

Reaction of Sulphene with 2-Hydroxyphenylethylideneanilines and Evaluation of the Products for Fungitoxicity

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Addition of sulphene, generated *in situ* from the reaction of methanesulphonylchloride and triethylamine, to 2-hydroxyphenylethylideneaniline and its N-phenyl derivatives (I–V) resulted in the formation of cycloadducts (Ia–Va). The crude products were recrystallized from ethanol. Elemental analysis of the products checks well with 1 : 1 addition product. On the basis of spectral studies, the products have been assigned 1,4-benzothiazine structure. The synthesized 1,4-benzothiazines (Ia–Va) were tested against *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum* and *Myrothecium roridum* for their antifungal activity by spore germination inhibition method at various concentrations. Some of the compounds have shown promising antifungal activity.

Key Words: Reaction, Sulphene, 2-Hydroxyphenylethylideneanilines, Fungitoxicity.

INTRODUCTION

1,4-Benzothiazines, the heterocyclic compounds containing sulphur and nitrogen in six-membered ring have been reported to possess biological activity^{1,2}. The synthesis of these compounds involves the addition of sulphene³ to aldimines or ketimines. It has been reported that the heterocyclic compounds derived from ketimines possess better fungitoxicity⁴. The aim of the present investigation is to prepare 1,4-benzothiazines from hydroxy substituted ketimines and to evaluate these synthesized compounds for their fungitoxicity. The results of this study are presented in this paper.

EXPERIMENTAL

Synthesis of 2-hydroxyphenylethylideneanilines

2-Hydroxyphenylethylideneanilines (I–V) were prepared by condensing 2-hydroxyacetophenone with aniline, 4-toluidine, 4-anisidine, 4-phenitidine and 4-chloroaniline respectively in equimolar ratio.

Synthesis of 1,4-benzothiazines

2-Hydroxyphenylethylideneaniline (I) (2.11 g, 0.01 mole) was dissolved in dry ether (25 mL) in a conical flask (100 mL) by warming the contents. To this was added, slowly and with constant shaking, sulphene generated *in situ* from

methanesulphonyl chloride (1.5 mL, 0.01 mole) and triethylamine (1.5 mL) in dry ether (25 mL). The triethylamine hydrogenchloride, which separated out, was filtered. The contents were kept overnight and were washed with water and dried over anhydrous magnesium sulphate. Removal of the ether yielded crude product which was filtered and recrystallized from petroleum ether and benzene mixture to get green crystals of 1,4-benzothiazine derivative (**Ia**) (Fig. 1): m.p. 140°C; yield 45%.

Addition of sulphene to ketimines (**II-V**) were carried out in the same manner to get 1,4-benzothiazine derivatives (**IIa-Va**) respectively.

Antifungal activity of 1,4-benzothiazines

The stock solutions (2000 ppm) of the compounds were prepared by dissolving the compound (20 mg each) in minimal quantity of ethanol (0.5 mL) and the volume was made up to 10 mL by adding sterilized distilled water. The stock solutions were serially diluted to 1000, 500, 250, 100, 50 and 25 ppm. The cavity slides were used to study the spore germination inhibition by these compounds at different concentrations. The treated spores in cavity slides were kept in petri plates lined with moist filter paper discs and incubated at $24 \pm 1^\circ\text{C}$ for 20 h.

The per cent spore germination inhibition was recorded by the following formula:

Percent spore germination inhibition

$$= \frac{\text{Spore germination in control} - \text{spore germination in treatment}}{\text{Spore germination in control}} \times 100$$

Dithane M-45 and Bavistin were used as checks to compare the activity of the compounds. The fungitoxicity has been expressed in terms of ED₅₀ value (effective dose to inhibit 50 per cent spore germination) and calculated by plotting the spore germination inhibition values against different concentrations of the compounds on graph paper.

RESULTS AND DISCUSSION

The reaction of 2-hydroxyphenylethylideneaniline (**I**) with sulphene, generated *in situ* from methanesulphonylchloride and triethylamine, under forcing conditions, gives a solid product. The compound has been characterized as 1,4-benzothiazine derivative (**Ia**) on the basis of elemental analysis and spectral studies. The IR spectrum of the product shows broad band at 3250 cm^{-1} indicating the presence of —NH group. The presence of this band suggests the migration of sulphene group from the nitrogen atom of the aniline moiety to the *ortho* position of N-phenyl ring followed by a protonic shift from the ring to the nitrogen atom under reaction conditions, thus rearranging the four membered 2-thiazetidine ring into a six membered 1,4-benzothiazine ring. Such migrations have already been reported in case of sulphene adducts of nitrones⁵ and aldimines⁶.

Further support for the 1,4-benzothiazine structure comes from PMR studies. PMR spectrum of **IVa** (R = OC₂H₅) indicates two proton quartet at 4.2 δ due to —OCH₂— protons, three proton triplet at 1.5 δ due to —CH₃ protons, a seven

proton multiplet between 6.7 to 7.8 δ . The protons of the ring are indicated at 2.1 δ (NH), 2.5 δ (CH_2) and 2.8 δ (CH) all as singlets.

The addition of sulphene to ketimines (**II-V**) with substituents methyl, methoxy, ethoxy and chloro in *para* position of N-phenyl ring has also resulted in the formation of 1,4-benzothiazines (**IIIa-Va**) respectively. 1,4-Benzothiazines synthesized by the addition of sulphene to ketimines along with their characteristics are recorded in Table-1.

TABLE-1
CHARACTERISTICS OF 1,4-BENZOTHAZINES

| Compound | R | Colour | Yield (%) | m.p.* (°C) | m.f.† |
|----------|-------------------------|--------------|-----------|------------|---|
| Ia | H | Greenish | 45 | 140 | $\text{C}_{15}\text{H}_{15}\text{NO}_3\text{S}$ |
| IIa | CH_3 | Light yellow | 50 | 158 | $\text{C}_{16}\text{H}_{17}\text{NO}_3\text{S}$ |
| IIIa | OCH_3 | Brown | 62 | 172 | $\text{C}_{16}\text{H}_{17}\text{NO}_4\text{S}$ |
| IVa | OC_2H_5 | Brown | 58 | 180 | $\text{C}_{17}\text{H}_{19}\text{NO}_4\text{S}$ |
| Va | Cl | Light brown | 66 | 187 | $\text{C}_{15}\text{H}_{14}\text{NO}_3\text{SCl}$ |

*All the melting points are uncorrected.

†All the compounds gave satisfactory elemental analysis.

The synthesized 1,4-benzothiazines were screened for their fungitoxicity against *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporum* and *Myrothecium roridum* by standard spore germination inhibition method⁷. The results have been expressed in terms of ED_{50} value (Table-2). All the compounds except **IIa** have shown promising antifungal activity. The compounds **Ia** and **Va** have been found to be most effective against *C. lunata* with ED_{50} value of 150 ppm each whereas compounds **IIIa** and **Va** were found to be most effective against *A. alternata* each exhibiting ED_{50} value of 180 ppm. The compound **IIIa** has shown most promising antifungal activity against *F. oxysporum* and *M. roridum* with ED_{50} value of 150 ppm and 160 ppm respectively. In general, compounds **Ia** and **IIIa** have been found to be most effective against all the test fungi with ED_{50} values less than 200 ppm.

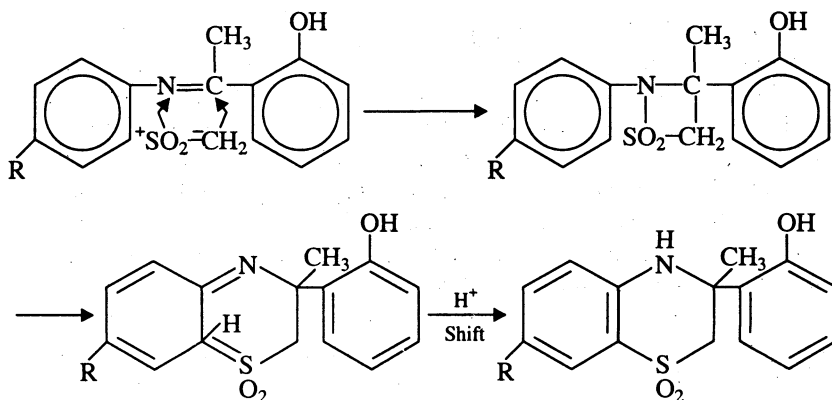


Fig. 1. Reaction sequence for formation of 1,4-benzothiazines.

TABLE-2
ANTIFUNGAL ACTIVITY OF 1,4-BENZOTHAZINES

| Compound | ED ₅₀ Values (ppm) against | | | |
|---------------|---------------------------------------|-----------------------------|---------------------------|----------------------------|
| | <i>Curvularia lunata</i> | <i>Alternaria alternata</i> | <i>Fusarium oxysporum</i> | <i>Myrothecium roridum</i> |
| Ia | 150 | 190 | 180 | 180 |
| IIa | 710 | * | * | 620 |
| IIIa | 180 | 180 | 150 | 160 |
| IVa | 500 | 270 | 340 | 440 |
| Va | 150 | 180 | 200 | 250 |
| Bavistin† | — | — | 9 | — |
| Dithane‡ M-45 | 39 | 35 | — | 30 |

*More than 1000 ppm

†Standard fungicide for *F. oxysporum*

‡Standard fungicide for *A. alternata*, *C. lunata*, *M. roridum*

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