Chapter 1

Preparation of starting materials
PREPARATION OF 3-ARYLSYDNONES

The 3-arylsydnones required for the present work were prepared according to the literature methods involving the following stages.

1) Preparation of N-aryl glycines

The aryl glycines were prepared by condensing anilines with chloroacetic acid or with ethylchloro/bromoacetate followed by hydrolysis.

\[
\begin{align*}
\text{ClCH}_2\text{COONa} & \quad \text{ClCH}_2\text{CO}_2\text{Et} \\
\text{R}_1\text{R}_2\text{NH}_2 & \quad \text{R}_1\text{R}_2\text{NHCH}_2\text{CO}_2\text{H} \\
\text{ClCH}_2\text{CO}_2\text{Et} & \quad \text{NaOH} \\
\text{R}_1\text{R}_2\text{NHCH}_2\text{CO}_2\text{Et} & 
\end{align*}
\]

2) Nitrosation of N-aryl glycines to N-nitroso-N-aryl glycines

Nitrosation was carried out by using sodium nitrite in water or aqueous hydrochloric acid.

\[
\begin{align*}
\text{R}_1\text{R}_2\text{NHCH}_2\text{CO}_2\text{H} & \quad \text{NaNO}_2 \\
\text{HCl or H}_2\text{O} & \quad 0\text{-}5^\circ\text{C} \\
\text{R}_1\text{R}_2\text{NHCH}_2\text{CO}_2\text{H} & \quad \text{R}_1\text{R}_2\text{NCH}_2\text{CO}_2\text{H}
\end{align*}
\]
3) Cyclisation of N-nitroso-N-aryl glycines to arylsydnones

The N-nitroso glycines were cyclised to the corresponding sydnones by heating with acetic anhydride on a water-bath (Table 1).

\[
\begin{align*}
\text{R}_1 & \quad \text{NCH}_2\text{CO}_2\text{H} & \xrightarrow{\text{Ac}_2\text{O}} & \text{R}_1 \quad \text{NCH}_2\text{CO}_2\text{H} + \text{H}_2\text{O} \\
\text{R}_2 & & \text{80-100°C} & \\
\end{align*}
\]

(1 a-h)

Preparation of 3-aryl-5-methyl-1,3,4-oxadiazolin-2-ones by one-pot ring transformation of 3-aryl sydnones.

The oxadiazolinones were prepared according to the literature procedure from 3-aryl sydnones by the reaction of bromine in acetic anhydride. The initial step at 0°C gave the 3-aryl-4-bromosydnones which were then converted in situ into 3-aryl-5-methyl-1,3,4-oxadiazolin-2-ones by heating the solution at 60°C.

Preparation of 4-amino-2-aryl-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-ones from Oxadiazolin-2-ones.

Scheme 1

\[
\begin{align*}
\text{R}_1 & \quad \text{NCH}_2\text{CO}_2\text{H} & \xrightarrow{\Delta} & \text{R}_1 \quad \text{NCH}_2\text{CO}_2\text{H} + \text{H}_2\text{O} \\
\text{R}_2 & & \text{NH}_2\text{NH}_2 & \\
\end{align*}
\]

(18 a-h) (19 a-h)
A probable mechanism (Scheme 2) involves initial attack of the nucleophile on C-2, which is the most reactive site in the 1,3,4-oxadiazolinone ring. This adduct formation would be followed by ring opening, breaking the O(1)-C(2) bond, resulting in the formation of open chain intermediate (A) (not isolated). Subsequent ring closure by an internal $S_N2$ attack results in the formation of the title compounds (19). This mechanism, which involves the addition of a nucleophile, ring opening and then ring closure of an open chain intermediate by intramolecular nucleophilic attack, may be described as an ANRORC type of ring conversion$^2$.

Scheme 2
Experimental

Preparation of 3-aryl-5-methyl-1,3,4-oxadiazolin-2-ones (18 a-h)

3-Arylsydnone (1.0 g) was suspended in acetic anhydride (5 ml) at 0°C and an ice-cold solution of bromine (0.5 ml) in acetic anhydride (5 ml) was added with stirring and cooling. 4-Bromosydnone separates after the addition. The reaction mixture was then heated on a water-bath, gradually increasing the temperature to 50-60°C for about 30 minutes. Vigorous evolution of CO$_2$ was observed. The solution was then diluted with water. The solid obtained was filtered, washed with water and crystallised from ethanol (Table 2).

Preparation of 4-amino-2-aryl-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-ones (19 a-h)

A mixture of oxadiazolinone (0.01 mol) and hydrazine hydrate (1.5 ml) was suspended in dry alcohol (15 ml) and refluxed on a water-bath for 8 hours. The reaction mixture was then diluted with water and the solid obtained was filtered and crystallized from ethanol (Table 3).
CHARACTERISATION DATA OF COMPOUNDS

Table 1

3-Arylsydnones (1 a-h)

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>Yield %</th>
<th>Melting Point °C</th>
<th>References</th>
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<tbody>
<tr>
<td>a</td>
<td>H</td>
<td>70</td>
<td>133-135</td>
<td>3</td>
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<tr>
<td>b</td>
<td>H</td>
<td>80</td>
<td>144-145</td>
<td>4</td>
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<tr>
<td>c</td>
<td>H</td>
<td>56</td>
<td>78-80</td>
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<tr>
<td>d</td>
<td>H</td>
<td>80</td>
<td>125-126</td>
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<tr>
<td>e</td>
<td>H</td>
<td>90</td>
<td>113-114</td>
<td>4</td>
</tr>
<tr>
<td>f</td>
<td>H</td>
<td>89</td>
<td>145-146</td>
<td>7</td>
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<tr>
<td>g</td>
<td>H</td>
<td>72</td>
<td>137-138</td>
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<tr>
<td>h</td>
<td>3-Cl</td>
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**CHARACTERISATION DATA OF COMPOUNDS**

Table 2

![Chemical Structure](image)

3-Aryl-5-methyl-1,3,4-oxadiazol-2-ones (18 a-h)

<table>
<thead>
<tr>
<th></th>
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<th>Yield</th>
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<td>4-CH₃</td>
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### CHARACTERISATION DATA OF COMPOUNDS

Table 3

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EVAULTION OF ANTIMICROBIAL ACTIVITY

(General procedure for all the compounds screened in the present investigation)

All the newly synthesised compounds were evaluated for their antimicrobial activity against Gram-negative bacterium *Escherichia coli*, Gram-Positive bacterium *Bacillus cirroflagellosus* and Fungi – *Aspergillus niger* and *Pencillium notatum*.

ANTIBACTERIAL ACTIVITY

(Evaluation of antibacterial activity by cup-plate method)

Preparation of nutrient broth

Nutrient broth was prepared by dissolving peptone (0.5%), yeast extract (0.15%), beef extract (0.15%), sodium chloride (0.36%), monopotassium phosphate (0.13%), in distilled water (100 mL). The pH of solution was adjusted to 7.2 by adding sodium hydroxide (4%) and the resulting solution was autoclaved for 20 minutes at 15psi.

Preparation of sub cultures (Inoculums)

One day prior to the experiment, the cultures of *Escherichia coli* and *Bacillus cirroflagellosus* were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37°C.

Preparation of nutrient agar medium

Nutrient agar medium was prepared by dissolving peptone (1%), yeast extract (0.6%), beef extract (0.5%), sodium chloride (0.5%), in distilled water. The pH of the solution was adjusted to 7.2 by adding 4% aqueous sodium hydroxide
solution. Agar (2.4%) was then added and the whole solution was autoclaved for 20 minutes at 15psi.

**Preparation of test sample solution**

Each test sample (5 mg) was dissolved in DMSO (1 mL) and 0.1 mL of this solution (50µgm) was used for testing.

**Method of testing**

Inoculation medium containing 24 hours grown culture was added aseptically to the nutrient medium and mixed thoroughly to get the uniform distribution. This solution was poured (25 mL in each dish) into Petri dishes and then allowed to attain room temperature. Thereafter, punching the set of agar with a sterile cork borer and scooping out the punched part made the cups. The diameter of each cup was 5mm. Ciprofloxacin was used as standard and DMSO as solvent control.

The entire test samples and the solvents were tested at a concentration of 50 µgm. The plates were allowed to stand for an hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37°C for 48 hours. The zone of inhibition against all the microorganisms was measured in millimetres.

**ANTIFUNGAL ACTIVITY**

*(Evaluation of antifungal activity by cup-plate method)*

**Preparation of subculture**

The inoculation medium was prepared by dissolving peptone (1%), yeast extract (0.3%), sodium chloride (0.5%), monopotassium phosphate (0.3%), and glucose (2%), in distilled water (100 mL). The pH of the solution was adjusted to 7.2
by adding sodium hydroxide solution (4%) and the solution was autoclaved for 20 minutes at 15psi.

One and half day prior to the experiment, the fungal cultures of Aspergillus niger and Pencillium notatum prepared in the inoculation medium were incubated at 37°C for 36 hours.

**Preparation of fungal medium**

The fungal medium was prepared by dissolving peptone (0.5%), sodium chloride (0.36%), monopotassium phosphate (0.13%) and glucose (2%) in distilled water (100 mL). The pH of the solution was adjusted to 7.2 by adding sodium hydroxide solution (4%) and the solution was autoclaved for 20 minutes at 15psi. This was cooled to 45-50°C with gentle shaking. One and half day grown cultures was added aseptically to this medium and mixed thoroughly to get uniform distribution.

**Medium of testing**

The solutions of test samples and standard were evaluated for antifungal activity by cup-plate method at a concentration of 50 µmg. The zone of inhibition was measured in millimetres for the particular test sample with each organism 48 hours interval. Griseofulvin was used as the standard.
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Concentration of the test sample used in each cup: 50 μgm

Concentration of the standard used: 50 μgm

Solvent control used: DMSO

Zone of inhibition of control: 8 mm

Zone of inhibition of Ciprofloxacin against E.coli: 15 mm

Zone of inhibition of Ciprofloxacin against B.cirroflagellosus: 22 mm

Zone of inhibition of Griseofulvin against P. notatum: 18 mm

Zone of inhibition of Griseofulvin against A.niger: 12 mm
REFERENCES