Synthesis, Characterization and Biological activities of Hydrazide Schiff's Bases

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Abstract

A series of hydrazide Schiff's bases were synthesized from different types of hydrazides and aldehydes, which were characterized through IR, LCMS, ^{1}H and ^{13}C NMR spectral studies. The synthesized pure compounds were screened for antibacterial and antioxidant activities. The S4 and S5 samples showed good antibacterial activity and maximum zone of inhibition of 18 mm and 12 mm against S. aureus and E. coli, respectively at 0.6 mg/disc concentration. S4 and S6 showed appreciable activity towards antioxidant DPPH and ABTS assay. Because of the biological activity of hydrazide Schiff's bases, they became important in medicinal chemistry.

Key words: Hydrazides, Schiff's base, imines, antibacterial, antioxidant, DPPH, ABTS assay.

Introduction

Schiff's base or Imine is one of the largely used families in organic synthesis, in the form of artificial intermediate or as a ligand in coordination chemistry. The first ever effort of synthesis of imine was done by Hugo Schiff in 1864 [1]. Imines are the functional groups or compounds containing a carbon nitrogen double bond, formed by the condensation of primary amine and carbonyl compounds such as aldehydes and ketones under different conditions with different solvents by the elimination of water molecules. Because of the presence of pai bond and lone pair of electrons present on the Nitrogen atoms, it shows variety of biological activities.

Schiff's bases of various compounds are reported to possess antiproliferative [2], Anticonvulsant [3], cytotoxic [4], anticancer [5], antifungal and anti HIV activities [6]. Schiff's bases and their metal complexes show very good antibacterial activities against *E. coli* and *B. subtilis.* Pyrazine and amino acid based moieties of Schiff's bases show antibacterial activity [7-9]. Schiff's bases derived from 5-chloro salicylaldehyde shows very good antimicrobial activity against *E. coli, Bacillus subtilies* and *Staphylococcus aureus* [10]. Some of the Schiff's bases of heterocyclics, such as quinazolinones, toluidinones, benzimidazole, thiazole, glucosamine pyrazolon, hydrazide furforaldiamine, halogenated

thiazolidiones, indole *p*-fluoro benzaldehyde show remarkable biological activities [11]. Schiff's bases derived from N-substituted and quaternized chitosan show good antifungal activity [12]. Many hydrazide Schiff's bases derived from 4-chloro benzaldehyde, substituted hydrazide Schiff's bases of carboxy methyl chitosan show anti-oxidant activity by the DPPH method and NO scavenging method [13-14].

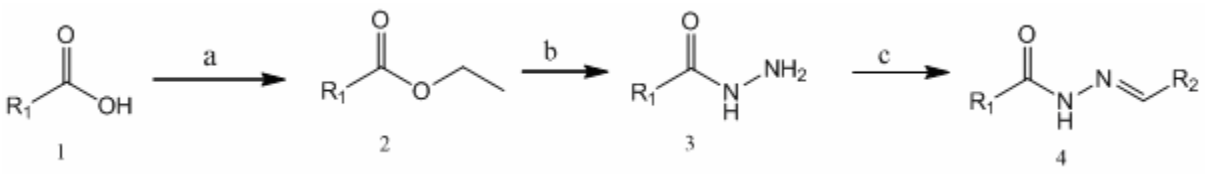
A number of acyl hydrazide Schiff's bases have shown interesting biological activities such as antibacterial, antifungal, anticonvulsant, anti-inflammatory, antimalarial, analgesic, antiplatelets, antituberculor activities [15-22]. Inspired by the above facts, it was planned for synthesis of the new series of hydrazide Schiff's bases, characterized through IR, LCMS, ^{1}H and ^{13}C NMR spectral studies and evaluated for antibacterial, antioxidant activities.

Materials and methods

Reactions were performed in two necked 100 mL round bottomed flask. The glass wares were previously rinsed with acetone and dried in hot air oven. The chemicals were purchased from Oakwood and aldehydes from Aldrich. The monitoring of reaction was carried out by Thin Layer Chromatography (TLC), silica coated aluminium plates, obtained from Merck, by using appropriate solvent mixtures. The melting point (mp) was determined by open capillary method and was uncorrected. The product was confirmed by LCMS and ^{1}H & ^{13}C NMR spectral studies. NMR spectrum was recorded by Agilent 400 MHz instrument. LCMS were recorded by positive mode, using solvents 0.1% formic acid in Acetonitrile. Antimicrobial activity was carried out by disc diffusion method. Antioxidant activity was found out by DPPH method, using 96-well micro titer plate and readings were taken from Varioskan Flash instruments.

Experimental Section:

Reaction Scheme:



Scheme 1 Synthesis of new hydrazide Schiff's bases

where (a) Ethanol, reflux for 5 hrs

(b) Hydrazine hydrate, Ethanol, Reflux for 6 hrs

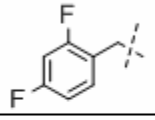
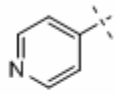
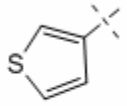
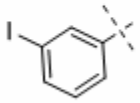
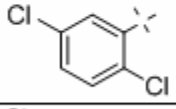
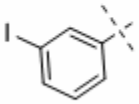
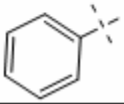
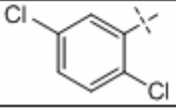
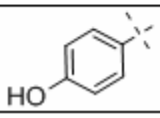
(c) R_2-CHO, ethanol, reflux for 4 hrs

List of substituents R_1 & R_2 are shown in the Table 1.

Chemistry: In the first step substituted carboxylic acids were converted into their ester derivatives (2), by the esterification reaction using known procedure [23]. Further this ester was converted into hydrazide derivative (3), by refluxing with hydrazine hydrate in ethanol medium. The title compounds (S1-S6) were

obtained by refluxing the hydrazide derivatives and suitable aldehydes in ethanol for 5 hrs in acidic medium (Scheme 1) and their physical data is shown in Table 2.

Table 1 R1 & R2 substituents of synthesized hydrazide Schiff's bases

Comp Id	R_1	R_2	Comp Id	R_1	R_2
S1			S4		
S2			S5		
S3			S6		

General procedure for synthesis of esters

To a mixture of substituted carboxylic acid (1 mmol) in ethanol (10 mL), was added Conc. Sulphuric acid (0.5 mL) and refluxed for 5 hrs. After completion of the reaction (checked by TLC), the reaction mixture was concentrated, the solid separated was filtered, washed with water and recrystallized from ethanol to give substituted ester (yield 92-96 %).

General procedure to synthesize the hydrazide from the ester

Ester obtained from the first step (1 mmol) was dissolved in ethanol (10 mL), and hydrazine hydrate (1.5 mmol) was refluxed for 6 hrs. After completion of the reaction (checked by TLC), the reaction mixture was evaporated and the solid was washed with water and recrystallized from ethanol (yield 88-91 %).

Table 2 Physical data of synthesized hydrazide Schiff's base derivatives.

Comp Id	R_t Value in LCMS (in min)	Yield* (in %)	Melting Point (in °C)
S1	0.14	95.1	145-146
S2	1.43	91.2	144-146
S3	0.54	94.6	112-113
S4	0.27	82.3	165-166
S5	1.71	92.6	194-195
S6	0.79	93.4	241-242

*calculated after Purification, all the compounds have the purity >98

General procedure to synthesize the Schiff's bases

An equimolar mixture of hydrazide and aromatic aldehydes were refluxed with ethanol in presence of catalytic amount of Con. H_2SO_4 for 4 hrs. After completion of the reaction (checked by TLC), it was

allowed to cool and solid separated out was filtered, dried, recrystallized from mixture of diethyl ether and ethyl acetate (yield 82-95 %).

Synthesis of (E)-2-(2,4-difluoro phenyl)-N'-(pyridin-4-ylmethylene) acetohydrazide (S1)

FT-IR (KBr, γ cm^{-1}): 3120 (-NH), 2966 (Ar C-H), 2864 (Alkyl C-H), 1693 (C=O Amide), 1675 (C=C-N), 1525 (Ar C=C), 1090 (C-F). ^{1}H NMR ($CDCl_3$, 400 MHz), δ 10.29 (s, 1H), 8.68 (s, 2H), 7.76 (s, 1H), 7.53 (s, 2H), 7.29-2.27 (d, *J* = 8.8 Hz, 1H), 6.87-6.85 (d, *J*= 5. 6 Hz, 2H), 4.12 (s, 2H). ^{13}C NMR: (400 MHz, $CDCl_3$), δ 172.9, 163.9, 150.4, 141.5, 140.8, 132.0, 120.9, 111.4, 111.2, 104.1, 103.9, 32.4. LCMS found: *m/z* 276.0 for [M^++1] peak, calculated for $C_{14}H_{11}F_2N_3O$ 275.09. Elemental analysis for $C_{14}H_{11}F_2N_3O$ by calculation C, 61.09; H, 4.03; N, 15.27 % and experimentally found C, 61.05; H, 4.07; N, 15.30 %.

Synthesis of (E)-N'-(3-iodo benzylidene) iso butyrohydrazide (S2)

FT-IR (KBr, γ cm^{-1}): 3133 (-NH), 3005 (Ar C-H), 2975 (Alkyl C-H), 1685 (C=O Amide), 1652 (C=C-N), 1275 (-C-C), 628 (C-I). ^{1}H NMR ($CDCl_3$, 400 MHz), δ 9.87-9.86 (d, *J* = 5.2 Hz, 1H), 8.01-8.0 (t, *J* = 1.6, 3.2 Hz, 1H), 7.71-7.68 (m, 2H), 7.6-7.58 (d, *J* = 8.0 Hz, 2H), 7.14-7.09 (t, *J* = 8.0, 15.6 Hz, 1H), 3.51-3.45 (m, 1H), 1.23-1.22 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR: (400 MHz, $CDCl_3$), δ 180.2, 141.5, 138.7, 136.1, 135.6, 130.3, 126.4, 94.5, 19.5, 18.7. LCMS found: *m/z* 316.8 for [M^++1] peak, calculated for $C_{11}H_{13}IN_2O$ 316.01. Elemental analysis calculated for $C_{11}H_{13}IN_2O$ C, 41.79; H, 4.14; N, 8.86 % and experimentally found C, 41.83; H, 4.18; N, 8.92 %.

Synthesis of (E)-N'-benzylidene isobutyro hydrazide (S3)

FT-IR (KBr, γ cm^{-1}): 3138 (-NH), 3010 (Ar C-H), 2989 (Alkyl C-H), 1678 (C=O Amide), 1668 (C=C-N), 1582 (Ar C=C), 1270 (C-C). ^{1}H NMR ($CDCl_3$, 400 MHz), δ 10.12-10.10 (d, *J* = 7.6 Hz, 1H), 7.85 (s, 14), 7.67-7.65 (m, 2H), 7.40-7.34 (m, 3H), 3.56-3.48 (m, 1H), 1.24-1.23 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR: (400 MHz, $CDCl_3$), δ 180.4, 147.8, 134.1, 129.9, 128.7, 127.1, 30.4, 19.5. LCMS found: *m/z* 191.0 for [M^++1] peak, calculated for $C_{11}H_{14}N_2O$ 190.11. Elemental analysis calculated for $C_{11}H_{14}N_2O$ C, 69.45; H, 7.42; N, 14.73 % and experimentally found C, 69.39; H, 7.38; N, 14.80 %.

Synthesis of (E)-2-cyano-N'-(thiophen-3-ylmethylene) acetohydrazide (S4)

FT-IR (KBr, γ cm^{-1}): 3140 (-NH), 2863 (Alkyl C-H), 2265 (-C≡N), 1669 (C=O Amide), 1662 (C=C-N), 1605 (Ar C=C), 735 (C-S). ^{1}H NMR (DMSO-d6, 400 MHz), δ 11.66 (s, 1H), 8.01 (s, 1H), 7.88-7.87 (m, 1H), 7.59-7.58 (m, 1H), 7.45-7.44 (m, 1H), 4.13 (s, 2H). ^{13}C NMR: (400 MHz, DMSO-d6), δ 165.1, 140.5, 137.5, 129.3, 128.7, 125.1, 116.5, 24.6. LCMS found: *m/z* 194.2 for [M^++1] peak, calculated for $C_8H_7N_3OS$ 193.03. Elemental analysis calculated for $C_8H_7N_3OS$ C, 49.73; H, 3.65; N, 21.75 % and experimentally found C, 49.77; H, 3.69; N, 21.71 %.

Synthesis of (E)-2, 5-dichloro-N'-(3-iodo benzylidene) benzohydrazide (S5)

FT-IR (KBr, γ cm^{-1}): 3210 (-NH), 3020 (Ar C-H), 1668 (C=O Amide), 1650 (C=C-N), 1642 (Ar C=C), 675 (C-I), 845 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz), δ 9.66 (s, 1H), 8.18-8.17 (d, *J* = 4.4 Hz, 1H), 7.79-7.07 (m, 2H), 7.45-7.44 (d, *J* = 1.2 Hz, 1H), 7.43-7.40 (d, *J* = 10 Hz, 2H), 7.18-7.13 (t, *J* = 8.0 Hz, 15.6 Hz, 1H), 7.08-7.04 (t, *J* = 8.0,15.6 Hz, 1H). ^{13}C NMR: (400 MHz, $CDCl_3$), δ. 164.1, 146.2, 140.1, 139.1, 134.3, 133.2, 133.0, 132.8, 130.4, 129.6, 129.2, 129.0, 97.3. LCMS found: *m/z* 418.7 for [M^++1] peak, calculated for $C_{14}H_9Cl_2IN_2O$ 417.91. Elemental analysis calculated for $C_{14}H_9Cl_2IN_2O$ C, 40.13; H, 2.16; N, 6.69 % and experimentally found C, 40.08; H, 2.09; N, 6.6 %.

Synthesis of (E)-2, 5-dichloro-N'-(4-hydroxy benzylidene) benzohydrazide (S6)

FT-IR (KBr, γ cm^{-1}): 3385 (-OH), 3190 (-NH), 3024 (Ar C-H), 1670 (C=O Amide), 1664 (C=C-N), 1575 (Ar C=C), 840 (C-Cl). ^{1}H NMR ($CDCl_3$, 400 MHz), δ 9.69 (s, 1H), 8.17-8.15 (d, *J* = 4.4 Hz, 1H), 7.98-7.97 (d, *J* = 4.0 Hz, 1H), 7.89 (s, 1H), 7.61-7.59 (d, *J* = 8.2 Hz, 1H), 7.57-7.55 (d, *J* = 4.4 Hz, 1H), 6.85-6.84 (d, *J* = 4.2 Hz, 2H), 5.21 (s, 1H). ^{13}C NMR: (400 MHz, DMSO-d6), δ 163.2, 160.8, 146.8, 133.7, 132.7, 132.6, 132.5, 130.6, 129.0, 128.9, 126.3, 116.0. LCMS found: *m/z* 308.9 for [M^++1] peak, calculated for $C_{15}H_{12}Cl_2N_2O_2$ 308.01. Elemental analysis calculated for $C_{14}H_{10}Cl_2N_2O_2$ C, 54.39; H, 3.26; N, 9.06 % and experimentally found C, 54.32; H, 3.23; N, 9.11 %.

Biological Activity Studies

Antibacterial Activity

The antibacterial activity of synthesized compounds S1-S6 were determined by disc diffusion method [24] (Singh *et al.,* 2009), with slight modifications against pathogenic Gram-positive (*Staphylococcus aureus* MTCC 7443, *Bacillus subtilis* MTCC 121) and Gram-negative (*Escherichia coli* MTCC 7410, *Salmonella typhi* MTCC 733) bacteria. Gentamycin and DMSO were used as control. 100 µL of all the test bacterial cultures were adjusted to 0.5 McFarland standards and aseptically surface spread on 20 mL solidified nutrient agar media and the plates were allowed to stand for 10 min at room temperature. Sterile discs of 6 mm diameter were loaded with a 10, 20 and 30 µL volume of synthesized compounds along with control. All the plates were incubated at 37^{0}C for 24 hrs. Antibacterial activity of synthesized compounds were evaluated by measuring the inhibition zone surrounding the disc and expressed in mm. Each experiment was repeated three times, the antibacterial activity studies are shown in Table 3.

Antioxidant assays

Free radical scavenging ability by DPPH radical assay (1, 1-diphenyl-2-picryl hydrazyl)

The free radical scavenging activities of the synthesized compounds S1-S6 were determined by using DPPH method [25]. Various concentrations of the synthesized compounds in an aliquot of 100 µL were mixed with 100 µL of 40 µM methanolic solution of DPPH (Himedia, Mumbai, India) in a 96-well micro titer plate. The decrease in absorbance at 517 nm was recorded after the incubation for 15 min. at room

temperature and the absorbance of the DPPH solution with only methanol and without sample was used as the control. The Ascorbic acid (AA, Himedia, Mumbai) was used as a standard to compare the compounds activity. Since the tested compound was yellow colored, appropriate blank readings at 517 nm were recorded for each tested dilution. The assay was carried out in triplicate. The percentage inhibition of the DPPH radical by the synthesized compound was calculated using the formula and the results are shown in the figure 2.

$$\text{Percentage of inhibition} = [(A_C - A_S)/A_C)] \times 100$$

where A_C is the absorbance of the control and A_S is the absorbance of the sample/standard at 15 min. The IC_{50} value was calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals.

Table 3 Antibacterial activity of synthesized compounds by disc diffusion method (inhibition zone in mm)

Synthesized compound	Concentration (mg/ disc)	*Bacillus subtilis*	*Staphylococcus aureus*
S4	0.2	-	-
	0.4	-	12
	0.6	-	18
S5	0.2	-	-
	0.4	-	-
	0.6	12	-
Gentamycin		28	22

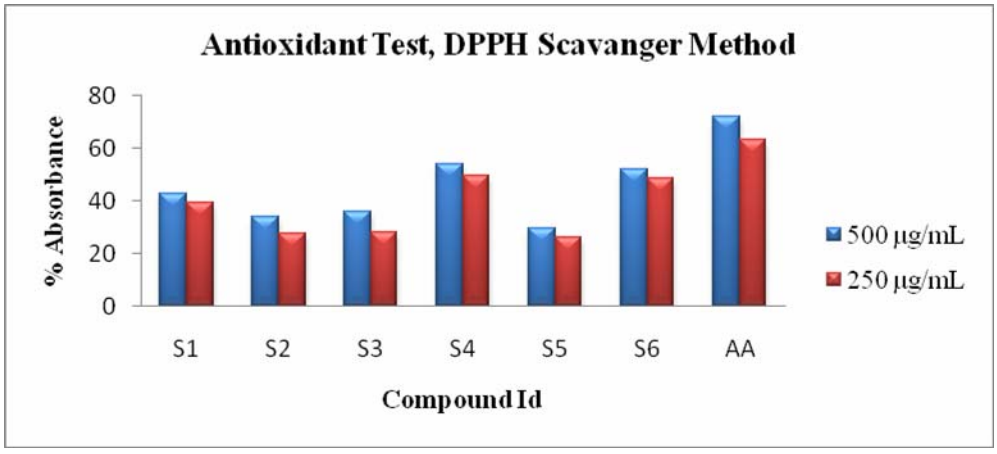


Figure 1. Antioxidant activity by DPPH scavenger method

ABTS [2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)] radical scavenging activity

ABTS assay is also one of the method to calculate the antioxidant ability [26]. The ABTS stock solution was prepared by mixing equal volumes of 7.4 mM ABTS (Sigma-Aldrich, USA) solution and 2.6 mM

potassium per sulfate solution followed by incubation for 12 hrs at room temperature in the dark. The reaction mixture consisted of 50 µL of the synthesized compounds at different concentrations (0.49-250 µg/mL in respective solvents) and 150 µL standardized ABTS solution. The decrease in absorbance was measured at 734 nm after 15 min. of incubation. Data for each assay was recorded in triplicate. Ascorbic acid was used as positive control. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula and the results are shown in the figure 3.

$$\text{Percentage of scavenging} = [(A_C - A_S)/A_C] \times 100$$

where A_C is absorbance of the control, A_S is absorbance of the synthesized compounds. The IC_{50} value was calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals.

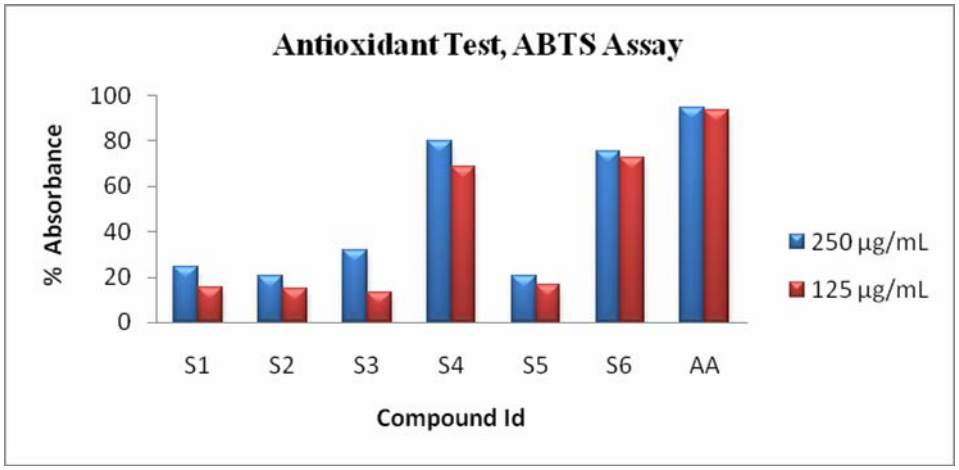


Figure 3. Antioxidant Activity by ABTS assay

Determination of IC_{50} Values:

Compounds A4 and A5 showed good activity both in DPPH Scavenging radical assay and ABTS assay, where as other comounds showed comparitively less activity. So, their IC_{50} values were calculated graphically based on capacity of compound concentration to scavenge 50 % of free radicals as is shown in Table 4.

Table 4 Antioxidant activities of synthesized compounds and their IC_{50} values.

Compound Id	IC_{50} Values*	
	DPPH Method	ABTS Assay
S4	343.76±0.27	43.88±0.26
S6	362.18±0.13	36.45±0.36
AA	16.07±0.11	2.28±0.02

* The IC_{50} was expressed as an average fromthree experiments ± standard deviation.

Results and Discussions:

In the present work, we were able to synthesize the hydrazide Schiff's bases by refluxing the equimolar mixture of Hydrazides and aldehydes by adding catalytic amount of acetic acid over a period of 4 hrs, the reactions were monitored by TLC. The synthesized compounds were confirmed by *m/z* value of molecular ion peak in mass spectroscopy. The appearance of singlet peak at δ 8.3 in ^{1}H NMR and peak at 145 ppm in ^{13}C NMR spectrum indicates the formation of imines. Finally, it was characterized through elemental analysis and their melting points were recorded. The fine products were screened for antibacterial and anti oxidant activities.

All the newly synthesized Schiff's bases were screened for their antibacterial activity by disc diffusion method. The results of antibacterial activity study revealed that, compounds S4 and S5 showed a maximum zone of inhibition of 18 mm and 12 mm against *S. aureus* and *E. coli*, respectively at 0.6 mg/ disc concentration. Further, the remaining compounds did not reveal any antibacterial activities.

The Schiff's bases were also screened for antioxidant activities by DPPH and ABTS assay. Absorbance of the different concentration was determined using ELISA instruments and its IC_{50} values were calculated by graphical method. Compounds S4 and S5 showed good activites and their IC_{50} values are 343.76±0.27, 362.18±0.13 and 43.88±0.26, 36.45±0.36 by DPPH and ABTS assay methods respectively.

Conclusions

The synthesis of hydrazide Schiff's bases (S1-S6) was achieved by an efficient and simple approach. The results of present investigation from the antibacterial study clearly indicate that Schiff's bases containing thiophene group (S4) and Iodo group (S5) are active against *S. aureus* and *E. coli*, respectively. From the antioxidant activity, it clear that, compounds with nitrile and thiophene (S4) and compound with hydroxyl group (S6) act as antioxidant both in DPPH Scavenging method and ABTS assay.

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