Synthesis, Characterization and Biological Evaluation of Schiff Bases of Propanedihydrazide

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Abstract: A Schiff base series of malonohydrazide was synthesized by reaction of malonohydrazide with various aldehydes. Hydrazine hydrate was reacted with ethyl malonate in ethanol to give malonohydrazide in very good yields. The synthesized compounds were characterized using FTIR and NMR spectroscopies. The biological evaluation of the Schiff bases was carried against Gram +tive and Gram -tive bacterial strains. The investigated bacterial strains were Staphylococcus aureus, Bacillus anthraces, Cyanobacteria bovius and Escherichia coli. Antioxidant evaluation of the synthesized compounds was done by means of DPPH method. The results showed that compound 4 showed good antimicrobial activities.

Key words: Malonic acid • Schiff bases • Antimicrobial activity • Antioxidant

INTRODUCTION

Carbohydrazides and their Schiff bases are very important biological compounds with reference to various biological activities such as antitumor, fungicidal, herbicidal, tuberculostatic activity, analgesic, anti-inflammatory, anti-diabetic, antithrombotic and antimicrobial properties. Substitution of the pyrazole ring at position C-3 with the carbohydrazide gives derivatives which show various biological activities such as antitumor and cannabinoind antagonist [1-4]. Substitution with a carbohydrazide moiety at position C-5 of the pyrazole ring seems to provide derivatives that exhibit antitumor activity as well as fungidal and herbicidal activities [5-11]. Pharmacophore (-CO-NHN=C) is reported to be the cause of biological activity of Schiff bases of hydrazones [12]. Consequently, all the compounds containing this structural feature constitute an important group of medicinal and pharmaceutical agents [13-15]. Dehydroacetic acid derived Schiff bases have been studied to possess antimicrobial activity [16-18].

Mechanism of action of vitamin B₃ containing enzymes has been reported to be recognized by the Schiff base hydrazones of pyridoxal phosphate and its analogous [19]. Dopamine-hydroxylase and tyrosine hydroxylase inhibition has been reported to be shown by a series of pyridazinyl hydrazones in vivo and in vitro [20]. Some of the genetic disorders such as thalassemia can be cured by tridentate hydrazones which can act as potential oral iron-chelating drugs [21]. Similarly Salicylaldehyde acetyl hydrazone also exhibits radiation protective properties as well as show very good biological activities [22].

In the light of previous work, it was worthwhile to study such compounds for the biological importance. Thus, the present work was mainly aimed to prepare and characterize Schiff bases of malonohydrazide and to evaluate for their biological applications.

MATERIALS AND METHODS

Materials Reagents: Hydrazine, diethyl mlonate, nicotininaldehyde, isonicotinealdehyde, p-nitrobenzaldehyde, p-dimethylaminobenzaldehyde, p-bromobenzaldehyde and DPPH obtained from Aldrich (USA), were used as received. Solvents (E. Merck, Germany) were used after drying according to the reported procedures.

Physical Measurements: Gallenkamp (U.K.) electrothermal melting point apparatus was used to determine the melting point through capillary tube. Bruker-300 MHz FT-NMR Spectrometer was used to record the NMR (H) and CDCl₃ (H = 7.25) used as an internal reference.
Scheme 1: Synthesis of propanedithyldrazide.

Scheme 2: Synthesis of Schiff bases of propanediylhydrazide.

Synthesis of Propanediylhydrazide: Diethyl malonate (1.52ml, 10mmol) was dissolved in ethanol (10ml) in a round bottom flask. Hydrazine (0.626ml, 20mmol) was added slowly to the solution of diethyl malonate. The mixture was refluxed for 2 hours at 78°C. Solvent was evaporated under vacuum. The obtained white solid was filtered, washed with distilled ethanol and re-crystallized from water to get colorless crystals of propanediylhydrazide (23).

Synthesis of Schiff Bases of Propanediylhydrazide: Stoichiometric amounts of propanediylhydrazide and various aldehydes were taken in methanol in a round bottom flask. The mixture was allowed to stir overnight. Precipitates obtained were filtered and washed with water.

N,N'-Bis(pyridin-3-ylmethylene)malonohydrazide (2) Yield: 76%, Melting point: Decomposed at 218°C, IR (cm⁻¹): 1629 (C=N), 1678 (C=O), 3198 (N-H), 1046 (N-N), 1587(C=N), 2910 (H-C saturated), H NMR (300 MHz, CDCl₃); δ (ppm) 3.07 (2H, s, CH₂), 8.0 (2H, s, 2NH), 7.89-8.29 (8H, m, 2C₆H₅N), 7.5 (1H, s, CH)

N,N'-Bis(pyridin-4-ylmethylene)malonohydrazide (3) Yield: 65%, Melting point: Decomposed at 210°C, IR (cm⁻¹): 1627 (C=N), 1678 (C=O), 3198 (N-H), 1046 (N-N), 1587(C=N), 2900 (H-C saturated), H NMR (300 MHz, CDCl₃); δ (ppm) 3.07 (2H, s, CH₂), 7.0 (2H, s, 2NH), 7.57-8.93 (8H, m, 2C₆H₅N), 7.5 (1H, s, CH)

N,N'-Bis(4-nitrobenzylidene)malonohydrazide (4) Yield: 79%, Melting point: Decomposed at 240°C, IR (cm⁻¹): 1635 (C=N), 1680 (C=O), 3190 (N-H), 1049 (N-N), 2910 (H-C saturated), H NMR (300 MHz, CDCl₃); δ (ppm) 3.07 (2H, s, CH₂), 8.0 (2H, s, 2NH), 7.89-8.29 (8H, m, 2C₆H₅N), 8.1 (1H, s, CH)
N',N'-Bis(4-N, N-dimethylbenzylidene)malonohydrazide (5)
Yield: 76%, Melting point: Decomposed at 208°C, IR (cm⁻¹): 1629 (C=N), 1676 (C=O), 3194 (N-H), 1043 (N-N), 2901 (H-C saturated), H NMR (300 MHz, CDCl₃): δ (ppm) 3.07 (2H, s, CH₂), 8.0 (2H, s, 2NH), 6.57-7.46 (8H, m, 2C₆H₄N), 2.9 (12H, s, 4CH₂), 8.1 (1H, s, CH)

N',N'-Bis(4-bromobenzylidene)malonohydrazide (6)
Yield: 85%, Melting point: Decomposed at 261°C, IR (cm⁻¹): 1630 (C=N), 1678 (C=O), 3190 (N-H), 1045 (N-N), 2905 (H-C saturated), H NMR (300 MHz, CDCl₃): δ (ppm) 3.07 (2H, s, CH₂), 8.0 (2H, s, 2NH), 7.5-8.18 (8H, m, 2C₆H₄N), 8.1(1H, s, CH)

Antibacterial Activity: The antibacterial test was performed according to the disc diffusion method [23, 24]. Compounds 1-6 were assayed for their antimicrobial activity in vitro against four strains of bacteria, gram negative (Escherichia coli) and gram positive (Staphylococcus aureus, Bacillus antherasis, Crynibateria bovius). Prepared agar and petridishes were sterilized by autoclaving for 15min at 121°C. The agar plates were surface inoculated uniformly from broth culture of the tested microorganisms. In the solidified medium suitably spaced apart filter discs, 6mm in diameter (dipped with the sample solution) were placed on pre-inoculated plates. The sample solution diffused in a circular zone and killed the microbes or inhibits their growth. The diameter of the zone was calculated to know the activity of the sample. Cefixime was used as references antibiotic drugs. All the experiments were performed in triplicate and SD (+) were calculated using MS Excel (2007).

DPPH Scavenging Activity: Free radical scavenging activities of the synthesized compounds 1-6 were determined using DPPH [25]. 100µl of solution of synthesized compounds were added to 2ml of 0.2mM DPPH in ethanol. The solutions were incubated for 20 minutes at 37°C. Absorbance of the solutions was measured at 516 nm with double beam spectrophotometer. 1mM ascorbic acid was used as a positive control.% age scavenging of the compounds was calculated by the following formula [26].

% Scavenging = (A, - A₀/A₀) × 100

where A₀ = Absorbance of DPPH solution A₅ = Absorbance of Sample solution

RESULTS AND DISCUSSION

Compounds (1-6) were synthesized according to Schemes 1 and 2. Malonohydrazide (1) was synthesized by reported procedure by the reaction of diethyl malonate with hydrazine in ethanol with refluxing for two hours. The melting point of 1 was in good agreement with the reported one and product was obtained in good yield (90%). Schiff bases of the propanedihydrazide (2-6) were synthesized by reacting propanedihydrazide with different aldehydes (nicotinaldehyde, Isonicotinaldehyde, p-nitrobenzaldehyde, p-dimethylaminobenzaldehyde and p-bromobenzaldehyde) in methanol. The reaction mixtures were allowed to stir overnight to get precipitates, which were filtered and washed with methanol. All the Schiff bases of malonohydrazide were characterized using FTIR and NMR analysis. FTIR spectrum of all the Schiff bases gave clear peak in the region 1670-1680

Absorbance of 0.2mM DPPH = 0.931

Fig. 1: Inhibition zone diameter values (IZD) in mm of compounds 1-6 (at conc. 1µg/µl) and reference drug (0.5µg/µl) against four different bacterial strains i.e. SA, BA, CB and EC.
indicating the presence of carbonyl group, while peak in the range 1620-1630 cm\(^{-1}\) for C\(=\)N group confirming the formation of Schiff bases. \(^1\)H NMR spectrum of all the Schiff bases of malonohydrazide showed a very clear singlet peak at \(\delta = 3.07\) ppm for methylene protons of malonohydrazide along with a singlet peak at \(\delta = 7.5\) ppm corresponding to methine proton of the imine group (HC\(=\)N) in the spectra of 2 and 3 which is shifted to low field around at \(\delta = 8.1\) ppm for 4, 5 and 6 confirmed the formation of the Schiff bases of malonohydrazide.

The synthesized compounds were checked for their antimicrobial activities against the following microorganisms, *Staphylococcus aurus* (SA), *Bacillus antherasis* (BA) and *Crynibacteria bovius* (CB) (gram +tive strains) and *E. coli* (EC) (gram –ive strain). The screening of these compounds was carried out using disc diffusion method and results were taken in triplicate. Compound 2 showed maximum activity against *E. coli* while 6 showed maximum activity against SA. Compound 4 and 6 showed good activity against tested bacterial strains Fig. 1.

The synthesized compounds were checked for their antioxidant activities using DPPH as free radical scavenger. The results showed an increase in the antioxidant activity by increasing the concentration of compound. Compound 1 showed good antioxidant activity and % inhibition was 50% at maximum concentration level. In synthesized Schiff bases, compound 6 and 4 showed good results at higher concentration with % inhibition 47% and 39% respectively Fig. 2.

**CONCLUSIONS**

Malonohydrazide Schiff bases were synthesized in good yield and characterized by FTIR and NMR spectra. The synthesis compounds showed low to good antibacterial and antioxidant activities. Nitro and bromo substituted derivatives showed good results and may further be investigated to get comprehensive biological potential of the compounds.

**REFERENCES**


