

Article

Synthesis of Some New Pyridine-2,6-carboxamide-derived Schiff Bases as Potential Antimicrobial Agents

Mohamed A. Al-Omar and Abd El-Galil E. Amr *

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

* Author to whom correspondence should be addressed; E-Mail: aamr1963@yahoo.com.

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Abstract: A series of pyridine-bridged 2,6-bis-carboxamide Schiff's bases has been prepared starting from 2,6-pyridinedicarbonyl dichloride (**1**) and L-alanine or 2-methylalanine methyl ester. The coupling of acid chloride **1** with L-alanine methyl ester hydrochloride -or 2-methylalanine methyl ester hydrochloride gave the corresponding 2,6-bis-carboxamide pyridine methyl esters **2a,b**. Hydrazonolysis of **2** with hydrazine hydrate afforded the corresponding bis-hydrazides **3a,b**. Treatment of **3a,b** with appropriate aromatic or heterocyclic aldehydes afforded the corresponding pyridine- bridged 2,6-bis-carboxamide Schiff's bases **4a-f** and **5a-f**, respectively. The newly synthesized compounds **2-5** were screened for their bactericidal and fungicidal activities. Many of the obtained compounds exhibited significant antimicrobial activity, comparable to streptomycin and fusidic acid, which were used as reference antibiotic drugs.

Keywords: 2,6-pyridinedicarbonyl dichloride; pyridine-2,6-carboxamides; Schiff's base; antimicrobial activity

1. Introduction

In our previous work, we have reported that certain of substituted pyridine and Schiff base derivatives as antimicrobial, anti-inflammatory and anticancer agents [1-6]. Also, Schiff base and other heterocyclic derivatives were reported to possess diverse biological activities, such as antibacterial

[7-10] and anti-inflammatory [11-13] properties. In addition, several substituted pyridines and their derivatives were reported to exhibit significant antimicrobial [14], anti-inflammatory [15] and anticancer activities [16]. In continuation of our interest in the chemical and pharmacological properties of disubstituted pyridine derivatives [17-20], we report herein the synthesis and antimicrobial activities of a new series of hydrazides and their corresponding N^2,N^6 -bis(1-oxo-1-(2-(substituted-benzylidene)-hydrazinyl)propan-2-yl)pyridine-2,6-di-carboxamide derivatives (Schiff's bases).

2. Results and Discussion

2.1. Chemistry

L-Alanine and/or 2-methylalanine methyl esters were initially coupled with 2,6-pyridinedicarbonyl dichloride (**1**) (acid chloride method) [21] to give the corresponding 2,6-bis-carboxamide pyridine methyl esters **2a,b**. Treatment of 2,6-bis-esters **2a,b** with hydrazine hydrate in absolute ethanol afforded the corresponding 2,6-bis-hydrazides **3a,b** (Scheme 1). Some physical properties of these compounds are listed in Table 1.

Scheme 1. Synthetic Pathway for Compounds **2a,b** and **3a,b**.

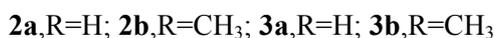
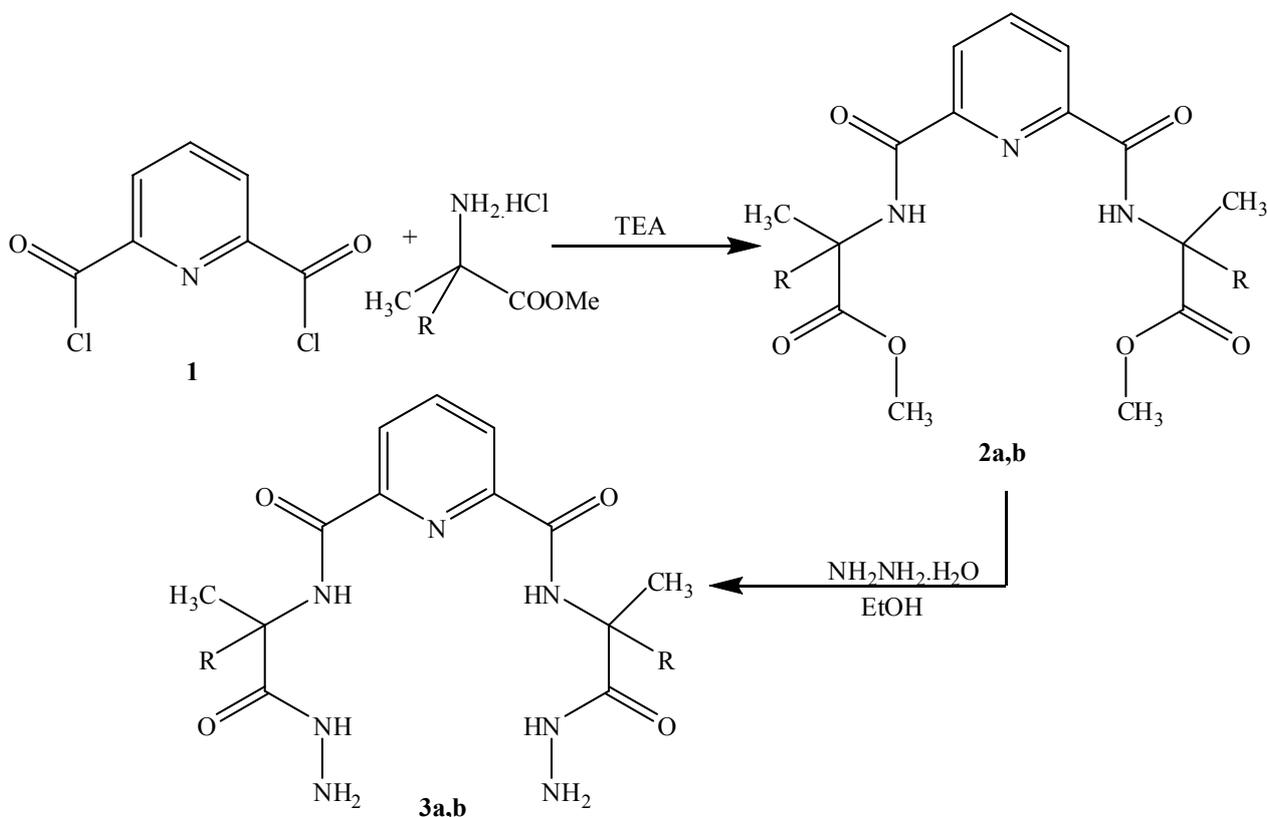
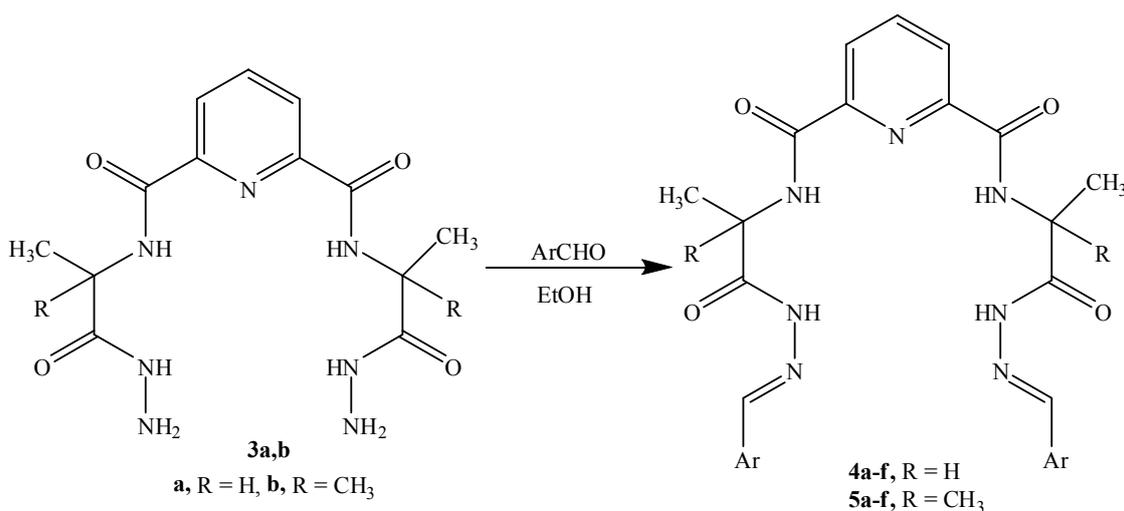


Table 1. Melting points, crystallization solvents, yields, molecular formulae and molecular weights of compounds **2a,b** and **3a,b**.

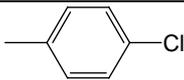
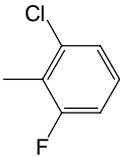
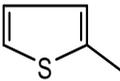
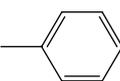
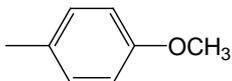
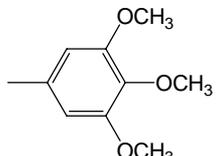
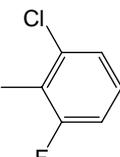
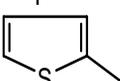
Comp. No.	R	Mp (°C)	Cryst. Solv.	Yield (%)	$[\alpha]^{30}_D$	Molecular Formula (Mol. Wt.)
2a	H	182-184	EtOH	75	+15 (DMF)	C ₁₅ H ₁₉ N ₃ O ₆ (337.33)
2b	CH ₃	196-198	EtOH	68	-	C ₁₇ H ₂₃ N ₃ O ₆ (365.38)
3a	H	252-254	AcOH/H ₂ O	82	+56 (DMF)	C ₁₃ H ₁₉ N ₇ O ₄ (337.33)
3b	CH ₃	246-248	AcOH/H ₂ O	85	-	C ₁₅ H ₂₃ N ₇ O ₄ (365.39)

Compounds **4a-f** and **5a-f** are new, and were synthesized via simple condensation of the hydrazides **3a,b** with appropriate aromatic or heterocyclic aldehydes, namely, benzaldehyde, *p*-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, *p*-chlorobenzaldehyde, 2-chloro-6-fluorobenzaldehyde, and/or 2-thiophenealdehyde in refluxing absolute ethanol giving the corresponding N²,N⁶-bis(1-(2-(substituted benzylidene)hydrazinyl)-1-oxopropan-2-yl)pyridine-2,6-dicarboxamides **4a-f** and N²,N⁶-bis(1-(2-(substituted benzylidene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamides **5a-f** (Scheme 2).

Scheme 2. Synthetic Pathway for Compounds **4a-f** and **5a-f**.**Table 2.** Melting points, crystallization solvents, yields, specific rotation, molecular formulae and molecular weights of compounds **4a-h** and **5a-h**.

Comp. No.	Ar	Mp (°C)	Cryst. Solv.	Yield (%)	$[\alpha]^{30}_D$ (DMF)	Molecular Formula (Mol. Wt.)
4a		122-124	EtOH/ <i>n</i> -hexane	68	+ 18	C ₂₇ H ₂₇ N ₇ O ₄ (513.55)
4b		210-212	AcOH/H ₂ O	75	+ 32	C ₂₉ H ₃₁ N ₇ O ₆ (573.60)
4c		148-150	AcOH	80	+ 24	C ₃₃ H ₃₉ N ₇ O ₁₀ (693.70)

Table 2. Cont.

4d		205-207	EtOH/ <i>n</i> -hexane	65	+ 54	C ₂₇ H ₂₅ Cl ₂ N ₇ O ₄ (582.44)
4e		168-170	AcOH/H ₂ O	72	+ 12	C ₂₇ H ₂₃ Cl ₂ F ₂ N ₇ O ₄ (618.42)
4f		185-187	EtOH/ <i>n</i> -hexane	60	+ 16	C ₂₃ H ₂₃ N ₇ O ₄ S ₂ (525.60)
5a		240-242	EtOH	70	-	C ₂₉ H ₃₁ N ₇ O ₄ (541.60)
5b		120-122	Dioxane	75	-	C ₃₁ H ₃₅ N ₇ O ₆ (601.65)
5c		135-137	AcOH/H ₂ O	66	-	C ₃₅ H ₄₃ N ₇ O ₁₀ (721.76)
5d		155-157	AcOH/H ₂ O	78	-	C ₂₉ H ₂₉ Cl ₂ N ₇ O ₄ (610.49)
5e		213-215	AcOH/H ₂ O	86	-	C ₂₉ H ₂₇ Cl ₂ F ₂ N ₇ O ₄ (646.47)
5f		220-222	EtOH	60	-	C ₂₅ H ₂₇ N ₇ O ₄ S ₂ (553.66)

The structures of all the newly synthesized compounds **2a,b**, **3a,b**, **4a-f** and **5a-f** were confirmed by their IR, ¹H-NMR, ¹³C-NMR and mass spectra.

2.2. Antimicrobial testing

Preliminary biological activity screening of the synthesized compounds has been performed at 50 µg/mL against microorganisms representing Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*) and fungi (*Candida albicans* and *Aspergillus niger*), using the bioassay technique for antibiotics [22] specified in the US Pharmacopeia. From Table 3 it appears that the Schiff's bases **4b-f** and **5b-f** have significant antimicrobial activities. Among these Schiff's bases, the 4-methoxy- **4b,5b**, 3,4,5-trimethoxy- **4c,5c**, 4-chloro-**4d,5d**, 2-chloro-6-fluoro-**4e,5e** and 2-thienyl- derivatives **4f,5f** have antimicrobial activities higher those of **4a,5a** with an unsubstituted phenyl group. The hydrazides **3a,b** were found to have lower antimicrobial activities, while the esters **2a,b** didn't show any antifungal activity. Streptomycin and fusidic acid were used as antibacterial and antifungal reference drugs, respectively.

Table 3. Antimicrobial activities of the new synthesized compounds **2a,b**, **3a,b**, **4a-f** and **5a-f**.

Comp. No.	Inhibition zones (cm)				
	Gram-positive		Gram-negative	Fungi	
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
2a	15	18	16	-	-
2b	14	13	15	-	-
3a	1	12	13	14	10
3b	12	14	15	12	11
4a	14	15	16	14	12
4b	20	19	19	16	15
4c	21	18	20	17	16
4d	20	19	21	17	14
4e	22	20	20	18	16
4f	21	18	18	17	16
5a	16	15	17	12	10
5b	21	18	20	14	15
5c	19	20	19	16	16
5d	20	17	18	16	14
5e	22	20	20	17	14
5f	20	19	21	16	15
Streptomycin	22	21	22	-	-
Fusidic acid	-	-	-	18	17

3. Experimental

3.1. General

Melting points (°C) were measured in open glass capillaries using a Barnstead 9001 Electrothermal melting point apparatus and are uncorrected. NMR spectra were obtained on a Bruker AC 500 Ultra Shield NMR spectrometer (Bruker, Fällanden, Switzerland) operating at 500.13 MHz for ¹H and 125.76 MHz for ¹³C; the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) used as internal standard. Electrospray ionization mass spectra (ESI-MS) were recorded on a Waters QuatroMicro triple quadrupole tandem mass spectrometer at 4.0 and 3.5 kV for positive and negative ions, respectively. Elemental analyses (C, H, N, Cl, S) were in full agreement with the proposed structures within ± 0.4% of the theoretical values. Monitoring of reactions and checking of purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminum sheets (60 F254, Merck) and visualization with ultraviolet light (UV) at 365 and 254 nm.

3.2. Chemistry

3.2.1. *N*²,*N*⁶-Bis(1-methoxy-oxopropan-2-yl)pyridine-2,6-dicarboxamides **2a,b**

To a solution of L-alanine and/or 2-methylalanine methyl esters (2 mmol), 2,6-pyridinedicarboyl dichloride **1** (0.204 g, 1 mmol) in dichloromethane (15 mL) was added at -10 °C with stirring. Triethylamine was added dropwise to the reaction mixture in order to keep the reaction mixture slightly basic (pH ~ 8). Stirring was continued for 3 h more at -15 °C and then 12 h at r.t. The reaction mixture was then washed with water, 1N hydrochloric acid, 1N sodium bicarbonate and finally with

water and dried over anhydrous calcium chloride. The solvent was evaporated under reduced pressure to dryness and the obtained solid was crystallized from the appropriate solvent indicated in Table 1 to give the corresponding bis-esters **2a,b**.

*N*²,*N*⁶-*Bis*(1-methoxy-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**2a**). IR (KBr, cm⁻¹): ν 3352-3268 (NH), 1745 (C=O, ester), 1678 (C=O, amide); ¹H-NMR (DMSO-d₆): δ 1.32 (d, 6H, 2 CH₃), 3.62 (s, 6H, 2OCH₃), 4.25 (m, 2H, 2CH), 8.18-8.26 (m, 3H, pyridine-H), 8.62 (s, 2H, 2NH exchangeable with D₂O); ¹³C-NMR: 17.32 (2C, 2CH₃), 47.88 (2C, 2CH), 56.15 (2C, 2OCH₃), 123.12, 138.54, 149.65 (5C, pyridine-C), 159.96, 171.86 (4C, 4C=O); MS, *m/z* (%): 337 (M⁺, 5), 306 (15), 275 (100), 219 (12), 163 (26), 133 (75), 105 (14), 77 (65).

*N*²,*N*⁶-*Bis*(1-methoxy-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**2b**). IR (KBr, cm⁻¹): ν 3332-3278 (NH), 1747 (C=O, ester), 1676 (C=O, amide); ¹H-NMR (DMSO-d₆): δ 1.46 (s, 12H, 4 CH₃), 3.56 (s, 6H, 2OCH₃), 8.15-8.28 (m, 3H, pyridine-H), 8.42 (s, 2H, 2NH exchangeable with D₂O); ¹³C-NMR: 23.78 (4C, 4CH₃), 55.18 (2C, NH-C(CH₃)₂CO), 123.18, 139.05, 149.72 (5C, pyr-C), 160.12, 172.65 (4C, 4C=O); MS, *m/z* (%): 365 (M⁺, 8), 334 (25), 303 (80), 218 (100), 148 (6), 133 (42), 105 (34), 77 (78).

3.2.2. *N*²,*N*⁶-*Bis*(1-hydrazinyl)pyridine-2,6-dicarboxamides **3a,b**

A mixture of bis-esters **2a** or **2b** (1 mmol) and hydrazine hydrate (0.8 mL, 16 mmol) in absolute ethanol (50 mL) was refluxed for 6 h. Excess solvent was evaporated under reduced pressure to dryness, the obtained residue was triturated with ethanol and the resulting solid was crystallized from the appropriate solvent to give bis-hydrazide derivatives **3a,b**, respectively (Table 1).

*N*²,*N*⁶-*Bis*(1-hydrazinyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**3a**). IR (KBr, cm⁻¹): ν 3465-3228 (NH, NH₂), 1680, 1675 (2C=O, amide); ¹H-NMR (DMSO-d₆): δ 1.36 (d, 6H, 2 CH₃), 4.12 (s, 4H, 2NH₂ exchangeable with D₂O), 4.62 (m, 2H, 2CH), 8.12-8.24 (m, 3H, pyridine-H), 8.68, 9.05 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 18.05 (2C, 2CH₃), 50.12 (2C, 2CH), 123.34, 139.05, 149.78 (5C, pyridine-C), 160.08, 171.24 (4C, 4C=O); MS, *m/z* (%): 337 (M⁺, 15), 321 (8), 305 (5), 275 (10), 219 (12), 176 (16), 133 (100), 105 (24), 77 (45).

*N*²,*N*⁶-*Bis*(1-hydrazinyl-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**3b**). IR (KBr, cm⁻¹): ν 3470-3218 (NH, NH₂), 1678, 1672 (2C=O, amide); ¹H-NMR (DMSO-d₆): δ 1.35 (s, 12H, 4 CH₃), 4.15 (s, 4H, 2NH₂ exchangeable with D₂O), 8.18-8.26 (m, 3H, pyridine-H), 8.18, 8.98 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 25.86 (4C, 4CH₃), 59.64 (2C, NH-C(CH₃)₂CO), 123.42, 139.00, 149.88 (5C, pyridine-C), 160.08, 178.65 (4C, 4C=O); MS, *m/z* (%): 365 (M⁺, 4), 333 (5), 233 (100), 148 (65), 133 (48), 105 (56), 77 (76).

3.2.3. General procedure for the synthesis of N^2, N^6 -bis(1-(substituted)pyridine-2,6-dicarboxamides **4a-f** and **5a-f**

A mixture of the hydrazide derivative **3a** or **3b** (1 mmol) and the appropriate aldehyde, namely benzaldehyde, *p*-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, *p*-chlorobenzaldehyde, 2-chloro-6-fluorobenzaldehyde, and/or 2-thiophenealdehyde (2 mmol) in absolute ethanol (25 mL) was heated under reflux for 4–6 h. The excess solvent was evaporated under reduced pressure, the residue was washed with *n*-hexane and triturated with diethyl ether. The obtained solid was filtered off, washed with ether, and crystallized from the appropriate solvent (see Table 2) to give the corresponding dicarboxamide derivatives **4a-f** and **5a-f**, respectively.

N^2, N^6 -Bis(1-(2-benzylidenehydrazinyl)-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**4a**). IR (KBr, cm^{-1}): ν 3356–3198 (NH), 1675, 1674 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.32 (d, 6H, 2 CH₃), 4.56 (m, 2H, 2CH), 7.15–7.76 (m, 10H, 2Ph-H), 8.16–8.35 (m, 5H, pyridine-H + 2CH=N), 8.72, 10.54 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 17.76 (2C, 2CH₃), 50.85 (2C, 2CH), 127.16, 128.60, 130.48, 132.98 (12C, 2Ph), 123.36, 138.70, 149.56 (5C, pyridine-C), 143.75 (2C, 2 CH=N), 160.28, 176.86 (4C, 4C=O); MS, m/z (%): 514 (M^+ +1, 12), 436 (4), 359 (16), 317 (24), 275 (100), 245 (65), 216 (32), 176 (46), 133 (90), 105 (64), 77 (52).

N^2, N^6 -Bis(1-(2-(4-methoxybenzylidene)hydrazinyl)-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**4b**). IR (KBr, cm^{-1}): ν 3348–3210 (NH), 1680, 1676 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.34 (d, 6H, 2 CH₃), 3.72 (s, 6H, 2OCH₃), 4.52 (m, 2H, 2CH), 7.18 (d, 4H, Ar-H), 7.78 (d, 4H, Ar-H), 8.18 (s, 2H, 2CH=N), 8.23–8.32 (m, 3H, pyridine-H), 8.68, 10.62 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 17.72 (2C, 2CH₃), 51.05 (2C, 2CH), 54.66 (2C, 2OCH₃), 114.05, 125.60, 129.78, 162.56 (12C, 2Ar-C), 123.30, 138.75, 149.50 (5C, pyridine-C), 144.00 (2C, 2 CH=N), 161.02, 176.82 (4C, 4C=O); MS, m/z (%): 574 (M^+ +1, 6), 542 (12), 511 (6), 353 (24), 322 (18), 220 (100), 204 (65), 189 (13), 133 (76), 118 (46), 105 (46), 77 (44).

N^2, N^6 -Bis(1-oxo-1-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)propan-2-yl)pyridine-2,6-dicarboxamide (**4c**). IR (KBr, cm^{-1}): ν 3354–3218 (NH), 1677, 1674 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.28 (d, 6H, 2 CH₃), 3.76 (s, 18H, 6OCH₃), 4.48 (m, 2H, 2CH), 7.12 (s, 4H, Ar-H), 8.14 (s, 2H, 2CH=N), 8.24–8.36 (m, 3H, pyridine-H), 8.72, 10.76 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 18.05 (2C, 2CH₃), 51.55 (2C, 2CH), 55.10 (4C, 4OCH₃), 58.72 (2C, 2OCH₃), 104.52, 127.65, 140.68, 152.82 (12C, 2Ar-C), 123.45, 139.06, 149.65 (5C, pyridine-C), 145.18 (2C, 2 CH=N), 161.25, 176.88 (4C, 4C=O); MS, m/z (%): 694 (M^+ +1, 4), 662 (8), 631 (12), 526 (18), 412 (28), 280 (100), 204 (45), 189 (8), 167 (34), 133 (82), 105 (66), 77 (56).

N^2, N^6 -Bis(1-(2-(4-chlorobenzylidene)hydrazinyl)-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**4d**). IR (KBr, cm^{-1}): ν 3352–3198 (NH), 1677, 1675 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.28 (d, 6H, 2 CH₃), 4.48 (m, 2H, 2CH), 7.42 (d, 4H, Ar-H), 7.65 (d, 4H, Ar-H), 8.16–8.35 (m, 5H, pyridine-H + 2CH=N), 8.72, 10.82 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 18.04 (2C, 2CH₃), 50.95 (2C, 2CH), 127.82, 128.62, 130.88, 135.76 (12C, 2Ar-C), 123.45, 139.08, 149.45 (5C, pyridine-C), 143.86

(2C, 2 CH=N), 160.94, 176.55 (4C, 4C=O); MS, m/z (%): 582 (M^+ , 6), 584 ($M^+ + 2$, 2), 548 (12), 546 (4), 511 (15), 435 (18), 359 (16), 204 (100), 133 (65), 105 (32), 77 (78).

*N*²,*N*⁶-Bis(1-(2-(2-chloro-6-fluorobenzylidene)hydrazinyl)-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**4e**). IR (KBr, cm^{-1}): ν 3410-3235 (NH), 1682, 1674 (2C=O, amide); ¹H-NMR (DMSO- d_6): δ 1.34 (d, 6H, 2 CH₃), 4.55 (m, 2H, 2CH), 7.24-7.72 (m, 6H, Ar-H), 8.15-8.38 (m, 5H, pyridine-H + 2CH=N), 8.80, 10.74 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 17.88 (2C, 2CH₃), 51.10 (2C, 2CH), 112.68, 117.78, 124.86, 133.56, 134.48, 160.65 (12C, 2Ar-C), 124.00, 139.24, 149.56 (5C, pyridine-C), 142.94 (2C, 2 CH=N), 160.76, 176.55 (4C, 4C=O); MS, m/z (%): 618 (M^+ , 15), 620 ($M^+ + 2$, 6), 488 (13), 490 (4), 359 (100), 318 (66), 275 (88), 204 (96), 133 (45), 105 (86), 77 (84).

*N*²,*N*⁶-Bis(1-oxo-1-(2-(thiophen-2-ylmethylene)hydrazinyl)propan-2-yl)pyridine-2,6-dicarboxamide (**4f**). IR (KBr, cm^{-1}): ν 3390-3212 (NH), 1680, 1675 (2C=O, amide); ¹H-NMR (DMSO- d_6): δ 1.42 (d, 6H, 2 CH₃), 4.46 (m, 2H, 2CH), 7.10-7.65 (m, 6H, thiophene-H), 8.22-8.35 (m, 5H, pyridine-H + 2CH=N), 8.76, 10.65 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 17.92 (2C, 2CH₃), 51.08 (2C, 2CH), 126.76, 127.65, 139.32, 143.84 (8C, 2 thiophene-C), 124.05, 139.32, 149.48 (5C, pyridine-C), 132.88 (2C, 2 CH=N), 160.65, 176.62 (4C, 4C=O); MS, m/z (%): 525 (M^+ , 6), 442 (14), 400 (32), 329 (10), 317 (4), 196 (75), 204 (86), 133 (100), 105 (68), 77 (72).

*N*²,*N*⁶-Bis(1-(2-benzylidenehydrazinyl)-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**5a**). IR (KBr, cm^{-1}): ν 3360-3210 (NH), 1678, 1674 (2C=O, amide); ¹H-NMR (DMSO- d_6): δ 1.42 (s, 12H, 4 CH₃), 7.25-7.82 (m, 10H, 2Ph-H), 8.12-8.25 (m, 3H, pyridine-H), 8.28 (s, 2H, 2CH=N), 8.45, 10.65 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 25.48 (4C, 4CH₃), 59.78 (2C, 2 NHC(CH₃)₂CO), 128.10, 128.90, 130.48, 133.05 (12C, 2Ph-C), 124.12, 139.01, 149.48 (5C, pyridine-C), 143.65 (2C, 2 CH=N), 160.12, 178.86 (4C, 4C=O); MS, m/z (%): 541 (M^+ , 6), 464 (4), 387 (12), 345 (24), 303 (100), 218 (45), 133 (65), 105 (74), 77 (48).

*N*²,*N*⁶-Bis(1-(2-(4-methoxybenzylidene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**5b**). IR (KBr, cm^{-1}): ν 3354-3212 (NH), 1680, 1678 (2C=O, amide); ¹H-NMR (DMSO- d_6): δ 1.30 (s, 12H, 4 CH₃), 3.68 (s, 6H, 2OCH₃), 7.08 (d, 4H, Ar-H), 7.72 (d, 4H, Ar-H), 8.16 (s, 2H, 2CH=N), 8.18-8.30 (m, 3H, pyridine-H), 8.42, 10.68 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 25.36 (4C, 4CH₃), 59.64 (2C, 2 NHC(CH₃)₂CO), 54.55 (2C, 2OCH₃), 113.98, 125.56, 129.88, 162.34 (12C, 2Ar-C), 123.48, 139.05, 149.35 (5C, pyridine-C), 143.89 (2C, 2 CH=N), 160.75, 178.85 (4C, 4C=O); MS, m/z (%): 602 ($M^+ + 1$, 16), 570 (22), 539 (18), 336 (100), 218 (10), 203 (67), 205 (54), 133 (18), 118 (45), 105 (42), 77 (32).

*N*²,*N*⁶-Bis(2-methyl-1-oxo-1-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)propan-2-yl)pyridine-2,6-dicarboxamide (**5c**). IR (KBr, cm^{-1}): ν 3362-3210 (NH), 1679, 1675 (2C=O, amide); ¹H-NMR (DMSO- d_6): δ 1.34 (s, 12H, 4 CH₃), 3.72 (s, 18H, 6OCH₃), 7.10 (s, 4H, Ar-H), 8.22 (s, 2H, 2CH=N), 8.18-8.28 (m, 3H, pyridine-H), 8.62, 10.48 (2s, 4H, 4NH exchangeable with D₂O); ¹³C-NMR: 25.67 (4C, 4CH₃), 55.82 (4C, 4OCH₃), 59.72 (2C, 2 NHC(CH₃)₂CO), 59.88 (2C, 2OCH₃), 104.05, 127.64, 141.08, 153.28 (12C, 2Ar-C), 123.75, 139.12, 149.70 (5C, pyridine-C), 145.22 (2C, CH=N), 160.98,

179.12 (4C, 4C=O); MS, m/z (%): 722 ($M^+ + 1$, 14), 690 (18), 660 (6), 524 (18), 388 (12), 345 (10), 303 (100), 218 (45), 133 (56), 77 (66).

N^2, N^6 -Bis(1-(2-(4-chlorobenzylidene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**5d**). IR (KBr, cm^{-1}): ν 3360-3205 (NH), 1678, 1674 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.32 (s, 12H, 4 CH₃), 7.44 (d, 4H, Ar-H), 7.66 (d, 4H, Ar-H), 8.22-8.37 (m, 5H, pyridine-H + 2CH=N), 8.64, 10.75 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 26.01 (4C, 4CH₃), 59.68 (2C, 2 NHC(CH₃)₂CO), 127.66, 128.88, 130.84, 135.92 (12C, 2Ar-C), 123.65, 139.10, 149.34 (5C, pyridine-C), 143.90 (2C, 2 CH=N), 160.86, 179.55 (4C, 4C=O); MS, m/z (%): 610 (M^+ , 10), 612 ($M^+ + 2$, 3), 498 (18), 500 (5), 463 (6), 387 (24), 345 (35), 303 (78), 218 (100), 133 (25), 105 (30), 77 (82).

N^2, N^6 -Bis(1-(2-(2-chloro-6-fluorobenzylidene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)pyridine-2,6-dicarboxamide (**5e**). IR (KBr, cm^{-1}): ν 3410-3232 (NH), 1680, 1676 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.35 (s, 12H, 4 CH₃), 7.28-7.78 (m, 6H, Ar-H), 8.16-8.36 (m, 5H, pyridine-H + 2CH=N), 8.72, 10.70 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 26.01 (4C, 4CH₃), 59.56 (2C, 2 NHC(CH₃)₂CO), 112.62, 117.66, 124.72, 133.46, 134.52, 160.60 (12C, 2Ar-C), 123.96, 139.12, 149.50 (5C, pyridine-C), 142.67 (2C, 2 CH=N), 160.66, 179.55 (4C, 4C=O); MS, m/z (%): 646 (M^+ , 6), 648 ($M^+ + 2$, 2), 516 (18), 518 (6), 387 (45), 345 (100), 303 (16), 133 (75), 105 (66), 77 (80).

N^2, N^6 -Bis(2-methyl-1-oxo-1-((*E*)-2-(thiophen-2-ylmethylene)hydrazinyl)propan-2-yl)pyridine-2,6-dicarboxamide (**5f**). IR (KBr, cm^{-1}): ν 3392-3208 (NH), 1680, 1676 (2C=O, amide); $^1\text{H-NMR}$ (DMSO- d_6): δ 1.35 (s, 12H, 4 CH₃), 7.14-7.62 (m, 6H, thiophene-H), 8.24-8.38 (m, 5H, pyridine-H + 2CH=N), 8.36, 10.15 (2s, 4H, 4NH exchangeable with D₂O); $^{13}\text{C-NMR}$: 26.02 (4C, 4CH₃), 59.52 (2C, 2 NHC(CH₃)₂CO), 126.64, 127.55, 139.28, 143.92 (8C, 2 thiophene-C), 123.98, 139.36, 149.42 (5C, pyridine-C), 128.10 (2C, 2 CH=N), 160.55, 179.86 (4C, 4C=O); MS, m/z (%): 553 (M^+ , 16), 470 (24), 387 (13), 345 (12), 303 (24), 218 (100), 133 (860), 105 (64), 77 (54).

4. Conclusions

A series of pyridine-bridged 2,6-bis-carboxamide Schiff's bases was prepared starting from 2,6-pyridinedicarbonyl dichloride (**1**) and L-alanine or 2-methylalanine methyl ester. The structural assignments of the new compounds were based on chemical and spectroscopic evidence. The newly synthesized compounds **2-5** have been screened for their bactericidal and fungicidal activities, and the Schiff's bases **4b-f** and **5b-f** have significant antimicrobial activities compared to streptomycin and fusidic acid which were used as antibacterial and antifungal reference drugs, respectively. The substituted 4-methoxy- **4b,5b**, 3,4,5-trimethoxy-**4c,5c**, 4-chloro-**4d,5d**, 2-chloro-6-fluoro-**4e,5e** and 2-thienyl- derivatives **4f,5f** have antimicrobial activities higher than that of **4a,5a** with an unsubstituted phenyl group.

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Sample Availability: Samples of the compounds are available from the authors.

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