Synthesis and Antioxidant Evaluation of Schiff Bases Derived from 2,6-Pyridinedicarboxylic Acid

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Received November 15, 2011: Revised February 25, 2012: Accepted May 03, 2012

Abstract: A series of novel Schiff bases derived from dipicolinic acid was synthesized and evaluated for antioxidant and iron chelating activity. In most cases Schiff bases derived from di-hydrazide showed higher antioxidant activity than the ones derived from mono-hydrazide and some of them are very promising considering the fact of being better antioxidants than the standards used in the investigation. For iron chelating activity substituents had a greater impact on the activity than mono or di-hydrazide skeleton.

Keywords: Antioxidant, dipicolinic acid, metal chelating, Schiff base.

1. INTRODUCTION

Dipicolinic acid (2,6-pyridinedicarboxylic acid) was first discovered in a biological system in 1936 and is now known to be a major component of bacterial spores [1], where it occurs in substational amounts (5-14% dry weight), including the species of *Bacillus* and *Clostridium* [2]. It appears to contribute to the resistance of the spores to UV radiation [1] and to be important for their stability and germination. Dipicolinic acid is present in nature as an oxidative degradation product of vitamins, coenzymes and alkaloid and is a component of fulvic acid [3] and it has frequently been cited in the literature as a plant sterilizing and water germicidal agent [3].

It shows various biological functions including activation/inactivation of some metalloenzymes, inhibition of electron transport system [4], inhibition of lipid peroxidation and protection of glutathione reductase from the copper-dependent inactivation [4] and is known to be an antioxidant for ascorbic acid in foods as well [3].

Dipicolinic acid, synthesized in large amount in the spore of genus *Bacillus*, is a potent metal-chelator, so the complexation of metal ions by 2,6-pyridinedicarboxylic acid has been extensively investigated and due to its low toxicity and amphophilic nature, it is a desirable metal ion ligand [5]. Dipicolinic acid is known to form stable chelates with metal ions and oxometal cations and can display widely varying coordination demeanor functioning as a bidentate, tridentate, meridian or bridging ligand [6].

Other interesting properties are its biological activity [7], like its ability to stabilize unusual oxidation states [8]. The iron complexes have also been used as well as specific molecular tools in DNA cleavage [9]. In general, substituted pyridine derivatives show antimicrobial, anti-inflammatory and antitumor activities and various 2,6-bis-substituted pyridine derivatives also show antibacterial activities [10].

Compounds with the structure of –C=N– (azomethine group) are known as Schiff bases, which are usually synthesized from the condensation of primary amines and active carbonyl groups and they have been of great interest for many years. They represent an important class of compounds in medicinal and pharmaceutical field, showing biological applications including antibacterial [11-14], antifungal [12-15] and antitumor activity [11]. These compounds play an important role in the development of coordination chemistry related to catalysis and enzymatic reactions, magnetism and molecular architectures [16]. A. González, *et al.* [17] have synthesized tin(IV) complexes derived from pyridine Schiff bases and some of them have shown an excellent antioxidant activity in rat brain homogenate on inhibition of thiobarbituric acid reactive substances.

Our work was based on the fact that reactive oxygen species (ROS), which are generated in living organisms as by-products of many metabolic reactions, can cause oxidative damage which is associated with many diseases. Also, transition metals can stimulate lipid peroxidation, thus the chelating of such species would have a significant role in lipid peroxidation prevention. So, except for the synthesis of dipicolinic acid derivatives, our investigation comprised of antioxidant and metal chelating investigation of these compounds.

2. CHEMISTRY

The synthesis of the target compounds was carried out as outlined in Fig. (**1**). The starting compound (dimethyl pyridine-2,6-dicarboxylate) (**2**) was prepared by esterification of pyridine-2,6-dicarboxylic acid (**1**). Compound **2** in reaction with 86% hydrazine hydrate in methanol at room temperature gave a compound, which was a mixture of the major product, 6-methyloxycarbonyl-2-pyridinecarboxylic acid hydrazide (so-called mono-hydrazide), and a minor product,

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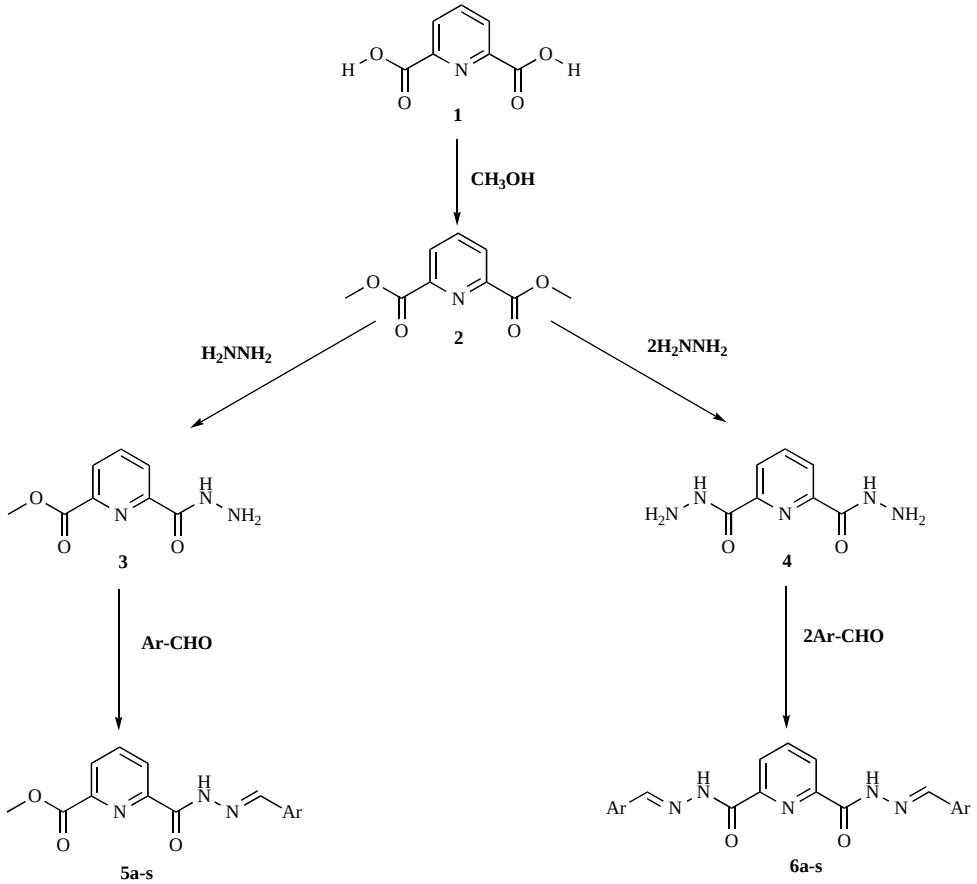

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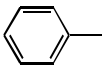
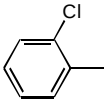
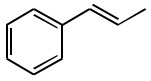
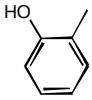
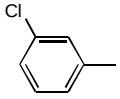
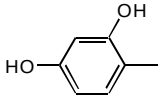
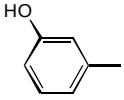
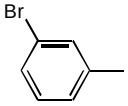
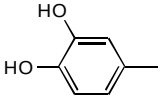
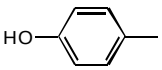
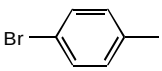
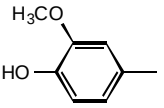
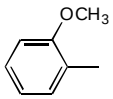
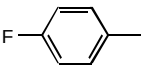
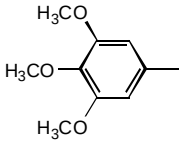
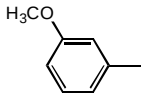
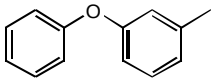
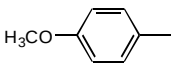
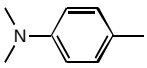
a		**h**		**o**	
b		**i**		**p**	
c		**j**		**q**	
d		**k**		**r**	
e		**l**		**s**	
f		**m**			
g		**n**			

Fig. (1). Synthesis of Schiff bases derived from dipicolinic acid.

2,6-pyridinedicarboxylic acid di-hydrazide (so-called di-hydrazide). Mono-hydrazide was separated from di-hydrazide by recrystallization from ethanol. Both, mono (**3**) and di-hydrazide (**4**), in reaction with aromatic aldehydes gave Schiff bases **5a-s** and **6a-s**.

3. PHARMACOLOGY

3.1. Scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl radical)

Determination of antioxidant activity was performed according to the procedure described in the literature [18, 19] with some modifications. DMSO (dimethyl sulfoxide) was used as a solvent [20], due to the low solubility of synthesized compounds in ethanol and methanol.

DMSO solution of the corresponding synthesized compound (0.75 mL; 0.2 mM) was added to a DMSO solution of DPPH radical (0.2 mM), so that the final concentration of DPPH radical and the synthesized compound in a solution was 0.1 mM. The mixture was shaken and allowed to stand at room temperature. After 30 min the absorbance at 517 nm was determined and the scavenging activity was calculated according to the formula below. Ascorbic acid (AA) was used as a reference compound.

$$\text{scavenging actvitiy (\%)} = ((A_b + A_s - A_m)/A_b)*100$$

A_b – absorbance of 0.1 mM DMSO solution of DPPH radical at 517 nm

A_s – absorbance of 0.1 mM DMSO solution of test compound at 517 nm

A_m – absorbance of DMSO mixture of test compound and DPPH radical at 517 nm

3.2. Evaluation of Antioxidant Activity by Phosphomolybdenum Method

The antioxidant activity of tested dipicolinic acid derivatives was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999) [21]. This method is based on the reduction of Mo(VI) to Mo(V) by the tested compounds followed by formation of green phosphate/Mo(V) complex at acid pH. An aliquot of 100 μL of sample solution (2 mM in DMSO) is mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples are incubated in a water bath at 95 °C for 90 minutes. Samples are cooled to room temperature and the absorbance was measured at 695 nm. The antioxidant activity was expressed relative to the antioxidant activity of the same concentration of ascorbic acid, on molar basis.

3.3. Iron Chelating Activity

The chelating activity of dipicolinic acid derivatives for ferrous ions Fe^{2+} was measured according to the literature method [22] with some modifications. Solution of $FeCl_2$ (0.025 mL; 2 mM) was added to 1 mL 2 mM methanol/DMSO solution (4:1) of the compound investigated. After 30 s, 0.05 mL of ferrozine (5 mM) was added. Samples were incubated at room temperature for 10 min and the absorbance of the complex formed between Fe^{2+} and ferrozine was measured at 562 nm. Metal chelating efficiency of samples was compared to the chelating activtiy of EDTA disodium salt. The chelating activity of the compound for Fe^{2+} was calculated as:

$$\text{chelating activity} = [(A_0 - A_1)/ A_0]\cdot 100\%$$

A_0 - absorbance of the control (blank, without samples) at 562 nm

A_1 - absorbance in the presence of the methanol/DMSO sample solution at 562 nm

4. RESULTS AND DISCUSSION

4.1. Antioxidant Activity

In the DPPH and phosphomolybdenum assay methods hydrogen and electron transfer from antioxidants to $DPPH^{\cdot}$ and Mo(VI) complex occurs. These transfers depened on the structure of antioxidants. The reason we used ascorbic acid as standard in both antioxidant investigations is that ascorbic acid has the ability to donate hydrogen and electrons and can be detected by both assay models.

4.1.1. Scavenging of 1,1-diphenyl-2-picrylhydrazyl Radical

DPPH free radical, which possesses an odd electron, gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The method used in this work applies to the overall antioxidant capacity of the sample after 30 minutes of incubation. DPPH scavenging activity was determined against ascorbic acid as a standard compound.

Data in Table **1** show that most of the Schiff bases derived from dipocolinic acid do not show significant scavenging activity on DPPH radical. The only exceptions are compounds **6q** and **5q** with scavenging activity around 50% and **6r** and **5r** with a scavenging activity 42% and 27%. In both cases, if we compare Schiff bases with the same -Ar, the ones derived from di-hydrazide showed higher scavenging activity than the ones derived from mono-hydrazide. The best scavenging activity is accomplished with the compound **6q**, Schiff base with 3,4-dihydroxyphenyl substituent, followed by the compound **5q**, with the same substituent, but derived from mono-hydrazide. It is obvious that in case of dipicolinic acid Schiff bases, *m*-, *p*-substitution on phenyl ring, with electron donating groups, is important for DPPH scavenging activity, since, only the compounds with this kind of substitution show moderate scavenging activity in comparison with all the rest, whose activity is not significant.

4.1.2. Evaluation of Antioxidant Activity by Phosphomolybdenum Method

Data in Fig. (**2**) show that substituents on phenyl ring have a great influence on antioxidant activity. The effects of various substituents on phenyl ring of Schiff bases derived from mono-hydrazide in descending order were found to be: 2-OCH_3 (**5e**) > H (**5a**) > 3-OH (**5c**) > 3-OCH_3 (**5f**) > 3-Cl (**5i**) > 4-OCH_3 (**5g**) > styryl (**5o**) > 2-OH (**5b**) > 4-F (**5l**) >

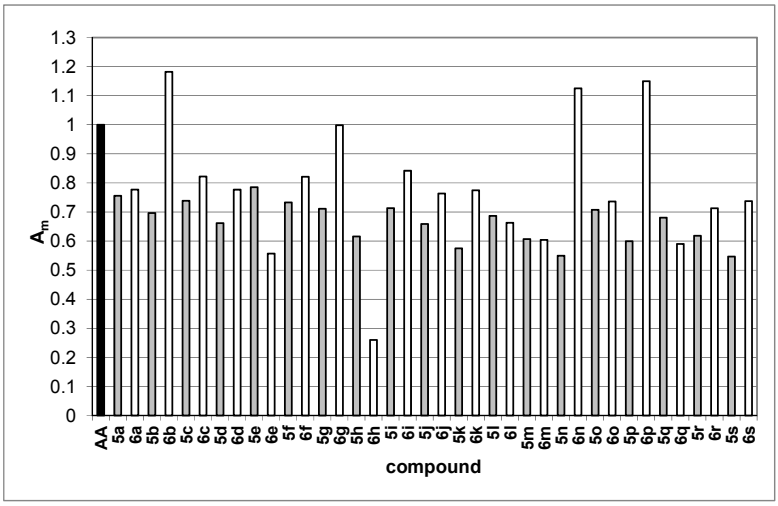


Fig. (2). Antioxidant activities of Schiff bases derived from dipicolinic acid relative to ascorbic acid (A_m – activity relative to ascorbic acid (AA) on a molar basis).

Table 1. DPPH Radical Scavenging Activity of Dipicolinic Acid Derivatives 5a-s and 6a-s [a]

Compound	Scavenging Activity on DPPH (%)	Compound	Scavenging Activity on DPPH (%)
AA[b]	**90.01**	**AA[b]**	**90.01**
5a	-	**6j**	-
6a	**0.49**	**5k**	**0.32**
5b	**7.84**	**6k**	-
6b	**6.06**	**5l**	-
5c	**3.82**	**6l**	-
6c	**5.02**	**5m**	-
5d	**7.00**	**6m**	**0.14**
6d	**6.83**	**5n**	**9.02**
5e	**6.11**	**6n**	**7.49**
6e	**4.69**	**5o**	-
5f	**4.79**	**6o**	**0.38**
6f	**5.60**	**5p**	**7.17**
5g	**5.07**	**6p**	**7.49**
6g	**5.86**	**5q**	**49.24**
5h	-	**6q**	**53.99**
6h	-	**5r**	**26.89**
5i	**4.53**	**6r**	**41.74**
6i	**4.05**	**6s**	**5.45**
5j	-	**5s**	-

[a]Data are means of three replicates.
[b]Ascorbic acid is used as standard.
- Indicated compounds are inactive.

3,4-$(OH)_2$ (**5q**)> 4-OH (**5d**) > 3-Br (**5j**) > 4-OH-3-OCH_3 (**5r**) > 2-Cl (**5h**) > 3-phenoxy (**5m**)> 2,4-$(OH)_2$ (**5p**) > 4-Br (**5k**) > 4-N,N-$(CH_3)_2$ (**5n**) > 3,4,5-$(OCH_3)_3$ (**5s**).

The effects of various substituents on phenyl ring of Schiff bases derived from di-hydrazide in descending order were found to be: 2-OH (**6b**) > 2,4-$(OH)_2$ (**6p**) > 4-N,N-$(CH_3)_2$ (**6n**) > 4-OCH_3 (**6g**) > 3-Cl (**6i**) > 3-OH (**6c**) > 3-OCH_3 (**6f**) > H(**6a**) > 4-OH (**6d**) > 4-Br (**6k**)> 3-Br (**6j**) > 3,4,5-$(OCH_3)_3$ (**6s**) > styryl (**6o**) > 4-OH-3-OCH_3 (**6r**) > 4-F (**6l**)> 3-phenoxy (**6m**) > 3,4-$(OH)_2$ (**6q**) > 2-OCH_3 (**6e**) > 2-Cl(**6h**).

In general, Schiff bases derived from di-hydrazide show better antioxidant activity, than the ones derived from mono-hydrazide, with the exception of **6l**, **6m**, **6q**, **6e** and **6h**.

Schiff bases **6b**, **6p**, **6n** and **6g** showed excellent antioxidant activity, compounds **6b**, **6p**, **6n** better than ascorbic acid, 1.18, 1.5 and 1.13 times better, respectively, and **6g** the same as ascorbic acid. None of Schiff bases derived from mono-hydrazide showed better antioxidant activity than ascorbic acid. All of the compounds having greater antioxidant activity in comparison to ascorbic acid, posses one or two electron donating groups in *ortho* (**6b**) or *para* (**6n**) position or in *ortho* and *para* position (**6p**). Considering all data above, it is obvious that substituents on phenyl ring have a different influence on antioxidant activity in Shiff bases derived from mono-hydrazydes and ones derived from di-hydrazides.

When comparing the results gained by two different antioxidant assays performed in this work, DPPH and phosphomolybdenum assay respectively, they appeared to be different. It is expected because completely different and complex mechanisms and conditions are involved and thus the results are different, which is also in agreement with other authors [23, 24].

4.2. Iron Chelating Activity

This investigation is based upon complex formation between ferrozine and Fe^{2+}, which is red in colour. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Therefore, the chelating activity of the coexisting chelator is estimated by measurement of colour reduction at 562 nm.

Table 2. Ferrous ion Chelating Activity of Dipicolinic Acid Derivatives 5a-s and 6a-s [a,c]

Compound	Chelating Activity (%)
EDTA[b]	97.43
5a	8.57
5b	25.14
5c	10.24
6c	4.15
5d	5.68
5e	0.56
6e	7.19
5f	4.80
5g	3.12
6g	11.14
6i	7.66
5j	11.27
6j	1.90
5k	6.25
5l	4.89
5o	0.38
5p	63.74
6p	20.48

[a]Data are means of three replicates.
[b]EDTA disodium salt is used as standard.
[c]Compounds not listed in the Table did not show chelating activity.

The formation of red coloured complex was inhibited by compounds **5p**, **5b**, **6p**, **5j**, **6g**. These compounds showed moderate chelating activity, but not comparable with EDTA. All of these compounds possess one or two electron donating groups in *o*- and *p*- position, with the exception of **5j**, which possesses electron withdrawing –Br group in *m*- position. Addition of electron donating groups has been proven to enhance iron chelating activity [25]. Compounds showing the best activity, **5p**, **5b** and **6p,** bear hydroxyl group in position 2 of aryl ring, which could be significant because lots of synthetic iron chelators posses the same moiety in the same position [25]. Other compounds showed low or no chelating activity under these conditions.

5. CONCLUSION

The present work comprises the synthesis of dipicolinic acid derivatives known as Schiff bases. The investigation includes antioxidant and chelating activity determination of the compounds above. For DPPH scavenging activity as well as antioxidant activity determined by phosphomolybdenum method compounds derived from di-hydrazide showed higher scavenging activity than the ones derived from mono-hydrazide. It is obvious that the position and electron donating groups had a significant influence on all the activities investigated.

6. EXPERIMENTAL

6.1. Materials and Methods

Melting points were determined on Electrothermal Capillary melting point apparatus and are uncorrected. Thin-layer chromatography was performed with fluorescent silica gel plates HF_{254} Merck, which were checked under UV 254 and 365 nm light, using benzene:acetone:acetic acid (8:1:1) as a solvent. The elemental analysis for C, H and N was done on a Perkin-Elmer Analyzer 2440. Infrared spectra (ν_{max}-cm^{-1}) were recorded on a Beckmann FT-IR 3303, using KBr disks. ^1H NMR spectra were recorded on JEOL EX-270 MHz NMR Spectrometer at 293 K in DMSO-d6. The MS spectra were recorded on LC/MS/MS (API 2000). The absorbance was measured on UV visible spectrophotometer Helios γ, (Thermo Spectronic, Cambridge UK).

6.2. Preparation of Dimethyl pyridine-2,6-dicarboxylate(2)

To a solution of dipicolinic acid (10 mmol) in 40 mL of methanol, 1.5-1.8 mL of sulphuric acid is added. Mixture is refluxed for 2-3 hours and left at room temperature, until crystals are formed. Crystals are filtered and recrystallized from ethanol.

Yield (94%), M.p. 121 ℃, R_f = 0.57. FT-IR (KBr) ν_{max} (cm^{-1}): 3450, 3063, 2968, 1742, 1572, 1450, 1290, 1244, 1165, 995, 758 and 696; ^1H NMR δ (ppm): 3.68 (s, 6H, $2OCH_3$), 8.03 (t, 1H, py-H); 8.26 (d, 2H, py-H), MS *m/z:* 196.0 [M + H^+], (M =195.18); Anal. Calcd. for $C_9H_9NO_4$ (195.05): C, 55.39; H, 4.65; N, 7.18%; Found: C, 55.30; H, 4.61; N, 7.21.

6.3. Preparation of 6-methyloxycarbonyl-2-pyridinecarboxylic acid hydrazide(3) and pyridine-2,6-dicarbohydrazide (4) [26]

Anhydrous hydrazine (98 %, 3.27 g, 0.200 mol in 150 mL methanol) was added dropwise to a stirred warm solution of dimethylpyridine-2,6-dicarboxylate (19.8 g; 0.102 mol in 500 mL methanol) over a period of 18 h. A white crystalline solid formed during the reaction, was filtered off, washed with methanol and dried under vacuum. The resulting compound is a mixture of 6-methyloxycarbonyl-2-pyridinecarboxylic acid hydrazide (mono-hydrazide) and pyridine-2,6-dicarbohydrazide (di-hydrazide), approximately 9:1 and they are separated by recrystallization from ethanol.

6.3.1. 6-Methyloxycarbonyl-2-pyridinecarboxylic acid hydrazide(3)

Yield (85%), M.p. 183-184 ℃, R_f = 0.14. FT-IR (KBr) ν_{max} (cm^{-1}): 3433, 3371, 3043, 1740, 1686, 1510, 1428, 1274, 1250, 1158, 987, 739 and 664; ^{1}H NMR δ (ppm): 3.69 (s, 3H, OCH_3), 4.52 (d, 2H, NH_2), 8.12 (t, 1H, py-H), 8.16 (d, 2H, py-H), 9.86 (s, 1H, NH); MS *m/z:* 196.0 [M + H^+], (M =195.18); Anal. Calcd. for $C_7H_9N_5O_2$ (195.08): C, 43.08; H, 4.65; N, 35.88%; Found: C, 43.01; H, 4.63; N, 35.91%.

6.3.2. Pyridine-2,6-dicarbohydrazide(4)

Yield (10%), M.p. 284 ℃, R_f = 0.03. FT-IR (KBr) ν_{max} (cm^{-1}): 3333, 3271, 3063, 1747, 1676, 1514, 1438, 1294, 1255, 1157, 989, 729 and 665; ^{1}H NMR δ (ppm): 4.53 (d, 4H, $2NH_2$), 8.01 (t, 1H, py-H); 8.26 (d, 2H, py-H), 9.96 (s, 2H, 2NH); MS *m/z:* 196.1 [M + H^+], (M =195.18); Anal. Calcd. for $C_7H_9N_5O_2$ (195.08): C, 43.08; H, 4.65; N, 35.88%; Found: C, 43.01; H, 4.63; N, 35.91%.

6.4. Preparation of Schiff Bases from Mono-hydrazide (5a – s)

Equimolar amount of 6-methyloxycarbonyl-2-pyridinecarboxylic acid hydrazide (**3**) and appropriate aromatic aldehyde (**a – s**) in ethanol, with a few drops of acetic acid, is refluxed for 3 hours. After cooling, the solution is poured onto crushed ice and filtered. Crude product is recrystallized from ethanol.

6.4.1. 6-Benzylidenehydrazinocarbonyl-2-methoxycarbonyl-pyridine(5a)

Yield (83%), M.p. 105 ℃, R_f = 0.63. FT-IR (KBr) ν_{max} (cm^{-1}): 3518, 3468, 3201, 3049, 1728, 1670, 1537, 1437, 1367, 1300, 1240, 1165, 1108 and 685; ^{1}H NMR δ (ppm): 3.69 (s, 3H,

OCH_3), 7.22-7.80 (m, 5H, arom.), 8.12 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.56 (s, H, N=CH), 11.81 (s, H, NH); MS *m/z:* 284.0 [M + H^+], (M = 283.28); Anal. Calcd. for $C_{21}H_{17}N_5O_2$ (283.14): C, 67.91; H, 4.61; N, 18.86%; Found: C, 67.89; H, 4.62; N, 18.80%.

6.4.2. 6-[(2-hydroxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine(5b)

Yield (88%), M.p. 164 ℃, R_f = 0.46. FT-IR (KBr) ν_{max} (cm^{-1}): 3516, 3437, 2997, 1724, 1668, 1620, 1535, 1240, 1157, 750 and 684; ^{1}H NMR δ (ppm): 3.70 (s, 3H, OCH_3), 7.02-7.66 (m, 4H, arom.), 7.92 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.78 (s, H, N=CH), 11.56 (s, H, OH), 11.88 (s, H, NH); MS *m/z:* 298.0 [M - H^+], (M = 299.28); Anal. Calcd. for $C_{21}H_{17}N_5O_4$ (299.28): C, 62.53; H, 4.25; N, 17.36%; Found: C, 62.50; H, 4.27; N, 17.31%.

6.4.3. 6-[(3-hydroxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5c)

Yield (99%), M.p. 249 ℃, R_f = 0.22. FT-IR (KBr) ν_{max} (cm^{-1}): 3433, 3281, 1710, 1697, 1579, 1529, 1492, 1435, 1313, 1286, 1249, 1145, 790 and 690; ^{1}H NMR δ (ppm): 3.68 (s, 3H, OCH_3), 7.02-7.60(m, 4H, arom.), 7.92 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.46 (s, H, N=CH), 9.83 (s, H, OH), 11.80 (s, H, NH); MS *m/z:* 298.0 [M - H^+], (M = 299.28); Anal. Calcd. for $C_{21}H_{17}N_5O_4$ (299.28): C, 62.53; H, 4.25; N, 17.36%; Found: C, 62.50; H, 4.28; N, 17.31%.

6.4.4. 6-[(4-hydroxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5d)

Yield (92%), M.p. 170 ℃, R_f = 0.22. FT-IR (KBr) ν_{max} (cm^{-1}): 3584, 3394, 3219, 1718, 1662, 1603, 1541, 1516, 1440, 1365, 1298, 1281, 1246, 1165, 844 and 679; ^{1}H NMR δ (ppm): 3.69 (s, 3H, OCH_3), 6.82-7.70 (m, 4H, arom.), 7.92 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.64 (s, H, N=CH), 9.54 (s, H, OH), 11.85 (s, H, NH); MS *m/z:* 298.0 [M - H^+], (M = 299.28); Anal. Calcd. for $C_{21}H_{17}N_5O_4$ (299.28): C, 62.53; H, 4.25; N, 17.36%; Found: C, 62.50; H, 4.28; N, 17.31%.

6.4.5. 6-[(2-methoxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5e)

Yield (92 %), M.p. 117 ℃, R_f = 0.39. FT-IR (KBr) ν_{max} (cm^{-1}): 3452, 3182, 3047, 2960, 1741, 1680, 1601, 1560, 1437, 1290, 1247, 1163, 997 and 756; ^{1}H NMR δ (ppm): 3.88 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 7.02-7.78 (m, 4H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.84 (s, H, N=CH), 11.91 (s, H, NH); MS *m/z:* 314.0 [M + H^+], (M = 313.31); Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (313.11): C, 64.03; H, 4.91; N, 16.23%; Found: C, 64.00; H, 5.09; N, 16.16%.

6.4.6. 6-[(3-methoxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5f)

Yield (98%), M.p. 111-117 ℃, R_f = 0.42. FT-IR (KBr) ν_{max} (cm^{-1}): 3529, 3470, 3255, 2978, 1726, 1693, 1583, 1535, 1433, 1307, 1236, 1157, 1078, 989 and 749; ^{1}H NMR δ (ppm): 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.02-7.56 (m, 4H, arom.), 7.95 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.74 (s, H, N=CH), 11.78 (s, H, NH); MS *m/z:* 314.0 [M + H^+], (M = 313.31); Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (313.11): C, 64.03; H, 4.91; N, 16.23%; Found: C, 63.96; H, 4.88; N, 16.24%.

6.4.7. 6-[(4-methoxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5g)

Yield (95%), M.p. 109-111 ℃, R_f = 0.40. FT-IR (KBr) ν_{max} (cm^{-1}): 3520, 3450, 3230, 2920, 1728, 1688, 1530, 1440, 1300, 1240, 1180 and 1015; ^{1}H NMR δ (ppm): 3.73 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.02-7.84 (m, 4H, arom.), 7.98 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.84 (s, H, N=CH), 11.85 (s, H, NH); MS *m/z:* 314.0 [M + H^+], (M = 313.31); Anal. Calcd. for C $_{23}H_{21}N_5O_6$ (313.11): C, 64.03; H, 4.91; N, 16.23%; Found: 64.00; H, 4.98; N, 16.19%.

6.4.8. 6-[(2-chlorobenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5h)

Yield (89%), M.p. 145-151 ℃, R_f = 0.52. FT-IR (KBr) ν_{max} (cm^{-1}): 3547, 3476, 3207, 2953, 1732, 1676, 1637, 1585, 1537, 1442, 1365, 1300, 1244, 1159, 767 and 682; ^{1}H NMR δ (ppm): 3.69 (s, 3H, OCH_3), 7.40-7.87 (m, 4H, arom.), 7.98 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.99 (s, H, N=CH), 11.81 (s, H, NH); MS *m/z:* 315.9 [M - H^+], (M = 317.73); Anal. Calcd. for $C_{21}H_{15}Cl_2N_5O_2$ (317.06): C, 57.29; H, 3.43; N, 15.91%; Found: C, 57.31; H, 3.42; N, 16.01%.

6.4.9. 6-[(3-chlorobenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5i)

Yield (92%), M.p. = 135 ℃, R_f = 0.54. FT-IR (KBr) ν_{max} (cm^{-1}): 3517, 3460, 3292, 3203, 1732, 1681, 1627, 1535, 1307, 1242, 1159, 787 and 684; ^{1}H NMR δ (ppm): 3.69 (s, 3H, OCH_3), 7.26-7.91 (m, 4H, arom.), 7.96 (t, 1H, py-H), 8.28 (d, 2H, py-H), 8.84 (s, H, N=CH), 11.76 (s, H, NH); MS *m/z:* 318.0 [M + H^+], (M = 317.73); Anal. Calcd. for $C_{21}H_{15}Cl_2N_5O_2$ (316.06): C, 57.29; H, 3.43; N, 15.91%; Found: C, 57.28; H, 3.40; N, 15.96%.

6.4.10. 6-[(3-bromobenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5j)

Yield (91%), M.p. 163 ℃, R_f = 0.59. FT-IR (KBr) ν_{max} (cm^{-1}): 3547, 3466, 3292, 3203, 1732, 1681, 1627, 1535, 1307, 1242, 1159, 787 and 684; ^{1}H NMR δ (ppm): 3.72 (s, 3H, OCH_3), 7.34-7.86 (m, 4H, arom.), .), 7.96 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.84 (s, H, N=CH), 11.91 (s, H, NH); MS *m/z:* 359.9 [M - H^+], (M = 362.18); Anal. Calcd. for $C_{15}H_{12}BrN_3O_3$ (361.18): C, 49.74; H, 3.34; Br, 22.06; N, 11.60%; Found: C, 49.78; H, 3.31; Br, 22.04; N, 11.68%.

6.4.11. 6-[(4-bromobenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5k)

Yield (90%), M.p. 162 ℃, R_f = 0.59. FT-IR (KBr) ν_{max} (cm^{-1}): 3433, 3173, 3026, 1728, 1674, 1585, 1537, 1440, 1361, 1298, 1246, 1151, 997, 736 i 686; ^{1}H NMR δ (ppm): 3.71 (s, 3H, OCH_3), 7.38-7.82 (m, 4H, arom.), 7.96 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.94 (s, H, N=CH), 11.84 (s, H, NH); MS *m/z:* 361.7 [M - H^+], (M = 362.18); Anal. Calcd. for $C_{15}H_{12}BrN_3O_3$ (361.18): C, 49.74; H, 3.34; Br, 22.06; N, 11.60; O, 13.25%; Found: C, 49.82; H, 3.33; Br, 22.10; N, 11.62; O, 13.23%.

6.4.12. 6-[(4-fluorobenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5l)

Yield (65%), M.p. 170-175 ℃, R_f = 0.53. FT-IR (KBr) ν_{max} (cm^{-1}): 3547, 3466, 3292, 3203, 1732, 1681, 1627, 1535, 1307, 1242, 1159, 787 and 684; ^{1}H NMR δ (ppm): 3.68 (s, 3H, OCH_3), 7.06-7.80 (m, 4H, arom.), 7.96 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.94 (s, H, N=CH), 11.88 (s, H, NH); MS *m/z:* 300.0 [M - H^+], (M = 301.27); Anal. Calcd. for $C_{15}H_{12}FN_3O_3$ (301.07): C, 59.80; H, 4.01; F, 6.31; N, 13.95; O, 15.93%; Found: C, 59.81; H, 4.03; F, 6.29; N, 13.92; O, 15.96%.

6.4.13. 6-[(3-phenoxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5m)

Yield (80%), M.p. 89-93℃, R_f = 0.60. FT-IR (KBr) ν_{max} (cm^{-1}): 3485, 3036, 1726, 1672, 1575, 1541, 1483, 1437, 1300, 1247, 1163, 954, 720 and 688; ^{1}H NMR δ (ppm): 3.89 (s, 3H, OCH_3), 7.11-7.85 (m, 9H, arom.), 7.96 (t, 1H, py-H), 8.25 (d, 2H, py-H),8.74 (s, H, N=CH), 11.89 (s, H, NH); MS *m/z:* 374.0 [M - H+], (M = 375.12); Anal. Calcd. for $C_{33}H_{25}N_5O_4$ (375.12): C, 71.34; H, 4.54; N, 12.61%; Found: C, 71.32; H, 4.50; N, 12.59%.

6.4.14. 6-[(4-dimethylaminobenzylidene)hydrazine-carbonyl]-2-methoxycarbonylpyridine (5n)

Yield (98%), M.p. 150 ℃, R_f = 0.25. FT-IR (KBr) ν_{max} (cm^{-1}): 3537, 3473, 3209, 1724, 1666, 1597, 1523, 1431, 1363, 1296, 1240, 1176, 815, 732 and 682; ^{1}H NMR δ (ppm): 3.02 (s, 6H, $N(CH_3)_2$), 3.69 (s, 3H, OCH_3), 6.81-7.50 (m, 4H, arom.),7.96 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.94 (s, H, N=CH), 11.80 (s, H, NH); MS *m/z:* 327.1 [M + H^+], (M = 326.35); Anal. Calcd. for $C_{25}H_{27}N_7O_2$ (326.35): C, 65.63; H, 5.95; N, 21.43%; Found: C, 65.60; H, 5.91; N, 21.39%.

6.4.15. 6-[(3-phenylallylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5o)

Yield (87%), M.p. 200 ℃, R_f = 0.37. FT-IR (KBr) ν_{max} (cm^{-1}): 3448, 3238, 3043, 1722, 1664, 1624, 1537, 1440, 1317, 1363, 1244, 1130, 987, 748 and 688; ^{1}H NMR δ (ppm): 3.89 (s, 3H, OCH_3), 6.82 (m, H, HC=CH), 7.12 (m, H, HC=CH), 7.34-7.72 (m, 5H, arom.),7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.50 (s, H, N=CH), 10.88 (s, H, NH); MS *m/z:* 307.9 [M - H^+], (M = 309.32). Anal. Calcd. for $C_{25}H_{21}N_5O_2$(309.32): C, 70.91; H, 5.00; N, 16.54%; Found: C, 70.89; H, 5.04; N, 16.51%.

6.4.16. 6-[(2,4-dihydroxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5p)

Yield (45%), M.p. 283 ℃, R_f = 0.14. FT-IR (KBr) ν_{max} (cm^{-1}): 3531, 3431, 3196, 1728, 1658, 1631, 1581, 1506, 1437, 1327, 1244, 976, 736 and 682; ^{1}H NMR δ (ppm): 3.69 (s, 3H, OCH_3), 6.41-7.65 (m, 3H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.74 (s, H, N=CH), 9.89 (s, H, OH), 11.80 (s, H, NH), 11.88 (s, H, OH); MS *m/z:* 314.0 [M - H^+], (M = 315.28); Anal. Calcd. for $C_{21}H_{17}N_5O_6$(315.28): C, 57.93; H, 3.94; N, 16.09%; Found: C, 57.91; H, 3.92; N, 16.00%.

6.4.17. 6-[(3,4-dihydroxybenzylidene)hydrazinocarbonyl]-2-methoxycarbonylpyridine (5q)

Yield (52%), M.p. 270 ℃, R_f = 0.1. FT-IR (KBr) ν_{max} (cm^{-1}): 3294, 3255, 2953, 1720, 1672, 1583, 1525, 1441, 1367, 1305, 1242, 1149, 993, 839, 736 and 672; ^{1}H NMR δ (ppm): 3.70 (s, 3H, OCH_3), 6.80-7.44 (m, 3H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.74 (s, H, N=CH), 9.48 (s, 2H, 2OH), 11.91 (s, H, NH); MS *m/z:* 314.0 [M - H^+], (M = 315.28); Anal. Calcd. for $C_{21}H_{17}N_5O_6$ (315.28): C, 57.93; H, 3.94; N, 16.09%; Found: C, 57.90; H, 3.94; N, 16.09%.

6.4.18. 6-[(3-hydroxy-4-methoxybenzylidene)hydrazine-carbonyl]-2-methoxycarbonylpyridine (5r)

Yield (93%), M.p. 187 ℃, R_f = 0.27. FT-IR (KBr): ν_{max} (cm^{-1}): 3581, 3462, 3196, 1728, 1674, 1599, 1543, 1512, 1429, 1244, 736 and 682; ^{1}H NMR δ (ppm): 3.73 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.90-7.48 (m, 3H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.94 (s, H, N=CH), 9.68 (s, H, OH), 11.90 (s, H, NH); MS *m/z:* 330.0 [M + H^+], (M =

329.31). Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (463.15): C, 59.61; H, 4.57; N, 15.11%; Found: C, 59.66; H, 4.53; N, 15.09%.

6.4.19. 6-[(3,4,5-trimethoxybenzylidene)hydrazine-carbonyl]-2-methoxycarbonylpyridine (5s)

Yield (96%), M.p. 182-185 °C, R_f = 0.36. FT-IR (KBr): ν_{max} (cm^{-1}): 3493, 2999, 2962, 2839, 1740, 1676, 1612, 1577, 1506, 1329, 1248, 1128, 999 and 738; ^{1}H NMR δ (ppm): 3.68 (s, 9H, $3OCH_3$), 3.89 (s, 3H, OCH_3), 7.04 (m, 2H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.74 (s, H, N=CH), 11.91 (s, H, NH); MS *m/z:* 374.0 [M + H^+], (M = 373.36); Anal. Calcd. for $C_{27}H_{29}N_5O_8$ (373.36): C, 58.80; H, 5.30; N, 12.70%; Found: C, 58.78; H, 5.31; N, 12.67%.

6.5. Preparation of Schiff bases from di-hydrazide (6a – s)

Ethanolic solution of 0.01 mole of pyridine-2,6-dicarbohydrazide (**4**) and 0.02. mole of aromatic aldehyde (**a – s**), with a few drops of acetic acid, is refluxed for 3 hours. After cooling, the solution is poured onto crushed ice, filtered and dried. Crude product is recrystallized from ethanol.

6.5.1. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-dibenzylidenepyridine-2,6-dicarbohydrazide (6a)

Yield (79 %), M.p. >300 °C; R_f = 0.42. FT-IR (KBr) ν_{max} (cm^{-1}): 3518, 3468, 3201, 3049, 1728, 1670, 1537, 1437, 1367, 1300, 1240, 1165, 1108 and 685; ^{1}H NMR δ (ppm): 7.22-7.86 (m, 10H, arom.), 7.96 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.84 (s, 2H, 2N=CH), 11.86 (s, 2H, 2NH), MS *m/z:* 372.2 [M + H^+], (M = 371.39); Anal. Calcd. for $C_{21}H_{17}N_5O_2$ (371.14): C, 67.91; H, 4.61; N, 18.86%; Found: C, 67.89; H, 4.62; N, 18.80%.

6.5.2. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(2-hydroxybenzylidene)pyridine-2,6-dicarbohydrazide (6b)

Yield (80%), M.p. >300 °C, R_f = 0.90. FT-IR (KBr) ν_{max} (cm^{-1}):3516, 3437, 2997, 1724, 1668, 1620, 1535, 1240, 1157, 750 and 684. ^{1}H NMR δ (ppm): 7.02-7.66 (m, 8H, arom.), 7.96 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.74 (s, 2H, 2N=CH), 11.26 (s, 2H, 2OH); 11.86 (s, 2H, 2NH), MS *m/z:* 402.1 [M - H^+], (M = 403.39); Anal. Calcd. for $C_{21}H_{17}N_5O_4$ (403.13): C, 62.53; H, 4.25; N, 17.36%; Found: C , 62.50; H, 4.27; N, 17.31%.

6.5.3. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(3-hydroxybenzylidene)pyridine-2,6-dicarbohydrazide (6c)

Yield (92%), M.p. >300 °C, R_f = 0.10. FT-IR (KBr) ν_{max} (cm^{-1}): 3433, 3281, 1710, 1697, 1579, 1529, 1492, 1435, 1313, 1286, 1249, 1145, 790 and 690. ^{1}H NMR δ (ppm): 7.12-7.48 (m, 8H, arom.), 7.96 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.64 (s, 2H, 2N=CH), 9.83 (s, 2H, 2OH), 11.96 (s, 2H, 2NH),; MS *m/z:* 402.0 [M - H^+], (M = 403.39); Anal. Calcd. for $C_{21}H_{17}N_5O_4$ (403.13): C, 62.53; H, 4.25; N, 17.36%; Found: , 62.50; H, 4.28; N, 17.31%.

6.5.4. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(4-hydroxybenzylidene)pyridine-2,6-dicarbohydrazide (6d)

Yield (98 %), M.p. >300 °C, R_f = 0.14. FT-IR (KBr) ν_{max} (cm^{-1}): 3584, 3394, 3219, 1718, 1662, 1603, 1541, 1516, 1440, 1365, 1298, 1281, 1246, 1165, 844 and 679; ^{1}H NMR δ (ppm): 6.85-7.88 (m, 8H, arom.), 7.95 (t, 1H, py-H), 8.28 (d, 2H, py-H), 8.66 (s, 2H, 2N=CH), 9.83 (s, 2H, 2OH), 11.82 (s, 2H, 2NH); MS *m/z:* 402.0 [M - H^+], (M = 403.39); Anal. Calcd. for $C_{21}H_{17}N_5O_4$ (403.13): C, 62.53; H, 4.25; N, 17.36%; Found: , 62.50; H, 4.28; N, 17.31%.

6.5.5. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(2-methoxybenzylidene)pyridine-2,6-dicarbohydrazide (6e)

Yield (93%), M.p. 228°C, R_f = 0.59. FT-IR (KBr) ν_{max} (cm^{-1}): 3452, 3182, 3047, 2960, 1741, 1680, 1601, 1560, 1437, 1290, 1247, 1163, 997 and 756; ^{1}H NMR δ (ppm): 3.80 (s, 6H, OCH_3), 7.18-7.72 (m, 8H, arom.), 7.95 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.78 (s, 2H, 2N=CH), 11.92 (s, 2H, 2NH),; MS *m/z:* 432.1 [M + H^+], (M = 431.44); Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (431.16): C, 64.03; H, 4.91; N, 16.23%; Found: C, 64.00; H, 5.09; N, 16.16%.

6.5.6. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(3-methoxybenzylidene)pyridine-2,6-dicarbohydrazide (6f)

Yield (86%), M.p. 285 °C, R_f = 0.42. FT-IR (KBr) ν_{max} (cm^{-1}): 3529, 3470, 3255, 2978, 1726, 1693, 1583, 1535, 1433, 1307, 1236, 1157, 1078, 989 and 749; ^{1}H NMR δ (ppm): 3.81 (s, 6H, OCH_3), 7.16-7.75 (m, 8H, arom.), 7.95 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.57 (s, 2H, 2N=CH), 11.93 (s, 2H, 2NH)); MS *m/z:* 432.0 [M + H^+], (M = 431.44); Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (431.16): C, 64.03; H, 4.91; N, 16.23%; Found: C, 63.96; H, 4.88; N, 16.24%.

6.5.7. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(4-methoxybenzylidene)pyridine-2,6-dicarbohydrazide (6g)

Yield (93%), M.p. 280 °C, R_f = 0.31. FT-IR (KBr) ν_{max} (cm^{-1}): 3520, 3450, 3230, 2920, 1728, 1688, 1530, 1440, 1300, 1240, 1180 and 1015; ^{1}H NMR δ (ppm): 3.78 (s, 6H, OCH_3), 7.14-7.86 (m, 8H, arom.), 7.95 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.62 (s, 2H, 2N=CH),11.80 (s, 2H, 2NH)); MS *m/z:* 432.1 [M + H^+], (M = 431.44); Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (431.16): C, 64.03; H, 4.91; N, 16.23%; Found: 64.00; H, 4.98; N, 16.19%.

6.5.8. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(2-chlorobenzylidene)pyridine-2,6-dicarbohydrazide (6h)

Yield (89%), M.p. >300 °C, R_f = 0.89. FT-IR (KBr) ν_{max} (cm^{-1}): 3547, 3476, 3207, 2953, 1732, 1676, 1637, 1585, 1537, 1442, 1365, 1300, 1244, 1159, 767 and 682; ^{1}H NMR δ (ppm): 7.36-7.75 (m, 8H, arom.), 7.95 (t, 1H, py-H), 8.24 (d, 2H, py-H), 8.76 (s, 2H, 2N=CH), 11.93 (s, 2H, 2NH); MS *m/z:* 439.9 [M - H^+], (M = 440.28); Anal. Calcd. for $C_{21}H_{15}Cl_2N_5O_2$ (439.06): C, 57.29; H, 3.43; N, 15.91%; Found: C, 57.31; H, 3.42; N, 16.01%.

6.5.9. ($N^{2'}E$,$N^{6'}E$)-$N^{2'}$,$N^{6'}$-bis(3-chlorobenzylidene)pyridine-2,6-dicarbohydrazide (6i)

Yield (78%), M.p. 292 °C, R_f = 0.42. FT-IR (KBr) ν_{max} (cm^{-1}): 3547, 3466, 3292, 3203, 1732, 1681, 1627, 1535, 1307, 1242, 1159, 787 and 684; ^{1}H NMR δ (ppm): 7.46-7.91 (m, 8H, arom.), 7.94 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.74 (s, 2H, 2N=CH), 11.88 (s, 2H, 2NH); MS *m/z:* 439.9 [M – H^+], (M = 440.28); Anal. Calcd. for $C_{21}H_{15}Cl_2N_5O_2$ (439.06): C, 57.29; H, 3.43; N, 15.91%; Found: C, 57.28; H, 3.40; N, 15.96%.

6.5.10. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(3-bromobenzylidene)pyridine-2,6-dicarbohydrazide (6j)

Yield (94%), M.p. 283-285 °C, R_f = 0.53. FT-IR (KBr) ν_{max} (cm^{-1}): 3527, 3446, 3281, 3213, 1730, 1684, 1630, 1535, 1303, 1240, 1158, 789 and 680; ^{1}H NMR δ(ppm): 7.41-7.82 (m, 8H, arom.), 7.94 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.72 (s, 2H, 2N=CH), 11.85 (s, 2H, 2NH); MS *m/z:* 527.9 [M - H^+], (M = 529.18); Anal. Calcd. for $C_{21}H_{15}Br_2N_5O_2$ (529.18): C, 47.66; H, 2.86; Br, 30.20; N, 13.23; O, 6.05%; Found: C, 47.56; H, 2.89; Br, 31.05; N, 13.30; O, 6.15%.

6.5.11. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(4-bromobenzylidene)pyridine-2,6-dicarbohydrazide (6k)

Yield (90%), M.p. 277 °C, R_f = 0.42. FT-IR (KBr) ν_{max} (cm^{-1}): 3538, 3456, 3279, 3208, 1734, 1680, 1636, 1538, 1304, 1242, 1159, 786 and 688; ^{1}H NMR δ (ppm): 7.48-7.76 (m, 8H, arom.), 7.94 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.64 (s, 2H, 2N=CH), 11.86 (s, 2H, 2NH); MS *m/z:* 527.8 [M - H^+], (M = 529.18); Anal. Calcd. for $C_{21}H_{15}Br_2N_5O_2$ (529.18): C, 47.66; H, 2.86; Br, 30.20; N, 13.23; O, 6.05%; Found: C, 47.58; H, 2.84; Br, 31.08; N, 13.32; O, 6.18%.

6.5.12. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(4-fluorobenzylidene)pyridine-2,6-dicarbohydrazide (6l)

Yield (73%), M.p. 277 °C, R_f = 0.35. FT-IR (KBr) ν_{max} (cm^{-1}): 3548, 3449, 3270, 3214, 1733, 1685, 1630, 1532, 1306, 1242, 1158, 786 and 684; ^{1}H NMR δ (ppm): 7.34-7.85 (m, 8H, arom.), 7.94 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.76 (s, 2H, 2N=CH), 11.88 (s, 2H, 2NH)); MS *m/z:* 406.0 [M - H^+], (M = 407.37); Anal. Calcd. for $C_{21}H_{15}F_2N5O_2$ (407.37): C, 61.91; H, 3.71; F, 9.33; N, 17.19; O, 7.85%; Found: C, 62.05; H, 3.65; F, 9.40; N, 17.24; O, 7.80%.

6.5.13. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(3-phenoxybenzylidene)pyridine-2,6-dicarbohydrazide (6m)

Yield (88%), M.p. 140 °C, R_f = 0.56. FT-IR (KBr) ν_{max} (cm^{-1}): 3485, 3036, 1726, 1672, 1575, 1541, 1483, 1437, 1300, 1247, 1163, 954, 720 and 688; ^{1}H NMR δ (ppm): 7.12-7.52 (m, 18H, arom.), 7.94 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.68 (s, 2H, 2N=CH), 11.86 (s, 2H, 2 NH),; MS *m/z:* 554.0 [M - H^+], (M = 555.58); Anal. Calcd. for $C_{33}H_{25}N_5O_4$ (555.19): C, 71.34; H, 4.54; N, 12.61%; Found: C, 71.32; H, 4.50; N, 12.59%.

6.5.14.($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(4-dimethylaminobenzylidene)pyridine-2,6-dicarbohydrazide (6n)

Yield (94%), M.p. >300 °C, R_f = 0.46. FT-IR (KBr): ν_{max} (cm^{-1}): 3547, 3473, 3209, 1724, 1666, 1597, 1523, 1431, 1363, 1296, 1240, 1176, 815, 732 and 682; ^{1}H NMR δ (ppm): 3.04 (s, 12H, $N(CH_3)_2$), 6.80-7.58 (m, 8H, arom.), 7.94 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.77 (s, 2H, 2N=CH), 11.89 (s, 2H, 2NH); MS *m/z:* 458.1 [M + H^+], (M = 457.53); Anal. Calcd. for $C_{25}H_{27}N_7O_2$ (457.22): C, 65.63; H, 5.95; N, 21.43%; Found: C, 65.60; H, 5.91; N, 21.39%.

6.5.15. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis((E)-3-phenylallylidene)pyridine-2,6-dicarbohydrazide (6o)

Yield (77%), M.p. 282 °C, R_f = 0.47. FT-IR (KBr) ν_{max} (cm^{-1}): 3448, 3238, 3043, 1722, 1664, 1624, 1537, 1440, 1317, 1363, 1244, 1130, 987, 748 and 688. ^{1}H NMR δ (ppm): 6.80 (m, 2H, HC=CH), 7.21 (m, 2H, HC=CH), 7.31-7.62 (m, 10H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 7.81 (s, 2H, 2 HC=N), 10.96 (s, 2H, HN-N); MS *m/z:* 422.0 [M - H^+], (M = 423.47); Anal. Calcd. for $C_{25}H_{21}N_5O_2$ (423.17): C, 70.91; H, 5-00; N, 16.54%; Found: C, 70.89; H, 5.04; N, 16.51%.

6.5.16. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(2,4-dihydroxybenzylidene) pyridine-2,6-dicarbohydrazide (6p)

Yield (45%), M.p. >300 °C, R_f = 0.56. FT-IR (KBr) ν_{max} (cm^{-1}): 3531, 3431, 3196, 1728, 1658, 1631, 1581, 1506, 1437, 1327, 1244, 976, 736 and 682; ^{1}H NMR δ (ppm): 6.38-7.63 (m, 6H, arom.), 7.95 (t, 1H, py-H), 8.27 (d, 2H, py-H), 8.74 (s, 2H, 2N=CH), 9.89 (s, 2H, OH), 11.85 (s, 2H, 2NH), 11.88 (s, 2H, OH); MS *m/z:* 434.0 [M - H^+], (M = 435.39); Anal. Calcd. for $C_{21}H_{17}N_5O_6$ (435.12): C, 57.93; H, 3.94; N, 16.09%; Found: C, 57.91; H, 3.92; N, 16.00%.

6.5.17. ($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(3,4-dihydroxybenzylidene) pyridine-2,6-dicarbohydrazide (6q)

Y = (63%), M.p. >300 °C, R_f = 0.38. FT-IR (KBr) ν_{max} (cm^{-1}): 3294, 3255, 2953, 1720, 1672, 1583, 1525, 1441, 1367, 1305, 1242, 1149, 993, 839, 736 and 672; ^{1}H NMR δ (ppm): 6.82-7.38 (m, 6H, arom.), 7.95 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.64 (s, 2H, 2N=CH), 9.68 (s, 4H, OH), 11.80 (s, 2H, 2NH); MS *m/z:* 434.0 [M - H^+], (M = 435.39); Anal. Calcd. for $C_{21}H_{17}N_5O_6$ (435.12): C, 57.93; H, 3.94; N, 16.09%; Found: C, 57.90; H, 3.94; N, 16.09%.

6.5.18.($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(3-hydroxy-4-methoxybenzylidene)pyridine-2,6-dicarbohydrazide (6r)

Yield (88%), M.p. 276-280 °C, R_f = 0.22. FT-IR (KBr) ν_{max} (cm^{-1}): 3581, 3462, 3196, 1728, 1674, 1599, 1543, 1512, 1429, 1244, 736 and 682; ^{1}H NMR δ (ppm): 3.81 (s, 6H, OCH_3), 6.92-7.62 (m, 6H, arom.), 7.94 (t, 1H, py-H), 8.26 (d, 2H, py-H), 8.65 (s, 2H, 2N=CH), 9.88 (s, 2H, OH), 11.86 (s, 2H, 2NH),); MS *m/z:* 462.6 [M - H^+], (M = 463.44); Anal. Calcd. for $C_{23}H_{21}N_5O_6$ (463.15): C, 59.61; H, 4.57; N, 15.11%; Found: C, 59.66; H, 4.53; N, 15.09%.

6.5.19.($N^{2'}E,N^{6'}E$)-$N^{2'},N^{6'}$-bis(3,4,5-trimethoxybenzylidene) pyridine-2,6-dicarbohydrazide (6s)

Yield (92%), M.p. >300 °C, R_f = 0.32. FT-IR (KBr) ν_{max} (cm^{-1}): 3493, 2999, 2962, 2839, 1740, 1676, 1612, 1577, 1506, 1329, 1248, 1128, 999 and 738; ^{1}H NMR δ (ppm): 3.84 (s, 18H, OCH_3), 7.04-7.42 (s, 4H, arom.), 7.94 (t, 1H, py-H), 8.25 (d, 2H, py-H), 8.56 (s, 2H, 2N=CH), 11.84 (s, 2H, 2NH); MS *m/z:* 552.0 [M + H^+], (M = 551.55). Anal. Calcd. for $C_{27}H_{29}N_5O_8$ (551.20): C, 58.80; H, 5.30; N, 12.70%; Found: C, 58.78; H, 5.31; N, 12.67%.

ACKNOWLEDGEMENT

Declared none.

CONFLICT OF INTEREST

Declared none.

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