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Synthesis and characterization of new Schiff bases containing pyridine moiety and their derivatives as antioxidant agents

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Abstract: In the present investigation, a series of newly synthesized Schiff bases of few nitrogen containing the above heterocyclic nucleus to evaluate for their possible antioxidant activity. The title compounds N[1]- (substituted benzilidine) – pyridin-3-yl-carbohydrazide (2a-n) were synthesized according to their standard procedures. The structures of the newly synthesized compounds have been confirmed on the basis of elemental analysis and spectral studies (IR, H^1NMR, C^{13}NMR and Mass).The synthesized compounds were screened for their antioxidant activity by using Nitric oxide (NO) radical scavenging. The synthesized compounds demonstrated a significant dose dependent antioxidant activity comparable with ascorbic acid using as a standard. Some of the compounds were existed encouraging results.
Keywords: Schiff base, pyridine and antioxidant activity.

INTRODUCTION

Schiff base which contain an azomethane group attract much interest in synthetic chemistry. Schiff base with donors (N,O,S) have structural similarities with natural biological systems and imports in elucidating the mechanism of transformations and reseminations reactions in biological systems due to presence of imine (-N=CH-) group[1].

Schiff base are used as substrate in the preparations of a number of industrial and biologically active compounds via closure, cycloaddition and replacement reactions. Moreover, Schiff base are also known to have biological activities such as antibacterial[2- 3], antifungal[4-5], antitumor[6-7] and antioxidant[8] activities. Schiff base have also been employed as ligands for complexation of metal ions. On the industrial scale, they have a wide range of applications such as dyes and pigments. Schiff base complexes play a vital role in designing metal complexes related to synthetic and natural oxygen carriers[9]. Metal complexes make the compounds effective as steriospecific catalysts towards oxidation, reduction, hydrolysis biological activity and other transformations of organic and inorganic chemistry. In organic compounds the presence of -N=C- along with other functional groups form more stable complexes compared to compounds with only –N=C- coordinating moiety. Similarly pyridine derivatives have been of great interest because of their role in natural and synthetic organic chemistry. Many products which contain a pyridine subunit exhibit biological activity such as antimicrobial[10] and antituberculosis[11] activities. So the pyridine containing Schiff bases are expected to have enhanced biological activities. It is well established that the biological activity associated with the hydrazone compound attributed to the presence of the active pharmacophore (-CONH-N=C-). Hence many hydrazone compounds containing this active moiety showed good biological activities according to the literature. In the present work, we have synthesized fifteen Schiff base from

nicotino hydrazide with substituted aromatic benzaldehydes afforded title compounds (2a-n) and evaluated their *in vitro* antioxidant activity.

EXPERIMENTAL

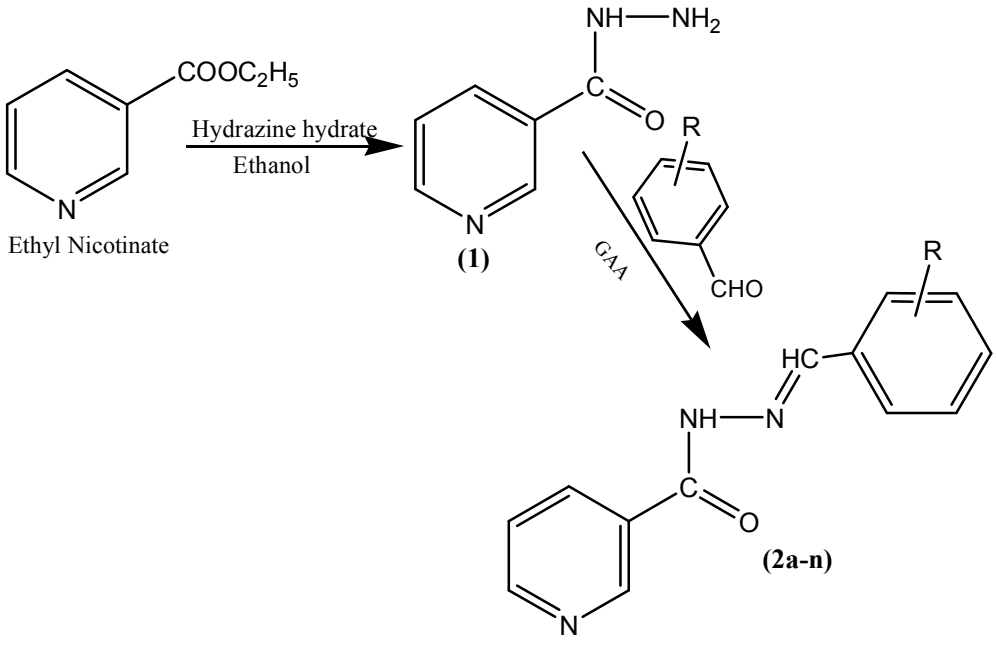
The melting points were determined in open capillaries on an electric melting point apparatus and are uncorrected. The purity of the compounds was confirmed by TLC using silica gel precoated plates (0.25mm, 60 F_{254}; Merck) using ethyl acetate and ethanol (2:3, v/v).The IR spectra were recorded on perkin-elmer BXF1, FTIR spectrophotometer using KBr discs method. H^1 & C^{13} NMR spectra were recorded on Bruker AMX, 400 MHz and using TMS as an internal standard. Chemical shifts are reported in parts per million (δ) and signals are described as singlet (s), doublet (d), broad and multiplet (m). FAB mass spectra were recorded on Angilent 1100 ESI-Mass (Turbo spray) spectrometer. Elemental analysis was carried out using Carlo Erba 1108 elemental analyzer. Solvents and reagents were purchased from the commercial vendors in the appropriate grade and were used as received.

Synthesis of nicotino hydrazide (1)

Mixture of ethyl nicotinate (0.1M) and hydrazine hydrate (0.2M) in ethanol and refluxed for 16hrs. The resulting reaction mixture was concentrated to half of the volume and poured it in to the crushed ice. The formed precipitate was separated and recrystallised from ethanol. m.p; 130-132^{0}C, yield; 86%, IR (KBr, cm-1); 3325 (N-H, str), 1661 (C=O, str), 1587 (C=N, str), H^1NMR (δ ppm); 9.58 (1H, s, NH), 4.58 (2H, s, NH_2), 7.42-8.98 (4H, m, Ar-H).

General procedure for the synthesis of Schiff bases of N^1- (substituted benzilidine) – pyridin-3-yl –carbohydrazide (2a-n)

Equimolar mixture of compound (1) and substituted aromatic benzaldehydes in ethanol by the addition of few drops of glacial acetic acid and were refluxed for 6hrs. The resulting mixture was poured in to ice cold water. The obtained product was filtered, dried and recrystallised from ethanol (**Scheme-I**).



(Scheme-1)

R:

2a: *p-fluro*; **2b**: *p-bromo*; **2c**: *p-methyl*; **2d**: *p-hydroxy*; **2e**: *o-hydroxy*; **2f**: *p-nitro*; **2g**: *5-nitro, 2-hydroxy*; **2h**: *p-dimethylamino*; **2i**: *p-chloro*; **2j**: *m-bromo*; **2k**: *o-chloro*; **2l**: *o-bromo*; **2m**: *p-methoxy*; **2n**: *o-nitro*

2a: N^1- (4^1 - fluoro benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 180-182^{0}C; yield (%)**:** 98.5; Rf**:** 0.71; molecular weight**:** 243; IR (cm-^{1}, KBr) : 3462 (N-H, str), 1566 (N-H; def), 3180 (=C-H, str), 1658 (C=O, str), 1595 (C=N, str), 1413 (C-N, str), 1133 (C-F, str); H^1 NMR (δ ppm): 12.04 (1H, s, N-H), 9.09 (1H, s, =C-H), 7.2-8.96 (8H, m, Ar-H); C^{13}NMR (δ ppm): 161.98 (C=O), 152.12 (C-1), 130 (C-2), 137 (C-3), 149 (C-5), 149 (=CH), 135 (C^1), 123 (C^2&C^6), 115 (C^3& C^5), 164 (C^4); Mass (*m/z*): 242, (M-H); Anal: Calcd. for $C_{13}H_{10}N_3OF$: C: 64.19, H: 4.11, N: 17.28, found: C: 64.22, H: 4.09, N: 17.30.

2b: N^1- (4^1 - bromo benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 190-192^{0}C; yield (%) : 98.2; Rf: 0.53; molecular weight: 304; IR (cm-1): 3379 (N-H, str), 3176 (=C-H, str), 1676 (C=O, str), 1589 (C=N, str), 667 (C-Br, str); H^1 NMR (δ ppm): 12.08 (1H, s, N-H), 9.07 (1H, s,=C-H), 7.5 - 8.7 (8H,m, Ar-H). Mass (*m/z*): 305, M+1. Anal: Calcd. for $C_{13}H_{10}N_3OBr$: C: 51.31, H: 3.28, N: 13.81, found: C: 51.33, H: 3.25, N: 13.83

2c: N^1- (4^1 - methyl benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 90-92^{0}C; yield (%) : 97.7; Rf: 0.65; molecular weight: 239; IR (cm-1): 3377 (N-H, str), 3184.5 (=C-H, str), 1671 (C=O, str), 1585 (C=N, str), 3020 (C-H, str); H^1 NMR (δppm) :11.95 (1H, s, N-H),9.08 (=C-H), 2.51 (3H, s,-CH_3),7.23-8.77 (8H, m, Ar-H). Mass (*m/z*): 240, M+1. Anal: Calcd. for $C_{14}H_{13}N_3O$: C: 70.29, H: 5.44, N: 17.57; found: C: 70.31, H: 5.46, N: 17.55.

2d: N^1- (4^1 - hydroxy benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 230-232^{0}C; yield (%) : 92.5; Rf: 0.81; molecular weight: 241; IR (cm-1): 3389 (N-H, str), 3073 (=C-H, str), 1657 (C=O, str), 1591 (C=N, str); H^1 NMR (δ ppm) : 11.81(1H, s, N-H), 9.06 (1H,s, =C- H), 9.95 (1H, s ,Ar- OH), 6.79-8.76 (8H, m, Ar-H). Mass (*m/z*): 242, M+1. Anal: Calcd. for $C_{13}H_{11}N_3O_2$: C: 64.73, H: 4.56, N: 17.43; found: C: 64.75, H: 4.58, N:17.42

2e: N^1- (2^1 - hydroxy benzilidine) – pyridin-3-yl - carbohydrazide:

m.p: 172-174^{0}C; yield (%) : 87.9; Rf: 0.45; molecular weight: 241; IR (cm-1): 3485 (N-H, str), 3056 (=C-H, str), 1644 (C=O, str), 1565 (C=N, str); H^1 NMR (δppm): 12.24 (1H, s, N-H), 9.10 (1H, s, =C-H), 11.15 (Ar-OH), 6.92-8.79 (8H,m,Ar-H). Mass (*m/z*): 242, M+1. Anal: Calcd. for $C_{13}H_{11}N_3O_2$: C: 64.72, H: 4.57, N: 17.42; found: C: 64.76, H: 4.59, N:17.41

2f: N^1- (4^1 - nitro benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 250-252^{0}C; yield (%) : 68; Rf: 0.78; molecularweight: 270; IR (cm-1): 3413 (N-H, str), 3184(=C-H, str), 1661 (C=O, str), 1572 (C=N, str), 1418 (Antisym, N=O), 1340 (Sym, N=O); H^1 NMR (δ ppm): 12.31 (1H, s, N-H), 9.1 (1H, s, =C-H), 7.58-8.79 (8H, m, Ar-H). Mass (*m/z*): 271, M+1. Anal: Calcd. for $C_{13}H_{10}N_4O_3$: C: 57.78, H: 3.70, N: 20.74; found: C: 57.80, H: 3.72, N: 20.69.

2g: N^1- (5^1 – nitro-2^1 –hydroxy benzilidine) – pyridin-3-yl -carbohydrazide

m.p: 260-262^{0}C; yield (%) : 78; Rf**:** 0.55; molecular weight: 286; IR (cm-1): 3303 (N-H, str), 3075 (=C-H, str), 1672 (C=O, str), 1552 (C=N, str), 1482 (Antisym, N=O), 1336 (Sym, N=O); H^1 NMR (δ ppm) 12.40 (1H, s, N-H), 9.12 (1H, s, =C-H), 7.13-8.76 (7H; Ar-H). Mass (*m/z*): 287, M+1. Anal: Calcd. for $C_{13}H_{10}N_4O_4$: C: 54.54, H: 3.50, N: 19.58; found: C: 54.56, H: 3.52, N: 19.60.

2h: N^1- (4^1 - dimethylamino benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 140-142^{0}C; yield (%) : 94; Rf: 0.67; molecular weight: 268; IR (cm-1): 3439 (N-H, str), 3188 (=C-H, str), 1680 (C=O, str), 1590 (C=N, str), 1365 (C-N, str); H^1 NMR (δ -ppm): 11.72 (N-H), 9.06 (=C-H), 2.51-3.35 (6H, S, (-CH_3)$_2$), 6.75-8.75 (8H; Ar-H). Mass (*m/z*): 269, M+1. Anal: Calcd. for $C_{15}H_{16}N_4O$: C: 67.16, H: 5.97, N:20.89; found: C: 67.18, H: 6.01, N:20.90

2i: N^1- (4^1 - chloro benzilidine) – pyridin-3-yl - carbohydrazide:

m.p: 230-232^{0}C; yield (%) 89.5; Rf**:** 0.38; molecular weight: 259; IR (cm-1): 3431 (N-H, str), 3256 (=C-H, str), 1660 (C=O, str),1592 (C=N, str), 705 (C-Cl, str); H^1 NMR (δ ppm): 12.08 (1H, S, N-H) , 9.08 (1H, S,=C-H), 7.53-8.78 (8H; Ar-H). Mass (*m/z*): 260, M+1. Anal: Calcd. for $C_{13}H_{10}N_3OCl$: C: 60.23, H: 3.86, N:16.21; found: C:60.25, H: 3.89, N: 16.22.

2j: N^1- (3^1 - bromo benzilidine) – pyridin-3-yl – carbohydrazide

m.p:100-102^{0}C; yield(%) : 88; Rf : 0.53; molecular weight : 304; IR (cm-1)**:** 3439 (N-H, str), 3152 (=C-H, str), 1668 (C=O, str), 1599 (C=N, str), 683 (C- Br, str); H^1 NMR (δ ppm) : 12.15(1H, S, N-H), 9.08 (1H, S,=C-H), 7.42-8.77 (8H; Ar-H). Mass (*m/z*): 305, M+1. Anal: Calcd. for C_{13} $H_{10}N_3OBr$: C: 51.31, H: 3.28, N: 13.81; found: C: 51.32, H: 3.30, N: 13.79.

2k: N^1- (2^1 - chloro benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 150-152^{0}C; yield (%): 99.3; Rf**:** 0.48; molecular weight: 259; IR (cm-1) 3568 (N-H, str), 3178 (=C-H, str), 1674 (C=O, str),1595 (C=N, str), 763 (C-Cl, str); H^1 NMR (δ ppm): 12.23 (1H, S, N-H), 9.10 (1H, S, =C-H), 7.42-8.87 (8H; Ar-H). Mass (*m/z*): 260, M+1. Anal: Calcd. for $C_{13}H_{10}N_3OCl$: C: 60.23, H: 3.86, N: 16.21; found: C: 60.25, H: 3.90, N: 16.19.

2l: N^1- (2^1 - bromo benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 160-162^{0}C; yield (%) : 56; Rf: 0.51; molecular weight: 304; IR (cm-1): 3380 (N-H, str), 3210 (=C-H,

str), 1686 (C=O, str), 1589 (C=N, str), 689 (C-Br, str); H^1 NMR (δ ppm): 12.02 (1H, s, N-H), 9.04 (1H, s,=C-H), 7.6 - 8.92 (8H,m,Ar-H). Mass (*m/z*): 305, M+1. Anal: Calcd. for $C_{13}H_{10}N_3OBr$: C: 51.28, H: 3.26, N: 13.80; found: C: 51.23, H: 3.23, N: 13.80.

2m: N^1- (4^1 - methoxy benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 241-243^{0}C; yield (%): 75; Rf**:** 0.42; molecular weight: 255; IR (cm-1) 3460 (N-H, str), 3158 (=C-H, str), 1665 (C=O, str),1585 (C=N, str); H^1 NMR (δ ppm): 12.03 (1H, s, N-H), 9.06 (1H, s, =C-H), 3.26 (3H, s, OCH3), 7.40-8.67 (8H, m, Ar-H). Mass (*m/z*): 256, M+1. Anal: Calcd. for $C_{14}H_{13}N_3O_2$: C: 65.88, H: 5.09, N: 16.47; found: C: 65.92, H: 5.12, N: 16.45.

2n: N^1- (2^1 - nitro benzilidine) – pyridin-3-yl – carbohydrazide

m.p: 186-188^{0}C; yield (%) : 88; Rf: 0.76; molecularweight: 270; IR (cm-1): 3413 (N-H), 1513 (N-H; def), 3184(=C-H), 1661 (C=O), 1572 (C=N), 1418 (Anti N=O), 1340 (Sym N=O); H^1 NMR (δ ppm): 12.31 (1H, S, N-H), 9.1 (1H, S, =C-H), 7.58-8.79 (8H; Ar-H). Mass (*m/z*): 271, M+1. Anal: Calcd. for $C_{13}H_{10}N_4O_3$: C: 57.77, H: 3.72, N: 20.64; found: C: 57.78, H: 3.64, N: 20.56.

ANTIOXIDANT ACTIVITY[12, 13]

All the newly synthesized compounds were tested for their in vitro free radical scavenging Nitric oxide (NO).

NITRIC OXIDE (NO) SCAVENGING

The nitric oxide radical scavenging activity was measured by using Griess' reagent. 5mL each of synthesized derivatives and ascorbic acid (standard) of different concentrations (25–200 μg/mL) in standard phosphate buffer solution (pH 7.4) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5 hours. Control was prepared without compound but with equivalent amount of buffer. After incubation, 0.5mL of the incubation mixture was mixed with 0.5 mL of Griess' reagent (1%Sulphanilamide, 2% o-phosphoric acid and naphthyl ethylene diamine dihydrochloride 0.1%) and the absorbance was measured at 546nm against blank (DMSO). From the absorbance the percent scavenging activity was calculated using the same formula as described above. The experiments were performed in triplicate.

RESULTS &DISCUSSION

In this study i reported the synthesis, characterization of some new Schiff bases and screened for their antioxidant activity. The nicotino hydrazide (1) was prepared by reacting ethylnicotinate and hydrazine hydrate in ethanol. The reaction mixture was refluxed for 16 hrs and it was confirmed by TLC and spectral data. The IR spectrum of the compound showed intence peaks at 3325 cm-1 for NH, 1661 cm-1 for carbonyl (C=O) stretching. H^1NMR spectra of the compound (1) showed a singlet at δ 9.58 indicating the presence of NH for proton (1H), singlet at δ 4.58 indicating the presence of NH_2 for protons (2H) and multiplet at δ 7.42-8.98 for aromatic protons (4H). Data from the elemental analysis and molecular ion recorded in the mass spectra further confirmed the structure of the compound (**1**).the Schiff bases of the title compounds (2a-k) were synthesized by condensation of nicotine hydrazide (**1**) with different substituted aromatic aldehydes in equimolar ratio. The formation of the title compounds is indicated by the disappearance of peak due to NH_2 of the starting material in IR and H^1NMR spectrum of all the compounds. The IR spectrum showed the presence of peak absorbed in between at 1552-1599 cm-1, 3055-3180 cm-1 due to -C=N stretching, =C-H stretching and H^1NMR spectrum showed the presence of singlet at δ 9.06-9.10 due to =CH group, and aryl groups of the all synthesized compounds. The mass spectrum of the compound showed molecular ion peaks corresponding to their molecular formula. The elemental (C, H, N) analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

All the synthesized compounds **(2a-n)** were evaluated for antioxidant activity using nitric oxide method. The nitric oxide assay has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. Nitric oxide generated as a result of decomposition of sodium nitroprusside in aq.medium, interacts with oxygen at physiological PH to produce nitrite ions, which are measured by using Griess' reagent. The nitrite ions were subjected to diazotization followed by azo coupling reaction to yield an azo dye, measured by an absorption band at 546nm. The scavenging ability of the synthesized compounds was compared with ascorbic acid as a standard. Compounds **2a**, **2c**, **2d**, **2e**, **2i** and **2m** produced better scavenging ability (**Table-1**). This may be due to the presence of *p-fluro, p-methyl, p-hydroxy, o-hydroxy*, *p-chloro and p-methoxy* groups. Rest of the compounds showed moderate radical scavenging activity was expressed as a percentage and was calculated using the following formula.

$$\% \text{ scavenging} = Ac - As/Ac \times 100$$

Whare as As = the absorbance of the test sample

Ac = the absorbance of the control.

Table:1. % scavenging activity of Schiff bases (2a-n) by Nitric Oxide method.

S.No	Concen. (µg/mL)	Standard	Control	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	2l	2m	2n
1	25	23.08	6.01	15.26	8.04	13.87	14.87	09.34	05.14	07.08	08.78	14.76	09.03	09.14	08.08	18.3	08.38
2	50	34.64	6.01	26.8	14.95	21.67	26.34	18.43	15.24	13.87	13.03	25.54	13.43	15.15	14.87	27.83	15.03
3	75	45.17	6.01	38.4	24.14	34.45	37.74	28.45	24.06	25.98	26.23	36.23	19.08	26.13	25.88	40.21	26.27
4	100	59.29	6.01	50.1	30.86	49.97	49.95	37.98	47.92	33.24	32.44	49.45	25.06	35.23	33.24	52.29	34.44
5	125	67.43	6.01	59.2	42.88	60.16	60.09	47.85	58.8	40.01	51.87	58.54	45.98	41.28	40.01	61.45	45.87
6	150	79.97	6.01	67.45	45.46	66.21	72.76	56.98	49.86	53.87	53.43	65.43	57.03	49.04	46.87	71.53	52.43
7	175	87.76	6.01	79.86	56.86	77.98	84.39	69.87	50.24	70.45	71.43	78.86	68.88	60.89	59.45	78.18	61.43
8	200	99.30	6.01	95.68	54.64	84.17	94.78	80.02	56.68	76.45	77.43	92.14	72.07	74.02	65.43	89.12	68.43

CONCLUSIONS

In conclusion, the synthesized new title compounds **(2a-n)** are characterized by spectral data and evaluate for their antioxidant activity. Among the synthesized compounds **2a**, **2c**, **2d**, **2e**, **2f** and **2i,** which are having electron donating groups on the phenyl ring exhibiting good activity with maximum scavenging free radical. Therefore, the series has opened new doors for possible modifications of the pharmacophoric replacements of antioxidant and future exploitation.

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