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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF CYANOPYRIDINYL TETRAHYDRONAPHTHALENE DERIVATIVES

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Abstract: A novel series of cyanopyridinyl tetrahydronaphthalene incorporated with different heterocycles were synthesized. The key compounds **2a**,**b** were condensed with chloroacetone and ethyl chloroacetate to give **3a**,**b** and **4a**,**b**, respectively. Also condensation of **4a**,**b** with hydrazine hydrate gave the corresponding hydrazide **5a**,**b**. Reaction of **5b** with different isothiocyanates gave the corresponding thiosemicarbazide derivatives **6a-c**. Also, condensation of **5a** with chloroacetic acid, methyl iodide and /or acetic anhydride yielded **7-9**, respectively. Moreover, reaction of **5a** with acetylacetone, ethyl acetoacetate, diethylmalonate, ethyl cyanoacetate, chloroacetone, ethyl chloroacetate, urea, phthalic anhydride, malic anhydride and/ or different aldehydes yielded the corresponding derivatives **10-18**, respectively. Newly synthesized compounds were screened for their antibacterial (*Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Escherichia* coli) and antifungal (*Saccharomyces cerevisiae and Candida albicans*) activity. The results revealed that some of novel compounds have exhibited significant biological activity against the tested microorganisms.

Keywords: tetrahydronaphthalene, cyanopyridine, antimicrobial activity

In the last twenty five years there has been a steep decline in the commercial output and research and development of antimicrobial agents by the major pharmaceutical companies due to the more attractive commercial returns that can be made for treatments of chronic human diseases. At the same time, there has been an explosion both in the numbers of pathogenic bacteria that have become resistant to antibiotics due to their widespread and misuse and of immuno-compromised patients that are particularly susceptible to opportunistic pathogens (1, 2). In addition, it is known that antifungal drugs do not have selective activity because of the biochemical similarity between human cell and fungi forms (3). Therefore, the discovery of new antimicrobial agents with novel modes of action and no cross-resistance with current antibiotics will be vital to meet the threats created by the emergence of bacteria resistant to the current therapeutic agents.

Literature survey revealed that tetralin is an important ring comprising different efficacious antimicrobial derivatives (4, 5). Additionally, the pyridine nucleus plays a key role of catalyzing both biological and chemical systems. In many enzymes of living organisms it is the prosthetic pyridine nucleotide (NADP) that is involved in various oxidation–reduction processes. It is also one of the most important heterocycles found in many antimicrobial pharmaceuticals (6, 7).

Since it is documented that the most effective antimicrobial compounds can be designed by joining two or more biologically active cyclic systems together in a single molecular framework, we report here the synthesis and antimicrobial evaluation of some novel structure hybrids incorporating both the tetralin moiety with pyridine heterocycle. This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities,

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hoping to add some synergistic biological significance to the target molecules.

EXPERIMENTAL

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at the Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, and were found to be within ± 0.5% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100 Fourier transform infrared spectrometer using the KBr disc technique at the Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^{1}H NMR spectra were determined by using a Jeol EX-270 NMR spectrometer at the Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Follow-up of the reactions and checking of the purity of the compounds were made by TLC on silica gel precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to a UV lamp at 254 nm for a few seconds. The chemical names for the prepared compounds are given according to the IUPAC system.

General procedure for the synthesis of 1,2-dihydro-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-substituted-2-oxopyridine-3-carbonitrile 1a,**b**

A mixture of 6-acetyl-1,2,3,4-tetrahydronaphthalene (6.0 g, 0.034 mol), the appropriate aldehyde namely: 3,4-dimethoxybenzaldehyde and/or 4-fluorobenzaldehyde (0.034 mol), ethyl cyanoacetate (3.8 mL, 0.034 mol) and ammonium acetate (21.0 g, 0.272 mol) in n-butanol (50 mL) was refluxed for 6 h. The formed precipitate was filtered, washed with ether, dried and recrystallized from acetic acid to give the title compounds **1a**,**b**, respectively.

1,2-Dihydro-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)-2-oxopyridine-3-carbonitrile (1a)

Yield 65%; m.p. 250-252^{o}C; IR (KBr, cm^{-1}): 2930, 2850 (CH_2 - tetrahydronaphthalene protons), 2219 (CN), 1637 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.74, 2.68 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.83 (6H, s, 2-OCH_3), 6.98-7.23 (7H, m, Ar-H and pyridone proton), 9.21 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 386 [M$^+$] (100); Analysis: calcd. for $C_{24}H_{22}N_2O_3$ (386.44): C, 74.59; H, 5.74; N, 7.25%; found: C, 74.89; H, 5.94; N, 7.35%.

4-(4-Fluorophenyl)-1,2-dihydro-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-2-oxopyridine-3-carbonitrile (8)

General procedure for the synthesis of 6-(1,2,3,4-tetrahydronaphthalen-6-yl)-2-mercapto-4-substituted-pyridine-3-carbonitrile 2a,b

A mixture of compounds **1a**,**b** (0.01 mol) and P_2S_5 (2.22 g, 0.01 mol) in pyridine (15 mL) was refluxed for 5 h. After cooling, the mixture was poured onto ice/cold water and acidified with hydrochloric acid. The formed precipitate was filtered, washed with water several times, dried and recrystallized from ethanol to give the title compounds **2a**,**b**, respectively.

6-(1,2,3,4-Tetrahydronaphthalen-6-yl)-2-mercapto-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (2a)

Yield 65%; m.p. 107-108^{o}C; IR (KBr, cm^{-1}): 2925, 2852 (CH_2 - tetrahydronaphthalene protons), 2215 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.72, 2.74 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.81 (6H, s, 2-OCH_3), 6.98-7.23 (7H, m, Ar-H and pyridine proton), 8.81 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 402 [M$^+$] (100); Analysis: calcd. for $C_{24}H_{22}N_2O_2S$ (402.51): C, 71.62; H, 5.51; N, 6.96; S, 7.79%; found: C, 71.32; H, 5.01; N, 6.76; S, 7.54%.

4-(4-Fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-2-mercaptopyridine-3-carbonitrile (2b)

Yield 55%; m.p. 95-96^{o}C; IR (KBr, cm^{-1}): 2930, 2850 (CH_2 - tetrahydronaphthalene protons), 2219 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.73, 2.69 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 6.81-7.54 (8H, m, Ar-H and pyridine proton), 8.75 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 360 [M$^+$] (100); Analysis: calcd. for $C_{22}H_{17}FN_2S$ (360.45): C, 73.31; H, 4.75; N, 7.77; S, 8.90%; found: C, 73.41; H, 4.82; N, 7.93; S, 8.75%.

General procedure for the synthesis of 2-(2-oxopropylthio)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-substituted-pyridine-3-carbonitrile 3a,b and ethyl 2-(3-cyano-6-(1,2,3,4-tetrahydronaphhalen-6-yl)-4-substituted-pyridin-2-ylthio)acetate 4a,**b**

A mixture of compounds **2a**,**b** (0.003 mol), anhydrous sodium carbonate (0.32 g, 0.003 mol)

and the appropriate halo derivatives, namely: chloroacetone and/or ethyl chloroacetate (0.003 mol) in DMF (20 mL) was refluxed for 4 h. The reaction mixture was cooled and poured onto ice/cold water and acidified with hydrochloric acid. The formed precipitate was filtered, washed with water several times, dried and recrystallized from methanol to give the title compounds **3a**,**b** and **4a**,**b**, respectively.

2-(2-Oxopropylthio)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (3a)

Yield 35%; m.p. 149-150°C; IR (KBr, cm^{-1}): 2929, 2853 (CH_2 - tetrahydronaphthalene protons), 2215 (CN), 1702 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70, 2.71 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.28 (3H, s, CH_3CO), 3.83 (6H, s, 2-OCH_3), 4.10 (2H, s, SCH_2), 6.72-7.91 (7H, m, Ar-H and pyridine proton); MS, m/z (%): 458 [M^+] (70), 459 [M^++1] (38), 73 [C_2H_3NS] (100); Analysis: calcd. for $C_{27}H_{26}N_2O_3S$ (458.57): C, 70.72; H, 5.71; N, 6.11; S, 6.99%; found: C, 70.43; H, 5.91; N, 6.61; S, 7.13%.

2-(2-Oxopropylthio)-4-(4-fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)pyridine-3-carbonitrile (3b)

Yield 40%; m.p. 114-115°C; IR (KBr, cm^{-1}): 2929, 2857 (CH_2 - tetrahydronaphthalene protons), 2216 (CN), 1709 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69, 2.68 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.17 (3H, s, CH_3CO), 3.79 (6H, s, 2-OCH_3), 4.12 (2H, s, SCH_2), 6.83-7.85 (8H, m, Ar-H and pyridine proton); MS, m/z (%): 416 [M^+] (100); Analysis: calcd. for $C_{25}H_{21}FN_2OS$ (416.51): C, 72.09; H, 5.08; N, 6.73; S, 7.70%; found: C, 72.31; H, 4.98; N, 6.82; S, 7.56%.

Ethyl 2-(3-cyano-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridin-2-ylthio)acetate (4a)

Yield 70%; m.p. 164-165°C; IR (KBr, cm^{-1}): 2931, 2834 (CH_2 - tetrahydronaphthalene protons), 2214 (CN), 1725 (CO, ester); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.29 (3H, t, -$COOCH_2\underline{CH_3}$), 1.69, 2.72 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.75 (6H, s, 2-OCH_3), 4.13 (2H, s, SCH_2), 4.16 (2H, q, -$COO\underline{CH_2}CH_3$), 7.05-7.99 (7H, m, Ar-H and pyridine proton); MS, m/z (%): 488 [M^+] (35), 115 (C_9H_7) (100); Analysis: calcd. for $C_{28}H_{28}N_2O_4S$ (488.60): C, 68.83; H, 5.78; N, 5.73; S, 6.56%; found: C, 68.83; H, 5.91; N, 5.63; S, 6.68%.

Ethyl 2-(3-cyano-4-(4-fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)pyridin-2-ylthio)acetate (4b)

Yield 68%; m.p. 131-132°C; IR (KBr, cm^{-1}): 2921, 2830 (CH_2 - tetrahydronaphthalene protons), 2217 (CN), 1740 (CO, ester); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.24 (3H, t, -$COOCH_2\underline{CH_3}$), 1.71, 2.67 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.78 (6H, s, 2-OCH_3), 4.11 (2H, s, SCH_2), 4.15 (2H, q, -$COO\underline{CH_2}CH_3$), 7.05-7.89 (8H, m, Ar-H and pyridine proton);; MS, m/z (%): 446 [M^+] (15), 357 [$C_{22}H_{14}FN_2S$] (100); Analysis: calcd. for $C_{26}H_{23}FN_2O_2S$ (446.54): C, 69.93; H, 5.19; N, 6.27; S, 7.18%; found: C, 70.28; H, 5.44; N, 6.35; S, 7.23%.

General procedure for the synthesis of 2-hydrazinyl-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(substituted phenyl)pyridine-3-carbonitrile 5a,b

Method A

A mixture of compound **2a**,**b** (0.01 mol) and hydrazine hydrate (1.0 mL, 0.02 mol) in absolute ethanol was refluxed for 4 h. After cooling the formed precipitate was filtered, dried and recrystallized from methanol to give the title compound **5a**,**b**.

Method B

A mixture of compounds **4a**,**b** (0.002 mol) and hydrazine hydrate (0.1 mL, 0.002 mol) in absolute ethanol (20 mL) was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from dioxane to give the title compounds **5a**,**b**, respectively.

2-Hydrazinyl-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (5a)

Yield 88%; m.p. 139-140°C; IR (KBr, cm^{-1}): 3245, 3177 (NH, NH_2), 2935, 2855 (CH_2 - tetrahydronaphthalene protons), 2216 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.67, 2.68 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.78 (6H, s, 2-OCH_3), 6.95-7.98 (7H, m, Ar-H and pyridine proton), 8.98, 9.01 (3H, s, $NHNH_2$, exchangeable by D_2O); MS, m/z (%): 400 [M^+] (32), 402 [M^+ + 2] (65), 370 [M^+-N_2H_2] (100); Analysis: calcd. for $C_{24}H_{24}N_4O_2$ (400.47): C, 71.98; H, 6.04; N, 13.99%; found: C, 71.74; H, 6.28; N, 13.68%.

4-(4-Fluorophenyl)-2-hydrazinyl-6-(1,2,3,4-tetrahydronaphthalen -6-yl)pyridine-3-carbonitrile (5b)

Yield 63%; m.p. 128-129°C; IR (KBr, cm^{-1}): 3336, 3208 (NH, NH_2), 2932, 2850 (CH_2 - tetrahy-

dronaphthalene protons), 2217 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69, 2.66 (8H, m, 4(CH_2) – tetrahydronaphthalene protons), 7.04-8.04 (8H, m, Ar-H and pyridine proton), 8.67, 9.22 (3H, s, $NHNH_2$, exchangeable by D_2O); MS, m/z (%): 358 [M^+] (95), 359 [M^+ + 1] (30), 77 [C_6H_5] (100); Analysis: calcd. for $C_{22}H_{19}FN_4$ (358.41): C, 73.72; H, 5.34; N, 15.63%; found: C, 73.51; H, 5.21; N, 15.78%.

General procedure for the synthesis of 1-(3-cyano-4-(4-fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)pyridin-2-yl)-4- substituted thiosemicarbazide 6a-c

A mixture of compound **5b** (0.87 g, 0.002 mol), the appropriate isothiocyanate, namely: methylisothiocyanate, ethylisothiocyanate and/or phenylisothiocyanate in dry benzene (20 mL) was refluxed for 6 h. The solvent was evaporated under reduced pressure and the remaining solid was recrystallized from methanol to give the title compounds **6a-c**, respectively.

1-(3-Cyano-4-(4-fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)pyridin-2-yl)-4-methylthiosemicarbazide (6a)

Yield 50%; m.p. 158-159°C; IR (KBr, cm^{-1}): 3372, 3256, 3151 (3NH), 2929, 2845 (CH_2 - tetrahydronaphthalene protons), 2216 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71, 2.82 (8H, m, 4(CH_2)-tetrahydronaphthalene protons), 2.56 (3H, s, -$NHCH_3$), 7.03-7.95 (8H, m, Ar-H and pyridine proton), 8.50, 8.68, 9.01 (3H, s, 3NH, exchangeable by D_2O); MS, m/z (%): 399 [M^+ - CH_6N] (30), 358 [$C_{22}H_{19}FN_4$] (100); Analysis: calcd. for $C_{24}H_{22}FN_5S$ (431.53): C, 66.80; H, 5.14; N, 16.23; S, 7.43%; found: C, 66.58; H, 5.29; N, 16.51; S, 7.57%.

1-(3-Cyano-4-(4-fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)pyridin-2-yl)-4-ethylthiosemicarbazide (6b)

Yield 45%; m.p. 215-216°C; IR (KBr, cm^{-1}): 3370, 3251, 3166 (3NH), 2932, 2851 (CH_2 - tetrahydronaphthalene protons), 2214 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.05 (3H, t, -$NHCH_2\underline{CH_3}$), 1.69, 2.78 (8H, m, 4(CH_2) – tetrahydronaphthalene protons), 3.51 (3H, q, -$NH\underline{CH_2}CH_3$), 7.07-8.10 (8H, m, Ar-H and pyridine proton), 8.41, 8.72, 9.21 (3H, s, 3NH, exchangeable by D_2O); MS, m/z (%): 418 [M^+ - C_2H_3] (6), 401 [M^+ - C_2H_5N] (100); Analysis: calcd. for $C_{25}H_{24}FN_5S$ (445.55): C, 67.39; H, 5.43; N, 15.72; S, 7.20%; found: C, 67.53; H, 5.34; N, 15.60; S, 7.44%.

1-(3-Cyano-4-(4-fluorophenyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)pyridin-2-yl)-4-phenylthiosemicarbazide (6c)

Yield 39%; m.p. 169-170°C; IR (KBr, cm^{-1}): 3362, 3279, 3181 (3NH), 2931, 2855 (CH_2 - tetrahydronaphthalene protons), 2218 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71, 2.73 (8H, m, 4(CH_2) – tetrahydronaphthalene protons), 7.12-7.97 (13H, m, Ar-H and pyridine proton), 8.38, 8.64, 8.89 (3H, s, 3NH, exchangeable by D_2O); MS, m/z (%): 399 [M^+ - C_6H_8N] (4), 77 [C_6H_5] (100); Analysis: calcd. for $C_{29}H_{24}FN_5S$ (493.60): C, 70.57; H, 4.90; N, 14.19; S, 6.50%; found: C, 70.29; H, 4.58; N, 14.31; S, 6.82%.

2-(2-Hydrazinyl-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carboyl)acetic acid (7)

A mixture of compound **5a** (2.0 g, 0.005 mol), chloroacetic acid (0.47 g, 0.005 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in acetic anhydride (25 mL) and glacial acetic acid (50 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured onto ice/cold water; the formed precipitate was filtered, dried, and recrystallized from ethanol to give compound **7**.

Yield 58%; m.p. 129-130°C; IR (KBr, cm^{-1}): 3417 (COOH), 2925, 2853 (CH_2 - tetrahydronaphthalene protons), 2214 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.68, 2.69 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.51 (2H, s, -CH_2), 3.76 (6H, s, 2-OCH_3), 6.88-8.01 (7H, m, Ar-H and pyridine proton), 8.23, 8.64, 11.21 (3H, s, 2NH, OH, exchangeable by D_2O); MS, m/z (%): 460 [M^+ + 2] (11), 441 [M^+ - OH] (38), 386 [M^+ - $C_2H_2NO_2$] (100); Analysis: calcd. for $C_{26}H_{26}N_4O_4$ (458.51): C, 68.11; H, 5.72; N, 12.22%; found: C, 68.24; H, 5.61; N, 12.43%.

2-(2-Methylhydrazinyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (8)

A mixture of compound **5a** (0.8 g, 0.002 mol), anhydrous sodium carbonate (0.21 g, 0.002 mol) and iodomethane (0.29 mL, 0.002 mol) in DMF (20 mL) was refluxed for 7 h. Then, the reaction mixture was cooled, poured onto ice/cold water. The formed precipitate was filtered, dried, and recrystallized from acetic acid to give the title compound **8**.

Yield 76%; m.p. 114-115°C; IR (KBr, cm^{-1}): 3352, 3215 (2NH), 2921, 2853 (CH_2 - tetrahydronaphthalene protons), 2216 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69, 2.72 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.56 (2H, s, -CH_3), 3.76 (6H, s, 2-OCH_3), 6.75-8.12 (7H, m, Ar-H and pyri-

dine proton), 8.11, 8.53 (2H, s, 2NH, exchangeable by D_2O); MS, m/z (%): 414 [M^+] (26), 416 [$M^+ + 2$] (76), 77 [C_6H_5] (100); Analysis: calcd. for $C_{25}H_{26}N_4O_2$ (414.50): C, 72.44; H, 6.32; N, 13.52%; found: C, 72.28; H, 6.52; N, 13.33%.

N'-(3-cyano-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridin-2-yl)acetohydrazide (9)

A mixture of compound **5a** (0.40 g, 0.001 mol) and acetic anhydride (0.11 mL, 0.001 mol) in acetic acid (10 mL) was refluxed for 6 h. The reaction mixture was cooled and poured onto ice/cold water. The formed precipitate was filtered, dried and recrystallized to give the title compound **9**.

Yield 65%; m.p. 101-103°C; IR (KBr, cm^{-1}): 3425, 3258 (2NH), 2930, 2857 (CH_2 - tetrahydronaphthalene protons), 2216 (CN), 1633 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.68, 2.59 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.12 (3H, s, CH_3), 3.73 (6H, s, 2-OCH_3), 6.88-7.74 (7H, m, Ar-H and pyridine proton), 7.91, 8.25 (2H, s, 2NH, exchangeable by D_2O); MS, m/z (%): 442 [M^+] (8), 415 [$C_{25}H_{25}N_3O_3$] (100); Analysis: calcd. for $C_{26}H_{26}N_4O_3$ (442.51): C, 70.57; H, 5.92; N, 12.66%; found: C, 70.41; H, 5.71; N, 12.43%.

6-(1,2,3,4-Tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)pyridine-3-carbonitrile (10)

Refluxing a mixture of compound **5a** (0.40 g, 0.001 mol) and acetylacetone (0.10 mL, 0.001 mol) in acetic acid (15 mL) for 6 h. The formed precipitate after cooling was filtered, dried and recrystallized from acetic acid to give the title compound **10**. Yield 52%: m.p. 114-115°C; IR (KBr, cm^{-1}): 2928, 2855 (CH_2 - tetrahydronaphthalene protons), 2214 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69, 2.71 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.94 (6H, s, 2CH_3-pyrazole ring), 3.78 (6H, s, 2-OCH_3), 6.01 (1H, s, pyrazole proton), 6.82-8.21 (7H, m, Ar-H and pyridine proton); MS, m/z (%): 414 [M^+ - C_4H_2] (42), 344 [M^+ - $C_6H_6N_3$] (50), 55 [C_4H_7] (100); Analysis: calcd. for $C_{29}H_{28}N_4O_2$ (464.56): C, 74.98; H, 6.08; N, 12.06%; found: C, 74.73; H, 5.94; N, 12.26%.

General procedure for the synthesis of compounds (11-13)

A mixture of compound **5a** (0.40 g, 0.001 mol) and active methylene compounds, namely: ethyl acetoacetate, diethylmalonate and/or ethyl cyanoacetate (0.001 mol) in ethanol (20 mL) containing few drops of piperidine was refluxed for 4 h. The formed precipitate was filtered, dried and recrystallized from methanol to give the title compounds **11-13**, respectively.

2-(4,5-Dihydro-3-methyl-5-oxopyrazol-1-yl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (11)

Yield 60%; m.p. 120-121°C; IR (KBr, cm^{-1}): 2928, 2857 (CH_2 - tetrahydronaphthalene protons), 2211 (CN), 1635 (CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69, 2.71 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.02 (3H, s, CH_3), 2.21 (2H, s, CH_2 - pyrazole proton), 3.79 (6H, s, 2-OCH_3), 6.80-8.32 (7H, m, Ar-H and pyridine proton); MS, m/z (%): 452 [M^+ - CH_2] (2), 402 [$C_{24}H_{26}N_4O_2$] (100); Analysis: calcd. for $C_{28}H_{26}N_4O_3$ (466.53): C, 72.09; H, 5.62; N, 12.01%; found: C, 72.35; H, 5.81; N, 12.27%.

6-(1,2,3,4-Tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)-2-(3,5-dioxopyrazolidin-1-yl) pyridine-3-carbonitrile (12)

Yield 75%; m.p. 107-108°C; IR (KBr, cm^{-1}): 3428 (NH), 2927, 2855 (CH_2 - tetrahydronaphthalene protons), 2215 (CN); 1735, 1633 (CO cyclic); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.72, 2.75 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.11 (2H, s, CH_2 - pyrazole proton), 3.79 (6H, s, 2-OCH_3), 6.95-8.42 (7H, m, Ar-H and pyridine proton), 8.69 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 443 [$C_{26}H_{25}N_3O_2$] (3), 56 [C_4H_8] (100); Analysis: calcd. for $C_{27}H_{24}N_4O_4$ (468.50): C, 69.22; H, 5.16; N, 11.96%; found: C, 69.39; H, 5.41; N, 11.75%.

2-(3-Amino-4,5-dihydro-5-oxopyrazol-1-yl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (13)

Yield 80%; m.p. 145-146°C; IR (KBr, cm^{-1}): 3386, 3124 (NH_2), 2928, 2855 (CH_2 - tetrahydronaphthalene protons), 2210 (CN), 1736 (CO, cyclic); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.72, 2.74 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.20 (2H, s, CH_2), 3.78 (6H, s, 2-OCH_3), 6.95-7.98 (7H, m, Ar-H and pyridine proton), 9.98 (2H, s, NH_2, exchangeable by D_2O); MS, m/z (%): 467 [M^+] (8), 402 [$C_{24}H_{26}N_4O_2$] (100); Analysis: calcd. for $C_{27}H_{25}N_5O_3$ (467.52): C, 69.36; H, 5.39; N, 14.98%; found: C, 69.61; H, 5.60; N, 14.77%.

3,4-Dihydro-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-8-(3,4-dimethoxyphenyl)-3-methyl-2H-pyrido[2,1-c][1,2,4]triazine-9-carbonitrile (14)

A mixture of compound **5a** (0.40 g, 0.001 mol) and chloroacetone (0.10 mL, 0.001 mol) in dry

Table 1. Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay.

Compound No.	Gram positive bacteria				Gram negative bacteria			Yeast	
	Staphylococcus aureus ATCC 29213	*Bacillus subtilis* ATCC6633	*Bacillus megaterium* ATCC 9885	*Sarcina lutea*	*Klebsiella pneumoniae* ATCC13883	*Pseudomonas aeruginosa* ATCC27953	*Escherichia coli* ATCC25922	*Saccharomyces cerevisiae*	*Candida albicans* NRRL Y-477
1a	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	21	20
2a	20	22	20	21	26	20	22	23	22
2b	27	24	29	20	26	28	25	33	32
3a	17	17	17	19	N.A.	N.A.	N.A.	N.A.	18
3b	20	20	18	22	22	18	20	21	25
4a	17	20	16	17	17	18	16	21	26
4b	17	N.A.	N.A.	16	16	16	17	18	20
5a	30	35	28	34	33	35	32	24	28
5b	17	25	N.A.	N.A.	30	28	32	29	25
6a	25	22	28	22	16	18	17	16	16
6b	15	26	28	29	24	20	20	29	23
6c	18	20	15	16	19	19	20	18	22
7	19	18	16	19	21	20	20	18	20
8	13	18	14	15	25	23	22	24	22
9	19	12	16	20	15	14	13	18	20
10	19	14	17	N.A.	18	17	17	23	22
11	17	15	16	17	19	18	16	20	20
12	17	17	16	16	17	18	17	25	21
13	15	17	14	88	19	19	18	20	18
14	17	16	15	18	15	13	14	21	22
15	18	15	15	19	N.A.	16	17	22	20
16	18	13	16	18	14	18	13	25	21

Table 1. cont.

Compound No.	Gram positive bacteria				Gram negative bacteria			Yeast	
	Staphylococcus aureus ATCC 29213	*Bacillus subtilis* ATCC6633	*Bacillus megaterium* ATCC 9885	*Sarcina lutea*	*Klebsiella pneumoniae* ATCC13883	*Pseudomonas aeruginosa* ATCC27953	*Escherichia coli* ATCC25922	*Saccharomyces cerevisiae*	*Candida albicans* NRRL Y-477
17a	14	14	15	15	26	25	27	29	26
18a	20	16	15	18	18	18	17	17	N.A.
18b	14	18	16	16	17	15	14	20	18
18c	23	17	13	27	18	18	19	17	18
18d	18.	17	15	16	16	16	17	18	15
Ciprofloxacin	20	22	24	20	25	24	23	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	23	22

The experiment was carried out in triplicate and the average zone of inhibition was calculated. N.A. = not active.

xylene (20 mL) was refluxed for 5 h. The formed precipitate that separated while hot was collected and recrystallized from ethanol to give the title compound **14**.

Yield 78%; m.p. 122-123°C; IR (KBr, cm^{-1}): 3423 (NH), 2922, 2852 (CH_2 - tetrahydronaphthalene protons), 2212 (CN); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.13 (3H, d, CH_3), 1.70, 2.67 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.79 (2H, m, CH_2 - triazine protons), 3.34 (1H, m, CH - triazine proton), 3.78 (6H, s, 2-OCH_3), 6.65-7.89 (7H, m, Ar-H and pyridine proton), 8.51 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 443 [M^+ + 3] (8), 57 [C_4H_9] (100); Analysis: calcd. for $C_{27}H_{28}N_4O_2$ (440.54): C, 73.61; H, 6.41; N, 12.72%; found: C, 73.50; H, 6.61; N, 12.51%.

3,4-Dihydro-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-8-(3,4-dimethoxyphenyl)-3-oxo-2H-pyrido[2,1-c][1,2,4]triazine-9-carbonitrile (15)

A mixture of compound **5a** (0.40 g, 0.001 mol) and ethyl chloroacetate (0.12 mL, 0.001 mol) in ethanol (20 mL) was refluxed for 6 h. The formed precipitate after cooling was filtered, dried and recrystallized form methanol to give the title compound **15**.

Yield 58%; m.p. 140-141°C; IR (KBr, cm^{-1}): 3448 (NH), 2928, 2852 (CH_2 - tetrahydronaphthalene protons), 2213 (CN), 1740 (CO, cyclic); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70, 2.67 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.51 (2H, s, CH_2 - triazine protons), 3.79 (6H, s, 2-OCH_3), 6.71-7.92 (7H, m, Ar-H and pyridine proton), 8.77 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 429 [$C_{26}H_{27}N_3O_3$] (2), 59 [C_4H_{11}] (100); Analysis: calcd. for $C_{26}H_{24}N_4O_3$ (440.49): C, 70.89; H, 5.49; N, 12.72%; found: C, 70.62; H, 5.38; N, 12.81%.

2,3-Dihydro-5-(1,2,3,4-tetrahydronaphthalen-6-yl)-7-(3,4-dimethoxyphenyl)-3-oxo-[1,2,4]triazolo[4,3-a]pyridine-8-carbonitrile (16)

A mixture of compound **5a** (0.40 g, 0.001 mol) and urea (0.01mol) was heated at 190-200°C for 6 h. After cooling, the reaction mixture was triturated with hot water, The formed precipitate was filtered, dried and recrystallized from ethanol to give the title compound **16**.

Yield 38%; m.p. 203-204°C; IR (KBr, cm^{-1}): 3204 (NH), 2927, 2854 (CH_2 - tetrahydronaphthalene protons), 2218 (CN), 1693 (CO, cyclic); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71, 2.70 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.83 (6H, s, 2-OCH_3), 6.97-7.91 (6H, m, Ar-H and pyridine proton), 8.64 (1H, s, NH, exchangeable by D_2O);

Table 2 Minimum inhibitory concentration (μg/mL) against the pathological strains based on twofold serial dilution technique.

Compound No.	Gram positive bacteria				Gram negative bacteria			Yeast	
	Staphylococcus aureus ATCC 29213	*Bacillus subtilis* ATCC6633	*Bacillus megaterium* ATCC 9885	*Sarcina lutea*	*Klebsiella pneumoniae* ATCC13883	*Pseudomonas aeruginosa* ATCC27953	*Escherichia coli* ATCC25922	*Saccharomyces cerevisiae*	*Candida albicans* NRRL Y-477
1a	-	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	100	100
2a	100	100	100	100	50	100	50	50	50
2b	50	50	50	50	50	50	50	25	25
3a	200	200	200	200	-	-	-	-	200
3b	20	20	18	22	22	18	20	100	50
4a	200	200	200	200	200	18200	200	100	100
4b	200	-	-	200	200	200	200	200	200
5a	25	25	25	25	25	25	25	50	50
5b	200	50	-	-	50	50	25	50	50
6a	50	100	50	100	200	200	200	200	200
6b	-	50	50	50	100	100	100	50	50
6c	200	200	-	200	200	200	200	200	100
7	200	200	200	200	200	200	200	200	200
8	-	200	-	-	100	100	100	100	100
9	200	-	200	100	-	-	-	200	100
10	200	-	200	-	200	200	200	100	50
11	200	-	200	200	200	200	200	200	200
12	200	200	200	200	200	200	200	50	50
13	-	200	-	200	200	200	200	200	200
14	200	200	-	200	-	-	-	100	100
15	200	-	-	200	-	200	200	100	100
16	200	-	200	200	-	200	-	50	100

Table 2. cont.

Compound No.	Gram positive bacteria				Gram negative bacteria			Yeast	
	Staphylococcus aureus ATCC 29213	*Bacillus subtilis* ATCC6633	*Bacillus megaterium* ATCC 9885	*Sarcina lutea*	*Klebsiella pneumoniae* ATCC13883	*Pseudomonas aeruginosa* ATCC27953	*Escherichia coli* ATCC25922	*Saccharomyces cerevisiae*	*Candida albicans* NRRL Y-477
17a	-	-	-	-	100	100	50	50	100
18a	100	200	-	200	200	200	200	200	N.A.
18b	-	200	200	200	200	-	-	200	200
18c	100	200	-	50	200	200	200	200	200
18d	200	200	-	200	200	200	200	200	-
Ciprofloxacin	25	25	25	25	25	25	25	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	25	25

N.A. = not active

MS, m/z (%): 430 [M^+ + 4] (10), 60 [C_3H_8O] (100); Analysis: calcd. for $C_{25}H_{22}N_4O_3$ (426.47): C, 70.41; H, 5.20; N, 13.14%; found: C, 70.59; H, 5.40; N, 13.26%.

General procedure for the synthesis compounds 17a, 17b

A mixture of compound **5a** (0.40 g, 0.001 mol), and an appropriate acid anhydride, namely: succinic anhydride and/or phthalic anhydride (0.001 mol) in acetic acid (10 mL) was refluxed for 6 h. The formed precipitate was filtered, dried and recrystallized to give the title compound **17a**,**b**, respectively.

2-(2,5-Dioxo-2H-pyrrol-1(5H)-ylamino)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (17a)

Yield 63%; m.p. 149-150°C; IR (KBr, cm^{-1}): 3256 (NH), 2929, 2854 (CH_2 - tetrahydronaphthalene protons), 2216 (CN), 1719 (2CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71, 2.76 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.76 (6H, s, 2-OCH_3), 6.79 (2H, s, CH-pyrrole protons), 7.08-7.85 (7H, m, Ar-H and pyridine proton), 8.34 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 476 [M^+ - 4] (2), 54 [C_4H_6] (100); Analysis: calcd. for $C_{28}H_{24}N_4O_4$ (480.51): C, 69.99; H, 5.03; N, 11.66%; found: C, 69.74; H, 5.24; N, 11.52%.

2-(1,3-Dioxoisoindolin-2-ylamino)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (17b)

Yield 73%; m.p. 189-190°C; IR (KBr, cm^{-1}): 3139 (NH), 2922, 2850 (CH_2 - tetrahydronaphthalene protons), 2214 (CN), 1722 (2CO); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.74, 2.75 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.79 (6H, s, 2-OCH_3), 6.93-7.94 (11H, m, Ar-H and pyridine proton), 8.51 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 501 [$C_{31}H_{25}N_4O_3$] (2), 76 [C_6H_4] (100); Analysis: calcd. for $C_{32}H_{26}N_4O_4$ (530.57): C, 72.44; H, 4.94; N, 10.56%; found: C, 72.20; H, 4.74; N, 10.69%.

General procedure for the synthesis of compounds 18a-d

A mixture of compound **5a** (0.40 g, 0.001 mol) and an appropriate aldehyde, namely: 4-chlorobenzaldehyde, anisaldehyde, 3-methylfurfural and/or 3-indolaldehyde (0.001 mol) in absolute ethanol (15 mL) was refluxed for 6 h. The reaction mixture was cooled and poured onto ice/cold water. The formed precipitate was filtered, dried and recrystallized

from methanol to give the title compound **18a-d**, respectively.

2-(2-(4-Chlorobenzylidene)hydrazinyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (18a)

Yield 48%; m.p. 111-112°C; IR (KBr, cm^{-1}): 3396 (NH), 2919, 2852 (CH_2 - tetrahydronaphthalene protons), 2215 (CN); ^1H NMR (DMSO-d_6, δ, ppm): 1.71, 2.76 (8H, m, 4(CH_2) – tetrahydronaphthalene protons), 3.80 (6H, s, 2-OCH_3), 7.01-7.98 (12H, m, Ar-H, CH=N and pyridine proton), 8.60 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 415 [M^+ - C_6HCl] (2), 165, 167 [$C_8H_6ClN_2$] (100, 31); Analysis: calcd. for $C_{31}H_{27}ClN_4O_2$ (523.02): C, 71.19; H, 5.20; N, 10.71%; found: C, 71.38; H, 5.31; N, 10.57%.

2-(2-(4-Methoxybenzylidene)hydrazinyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (18b)

Yield 55%; m.p. 120-121°C; IR (KBr, cm^{-1}): 3423 (NH), 2929, 2838 (CH_2 - tetrahydronaphthalene protons), 2214 (CN); ^1H NMR (DMSO-d_6, δ, ppm): 1.68, 2.61 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.79 (6H, s, 2-OCH_3), 3.82 (3H, s, -OCH_3), 7.05-8.03 (12H, m, Ar-H, CH=N and pyridine proton), 8.52 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 506 [$C_{31}H_{30}N_4O_3$] (2), 77 [C_6H_5] (100); Analysis: calcd. for $C_{32}H_{30}N_4O_3$ (518.61): C, 74.11; H, 5.83; N, 10.80%; found: C, 74.24; H, 5.91; N, 10.62%.

2-(2-((5-Methylfuran-2-yl)methylene)hydrazinyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (18c)

Yield 60%; m.p. 109-110°C; IR (KBr, cm^{-1}): 3422 (NH), 2921, 2851 (CH_2 - tetrahydronaphthalene protons), 2214 (CN); ^1H NMR (DMSO-d_6, δ, ppm): 1.72, 2.67 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 2.22 (3H, s, -CH_3), 3.79 (6H, s, 2-OCH_3), 5.82-7.95 (10H, m, Ar-H, CH=N and pyridine proton), 8.41 (1H, s, NH, exchangeable by D_2O); MS, m/z (%): 494 [M^+ + 2] (2), 79 [C_6H_7] (100); Analysis: calcd. for $C_{30}H_{28}N_4O_3$ (492.57): C, 73.15; H, 5.73; N, 11.37%; found: C, 73.30; H, 5.55; N, 11.12%.

2-(2-((1H-indol-3-yl)methylene)hydrazinyl)-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(3,4-dimethoxyphenyl)pyridine-3-carbonitrile (18d)

Yield 68%; m.p. 131-132°C; IR (KBr, cm^{-1}): 3361, 3201 (2NH), 2923, 2854 (CH_2 - tetrahydronaphthalene protons), 2216 (CN); ^1H NMR (DMSO-d_6, δ, ppm): 1.74, 2.67 (8H, m, 4(CH_2) - tetrahydronaphthalene protons), 3.80 (6H, s, 2-OCH_3), 6.87-7.87 (13H, m, Ar-H, CH=N and pyridine proton), 8.32, 9.21 (2H, s, 2NH, exchangeable by D_2O); MS, m/z (%): 496 [M^+ - OCH_3] (2), 144 [$C_9H_8N_2$] (100); Analysis: calcd. for $C_{33}H_{29}N_5O_2$ (527.62): C, 75.12; H, 5.54; N, 13.27%: found: C75.27; H, 5.62; N, 13.10%.

Antimicrobial studies

The compounds were individually tested against a panel of Gram positive and Gram negative bacterial pathogens, yeast and fungi (*Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Sarcina lutea, Klebseilla pneumoniae, Pseudomonas aeruginosa*, *Escherichia coli, Saccharomyces cerevisiae and Candida albicans*). Antimicrobial tests were carried out by the agar well diffusion method (9) using 100 µL of suspension containing 1 × 10^8 CFU/mL of pathological tested bacteria and 1 × 10^6 CFU/mL of yeast spread on nutrient agar (NA) and Sabouraud dextrose agar (SDA), respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 200 mg of the chemical compound in 1 mL of dimethyl sulfoxide (DMSO). The inculcated plates were then incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. (ciprofloxacin (50 mg/mL) and ketoconazole (50 mg/mL)) were used as standard for antibacterial and antifungal activity, respectively. Ciprofloxacin and ketoconazole were used as standards because they have abroad spectra antibiotic widely used. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal inhibitory concentration (MIC) measurement

The bacteriostatic activity of the active compounds (having inhibition zones (IZ) = 16 mm) was then evaluated using the twofold serial dilution technique (10). Twofold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions were 200,100, 50, 25 µg /mL. Each 5 mL

received 0.1 mL of the appropriate inoculums and incubated at 37°C for 24 h for bacteria and 48 h at 28°C for fungi. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC) (Table 2).

RESULTS AND DISCUSSION

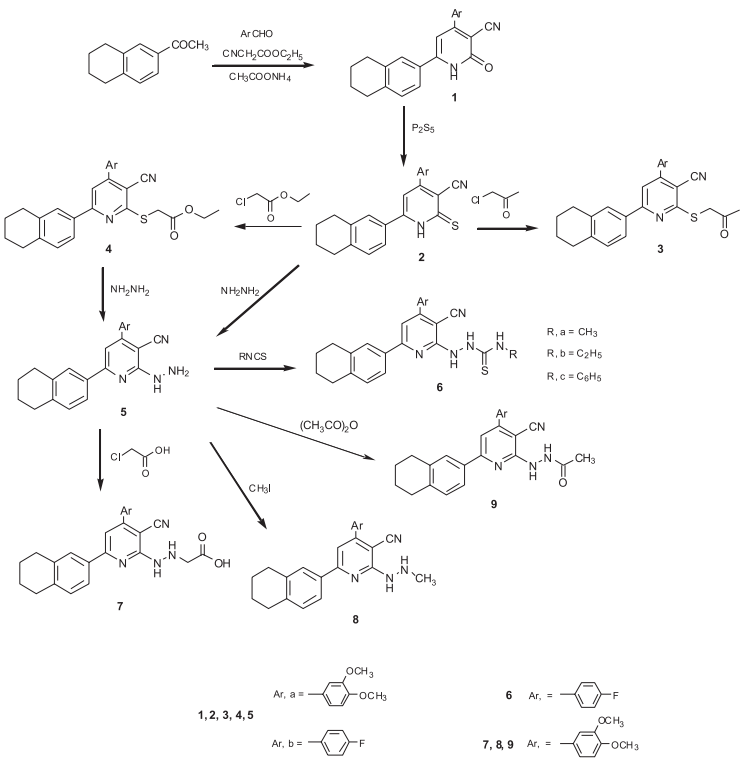
Chemistry

The target compounds were synthesized according to steps outlined in Schemes 1 and 2. The key intermediates 6-(1,2,3,4-tetrahydronaphthalen-6-yl)-2-mercapto-4-(3,4-substituted-phenyl) pyridine-3-carbonitrile **2a**,**b** were prepared by the treatment of 1,2-dihydro-6-(1,2,3,4-tetrahydronaphthalen-6-yl)-4-(substituted phenyl)-2-oxopyridine-3-carbonitrile **1a**,**b** with phosphorus pentasulfide in dry pyridine. Mass spectra of compounds **2a**,**b** showed molecular ion peak at 402 and 360, respectively.

Treatment of the key intermediates **2a**,**b** with chloroacetone and/or ethyl chloroacetate produced the corresponding derivatives **3a**,**b** and **4a**,**b**, respectively. IR spectra of compounds **3a**,**b** showed bands at 1702, 1709 cm^{-1} due to (CO) group and its ^1H NMR showed singlet signals at δ 2.28, 2.17 ppm due to methyl group and also singlet signals at δ 4.10, 4.12 ppm of methylene group. However, IR spectra of compounds **4a**,**b** showed bands at 1725 and 1740 cm^{-1} due to (CO) ester group and its ^1H NMR spectra exhibited the ester group signals at δ 1.29, 4.16 and 1.24, 4.13 ppm, respectively.

Condensation of **4a**,**b** with hydrazine hydrate afforded hydrazide derivatives **5a**,**b** and their IR spectra showed bands at 3245, 3177 and 3336, 3208 cm^{-1}, respectively, due to ($NHNH_2$) group. Compound **5a** was allowed to react with different substituted isothiocyanates, namely: methyl isothiocyanate, ethyl isothiocyanate and/or phenyl isothiocyanate, to give the corresponding substituted thiosemicarbazides **6a**-**c**. ^1H NMR of compound **6a** showed singlet signal at δ 2.56 ppm due to methyl group and **6b** showed signals at δ 1.05 and 3.51 ppm due to ethyl group.

Moreover, **5a** undergo reaction with chloroacetic acid to give the corresponding derivative **7** and its ^1H NMR showed singlet signal at δ 3.51 ppm due to methylene group. Also, reaction of compound **5a** with iodomethane gave the methylhydrazide derivative **8**. ^1H NMR showed singlet signal



Scheme 1. Synthesis of hydrazide pyridines of tetrahydronaphthalene

at δ 2.56 ppm due to methyl group. In addition, reaction of **5a** with acetic anhydride yielded acetohydrazide derivative **9**. ^{1}H NMR showed singlet signal at δ 2.21 ppm due to methyl group. (Scheme 1).

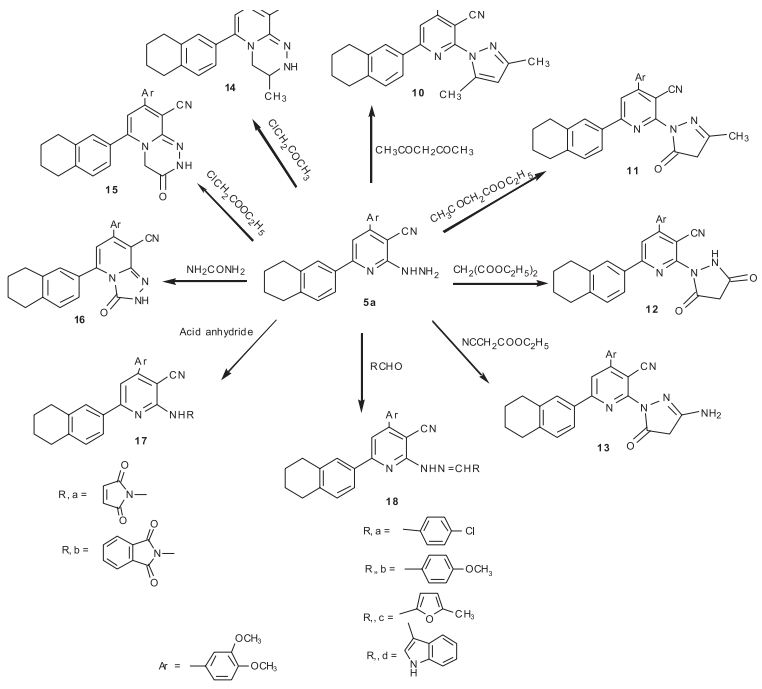
The hydrazide derivative **5a** is very useful intermediate for further cyclocondensation reactions. Thus, cyclocondensation of **5a** with active methylene derivatives, namely; acetylacetone, ethyl acetoacetate, diethylmalonate and ethyl cyanoacetate, yielded the corresponding pyrazole derivatives **10**-**13**, respectively. ^{1}H NMR spectrum of compound **10** showed singlet signals at δ 2.94 and 6.01 ppm due to methyl group and CH of pyrazole moiety. Also, ^{1}H NMR spectrum of compound **11** showed singlet signal at δ 2.02 and 2.21 ppm due to methyl group and CH_2 of pyrazolinone moiety. IR spectrum of compound **12** showed bands at 1735 and 1633 cm^{-1} due to the two carbonyl groups of pyrazoldione moiety. IR spectrum of compound **13** showed band at 1736 cm^{-1} due to CO group. In addition, cyclocondensation of **5a** with chloroacetone and/or ethyl chloroacetate gave the corresponding triazine derivatives **14** and **15**, respectively. ^{1}H NMR spectrum of compound **14** showed doublet signal at δ 1.13 ppm and multiple signals at δ 2.79, 3.34 ppm due to CH_3, CH_2 and CH groups of triazine moiety. IR spectrum of compound **15** showed band at 1740 cm^{-1} due to carbonyl group of triazine moiety. Also, cyclocondensation of **5a** with urea afforded the corresponding triazole derivative **16**.

Moreover, condensation of **5a** with acid anhydrides, namely: maleic anhydride and/or phthalic anhydride yielded pyrrole and isoindoline derivative **17a**,**b**. respectively. A series of new Schiff bases **18a**-**d** were synthesized by the condensation of hydrazide derivative **5a** with various substituted aromatic aldehydes, namely: 4-chlorobenzaldehyde, anisaldehyde, 5-methylfurfural and/or indole-3-aldehyde. The ^{1}H NMR spectrum of **18b** showed the three methoxy groups at δ 3.79 and 3.82 ppm while ^{1}H NMR of **18c** showed signal at δ 2.22 ppm of methyl group of furan ring.

Antimicrobial activity

Most of the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC6633, *Bacillus megaterium* ATCC 9885 and *Sarcina lutea* as examples of Gram positive bacteria and *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas. aeruginosa* ATCC 27953 and *Escherichia coli* ATCC 25922 as examples of Gram negative bacteria. They were also evaluated for their *in vitro* antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans* NRRL Y-477.

Agar diffusion method was used for determination of the antimicrobial activity using ciproflox-



Scheme 2. Synthesis of different heterocycles of tetrahydronaphthalene

acin and ketoconazole as reference drugs. The results were recorded for each tested compounds as the average diameter as inhibition zones (IZ) of bacterial or fungal growth around the discs in mm (Table 1).

Minimum inhibitory concentration (MI) measurements were determined for the compounds (Table 2).

According to the obtained results (Tables 1, 2), it can be noticed that the parent starting compound 3,4-dimethoxyphenylpyridine **1a** and its thioxopyridine derivative **2a** produced weak to complete insensitivity towards the tested Gram positive and Gram negative bacterial strains. Also, the attachment of sulfur group of **2a** to ketone and ethyl ester side chains **3a**, **4a** did not improve the antibacterial activity. In comparison, the attachment of 4-fluorophenyl ring to the thioxopyridine ring in **2b** enhanced the activity of Gram positive and Gram negative bacterial growth inhibition to be half of that obtained by ciprofloxacin (MIC: 50, 25 μg/mL, respectively).

Broad spectrum of antibacterial activity greater than that obtained by the reference drugs was obtained upon the attachment of a methyl ketone side chain to the sulfur group of **2b** to get the derivative **3b** (MIC 18-22 μg/mL). A noteworthy enhancement in the activity against both Gram positive and Gram negative bacteria to be equal to that obtained by ciprofloxacin was gained by hydrazinolysis of the parent **2a** to give the hydrazine pyridine derivative **5a** (MIC: 25 μg/mL).

At the same time, the incorporation of a hydrazide side chain to the sulfur group **5b** produced sensitivity towards the tested Gram negative bacteria (MIC 25-50 μg/mL) but complete loss of activity against Gram positive bacterial strain, while the conversion of side chain to thiosemicarbazide **6a-c** enhanced the sensitivity of the compounds against Gram positive bacteria used in the experiment (MIC: 50-100 μg/mL), but unfortunately led to great reduction of the activity towards Gram negative bacteria.

It is noticeable that the incorporation of different heterocyclic rings to the parent 3,5-dimethoxypyridine ring such as oxadiazole, triazinephenyl, thiazolo, triazine, pyrazole and different substituted triazinephenyl rings did not offer any advantage in the antibacterial activity against both Gram positive and Gram negative bacteria.

The data obtained revealed that the listed compounds have weak antifungal activity except the parent 4-fluoropyridine derivative **2b**, which showed equipotent antifungal activity to that obtained by the reference ketoconazole (MIC: 25 μg/mL).

CONCLUSION

The antimicrobial evaluation of most of the newly prepared compounds exhibited that high broad spectrum activity against both Gram positive and Gram negative was gained by the parent 3,4-dimethoxypyridine derivative attached to hydrazine side chain **5a** and also by the parent thioxopyridine compound bearing 4-fluorophenyl group **2b** and its ketone derivative **3b**. Interestingly, **2b** is the only derivative that exhibited dual antibacterial and antifungal activity.

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