3.64, 3.52 (C-H, morpholine) | 3298.7 cm <sup>-1</sup> (C-H, adjacent to Cl), 1461.1 cm <sup>-1</sup> (C=N), 1177.8 cm <sup>-1</sup> (C-N) and bending vibrations at around 751.1 cm <sup>-1</sup> (C-N) | 441.10                 |
| VS <sub>3</sub> | 5.48, 6.02 (C-H, sydnone), 7.50 to 7.97 (C-H, aromatic), 6.89, 7.62 (C-H, chalcone), 3.64, 3.52 (C-H, morpholine) | 3337.8 cm <sup>-1</sup> (N-H), 1653.1 (C=O), 1459.3 cm <sup>-1</sup> (C=N), 1127.5 cm <sup>-1</sup> (C-N) and bending vibrations at around 749.2 cm <sup>-1</sup> (C-N)   | 485.10                 |
| VS <sub>4</sub> | 5.48, 6.02 (C-H, sydnone), 6.68 to 7.97 (C-H, aromatic), 6.89, 7.62 (C-H, chalcone),                              | 3371.4 cm <sup>-1</sup> (N-H), 1718.3 cm <sup>-1</sup> (C=O), 1436.9 cm <sup>-1</sup> (C=N), 1170.4 cm <sup>-1</sup> (C-N)  | 422.40                 |

|                 |  |   |        |
|-----------------|--|---|--------|
|                 | 4.40 (N-H, amine),<br>3.64, 3.52 (C-H,<br>morpholine)  | and bending<br>vibrations at around<br>775.3 cm <sup>-1</sup> (C-N)   |        |
| VS <sub>5</sub> | 5.48, 6.02 (C-H,<br>sydnone), 7.50 to 8.25<br>(C-H, aromatic), 7.03,<br>7.68 (C-H, chalcone),<br>3.64, 3.52 (C-H,<br>morpholine) | 3363.9 cm <sup>-1</sup> (C-H,<br>adjacent to NO <sub>2</sub> ),<br>1653.1 cm <sup>-1</sup> (C=O),<br>1459.3 cm <sup>-1</sup> (C=N),<br>1170.4 cm <sup>-1</sup> (C-N)<br>and bending<br>vibrations at around<br>812.6 cm <sup>-1</sup> (C-N) | 452.40 |

In the <sup>1</sup>HNMR spectra the peaks at chemical shift value of 5.48 and 6.02 corresponding to the proton of mesoionic sydnone ring (C-H), 6.89 and 7.59 corresponding to the proton of methylene groups of the chalcone, 3.52 and 3.64 corresponding to the morpholine protons and 7.38 to 8.25 corresponding to the protons of the aromatic rings were present in all the compounds.

### Antimicrobial activity

#### Zone of Inhibition

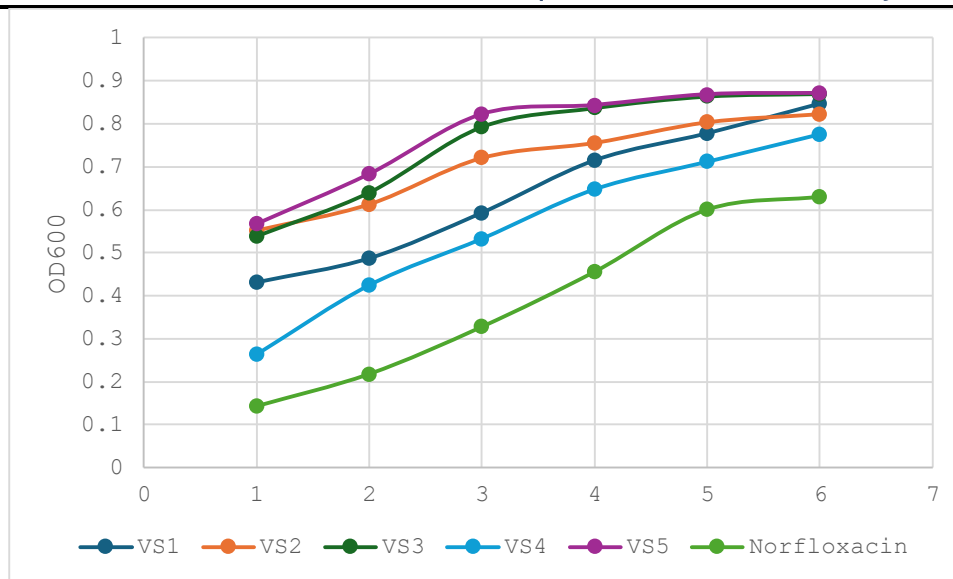
The zone of inhibition was measured to assess the preliminary antibacterial activity of the sydnone-morpholine derivatives. Four concentrations of the sydnone-morpholine derivatives were tested for antibacterial action (Table 3).

**Table 3. Zone of inhibition exhibited by compounds**

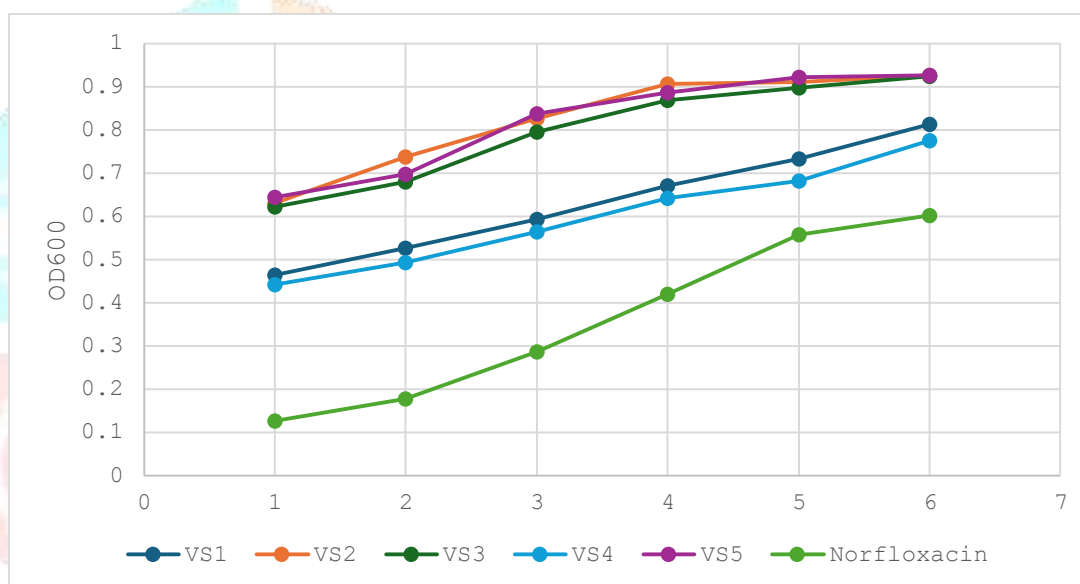
| Compound Code   | Zone of Inhibition (mm)* |      |           |           |                  |      |           |           |
|-----------------|--------------------------|------|-----------|-----------|------------------|------|-----------|-----------|
|                 | <i>E. coli</i>           |      |           |           | <i>S. aureus</i> |      |           |           |
|                 | 25µg                     | 50µg | 75µg      | 100µg     | 25µg             | 50µg | 75µg      | 100µg     |
| VS <sub>1</sub> | -                        | -    | 16        | 17        | -                | -    | 13        | 14        |
| VS <sub>2</sub> | -                        | -    | 13        | 14        | -                | -    | 11        | 12        |
| VS <sub>3</sub> | -                        | -    | 13        | 14        | -                | -    | 11        | 12        |
| VS <sub>4</sub> | -                        | -    | <b>19</b> | <b>24</b> | -                | -    | <b>16</b> | <b>18</b> |
| VS <sub>5</sub> | -                        | -    | 12        | 13        | -                | -    | 11        | 12        |

#### 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. The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Figure 1,2).



**Figure 1. Plot of optical density vs. concentration for *S. aureus***



**Figure 2. Plot of optical density vs. concentration for *E. coli***

As visible from the results, the IC<sub>50</sub> value for VS<sub>4</sub> was the lowest against both the pathogen (10 µg/mL for *E. coli* and <10 µg/mL for *S. aureus*). Also from the results it was very evident that attachment of electron withdrawing groups in the molecule increased the IC<sub>50</sub> (decreased antimicrobial potency) as seen in compounds VS<sub>2</sub>, VS<sub>3</sub> and VS<sub>5</sub>.

## Conclusion

The primary objective of the work was to synthesize novel sydnone-morpholine derivatives with good antimicrobial potency. The sydnone derivatives were found to possess antibacterial action against both gram negative and gram positive bacterial in less than 10 µg/mL concentration. The results led us to conclude that the sydnone derivatives were good antimicrobials and novel lead molecules may be generated by optimizing the derivatives using molecular modelling studies in future.

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