Synthesis, characterization of some 2-mercapto-5-methoxy-1H-benzimidazole and test with some plant pathogenic fungi

Ihmood Kh. Jebur¹ and Salih Mohammed Ismail²

¹ Dept. of Chem., College of science, University of Tikrit, Tikrit, Iraq.
² Dept. of plant protection, Collage of Agriculture, University of Tikrit, Tikrit, Iraq

Email: ihmood.jebur@yahoo.com

Abstract. In this research the new starting material 2-mercapto-5-methoxy-1H-benzimidazole (S1) was synthesized by the condensation reaction of 4-methoxy-o-phenylenediamine and (1mmol) and carbon disulfide (1 mmol) in ethanol (10 mL) under basic condition we used potassium hydroxide as base which produced MBI with high yield and purity. 2-mercapto-6-methoxy-1H-benzimidazole(S1), which on treatment with hydrazine hydrate in ethanol yielded hydrazino-6-methoxy-2-mercapto benzothiazole (S2). Condensation of compound (S2) with 4-(N,N-di Methylbenzaldehyde yielded 4-[(N,N-di Methyl benzylidine) hydrazino]- 6-methoxy -2-mercaptobenzothiazole (S3). The synthesized compounds were identified according to their physical properties, spectroscopic data (FT-IR, ¹H-NMR, and ¹³C-NMR) The study showed three chemical compounds test S1, S2, and S3 to three concentration (100,200,300) mg/L in inhibiting colony diameter of three phytopathogenic fungi Fusarium oxysporum fsp.watermilon, Macro phomina phasiolina and Rhizoctonia solani these compounds effectiveness of inhibition of S1 and to three concentrations of the high against growth of fungi, with an inhibitory rate of 89.39% While S2 was less effective at inhibition of 33.89%.

keywords: 1,3-benzimidazole, hydrazine, fungicidal, Macrophomina Rhizoctonia, Fusarium

Introduction:

Fused heterocyclic compounds are very important in the field of pharmaceutical chemistry because of their pharmacological properties which include wide applications in medicinal chemistry[1]. Substituted 1,3-benzimidazole derivatives are an important class of fused heterocyclic compounds. Benzimidazole derivatives possess a wide spectrum of biological activities such as antiviral [1,2], anticancer, antibacterial [3], proton pump blocker [4], anti hypertensive II, hypertension [5], antioxidant [6], antinociceptive[7], anti-inflammatory[7,8], analgesic [8], antiparasites [9], human cytomegalovirus (HCMV) replication inhibitor [10], fungicidal 11, and antihistamines [12,13], antifungal[14], antiparkinson [15], antidiabetic [16], anthemintic[17], anti-diabeticactivities [18].
Materials and Methods

Scheme:

Scheme (1): The reactions sequence for the synthesis of some new 6-methoxy-2-mercaptopbenzimidazole derivatives.

Synthesis of 6-methoxy-2-mercaptobenzimidazole MBI (S1)

A mixture of (21.8gm, 0.2 mole) (1.0gm, 0.0072mole) of ortho hydroxyl aniline in absolute ethanol (150ml), potassium hydroxide (11.3 gm ,0.2 mole) was added then carbon130disulphide (15.34 gm 0.2 mole, 12.38 ml) was added gradually with stirring. The mixture was refluxed for 6hours. till H2S gas ceased, then 1.5gm of charcoal was added and the reaction mixture was heated on a water bath at (60-70°C) for 15 minutes the charcoal is separated by filtration. The filtrate at a temperature between (65-75 C), 150ml of warm water (60-70°C) was added followed by 25ml of acetic acid with good stirring and the reaction mixture was acidified by dropwise addition of (6ml,1N) acetic acid. After completion of reaction the reaction mixture was filtered and the precipitated as white crystals. The solution was kept in the freezer till the solution completely froze and then allowed to melt; the precipitated is collected, filtered, then washed with cooled distilled water, dried and recrystallized from ethanol, yield 7% and m.p. 261-264.[19]

Synthesis of 6-methoxy (benzimidazole-2-y1) hydrazine (S2)

To a warm hydrazine hydrate solution of (0.02mol) 6-methoxy-2-mercaptopbenzimidazole (0.01mole, 1.93g) was mixed with 4-(N,N-diMethylbenzaldehyde (0.01mol) in ethanol (30ml), the reaction mixture was refluxed for 4hrs after the addition of 4 drops of glacial acetic acid. The resultant solution was poured onto crushed ice and solid product was filtered, washed with methanol, and then dried and crystallized from absolute ethanol. Yield: 64%, m.p.210-202 °C.

Synthesis of (2-acetamidobenzothiazole-2-y1)-6-phenyl hydrazine (S3)

6-methoxyhydrazinobenzothiazole (6) (0.01mole, 1.93g) was mixed with 4-(N,N-diMethylbenzaldehyde (0.01mol) in ethanol (30ml), the reaction mixture was refluxed for 4hrs after the addition of 4 drops of glacial acetic acid. The resultant solution was poured onto crushed ice and solid product was filtered, washed with methanol, and then dried and crystallized from absolute ethanol. Yield: 82%, m.p. 61-63 °C.

Isolation and diagnosis of plant pathogenic fungi

Isolation and diagnosis of pathogenic fungi of the plant Fusarium oxysporum f.sp.watermelon Macrophomina phasiolina and Rhizoctonia solani were isolated from the roots of the watermelon plant, sesame and tomato respectively, which showed the symptoms of wilt disease and were collected from their agricultural fields in Al-Sharqat district. The isolation process was carried out and their fungal colonies were isolated in a laboratory Plant Protection Diseases at the Faculty of Agriculture, University of Tikrit and used the isolation method referred to by [20]. The taxonomic keys developed by [21], were used to diagnose these fungi based on the phenotypic properties of their colonies and their multiplication structures on the nutrition medium potato dextrose Aigr (PDA) and then multiply the fungal colonies for use in the testing of some chemical compounds (S1, S2, S3) in the effectiveness of inhibition of diameter colony growth of those fungi.
Biologic efficacy test of chemical compounds

Thus study deals with the effect of chemical compounds (S1, S2, S3) of inhibiting the growth of the colony of three pathogenic fungi of economically important plants and using the method of poisoning the PDA nutrition medium with these chemical compounds and in three concentrations (100,200, 300 mg / L). After the completion of the period of sterilization in autoclaved at 121°C and 1.5 pound pressure and before the hardening of the PDA medium at 45°C, they were poisoned by adding chemical compounds individually and then teaching them. According to type and concentration of chemical compound is taken into consideration, leaving a flask of vials containing the sterile PDA medium without poisoning for use in the control treatment. Petri dish of 9 cm diameter were used and after hardening of the medium was inoculated by transfer a 0.5 cm diameter disc from the fungi colony to 72 hours old under sterilization conditions in the isolation chamber of the Plant Pathology Laboratory. The type of fungus colony and the chemical compound and the concentration used to poison the medium were recorded on each treatment containing three dishes and three replicates, taking into account the preparation of dishes that were not poisoned and cleared from the three fungal colonies separately. All dishes incubated at 27 ± 2°C and after the fungus colony is completed for the whole covered dish area in the control treatment. The readings of the fungi colony diameter were taken for each treatment by measuring the diameter rate and then calculate the inhibition ratio of each treatment for these chemical compounds according to the following equation used [22-23]. Inhibition percent = Growth colony diameter in control treatment - colony diameter in control treatment x 100

Results and discussion

In present work we synthesize some 2-mercapto-6-methoxy-benzimidazole derivatives (S1) as starting materials for preparing 2-mercapto-6-methoxy-benzimidazole (S1) MBI derivatives initially we conducted reaction between 6-methoxy-2-phenylenediamine (1mmol) and CS2 (1mmol) in ethanol (10 ml) under basic condition, we use potassium hydroxide as base which produced MBI with high yield and purity. Compound MBI was characterized by IR, 1H-NMR, 13C-NMR and mass spectroscopy. The FT-IR spectra of compound (S1) shows disappearance of absorption band at 2520-2565 cm\(^{-1}\) due to (S-H) and appearance of strong absorption bands at 3286 cm\(^{-1}\)(N-H) and the appearance of clear strong absorption band at (1625-1610) cm\(^{-1}\) due to (C=N) imidazole, while C-S-C bands are noticed at the range 650-665 cm\(^{-1}\)\([19]\).

1H-NMR spectrum of compound (S1) showed clear singlet signal at =12.41 ppm due to (NH) group proton, while signal at =12.50 ppm due to (S-H) signal at = 3.72 ppm due to (OCH) while, multiplet signals at = (6.5 -7.14) ppm for aromatic protons.

2-Hydraxino-6-methoxy-benzimidazole (S2) is prepared from the reaction of 2-mercapto 6-methoxy-benzimidazole (S1) with hydrazine hydrate in presence of sodium hydroxide in which the spectral data confirms formation of this compound. The IR spectrum compound (S2) shows absorption bands at 3359 and 3265 cm\(^{-1}\) due to stretching (-NH-NH\(_2\)) group in hydrazine with disappearance the bond of (SH) at (2520-2665) cm\(^{-1}\) while, absorption of C-H stretching at 2827-2860 cm and 2916-2920 cm\(^{-1}\) and absorption of C=N at 1596-1648 cm\(^{-1}\). Also two bands of absorption of aromatic C=C are noticed at 1494-1523 cm\(^{-1}\) and 1439-1450 cm\(^{-1}\).

\(^1\)H-NMR spectra of compound (S2) showed clear singlet signal at =2.08 ppm due to (CH\(_3\)) group protons, signals at = (3.5 and 3.8) ppm 132 due to (NH\(_2\)) and (NH) of hydrazine moiety while, multiplet signals at = (7.27-8.12) ppm for aromatic protons and singlet signal at = 8.61 ppm for imine proton (-N=CH\(_2\)).

The 2-(N,N-dimethylbenzylidene)-6-methoxy-2-hydrazide-benzo thiazole (S3) was synthesized from the reaction of compound (S2) with 4-N,N-dimethylbenzaldehyde. The IR spectra of the compound
(S₃) shows strong band in the region (1605-1630) cm⁻¹ as due to (C=N) stretching vibration imine, and two characteristic absorption bands disappeared at 3359 and 3265 cm⁻¹ due to of a symmetric and symmetric (-NH-NH₂) group stretching. ¹H-NMR spectra of compound (S₃) showed clear singlet signal at =2.08 ppm due to (CH₃) group protons, while, multiplet signals at = (7.27-8.12) ppm for aromatic protons and singlet signal at = 8.61 ppm for imine proton (-N=CH⁻).

Effectiveness of chemical compound against fungi

The results of the statistical analysis in Table (1), showed the difference in the effect of the chemical compounds and their concentrations used in the inhibition of the fungus colony. The S₁ compound was superior in inhibiting colony growth and the least inhibitory effect was achieved by the S₂ compound at 89.39 and 33.89% respectively . The difference in the effect of these compounds according to their three concentrations and achieved the highest rate of inhibition with the third concentration of 300 mg / L, which amounted to 73.63%, and is observed in the results of the table of the interference chemical compound and the concentration used significantly exceeds the third concentration 300 mg/ l and the compound S₁ has a rate of inhibition 97.85 and followed the two and the first to the same chemical compound as it reached 89.41, 80.91% respectively, due to SH group in it and working on the union with the enzymes produced by fungi supplies growth that discourage their effectiveness and thus hinder its colonies growth requirements [20]. It was noted that the results of the triangular interference between the compound and the concentration used and the fungus type achieved the highest percentage of inhibition of the colony of the fungus Rhizoctonia solani with the third concentration of the compound S₁, which did not differ significantly from the second concentration of the same compound in the inhibitory effect of 100, 97.4%, respectively, while the least inhibition with fungus Fusarium oxysporum and for compound S₂ and all three concentrations.

Table (1) :-Effect of chemical compounds in the inhibition ratio of colony growth fungi

| Chemical Compound (s) | Concentration (c) | Fungi (F) |
|-----------------------|------------------|-----------|
| S₃                    | 100              | F1        |
| S₂                    | 82.22 de         |           |
| S₁                    | 80.11 e          |           |
| S₃                    | 80.40 e          |           |
| S₂                    | 61.56 c          |           |
| S₁                    | 52.20 igh        |           |
| S₃                    | 59.77 c          |           |
| S₂                    | 70.61 a          |           |
| S₁                    | 70.61 a          |           |
| S₃                    | 74.22 b          |           |
| S₂                    | 52.25 igh        |           |
| S₁                    | 97.40 ab         |           |
| S₃                    | 75.93 a          |           |
| S₂                    | 53.56 gh         |           |
| S₁                    | 100 a            |           |

Fungi effect

| S₃                    | 46.63 f          | Fusarium oxysporum F1 |
| S₂                    | 50.07 ih         | Macrophomina phasiolina F2 |
| S₁                    | 47.95 g          | Rhizoctonia solani F3 |

Concentrati on effect

| S₃                    | 48.43 c          | Chemical compounds |
| S₂                    | 59.30 b          | S1 |
| S₁                    | 73.63 a          | S2 |
| S₃                    | 58.04 b          | S3 |

*the similar characters indicate that they are not significantly different below the probability level 5% to Dinkn test
Conclusion

The present work deals with the preparation of 2-mercapto-6-methoxy benzimidazole by treating with 4-methoxy-o-phenylene diamine and (1 mmol) and carbon disulfide (1 mmol) in ethanol (10 mL) under basic conditions. This method of synthesis is accurate and gives high percent purity with a greater yield. All the derivatives prepared by this method are analysed by Mass and IR. In conclusion, synthesis at different positions of benzimidazole give a wide variety of compounds with broad spectrum of pharmacological activity. Through the study, it becomes clear that the S1 compound is superior in inhibiting against three types of fungi affect plants (Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseolina) as shown in table (1). This superiority is due to the existence of free SH group in compound.

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