American-Eurasian J. Agric. & Environ. Sci., 15 (12): 2479-2483, 2015
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2015.15.12.12831

Synthesis, Characterization and Biological Evaluation of Schiff Bases of Propanedihydrazide

[1]Muhammad Nawaz Tahir, [2]Naghmana Kausar, [3]Aadil Abbas and [2]Shahzad Murtaza

[1]Department of Physics, University of Sargodha, Sargodha, Pakistan
[2]Department of Chemistry, Faculty of Science, University of Gujrat, Gujrat, Pakistan
[3]Institue of Chemistry,University of the Punjab, Lahore, Pakistan

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, antidiabetic, 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 cannabinoid 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 fungicidal 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 B_6 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].

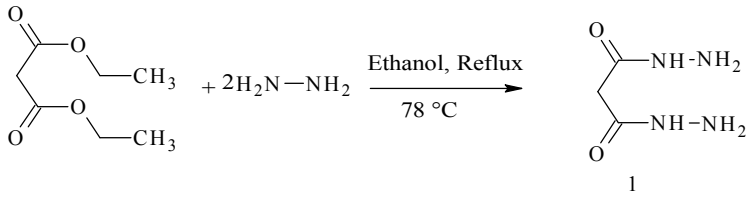
In the light of previous work, it was worthwhile to study such compounds for the biological importance. Thus, the present work was mainly aimed to prepare and characterize Schiff bases of malonohydrazide and to evaluate for their biological applications.

MATERIALS AND METHODS

Materials Reagents: Hydrazine, diethyl mlonate, nicotinealdehyde, 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.

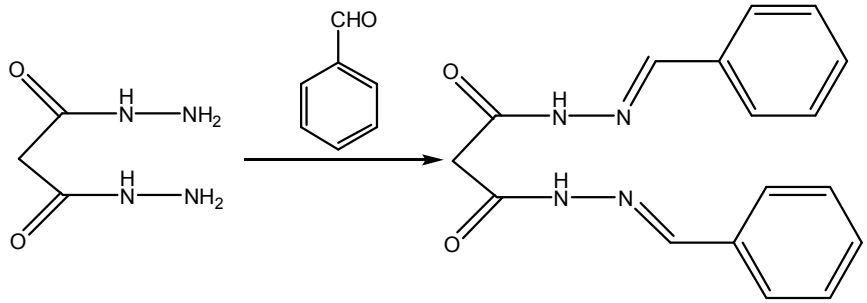
Physical Measurements: Gallenkamp (U.K.) electrothermal melting point apparatus was used to determine the melting point through capillary tube. Bruker-300 MHz FT-NMR Spectrometer was used to record the NMR (^{1}H) and $CDCl_3$ [^{1}H = 7.25] used as an internal reference.

Corresponding Author: Aadil Abbas, Institue of Chemistry,University of the Punjab, Lahore, Pakistan.

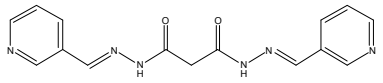


1

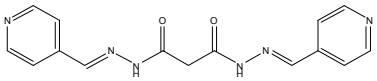
Scheme 1: Synthesis of propanedihydrazide.



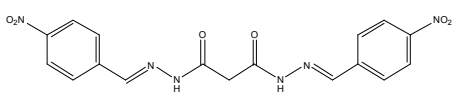
Scheme 2: Synthesis of Schiff bases of propanedihydrazide.



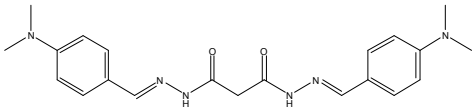
2



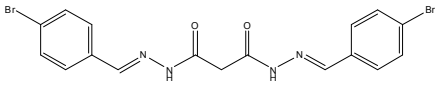
3



4



5



6

Synthesis of Propanedihydrazide: 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 propanedihydrazide [23].

Synthesis of Schiff Bases of Propanedihydrazide: Stoichiometric amounts of propanedihydrazide 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^{1},*N*^{3}-Bis(pyridin-3-ylmethylene)malonohydrazide (**2**) Yield: 76%, Melting point: Decomposed at 218°C, IR (cm^{-1}): 1629 (C=N), 1675 (C=O), 3194 (N-H), 1044 (N-N), 1583($C=N_{py}$), 2905 (H-C saturated), ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.07 (2H, s, CH_2), 7.0 (2H, s, 2NH), 7.33-8.9 (8H, m, $2C_5H_4N$), 7.5 (1H, s, CH)

N^{1},*N*^{3}-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($C=N_{py}$), 2900 (H-C saturated),^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.07 (2H, s, CH_2), 7.0 (2H, s, 2NH), 7.57-8.9 (8H, m, $2C_5H_4N$), 7.5 (1H, s, CH)

N^{1},*N*^{3}-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),^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.07 (2H, s, CH_2), 8.0 (2H, s, 2NH), 7.89-8.29 (8H, m, $2C_6H_4N$), 8.1 (1H, s, CH)

N^1,N^3-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), ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.07 (2H, s, CH_2), 8.0 (2H, s, 2NH), 6.57-7.46 (8H, m, $2C_5H_4N$), 2.9 (12H, s, $4CH_3$), 8.1 (1H, s, CH)

N^1,N^3-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), ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.07 (2H, s, CH_2), 8.0 (2H, s, 2NH), 7.5-8.18 (8H, m, $2C_5H_4N$), 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, Crynibacteria 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].

$$\% \text{ Scavenging} = (A_o - A_T/A_o) \times 100$$

where A_o = Absorbance of DPPH solution A_T = 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

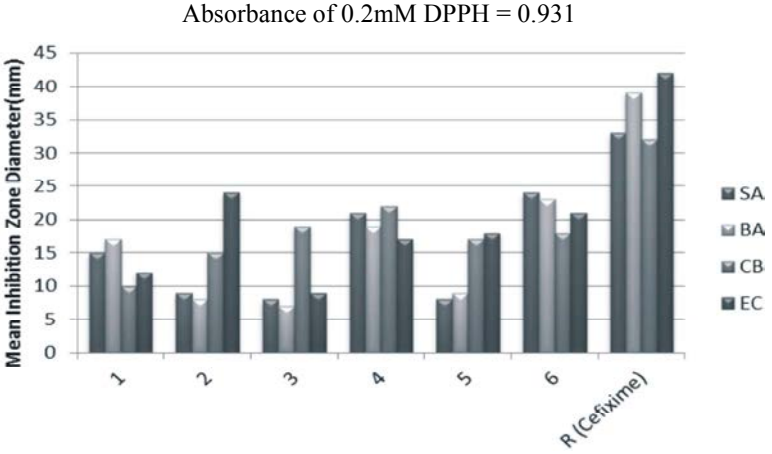


Fig. 1: Inhibition zone diameter values (IZD) in mm of compounds 1-6 (at conc. 1µg/µl) and reference drug (0.5µg/µl) against four different bacterial strains i.e. SA, BA, CB and EC.

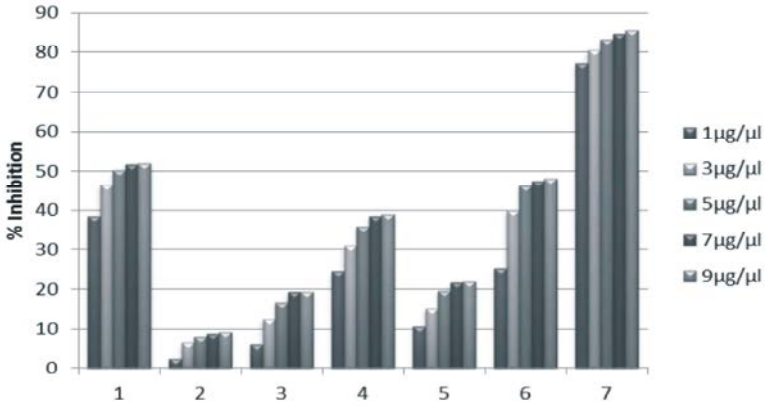


Fig. 2:%age scavenging of synthesized compounds 1-6.

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 δ = 3.07ppm for methylene protons of malonohydrazide along with a singlet peak at δ = 7.5ppm 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 δ =8.1ppm 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, *Staphylococus aurus* (SA), *Bacilus 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 activty against SA. Compound 4 and 6 showed good activty 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

1. Hegazi, B., H.A. Mohamed, K.M. Dawood and F.A.R. Badria, 2010. Cytotoxicity and utility of 1-indanone in the synthesis of some new heterocycles. Chem. Pharm. Bull., 58(1): 479-483.
2. Rostom, S.A.F., 2010. Pyrazole Compounds with Carbohydrazide Moiety Internalized- acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents. Bioorg. Med. Chem., 18: 2767-2776.
3. Francisco, M.E.Y., H.H. Seltzman, A.F. Gilliam, R.A. Mitchell, S.L. Rider, R.G. Pertwee, L.A. Stevenson and B.F. Thomas, 2002. 1-H-pyrazole derivatives 5-9 and 2,3-dihydro-1-H-pyrazole derivative 10 that exhibit biological activities. J. Med. Chem., 45: 2708-2719.
4. Padgett, L.W., 2005. Recent Developments in Cannabinoid Ligands. Life Sci., 77: 1767-179.
5. Xia, Y., X.D. Fan, B.X. Zhao, J. Zhao, D.S. Shin and J.Y. Miao, 2008. Synthesis and structure activity derivatives as potential agents against A549 lung cancer cells. Eur. J. Med. Chem., 43: 2347-2353.
6. Xia Y., Z.W. Dong, B.X. Zhao, X. Ge, N. Meng, D.S. Shinc and J.Y. Miaob. 2007. Synthesis and structure-activity relationships of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide derivatives as potential agents against A549 lung cancer cells. Bioorg. Med. Chem., 15: 6893-6899.

7. Fan, C.D., B.X. Zhao, F. Wei, G.H. Zhang, W.L Dong and J.Y. Miao. 2008. Synthesis and discovery of autophagy inducers for A549 and H460 lung cancer cells, novel 1-(20-hydroxy-30-aroxypropyl)-3-aryl-1H-pyrazole-5-carbohydrazide derivatives. Bioorg. Med. Chem. Lett., 18: 3860-3864.
8. Fan, C., J. Zhao, B. Zhao, S. Zhang and J. Miao. 2009. Novel complex of copper and a salicylaldehyde pyrazole hydrazone derivative induces apoptosis through up-regulating integrin in vascular endothelial cells. Chem Res. Toxicol., 22: 1517-1525.
9. Fan, C.D., H. Su, J. Zhao, B.X. Zhao, S.L. Zhang and J.Y. Miao.2010. A novel copper complex of salicylaldehyde pyrazole hydrazone induces apoptosis through up-regulating integrin b4 in H322 lung carcinoma cells. 2010. Eur. J. Med. Chem., 45: 1438-1446.
10. Li, Z.M., H.S. Chen, W.G. Zhao, K. Zhang and X.S. Huang. 1997. Synthesis and biological activity of pyrazole derivatives. Chem. J. Chin. Univ., 18: 1794-1799.
11. Chen, H.S. and Z.M. Li, 2002. Synthesis of some heteroaryl pyrazole derivatives and their biological activities. Chin. J. Chem., 18: 596-602.
12. Al-Amiery, A.A., Y.K. Al-Majedy, H.H. Ibrahim and A.A. Al-Tamimi. 2012. Antioxidant, antimicrobial and theoretical studies of the thiosemicarbazone derivative Schiff base 2-(2-imino-1-methylimidazolidin-4-ylidene) hydrazinecarbothioamide (IMHC). Org. Med. Chem. Lett., 2: 4.
13. Singh, V.P., A. Katiyar and S. Singh, 2008. Synthesis, characterization of some transition metal (II) complexes of acetone p-amino acetophenone salicyloyl hydrazone and their anti microbial activity, Biometals, 21: 491.
14. Pandeya, S.N., D. Sriram, G. Nath and E. De Clercq, 1999. Biological activities of isatin and its derivatives. Eur. J. Pharmacol. Sci., 9: 25.
15. Karthikeyan, M.S., D.J. Prasad, B. Poojary, K.B. Subramanya, B.S. Holl and N.S. Kumari, 2006. Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety. Bioorg Med Chem., 14: 7482-7489.
16. Shelke V.A., S.M. Jadhav, S.G. Shankarwar, A.S. Munde and T.K. Chondhekar, 2011. Synthesis, Spectroscopic Characterization and Antimicrobial Activities of Some Rare Earth Metal Complexes of Biologically Active Asymmetrical Tetradentate Ligand. J. Korean Chem. Soc., 55, 436.
17. Mane P.S., S.M. Salunka, B.S. More and A.M. Chougule, 2011. Synthesis and structural studies of transition metal complexes with bidentate Schiff base derived from 3-acetyl-6-methyl-(2H)-pyran-2,4(3H)-dione. Int. J. Basic Appl. Res., 01: 24.
18. Munde, A.S., V.A. Shelke, S.M. Jadhav, A.S. Kirdant, S.R. Vaidya, S.G. Shankarwar and T.K. Chondhekar. 2012. Synthesis, Characterization and Antimicrobial Activities of some Transition Metal Complexes of Biologically Active Asymmetrical Tetradentate Ligands. Adv. Appl. Sci. Res., 3: 175.
19. Bell, C.F. and D.R. Rose, 1965. Spectrophotometric determination of palladium with pyridine-2-aldehyde-2-pyridylhydrazone, Talanta, 12: 696-700.
20. Sears, J. K. And J.R. Darby, 1982. The Technology of Plasticizers, Wiley, New York, USA.
21. Massarani, E., D. Nardi, A. Tajana and L. Degen, 1971. Antibacterial nitrofuran derivatives. 5-Nitro-2-furaldehyde aminoacethydrazones. J. Med. Chem., 14: 633-635.
22. Arapov, O.V., O.F. Alferva, E.I. Levocheskaya and I. Krasilnikov, 1987. Radioprotective efficacy of acyl hydrazones. Radiohiologiya, 27: 843-846.
23. Subedi, A., M.P. Amatya, T.M. Shrestha, S.K. Mishra and B.M. Pokhre, 2012. Synthesis, analgesic and anti-inflammatory activities of new bisisatin malonohydrazides. J. Sci. Eng. and Tech., 8(1): 73-80.
24. Lillian, B., R. Calhelha, I. Ferreira, P. Baptista and L. Estevinho, 2007. Antioxidant and antibacterial activity of methanolic extract of Machilus odoratissima, Eur. Food Res. Technol., 225: 151-156.
25. Nagai, M.M. Tani, Y. Kishimoto, M. Lizuka, E. Saita, M. Toyazaki, T. Kamiya, M. Ikeguchi and K. Kondo, 2011. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms. J. Clin. Biochem., 48(3): 203-208.
26. Subedi, A., M.P. Amatya, T.M. Shrestha, S.K. Mishra and B.M. Pokhre, 2012. Sweet potato (*Ipomoea batatas* L.) leaves suppressed oxidation of low density lipoprotein (LDL) *in vitro* and in human subjects. J. Sci. Eng. and Tech., 8(1): 73-80.