ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
Research Article
www.ijptonline.com

SYNTHESIS OF NOVEL NAPHTHALENE-PYRIDINE HYBRID COMPOUNDS FOR ANTI-AVIAN INFLUENZA VIRUS (H5N1) AND ANTIMICROBIAL EVALUATION

Eman R. Kotb[1], Manal M. Anwar[2], Yasmin M Syam*[2], Ola Bagato[3], Shereen AbdelMoaz[4]
[1]Photochemistry Department, National Research Center, Cairo, Egypt
[2]Therapeutical chemistry Department, National Research Center, Cairo, Egypt
[3]Virology Laboratory, National Research Center, Cairo, Egypt
[4]Food Risk Analysis Group-Center of Excellence for Advanced Sciences, Dokki, Cairo, Egypt
E-mail: yasmin_syam@yahoo.com; Tel.:+2-(0)1005639588; Fax: + (202) 337-0931

Received on 12-07-2015 *Accepted on 29-07-2015*

Abstract

With the aim of developing potential anti-influenza and antimicrobial agents, a new series of naphthalenyl-pyridine hybrids were synthesized utilizing 6-(naphthalen-2-yl)-4-(4-nitrophenyl/-2-pyridinyl)-2-oxo-1,2-dihydropridine-3-carbonitriles **1a, b** as the starting compounds. Condensation of **1a, b** with the appropriate alkyl and/or aromatic acid chlorides in dry pyridine led to the formation of the corresponding N-substituted derivatives **2a,b - 6a, b**. The chloro-naphthalene derivatives **7a, b** were allowed to react with different sulfa drugs to yield the benzene-sulfonamide derivatives **12a,b - 14a, b**, while their cyclo-condensation with sodium azide afforded the corresponding tetrazolo[1,5-*a*]pyridine derivatives **11a, b**. New analogues of triazolo[4,3-*a*]pyridine and pyrido[2,1-*c*][1,2,4]triazine fused heterocyclic ring systems **16a, b**, **17a, b** and **19a, b** were also synthesized via the reaction of the hydrazinyl derivatives **15a, b** with ethylchloroformate, carbon disulfide and diethyl oxalate, respectively. Moreover, the compounds **15a, b** reacted with *N*,*N*-Dimethylformamide dimethyl acetal (DMF-DMA) to produce the corresponding imine derivatives that were condensed with urea, thiourea and guanidine to yield the formamidine analogues (**20a,b - 22a,b**). Antimicrobial evaluation of the synthesized compounds exhibited promising antibacterial activity against *S. aureus* bacterial strain specially compounds **13b, 14b** and **17b**. In addition, significant antifungal effect was also obtained specially against *C. albicans* in contrast to moderate anti-avian influenza virus (H5N1) activity.

Keywords: Naphthalene-pyridine hybrid. N,N-dimethylformamidines, Anti-avian influenza, Antimicrobial activity.

Introduction

Influenza is an acute infectious disease caused by RNA virus that belongs to the orthomyxovirus family: influenza virus A, B or to a much lesser extent, influenza virus C. It is the contagious etiologic agent that causes an acute respiratory infection. Despite influenza lasts for a week and does not pose any serious threat to the human health, it sometimes can be serious and results in death. There have been various pandemic outbreaks of influenza resulting to the death of millions of people worldwide [1-3]. In recent years, the appearance of the highly pathogenic influenza A virus subtype H5N1 that is an emerging avian influenza virus has been causing global concern as a potential pandemic threat. Although WHO currently recommend the two Neuraminidase inhibitors, oseltamivir and zanamivir are the licensed antiviral medications for the treatment and prevention of human infection with avian influenza A viruses in the United States, some evidence of resistance to the two drugs has been developed in H5N1 viruses isolated from some human cases [4-6]. So, the development of new classes of antiviral drugs is a significant and an urgent task.

Also, since the 1980s morbidity and mortality due to bacterial and fungal infections have been rising and nowadays this problem has become an increasing worldwide threat due to respiratory infections, AIDS and tuberculosis (TB). The resistance of common pathogens to the first-choice drugs increased up to 100% during the last decade and the resistance of some strains to the second- or third-choice drugs is a great problem [7]. Fungal infections and antibiotics resistance became very important complications caused in immunocompromised individuals such as those suffering from tuberculosis, cancer or AIDS and in organ transplant cases [8]. The success of treatment is also decreased by the development of cross-resistance or multidrug-resistant (MDR) strains. Methicillin-resistance of important Gram-positive pathogen, *Staphylococcus aureus* (MRSA), has become one of the most challenging and persistent worldwide health problems and even though originally limited to hospitals, nowadays MRSA is an increasing cause of infections in the community [9-11].

Literature survey has documented that various pyridine and 3-cyanopyridone congeners have been associated with anti-avian influenza virus (H5N1) [12], antibacterial and antifungal activities [13-16]. Also, several antimicrobial drugs containing naphthalene nucleus are available such as nafacillin, naftifine, tolnaftate and terbinafine[17-19]. Naftifine is an antifungal drug used for the topical treatment of tinea pedis, tinea cruris and tinea corporis[20].

Depending on the above knowledge, the search for potentiators of the activity of known antimicrobial agents and the development of new hybrid agents are continued to produce new leads of antiviral and antimicrobial activity. The idea of chemically fusing two antimicrobials with different mechanisms or an antimicrobial agent with a potentiating entity has been the subject of study for several decades, but no considerable success has been registered till date and the search of a new hybrid agents still continues [21, 22]. Thus, this work was focused on the synthesis of novel naphthalene-pyridine hybrids conjugated to various side chains and/or fused with different other heterocycles of documented antiviral and antimicrobial potency such sulfonamide derivatives, formamidine side chain, tetrazole, triazole and triazine ring systems [23-32]. Antiviral activity against H5N1 viral strain and antibacterial and antifungal potency of the synthesized derivatives were evaluated hoping to gain new anti-avian influenza and antimicrobial agents of optimized activity that is able to overcome the emergence of the resistance problem.

Materials and Methods

Chemistry

All melting points were uncorrected and determined on an electrothermal melting point apparatus (Stuart Scientific, UK). Elemental microanalyses (C, H, N) were performed on a model 2400 CHNSO analyzer (Perkin Elmer, USA). All compounds were within ±0.5 % of the theoretical values. Infrared spectra were recorded on a JASCO FT-IR 6100, Fourier transform, infrared spectrometer at cm-1 scale using KBr disc technique (JASCO, Japan). ^{1}H NMR and ^{13}C NMR spectra were determined by using a JEOL AS-500 NMR spectrometer (JEOL, Japan), chemical shifts were expressed in δ (ppm) downfield from TMS as an internal standard. The mass spectra were measured with a GC MS-Qp1000EX Shimadzu (Shimadzu, Japan). Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Germany) using chloroform/ petroleum ether 40-60 (5:1, V/V) and the spots were detected by exposure to UV lamp at λ 254 nanometer for few seconds and by iodine vapor.

The chemical names given for the prepared compounds are according to the IUPAC system.

General procedure for synthesis of 1,2-Dihydro-6-(naphthalen-2-yl)-4-substituted-2-oxopyridine-3-carbonitrile derivatives 1a,b

A mixture of 2-acetylnaphthalene (10 mmol), 4-nitrobenzaldehyde/2-pyridinaldehyde (10 mmol), ethyl cyanoacetate (10 mmol), and ammonium acetate (80 mmol) in methanol (40 mL) was refluxed for 3h. After cooling, the precipitate was filtered, dried, and recrystallized from methanol to give the corresponding derivatives **1a,b**.

1,2-Dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**1a**)

Yield: 90 %; mp (^{o}C):>300. IR (KBr, cm^{-1}): 3420 (NH), 2210 (C≡N), 1656 (C=O), 1623, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.88 (s, 1H, pyridine-H5), 7.27–7.90 (m, 11H, Ar-H), 9.51 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$367 (30 %).Anal.Calcd for $C_{22}H_{13}N_3O_3$ (367.36): C, 71.93; H, 3.57; N, 11.44. Found: % C, 71.51; H, 3.80; N, 11.03.

1,2-Dihydro-6-(naphthalen-2-yl)-2-oxo-4-(pyridin-2-yl)pyridine-3-carbonitrile (**1b**)

Yield: 85 %; mp (^{o}C):>300. IR (KBr, cm^{-1}): 3425 (NH), 2215 (C≡N), 1648 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 6.88 (s, 1H, pyridine-H5), 7.27–7.90 (m, 11H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$323 (40 %).Anal. Calcd for $C_{21}H_{13}N_3O$ (323.35): C, 78.00; H, 4.05; N, 13.00. Found: % C, 77.81; H, 4.50; N, 13.49.

General procedure for synthesis of 1-Acetyl or sulfonyl-1,2-dihydro-6-(naphthalen-2-yl)-4-substituted-2-oxopyridine-3-carbonitrile 2a,b-6a,b

A mixture of compounds of **1a,b** (10 mmol) and different chloro derivatives namely; acetyl chloride, chloroacetyl chloride, benzoyl chloride, benzenesulfonyl chloride and *p*-toluenesulfonyl chloride (10 mmol) in pyridine (20 mL) was refluxed for 6h. The reaction mixture was cooled and poured onto cold water, then acidified by dilute HCl (37%). The solid obtained was filtered, dried and crystallized from ethanol/water to give the compounds, **2a,b-6a,b** respectively.

1-Acetyl-1,2-dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**2a**)

Yield: 83 %; mp (^{o}C):>300. IR (KBr, cm^{-1}): 3132 (CH-aromatic), 2946 (CH-aliphatic), 2217 (C≡N), 1680 (C=O), 1649 (C=O), 1620, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 2.61 (s, 3H, -CO-CH_3), 6.91 (s, 1H, pyridine-H5), 7.27–7.90 (m, 11H, Ar-H). MS m/z: $(M+1)^{+\cdot}$410 (35 %), $M^{+\cdot}$409 (32.61 %).Anal.Calcd for $C_{24}H_{15}N_3O_4$ (409.39): % C, 70.41; H, 3.69; N, 10.26. Found: % C, 70.62; H, 3.83; N, 10.49.

1-Acetyl-1,2-dihydro-6-(naphthalen-2-yl)-4-(pyridin-2-yl)-2-oxopyridine-3-carbonitrile (**2b**)

Yield: 82 %; mp (oC):>300. IR (KBr, cm^{-1}): 3037 (CH-aromatic), 2950 (CH-aliphatic), 2200 (C≡N), 1700 (C=O), 1650 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 2.72 (s, 3H, -CO-CH_3), 6.56 (s, 1H, pyridine-H5), 7.19–8.08 (m, 11H, Ar-H). (^{13}C NMR (DMSO, δ ppm): 27.27 (CH_3), 107.12 (C≡N), 124.13, 127.56, 128.17, 128.77, 129.07, 129.37, 130.05, 136.55,

149.05, 151.60, 155.65 (aromatic-C), 162.63, 198.37 (2 C=O).MS m/z: $(M+1)^{+\cdot}$366 (31 %), M^{+}.365 (31).Anal.Calcd for $C_{23}H_{15}N_3O_2$ (365.38): % C, 75.60; H, 4.14; N, 11.50. Found: % C, 75.83; H, 3.94; N, 11.73.

1-(2-Chloroacetyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**3a**)

Yield: 78 %; mp (^{o}C):>300. IR (KBr, cm^{-1}): 3052 (CH-aromatic), 2916 (CH-aliphatic), 2217 (C≡N), 1698 (C=O), 1650 (C=O), 1625, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 4.24 (s, 2H, -CO-CH_2), 6.56 (s, 1H, pyridine-H5), 7.19–8.08 (m, 11H, Ar-H). MS m/z: $(M+2)^{+\cdot}$ 445 (21 %), $(M+1)^{+\cdot}$ 444 (100.00), $M^{+\cdot}$ 443 (65 %). Anal.Calcd for $C_{24}H_{14}ClN_3O_4$ (443.84): % C, 64.95; H, 3.18; N, 9.47. Found: % C, 65.24; H, 2.98; N, 9.73.

1-(2-Chloroacetyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(pyridin-2-yl)-2-oxopyridine-3-carbonitrile (**3b**)

Yield: 82 %; mp (^{o}C): 290-292. IR (KBr, cm^{-1}): 3093 (CH-aromatic), 2959 (CH-aliphatic), 2200 (C≡N), 1697 (C=O), 1650 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 4.37 (s, 2H, -CO-CH_2), 7.13 (s, 1H, pyridine-H5), 7.40–8.06 (m, 11H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 30.7 (CH_2-Cl), 107.1 (C≡N), 124.04, 124.13, 125.2, 128.10, 128.70, 128.77, 129.01, 129.09, 129.37, 130.05, 136.45, 149.04, 151.61, 152.63, 157.05 (aromatic-C), 162.63, 198.37 (2 C=O). MS m/z: $(M+2)^{+\cdot}$401 (21 %), $M^{+\cdot}$ 399 (65 %).Anal.Calcd for $C_{23}H_{14}ClN_3O_2$ (399.83): % C, 69.09; H, 3.53; N, 10.51. Found: % C, 69.32; H, 3.68; N, 10.73.

1-(Benzoyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**4a**)

Yield: 75 %; mp (^{o}C): 260-262. IR (KBr, cm^{-1}): 3056 (CH-aromatic), 2917 (CH-aliphatic), 2200 (C≡N), 1680 (C=O), 1647 (C=O) 1625, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.56 (s, 1H, pyridine-H5), 7.38–8.25 (m, 16H, Ar-H). MS m/z: $M^{+\cdot}$471 (50 %).Anal.Calcd for $C_{29}H_{17}N_3O_4$ (471.46): % C, 73.88; H, 3.63; N, 8.91. Found: % C, 74.06; H, 3.47; N, 9.15.

1-(Benzoyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(pyridin-2-yl)-2-oxopyridine-3-carbonitrile (**4b**)

Yield: 75 %; mp (^{o}C): 273-275. IR (KBr, cm^{-1}): 3048 (CH-aromatic), 2921 (CH-aliphatic), 2200 (C≡N), 1673 (C=O), 1650 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 6.58 (s, 1H, pyridine-H5), 7.18–8.25 (m, 16H, Ar-H). ^{13}C NMR (DMSO, d ppm): 107.12 (C≡N), 116.31, 123.31, 123.89, 124.15, 124.82, 125.50, 126.61, 127.56, 128.12, 128.70, 128.76, 129.01, 129.37, 130.04, 132.24, 133.91, 136.54, 149.05, 151.61, 152.33, 157.24 (aromatic-C), 162.63, 198.37 (2 C=O). MS m/z: $M^{+\cdot}$427 (62 %).Anal.Calcd for $C_{28}H_{17}N_3O_2$ (427.45): % C, 78.68; H, 4.01; N, 9.83. Found: % C, 78.79; H, 3.83; N, 9.62.

1-(Phenylsulfonyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**5a**)

Yield: 77 %; mp (^{o}C): 298-300. IR (KBr, cm^{-1}): 3055 (CH-aromatic), 2950 (CH-aliphatic), 2200 (C≡N), 1650 (C=O), 1340 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 7.16 (s, 1H, pyridine-H5), 7.21–8.00 (m, 16H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 110.73 (C≡N), 123.23, 124.53, 124.82, 125.21, 126.45, 127.61, 128.10, 128.25, 128.72, 129.01, 129.31, 130.45, 131.95, 132.08, 132.85, 145.82, 146.41, 146.91, 147.30, 148.25 (aromatic-C), 150.66 (C=O). MS m/z: $M^{+\cdot}$507 (75 %). Anal.Calcd for $C_{28}H_{17}N_3O_5S$ (507.52): % C, 66.26; H, 3.38; N, 8.28. Found: % C, 66.45; H, 3.47; N, 8.49.

1-(Phenylsulfonyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(pyridin-2-yl)-2-oxopyridine-3-carbonitrile (**5b**)

Yield: 70 %; mp (^{o}C): 188-200. IR (KBr, cm^{-1}): 3051 (CH-aromatic), 2920 (CH-aliphatic), 2214 (C≡N), 1648 (C=O), 1350 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.96 (s, 1H, pyridine-H5), 7.30–8.00 (m, 16H, Ar-H). MS m/z: $[M-2]^{+\cdot}$461 (15 %).Anal.Calcd for $C_{27}H_{17}N_3O_3S$ (463.51): % C, 69.96; H, 3.70; N, 9.07. Found: % C, 70.27; H, 3.58; N, 8.86.

1-(4-Methylphenylsulfonyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**6a**)

Yield: 85 %; mp (^{o}C):> 300. IR (KBr, cm^{-1}): 3051 (CH-aromatic), 2920 (CH-aliphatic), 2214 (C≡N), 1648 (C=O), 1350 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 2.13 (s, 3H, CH_3), 6.66 (s, 1H, pyridine-H5), 7.30–8.23 (m, 15H, Ar-H). MS m/z: $M^{+\cdot}$521 (49 %).Anal.Calcd for $C_{29}H_{19}N_3O_5S$ (521.54): % C, 66.78; H, 3.67; N, 8.06; S, 6.15. Found: % C, 66.59; H, 3.82; N, 8.25; S, 5.87.

1-(4-Methylphenylsulfonyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(pyridin-2-yl)-2-oxopyridine-3-carbonitrile (**6b**)

Yield: 82 %; mp (^{o}C): 242-244. IR (KBr, cm^{-1}): 3100 (CH-aromatic), 2925 (CH-aliphatic), 2200 (C≡N), 1650 (C=O), 1350 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 2.13 (s, 3H, CH_3), 6.56 (s, 1H, pyridine-H5), 7.26–8.14 (m, 15H, Ar-H). MS m/z: $M^{+\cdot}$477 (56 %).Anal.Calcd for $C_{28}H_{19}N_3O_3S$ (477.53): % C, 70.42; H, 4.01; N, 8.80; S, 6.71. Found: % C, 70.63; H, 4.26; N, 8.63; S, 6.54.

General procedure for synthesis of 2-Chloro-6-(naphthalen-2-yl)-4-substituted-pyridine-3-carbonitrile 7a,b

A suspension of compounds **1a,b** (10 mmol) and PCl_5 (0.5 g) in $POCl_3$ (8 mL) was heated under reflux for 2h on a water bath. After cooling, the reaction mixture was poured slowly on crushed ice, the solid formed was filtered, washed with cold water and dried to give the chloro derivatives **7a,b**.

2-Chloro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridine-3-carbonitrile (**7a**)

Yield: 76 %; mp (^{o}C): 178-180. IR (KBr, cm^{-1}): 3050 (CH-aromatic), 2856 (CH-aliphatic), 2200 (C≡N), 1626, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.76 (s, 1H, pyridine-H5), 7.26–8.14 (m, 11H, Ar-H). MS m/z: $(M+2)^{+\cdot}$387 (4 %),

$M^{+\cdot}$ 385 (13 %).Anal.Calcd for $C_{22}H_{12}ClN_3O_2$ (385.80): % C, 68.49; H, 3.14; N, 10.89. Found: % C, 68.53; H, 3.26; N, 11.06.

2-Chloro-6-(naphthalen-2-yl)-4-(pyridin-2-yl)pyridine-3-carbonitrile (**7b**)

Yield: 79 %; mp (^oC): 216-218. IR (KBr, cm^{-1}): 3050 (CH-aromatic), 2856 (CH-aliphatic), 2220 (C≡N). ^1H NMR (DMSO-d6, δ ppm): 6.71 (s, 1H, pyridine-H5), 7.26–8.20 (m, 11H, Ar-H). MS m/z: $(M+2)^{+\cdot}$343 (4.62 %), $M^{+\cdot}$ 341 (13.82 %).Anal.Calcd for $C_{21}H_{12}ClN_3$ (341.79): % C, 73.79; H, 3.54; N, 12.29. Found: % C, 73.42; H, 3.73; N, 11.95.

General procedure for synthesis of 1,2-Dihydro-1-(aminomethyl)-6-(naphthalen-2-yl)-4-substituted-2-oxopyridine-3-carbonitrile derivatives 8a,b-10a,b.

A solution of *p*-formaldehyde (0.90 g, 10 mmol) and the appropriate amine namely: 4-methylpiperidine, 4-methylpiperazine, morpholine (15 mmol) was refluxed in absolute ethanol (20 mL) for 30 min till complete solubilization of *p*-formaldehyde. Then, a solution of the pyridone derivatives **1a,b** (10 mmol) in absolute ethanol (10 mL) was added to the previous mixture and the reflux was continued for 8h. Upon cooling, the obtained product was filtered, dried and recrystallized from dioxane to give the corresponding mannich bases **8a,b-10a,b**.

1,2-Dihydro-1-[(4-methylpiperidin-1-yl)methyl]-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**8a**)

Yield: 89 %; mp (^oC): 296–298. IR (KBr, cm^{-1}): 3100 (CH-aromatic), 2925 (CH-aliphatic), 2200 (C≡N), 1650 (C=O). ^1H NMR (DMSO-d6, δ ppm): 1.13 (s, 3H, CH_3), 2.12 (m, 5H, β-2CH_2, δ-CH of piperidine ring), 3.12 (m, 4H, α-2CH_2 of piperidine ring), 4.21 (s, 2H, -CH_2-N-), 6.71 (s, 1H, pyridine-H5), 7.21–8.05 (m, 11H, Ar-H). MS m/z: $M^{+\cdot}$478 (56 %).Anal.Calcd for $C_{29}H_{26}N_4O_3$ (478.54): % C, 72.79; H, 5.48; N, 11.71. Found: % C, 72.92; H, 5.81; N, 12.06.

1,2-Dihydro-1-[(4-methylpiperidin-1-yl)methyl]-6-(naphthalen-2-yl)-2-oxo-4-(pyridin-2-yl)pyridine-3-carbonitrile (**8b**)

Yield: 89 %; mp (^oC): > 300. IR (KBr, cm^{-1}): 3050 (CH-aromatic), 2925 (CH-aliphatic), 2216 (C≡N), 1649 (C=O). ^1H NMR (DMSO-d6, δ ppm): 1.20 (s, 3H, CH_3), 2.34 (m, 5H, β-2CH_2, δ-CH of piperidine ring), 3.12 (m, 4H, α-2CH_2 of piperidine ring), 4.24 (s, 2H, -CH_2-N-), 6.75 (s, 1H, pyridine-H5), 7.21–8.31 (m, 11H, Ar-H). MS m/z: $M^{+\cdot}$434 (58 %).Anal.Calcd for $C_{28}H_{26}N_4O$ (434.53): % C, 77.39; H, 6.03; N, 12.89. Found: % C, 77.01; H, 5.91; N, 12.56.

1,2-Dihydro-1-[(4-methylpiperazin-1-yl)methyl]-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**9a**)

Yield: 82 %; mp (^oC): > 300. IR (KBr, cm^{-1}): 3150 (CH-aromatic), 2925 (CH-aliphatic), 2217 (C≡N), 1650 (C=O). ^1H NMR (DMSO-d6, δ ppm): 2.25 (s, 3H, CH_3), 3.11 (m, 8H, piperazine ring), 4.30 (s, 2H, -CH_2-N-), 6.75 (s, 1H, pyridine-

H5), 7.21–8.31 (m, 11H, Ar-H). MS m/z: $M^{+\cdot}$ 479 (58 %).Anal.Calcd for $C_{28}H_{25}N_5O_3$ (479.53): % C, 70.13; H, 5.25; N, 14.60. Found: % C, 70.46; H, 5.61; N, 14.86.

1,2-Dihydro-1-[(4-methylpiperazin-1-yl)methyl]-6-(naphthalen-2-yl)-2-oxo-4-(pyridin-2-yl)pyridine-3-carbonitrile (**9b**)

Yield: 86 %; mp (^oC): > 300. IR (KBr, cm^{-1}): 3150 (CH-aromatic), 2925 (CH-aliphatic), 2217 (C≡N), 1650 (C=O). ^1H NMR (DMSO-d6, δ ppm): 2.18 (s, 3H, CH_3), 3.24 (m, 8H, piperazine ring), 4.28 (s, 2H, -CH_2-N-), 6.65 (s, 1H, pyridine-H5), 7.21–8.12 (m, 11H, Ar-H). MS m/z: $M^{+\cdot}$435 (58 %).Anal.Calcd for $C_{27}H_{25}N_5O$ (435.52): % C, 74.46; H, 5.79; N, 16.08. Found: % C, 74.82; H, 5.91; N, 15.86.

1,2-Dihydro-1-(morpholinomethyl)-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**10a**)

Yield: 74%; mp (^oC):>300. IR (KBr, cm^{-1}): 3110 (CH-aromatic), 2930 (CH-aliphatic), 2220 (C≡N), 1650 (C=O). ^1H NMR (DMSO-d6, δ ppm): 2.85 (m, 4H, N-$(CH_2)_2$), 3.75 (m, 4H, O-$(CH_2)_2$), 4.31 (s, 2H, -CH_2-N-), 6.71 (s, 1H, pyridine-H5), 7.26–8.14 (m, 11H, Ar-H). MS m/z: $(M+1)^+$. 467 (33 %), $M^{+\cdot}$ 466 (15 %).Anal.Calcd for $C_{27}H_{22}N_4O_4$ (466.49): % C, 69.52; H, 4.75; N, 12.01. Found: % C, 69.73; H, 4.94; N, 12.35.

1,2-Dihydro-1-(morpholinomethyl)-6-(naphthalen-2-yl)-2-oxo-4-(pyridin-2-yl)pyridine-3-carbonitrile (**10b**)

Yield: 85%; mp (^oC):>300. IR (KBr, cm^{-1}): 3060 (CH-aromatic), 2920 (CH-aliphatic), 2218 (C≡N), 1647 (C=O). ^1H NMR (DMSO-d6, δ ppm): 2.80 (m, 4H, N-$(CH_2)_2$), 3.75 (m, 4H, O-$(CH_2)_2$), 4.28 (s, 2H, -CH_2-N-), 6.68 (s, 1H, pyridine-H5), 7.30–8.00 (m, 11H, Ar-H). ^{13}C NMR (DMSO, δ ppm): 53.27 (-N-$(CH_2)_2$, carbon-morpholine ring), 64.27 (CH_2, mannich side chain), 66.50 (O-$(CH_2)_2$, morpholine ring), 107.12 (C≡N), 116.57, 124.11, 124.83, 127.31, 128.13, 128.53, 128.74, 129.04, 129.35, 132.23, 136.52, 149.05, 151.60, 152.54, 153.44, 157.04 (aromatic carbons), 162.63 (C=O). MS m/z: $M^{+\cdot}$422 (81 %).Anal.Calcd for $C_{26}H_{22}N_4O_2$ (422.48): % C, 73.92; H, 5.25; N, 13.26. Found: % C, 74.32; H, 5.53; N, 13.06.

5-(Naphthalen-2-yl)-7-substituted-tetrazolo[1,5-a]pyridine-8-carbonitrile derivatives **11a,b**

A mixture of the chloro-derivatives **7a,b** (10 mmol) and sodium azide (0.65 g, 10 mmol) in glacial acetic acid (30 mL) was refluxed for 6h. The reaction mixture was cooled and poured onto ice/H_2O. The formed precipitate was filtered , dried and recrystallized from dioxane to give the desired compounds **11a,b**.

5-(Naphthalen-2-yl)-7-(4-nitrophenyl)tetrazolo[1,5-a]pyridine-8-carbonitrile (**11a**)

Yield: 83%; mp (^{o}C): 247-249. IR (KBr, cm^{-1}): 3059 (CH-aromatic), 2921 (CH-aliphatic), 2217 (C≡N), 1640 (C=N), 1625, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 7.01 (s, 1H, pyridine-H5), 7.57–8.20 (m, 11H, Ar-H). MS m/z: $(M+1)^{+\cdot}$ 393 (78 %), $M^{+\cdot}$392 (38 %).Anal.Calcd for $C_{22}H_{12}N_6O_2$ (392.37): % C, 67.34; H, 3.08; N, 21.42. Found: % C, 67.18; H, 3.24; N, 21.66.

5-(Naphthalen-2-yl)-7-(pyridin-2-yl)tetrazolo[1,5-*a*]pyridine-8-carbonitrile (**11b**)

Yield: 82%; mp (^{o}C): 155-157. IR (KBr, cm^{-1}): 3120 (CH-aromatic), 2921 (CH-aliphatic), 2218 (C≡N), 1640 (C=N). ^{1}H NMR (DMSO-d6, δ ppm): 6.98 (s, 1H, pyridine-H5), 7.27–8.20 (m, 11H, Ar-H). MS m/z: $(M+1)^{+\cdot}$349 (66 %), $M^{+\cdot}$ 348 (75 %).Anal.Calcd for $C_{21}H_{12}N_6$ (348.36): % C, 72.40; H, 3.47; N, 24.12 Found: % C, 72.73; H, 3.54; N, 24.07.

General procedure for synthesis of 2-(4-Aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-substituted-pyridine-3-carbonitrile derivatives12a,b-14a,b

A mixture of compounds of **7a,b** (10 mmol) and the appropriate sulfa drugs namely; sulfanilamide, sulfapyridine and sulfadiazine (10 mmol) in ethanol (20 mL) containing a few amount of TEA (5 mL) was refluxed for 6h. The reaction mixture was cooled and poured onto cold water, then acidified by dilute HCl (37%). The solid obtained was collected by filtration, dried and crystallized from ethanol/water to give the compounds, **12a,b-14a,b** respectively.

2-(4-Aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridine-3-carbonitrile (**12a**)

Yield: 78 %; mp (^{o}C): > 300. IR (KBr, cm^{-1}): 3423-3260 (NH_2, NH), 3050 (CH-aromatic), 2933 (CH-aliphatic), 2220 (C≡N), 1340, 1165 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 5.41 (s, 2H, NH_2, exchangeable with D_2O), 6.56 (s, 1H, pyridine-H5), 7.40–8.20 (m, 15H, Ar-H), 9.54 (s, 1H, NH, exchangeable with D_2O). MS m/z: $(M+1)^{+\cdot}$522 (100 %).Anal.Calcd for $C_{28}H_{19}N_5O_4S$, (521.55): % C, 64.48; H, 3.67; N, 13.43; S, 6.15. Found: % C, 64.72; H, 3.43; N, 13.17; S, 5.84.

2-(4-Aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(pyridine-2-yl)pyridine-3-carbonitrile (**12b**)

Yield: 85 %; mp (^{o}C): 222-224. IR (KBr, cm^{-1}): 3460, 3245 (NH_2, NH), 3040 (CH-aromatic), 2933 (CH-aliphatic), 2218 (C≡N), 1340, 1168 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 5.51 (s, 2H, NH_2, exchangeable with D_2O), 6.56 (s, 1H, pyridine-H5), 7.40–8.20 (m, 15H, Ar-H), 9.54 (s, 1H, NH, exchangeable with D_2O). MS m/z: $M^{+\cdot}$477 (83 %).Anal.Calcd for $C_{27}H_{19}N_5O_2S$ (477.54): % C, 67.91; H, 4.01; N, 14.67; S, 6.71. Found: % C, 67.45; H, 3.83; N, 14.26; S, 6.84.

2-(4-(Pyridin-2-yl)aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(4-nitrophenyl) pyridine-3-carbonitrile (**13a**)

Yield: 82 %; mp (^{o}C): 248-250. IR (KBr, cm^{-1}): 3443, 3345 (2NH), 3058 (CH-aromatic), 2998 (CH-aliphatic), 2215 (C≡N), 1345, 1163 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.53 (s, 1H, pyridine-H5), 7.40–8.20 (m, 19H, Ar-H), 9.41, 9.50 (2s, 2H, 2NH, exchangeable with D_2O). ^{13}C NMR (DMSO, δ ppm): 110.7 (C≡N), 123.71, 123.90, 124.33, 124.52, 125.62, 125.87, 126.52, 126.89, 127.61, 128.14, 128.65, 128.72, 129.31, 129.38, 131.32, 131.67, 132.85, 132.90, 144.34, 145.81,146.72, 146.95, 147.10, 147.45, 148.25, 152.41, 153.01, 156.51, 158.41 (aromatic-C). MS m/z: $M^{+\cdot}$ 598 (83 %).Anal.Calcd for $C_{33}H_{22}N_6O_4S$ (598.63): % C, 66.21; H, 3.70; N, 14.04; S, 5.36. Found: % C, 66.10; H, 3.48; N, 14.51; S, 5.14.

2-(4-(Pyridin-2-yl)aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(pyridin-2-yl)pyridine-3-carbonitrile (**13b**)

Yield: 84 %; mp (^{o}C): 185-187. IR (KBr, cm^{-1}): 3343, 3145 (2NH), 3058 (CH-aromatic), 2989 (CH-aliphatic), 2217 (C≡N), 1345, 1160 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.55 (s, 1H, pyridine-H5), 7.40–7.92 (m, 19H, Ar-H), 9.50, 9.62 (2s, 2H, 2NH, exchangeable with D_2O). MS m/z: $M^{+\cdot}$ 554 (42 %).Anal.Calcd for $C_{32}H_{22}N_6O_2S$ (554.62): % C, 69.30; H, 4.00; N, 15.15; S, 5.78. Found: % C, 69.15; H, 4.47; N, 15.56; S, 5.95.

2-(4-(Pyrimidin-2-yl)aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridine -3-carbonitrile (**14a**)

Yield: 82 %; mp (^{o}C): > 300. IR (KBr, cm^{-1}): 3365, 3245 (2NH), 3058 (CH-aromatic), 2990 (CH-aliphatic), 2200 (C≡N), 1340, 1160 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.56 (s, 1H, pyridine-H5), 7.40–8.20 (m, 18H, Ar-H), 9.54, 9.63 (2s, 2H, 2NH, exchangeable with D_2O). ^{13}C NMR (DMSO, δ ppm): 110.61 (C≡N), 124.32, 124.52, 125.62, 125.91, 126.31, 126.87, 127.21, 127.29, 128.14, 128.25, 128.65, 128.72, 129.31, 129.38, 130.21, 130.79, 132.85, 132.90, 148.25, 150.45, 152.41, 153.01, 156.51, 157.90, 158.41, 169.31 (aromatic-C). MS m/z: $M^{+\cdot}$599 (83 %).Anal.Calcd for $C_{32}H_{21}N_7O_4S$ (599.62): % C, 64.10; H, 3.53; N, 16.35; S, 5.35. Found: % C, 64.45; H, 3.83; N, 16.06; S, 5.14.

2-(4-(Pyrimidin-2-yl)aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(pyridine-2-yl)pyridine-3-carbonitrile (**14b**)

Yield: 79 %; mp (^{o}C): 252-254. IR (KBr, cm^{-1}): 3345, 3236 (2NH), 3058 (CH-aromatic), 2929 (CH-aliphatic), 2218 (C≡N), 1345, 1167 (SO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.56 (s, 1H, pyridine-H5), 7.39–8.12 (m, 18H, Ar-H), 9.51, 9.73 (2s, 2H, 2NH, exchangeable with D_2O). MS m/z: $M^{+\cdot}$ 555 (50 %).Anal.Calcd for $C_{31}H_{21}N_7O_2S$ (555.61): % C, 67.01; H, 3.81; N, 17.65; S, 5.77. Found: % C, 67.55; H, 3.53; N, 17.26; S, 5.54.

General procedure for synthesis of 2-Hydrazinyl-6-(naphthalen-2-yl)-4-substituted-pyridine-3-carbonitrile derivatives 15a,b

Hydrazine hydrate (99%) (1.6 mL, 50 mmol) was added to the chloro-compounds **7a,b** (10 mmol) dissolved in absolute ethanol (20 mL) and refluxed for 8h. The solid separated after concentration and cooling was filtered, dried and recrystallized from isopropanol/ petroleum ether to yield the hydrazine derivatives **15a,b**.

2-Hydrazinyl-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridine-3-carbonitrile (**15a**)

Yield: 85 %; mp (^{o}C): 105-107. IR (KBr, cm^{-1}): 3447-3132 (NH_2, NH), 3059 (CH-aromatic), 2920 (CH-aliphatic), 2200 (C≡N), 1625, 1262 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 5.51 (s, 2H, NH_2, D_2O exchangeable), 6.88 (s, 1H, pyridine-H5), 7.27–7.90 (m, 11H, Ar-H), 9.51 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 381 (30 %).Anal.Calcd for $C_{22}H_{15}N_5O_2$ (381.39): % C, 69.28; H, 3.96; N, 18.36. Found: % C, 69.42; H, 4.21; N, 18.25.

2-Hydrazinyl-6-(naphthalen-2-yl)-4-(pyridin-2-yl)pyridine-3-carbonitrile (**15b**)

Yield: 85 %; mp (^{o}C): 207-209. IR (KBr, cm^{-1}): 3380-3232 (NH_2, NH), 3050 (CH-aromatic), 2920 (CH-aliphatic), 2200 (C≡N). ^{1}H NMR (DMSO-d6, δ ppm): 5.56 (s, 2H, NH_2, D_2O exchangeable), 6.65 (s, 1H, pyridine-H5), 7.30–8.10 (m, 11H, Ar-H), 9.31 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 337 (59 %).Anal.Calcd for $C_{21}H_{15}N_5$ (337.38): % C, 74.76; H, 4.48; N, 20.76. Found: % C, 74.53; H, 4.62; N, 21.03.

General procedure for synthesis of 2,3-Dihydro-5-(naphthalen-2-yl)-7-substituted-3-oxo-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrile16a,b

A mixture of the hydrazinyl compounds **15a,b** (10 mmol) and ethylchloroformate (1.08 mL, 10 mmol) in pyridine (10 mL) was refluxed for 6h. The reaction mixture was cooled, poured onto ice/cold water containing few drops of HCl (37%). Then the obtained product was filtered, washed with water, dried and recrystallized from isopropanol to give the corresponding derivatives **16a,b**.

2,3-Dihydro-5-(naphthalen-2-yl)-7-(4-nitrophenyl)-3-oxo-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrile (**16a**)

Yield: 77 %; mp (^{o}C): 220-222. IR (KBr, cm^{-1}): 3305 (NH), 3055 (CH-aromatic), 2950 (CH-aliphatic), 2213 (C≡N), 1710 (C=O), 1631, 1260 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.53 (s, 1H, pyridine-H5), 7.30–8.10 (m, 11H, Ar-H), 9.35 (s, 1H, NH, D_2O exchangeable). MS m/z: $(M.+2)^{+\cdot}$ 409 (100%), $(M+1)^{+\cdot}$ 408 (57.63 %), $M^{+\cdot}$ 407 (19.73 %). Anal.Calcd for $C_{23}H_{13}N_5O_3$ (407.38): % C, 67.81; H, 3.22; N, 17.19. Found: % C, 67.95; H, 3.46; N, 17.24.

2,3-Dihydro-5-(naphthalen-2-yl)-7-(pyridin-2-yl)-3-oxo-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrile (**16b**)

Yield: 80 %; mp (^{o}C): 200-202. IR (KBr, cm^{-1}): 3305 (NH), 3055 (CH-aromatic), 2950 (CH-aliphatic), 2200 (C≡N), 1670 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 6.53 (s, 1H, pyridine-H5), 7.32–7.94 (m, 11H, Ar-H), 9.25 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 363 (13 %).Anal.Calcd for $C_{22}H_{13}N_5O$ (363.37): % C, 72.72; H, 3.61; N, 19.27. Found: % C, 72.92; H, 3.86; N, 19.58.

General procedure for synthesis of 3-Mercapto-5-(naphthalen-2-yl)-7-substituted-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrilederivatives17a,b

To a stirred suspension of the hydrazinyl-derivatives **15a,b** (22 mmol) in absolute ethanol (20 mL), ethanolic KOH (30 mL, 10 mmol) and CS_2 (2 mL) were added drop wisely. The reaction mixture was heated under reflux for 6h. Upon reaction completion, the excess solvent was evaporated and the formed precipitate was washed with water acidified with dil. HCl (37%), filtered, dried and crystallized from ethanol to produce the corresponding derivatives **17a,b**.

3-Mercapto-5-(naphthalen-2-yl)-7-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrile (**17a**)

Yield: 78 %; mp (^{o}C): 230-232. IR (KBr, cm^{-1}): 3408 (NH), 3052 (CH-aromatic), 2921 (CH-aliphatic), 2200 (C≡N), 1199 (C=S). ^{1}H NMR (DMSO-d6, δ ppm): 6.91 (s, 1H, pyridine-H5), 7.32–7.94 (m, 11H, Ar-H), 9.25 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 423 (20 %).Anal.Calcd for $C_{23}H_{13}N_5O_2S$ (423.45): % C, 65.24; H, 3.09; N, 16.54; S, 7.57. Found: % C, 65.52; H, 3.28; N, 16.73; S, 7.86.

3-Mercapto-5-(naphthalen-2-yl)-7-(pyridin-2-yl)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrile (**17b**)

Yield: 75 %; mp (^{o}C): 210-212. IR (KBr, cm^{-1}): 3300 (NH), 3020 (CH-aromatic), 2930 (CH-aliphatic), 2210 (C≡N), 1195 (C=S). ^{1}H NMR (DMSO-d6, δ ppm): 6.65 (s, 1H, pyridine-H5), 7.12–8.02 (m, 11H, Ar-H), 9.31 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$379 (34 %).Anal.Calcd for $C_{22}H_{13}N_5S$ (379.44): % C, 69.64; H, 3.45; N, 18.46; S, 8.45. Found: % C, 69.32; H, 3.65; N, 18.57; S, 8.46.

General procedure for synthesis of N'-(3-cyano-6-(naphthalen-2-yl)-4-substituted-pyridin-2-ylamino)- N,N-dimethylformamidine derivatives18a,b

A mixture of the hydrazinyl derivatives **15a,b** (10 mmol) and N,N-dimethylformamide-dimethylacetal (1.43 mL, 12 mmol) in dry toluene (10 mL) was refluxed for 6h. The reaction mixture was cooled, poured onto ice/cold water.

Then, the obtained product was filtered, washed with water, dried and recrystallized isopropyl/petroleum ether to give the derivatives **18a,b**, respectively.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridin-2-ylamino)-N,N-dimethylformamidine (**18a**)

Yield: 85 %; mp (^{o}C): 140-142. IR (KBr, cm^{-1}): 3431 (NH), 3055 (CH-aromatic), 2930 (CH-aliphatic), 2216 (C≡N), 1629, 1256 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 2.91 (s, 6H, $2CH_3$), 6.23 (s, 1H, HC=N-), 7.05 (s, 1H, pyridine-H5), 7.12–8.02 (m, 11H, Ar-H), 9.31 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$436 (14 %).Anal.Calcd for $C_{25}H_{20}N_6O_2$ (436.47): % C, 68.80; H, 4.62; N, 19.25. Found: % C, 68.57; H, 4.83; N, 19.38.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(pyridine-2-yl) pyridin-2-ylamino)-N,N-dimethylformamidine (**18b**)

Yield: 87 %; mp (^{o}C):>300. IR (KBr, cm^{-1}): 3304 (NH), 3055 (CH-aromatic), 2930 (CH-aliphatic), 2200 (C≡N). ^{1}H NMR (DMSO-d6, δ ppm): 3.12 (s, 6H, $2CH_3$), 6.45 (s, 1H, HC=N-), 6.65 (s, 1H, pyridine-H5), 7.12–8.02 (m, 11H, Ar-H), 9.52 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$392 (51 %).Anal.Calcd for $C_{24}H_{20}N_6$ (392.46): % C, 73.45; H, 5.14; N, 21.41. Found: % C, 73.86; H, 5.45; N, 21.69.

General procedure for synthesis of 3-hydroxy-6-(naphthalen-2-yl)-8-substituted-4-oxo-4H-pyrido[2,1-*c*][1,2,4]triazine-9-carbonitrile derivatives19a,b

A mixture of the hydrazine compounds 15a,b (10 mmol) and diethyl oxalate (1.46 mL, 10 mmol) in absolute ethanol (10 mL) was refluxed for 8h. The precipitate formed on cooling was filtered, dried and crystallized from isopropanol to give the derivatives **19a, b**, respectively.

3-Hydroxy-6-(naphthalen-2-yl)-8-(4-nitrophenyl)-4-oxo-4H-pyrido[2,1-*c*][1,2,4]triazine-9-carbonitrile (**19a**)

Yield: 65 %; mp (^{o}C): > 300. IR (KBr, cm^{-1}): 3423 (OH), 3055 (CH-aromatic), 2930 (CH-aliphatic), 2219 (C≡N), 1646 (C=O), 1629, 1256 (NO_2). ^{1}H NMR (DMSO-d6, δ ppm): 6.56 (s, 1H, pyridine-H5), 7.12–8.02 (m, 11H, Ar-H), 10.00 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6 δ ppm): 110.70 (C≡N), 124.32, 124.52, 125.62, 125.91, 127.21, 127.29, 128.14, 128.25, 129.31, 129.38, 132.85, 132.90, 148.25, 152.41, 153.01, 156.51, 157.90, 158.41, 163.13 (aromatic-C), 163.91 (C=O), 164.42 (C-OH). MS m/z: $M^{+\cdot}$435 (17 %).Anal.Calcd for $C_{24}H_{13}N_5O_4$ (435.39): % C, 66.21; H, 3.01; N, 16.09. Found: % C, 66.48; H, 3.31; N, 15.78.

3-Hydroxy-6-(naphthalen-2-yl)-4-oxo-8-(pyridin-2-yl)-4H-pyrido[2,1-*c*][1,2,4]triazine-9-carbonitrile (**19b**)

Yield: 65 %; mp (^{o}C): 281-283. IR (KBr, cm^{-1}): 3426 (OH), 3055 (CH-aromatic), 2950 (CH-aliphatic), 2213 (C≡N), 1650 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 6.58 (s, 1H, pyridine-H5), 7.12–8.32 (m, 11H, Ar-H), 10.05 (s, 1H, OH, D_2O exchangeable). MS m/z: $M^{+\cdot}$391 (25 %).Anal.Calcd for $C_{23}H_{13}N_5O_2$ (391.38): % C, 70.58; H, 3.35; N, 17.89. Found: % C, 70.35; H, 3.57; N, 18.08.

General procedure for synthesis of N'-(3-cyano-6-(naphthalen-2-yl)-4-substituted-pyridin-2-ylamino)-N-(urea/thiourea) formamidine derivatives 20a,b and 21a,b

A mixture of N,N-dimethylformamidine derivatives **18a,b** (1 mmol) and urea or thiourea (12 mmol) was dissolved in DMF (20 mL) followed by addition of dil. HCl (37%) (5 mL). The resulting mixture was stirred at 60°C for 7h. The solvent was evaporated and residue was treated with ice\water. The obtained solid was collected by filtration, washed with water, dried and crystallized from ethanol to give desired compounds **20a,b**, **21a,b** respectively.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridin-2-ylamino)-N-(urea)formamidine (**20a**)

Yield: 80 %; mp (^{o}C): 132-134. IR (KBr, cm^{-1}): 3422-3152 (NH_2, 2NH), 3055 (CH-aromatic), 2922 (CH-aliphatic), 2218 (C≡N), 1649 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 6.51 (s, 2H, NH_2, D_2O exchangeable), 6.98 (s, 1H, pyridine-H5), 7.12–8.32 (m, 12H, HC=N-, Ar-H), 3.70, 10.44 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$451 (63 %).Anal.Calcd for $C_{24}H_{17}N_7O_3$ (451.44): % C, 63.85; H, 3.80; N, 21.72. Found: % C, 63.93; H, 3.52; N, 21.95.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(pyridine-2-yl)pyridin-2-ylamino)-N-(urea)formamidine (**20b**)

Yield: 80 %; mp (^{o}C): > 300. IR (KBr, cm^{-1}): 3425-3160 (NH_2, 2NH), 3055 (CH-aromatic), 2922 (CH-aliphatic), 2200 (C≡N), 1649 (C=O). ^{1}H NMR (DMSO-d6, δ ppm): 6.58 (s, 2H, NH_2, D_2O exchangeable), 7.11 (s, 1H, pyridine-H5), 7.12–8.32 (m, 12H, HC=N-, Ar-H), 3.70, 10.44 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$407 (77 %).Anal.Calcd for $C_{23}H_{17}N_7O$ (407.43): % C, 67.80; H, 4.21; N, 24.06. Found: % C, 67.66; H, 3.94; N, 23.86.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridin-2-ylamino)-N-(thiourea)formamidine (**21a**)

Yield: 87 %; mp (^{o}C): 223-225. IR (KBr, cm^{-1}): 3425-3310 (NH_2, 2NH), 3051 (CH-aromatic), 2920 (CH-aliphatic), 2217 (C≡N), 1150 (C=S). ^{1}H NMR (DMSO-d6, δ ppm): 6.58 (s, 2H, NH_2, D_2O exchangeable), 7.11 (s, 1H, pyridine-H5), 7.32–8.51 (m, 12H, HC=N-, Ar-H), 3.70, 8.81 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d6 δ ppm): 110.70 (C≡N), 124.32, 124.52, 125.62, 125.91, 127.21, 127.29, 128.14, 128.25, 129.31, 129.38, 132.85, 148.25, 152.41, 153.01, 156.51,

157.90 (aromatic-C), 158.41 (-N=CH), 180.13 (-C=S). MS m/z: $M^{+\cdot}$467 (17 %).Anal.Calcd for $C_{24}H_{17}N_7O_2S$ (467.50): % C, 61.66; H, 3.67; N, 20.97; S, 6.86. Found: % C, 61.43; H, 3.83; N, 20.41; S, 6.67.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(pyridine-2-yl)pyridin-2-ylamino)-N-(thiourea)formamidine (**21b**)

Yield: 82 %; mp (^oC): 198-200. IR (KBr, cm^{-1}): 3450-3350 (NH_2, 2NH), 3051 (CH-aromatic), 2920 (CH-aliphatic), 2200 (C≡N), 1150 (C=S). ^1H NMR (DMSO-d6, δ ppm): 5.58 (s, 2H, NH_2, D_2O exchangeable), 7.10 (s, 1H, pyridine-H5), 7.21–8.34 (m, 12H, HC=N-, Ar-H), 3.70, 8.56 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$423 (73 %).Anal.Calcd for $C_{23}H_{17}N_7S$ (423.49): % C, 65.23; H, 4.05; N, 23.15; S, 7.57. Found: % C, 65.35; H, 3.88; N, 23.49; S, 7.28.

General procedure for synthesis of N'-(3-cyano-6-(naphthalen-2-yl)-4-substituted-pyridin-2-ylamino)-N-(guanidine)formamidine derivatives22a,b

Guanidine sulfate (0.19 g, 2 mmol) was added to the formamidine derivatives **18a,b** (1 mmol) dissolved in absolute ethanol (10 mL) in the presence of anhydrous K_2CO_3 (0.28 g, 2 mmol) and the mixture was refluxed for 10h. The mixture solution was allowed to cool to room temperature and diluted with water. The obtained product was collected by filtration, washed with water, dried and crystallized from ethanol to yield the derivatives **22a,b**, respectively.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(4-nitrophenyl)pyridin-2-ylamino)-N-(guanidine)formamidine (**22a**)

Yield: 82 %; mp (^oC): 198-200. IR (KBr, cm^{-1}): 3450-3350 (NH_2, 3NH), 3060 (CH-aromatic), 2921 (CH-aliphatic), 2200 (C≡N), 1630, 1262 (NO_2). ^1H NMR (DMSO-d6, δ ppm): 4.32 (s, 2H, NH_2, D_2O exchangeable), 7.10 (s, 1H, pyridine-H5), 7.21–8.51 (m, 12H, HC=N-, Ar-H), 3.71, 8.80, 10.21 (3s, 3H, 3NH, D_2O exchangeable). ^{13}C NMR (DMSO-d6 δ ppm): 110.70 (C≡N), 124.32, 124.52, 125.62, 125.91, 127.21, 127.29, 128.14, 128.25, 129.31, 129.38, 132.85, 148.25, 152.41, 153.01, 156.51, 157.90 (aromatic-C), 158.41 (-N=CH), 163.13 (NH=C=NH_2). MS m/z: $(M-1)^{+\cdot}$449 (66 %).Anal.Calcd for $C_{24}H_{18}N_8O_2$ (450.45): % C, 63.99; H, 4.03; N, 24.88. Found: % C, 63.86; H, 4.27; N, 24.51.

N'-(3-cyano-6-(naphthalen-2-yl)-4-(pyridine-2-yl)pyridin-2-ylamino)-N-(guanidine)formamidine (**22b**)

Yield: 80 %; mp (^oC): 212-214. IR (KBr, cm^{-1}): 3450-3390 (NH_2, 3NH), 3058 (CH-aromatic), 2921 (CH-aliphatic), 2200 (C≡N). ^1H NMR (DMSO-d6, δ ppm): 4.52 (s, 2H, NH_2, D_2O exchangeable), 7.10 (s, 1H, pyridine-H5), 7.32–8.51 (m, 12H, HC=N-, Ar-H), 3.80, 8.80, 10.21 (3s, 3H, 3NH, D_2O exchangeable). MS m/z: $(M-1)^{+\cdot}$405 (50 %).Anal.Calcd for $C_{23}H_{18}N_8$ (406.44): % C, 67.97; H, 4.46; N, 27.57. Found: % C, 67.72; H, 4.54; N, 27.74.

Biological evaluation

Antiviral screening

MTT cytotoxicity assay (TC50)

Samples were 10-fold serially diluted with Dulbecco's Modified Eagle's Medium (DMEM). Stock solutions of the test compounds were prepared in 10 % DMSO in dd H^2O. The cytotoxic activity of the extracts were tested in Madin Darby Canine kidney (MDCK) cells by using the 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method [33] with minor modification. Briefly, the cells were seeded in 96 well-plates (100 µL/well at a density of 3×105 cells/mL) and incubated for 24h at 37°C in 5% CO_2. After 24h, the cells were treated with various concentrations of the tested compounds in triplicates. After further 24h, the supernatant was discarded and the cell monolayers were washed with sterile phosphate buffer saline (PBS) three times and MTT solution (20 µL of 5 mg/mL stock solution) was added to each well and incubated at 37 °C for 4h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µL of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 mL HCL in 50 mL isopropanol). The absorbance of formazan solutions were measured at λmax 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation.

$$\% \text{ cytotoxicity} = \frac{(\text{Absorbance of cells without treatment}) - (\text{Absorbance of cells with treatment}) \times 100}{\text{Absorbance of cells without treatment}}$$

The plot of % cytotoxicity versus sample concentrations was used to calculate the concentration which exhibited 50% cytotoxicity (LD50).

Plaque reduction assay

Assay was carried out according to the method of [34] in a six well plate where MDCK cells (105 cells / ml) were cultivated for 24h at 37°C. A/CHICKEN/QALUBIA/1/2006 (H5N1) virus was diluted to give 104 PFU/ well and mixed with the safe concentration of the tested compounds, 1 µg/mL of L-1-(tosyl-amido-2-phenyl) ethyl chloromethyl ketone (TCPK) and incubated for 1h at 37 °C before being added to the cells. Growth medium was removed from the cell culture plates and virus-compounds or virus-extract and Virus-Zanamivir mixtures were inoculated (100 µL / well). After 1h contact time for virus adsorption, 3 mL of DMEM supplemented with 2% agarose was added onto the cell monolayer,

plates were left to solidify and incubated at 37 ^{o}C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for two hours then plates were stained with 0.1% crystal violet in distilled water. Control wells were included where untreated virus was incubated with MDCK cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following

% inhibition = viral count (untreated) - viral count (treated)/viral count (untreated) x 100

Antimicrobial screening

1. Preparation of microbial suspensions

Antimicrobial activities were carried out against highly pathogenic reference strains accused of causing food poisoning diseases from food of animal origin. Bacterial strains used were: gram positive bacteria; Enterotoxigenic S. aureus ATCC 13565, gram negative bacteria; Salmonella Typhimurium ATCC13311 and mycotic stain C. albicans EMCC105 and Asp. flavus isolate. Agar well diffusion (qualitative method) and minimum inhibitory concentration (MIC) (quantitative method) were used in this study. Wherein a suspension of bacterial and mycotic strains were freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 mL of Muller Hinton Broth for bacterial strains and Sabaroud Dextrose broth for mycotic strain. The inoculated tubes were incubated at 37°C and 28 °C for 24h, respectively. Serial dilutions were carried out for each strain, dilution matching with 0.5 Mc-Farland (about 1×108 cells/ml), was selected for screening of antimicrobial activities. Ciprofloxacin (5µg/disk) and Amphotericin (30 µg/disk) were used as reference drugs (Oxoid) for bacterial and mycotic strains, respectively, DMSO was used as a negative control.

2. Determination of antimicrobial activity by agar well diffusion method

In 3 replicates, the antimicrobial activity against the bacterial strains was evaluated by using the agar-well diffusion method [35, 36]. Hundred µL of cell culture suspension matching with 0.5 McFarland of the target reference strains were spread onto the plates. To investigate the antibacterial activity, 50 µL of different compounds at different concentrations were added in individual wells, Ciprofloxacin (5µg/disk) and Amphotericin (30 µg/disk) were added as control positive reference drugs as antibacterial and antimycotic, respectively. DMSO as a negative control were added into the wells of agar plates directly. Plates were left for 1h at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effects of variations in time between the applications of different solutions. The plates were re-incubated aerobically at 37

°C and 30°C for 24h for bacterial and mycotic strains, respectively. After incubation, plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for each of the tested samples. 3. Determination of Minimum Inhibitory Concentration (MIC).

Microtiter dilution plate quantitative method, i.e. the minimum inhibitory concentration (MIC) [37] was used for evaluation of the antimicrobial activity of testing compounds. Determination of MIC of extract against tested strains was achieved using 96-well sterile micro plates. The first well contain the concentrated form of the tested compound used in the agar disk diffusion method (10µg of the tested compound dissolved in 1mL DMSO), then two fold serial dilutions were carried out for the tested compounds, reference drugs (Ciprofloxacin and Amphotericin) and DMSO. Then the wells were inoculated with 100 µL of the tested isolates (0.5 Mc-Farland, about 1×108 cells/mL) and incubated at 37°C-28°C for 24 h for bacterial and fungal strains respectively. After incubation, plates were examined visually for bacterial or fungal growth precipitation. The experiment was repeated three times. The lowest concentration that showed complete hindrance of growth was taken as MIC.

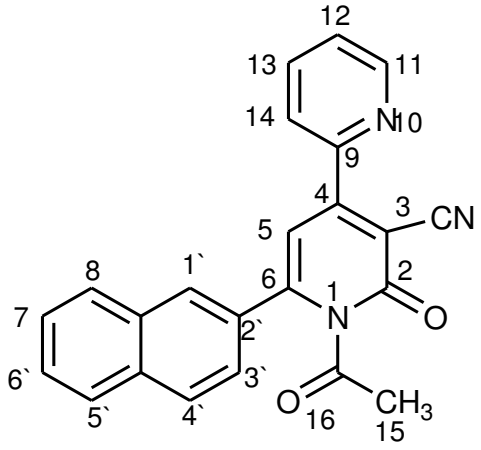
Results and discussion

Chemistry

The routes adopted for the synthesis of the new naphthalene-pyridine hybrid derivatives in this study were depicted in Schemes 1 and 2. The structural confirmation of the newly synthesized compounds was based on their elemental analyses and spectral data. The starting 6-(2-naphthyl)-2-oxo-4-(4-nitro phenyl/2-pyridinyl)-1,2-dihydropyridine-3-carbonitrile derivatives **1a,b** were synthesized following the literature method [15,38]. IR spectra of **1a,b** compounds showed absorption bands at 3425–3420 cm^{-1}, assignable to the –NH-stretching vibrations, characteristic bands at 2215, 2210 cm^{-1} corresponding to –CN groups, stretching bands at 1648, 1650 cm^{-1} referring to C=O groups in addition to two characteristic vibrational bands at 1623, 1260 due to NO_2 group of **1b** derivative. In addition, their ^{1}H-NMR spectra showed the aromatic protons as multiplet signals at δ 7.27-7.90 ppm, while NH protons appeared as D_2O exchangeable signals at 9.51, 9.56 ppm. Taking advantage of a good hydrogen atom mobility in NH group of the key starting compounds **1a,b**,[39, 40] thus upon N-substitution of **1a,b** in a basic medium with different acid chloride derivatives namely; acetyl chloride, chloroacetyl chloride and benzoyl chloride, the corresponding products **2a,b**, **3a,b** and **4a,b** were obtained. While the reaction of **1a,b** with benzenesulfonyl chloride and tosyl chloride, the N-sulfonylated products **5a,b**

and **6a,b** were formed respectively as depicted in **Scheme 1**. IR spectra of the new **2a,b-4a,b** derivatives revealed the appearance of new bands at the range 1700-1673 cm^{-1} due to C=O groups of the alkyl or aryl side chains in addition to the bands of C=O groups of the parent pyridone rings, while the sulfonyl analogues **5a,b, 6a,b** showed absorption bands at the range of 1350-1340 cm^{-1} due to SO_2 groups alongside with the main bands of the parent molecules. In ^{1}H-NMR spectra of compounds **2a,b**, the protons of the acetyl CH_3 groups appeared as singlet signals at δ 2.61, 2.72 ppm for **2a** and **2b**, respectively. In case of **3a,b**, the methylene protons of $–CH_2Cl$ displayed as singlet signals at δ 4.24, 4.37 ppm for **3a** and **3b**, respectively. Assignments of compounds **2b** and **5a** were aided by the use of 2D homonuclear chemical shift correlated ^{1}H-NMR (COSY) (DMSO, δ ppm). ^{1}H, ^{13}C and COSY NMR assignments for compounds **2b** and **5a** were provided in **tables 1,2**.

Table 1: ^{1}H, ^{13}C and COSY NMR assignments for compound 2b

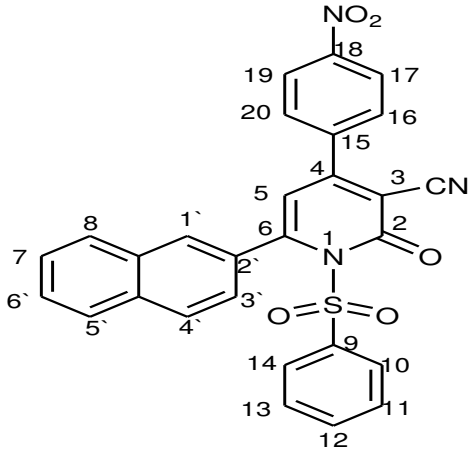


2b	^{13}C(δ_C)	^{1}H (δ_H)[a]	COSY		^{13}C(δ_C)	^{1}H (δ_H)[a]	COSY
1	-	-	-	6`	128.17	7.64	-
1`	127.56	8.78	H-3`	7	128.17	7.64	H-8
2	162.63	-	-	8	130.05	8.35	H-7
2`	129.37	-	-	9	151.60	-	-
3	127.13	-	-	10	-	-	-
3`	129.37	7.74	H-1`, H-4`	11	149.05	8.98	-
4	155.65	-	-	12	129.07	8.01	-

4`	127.56	8.78	H-3`	13	128.77	8.68	-
5	124.13	7.14	-	14	128.77	8.68	-
5`	130.05	8.35	H-6	15	27.7	2.72	-
6	136.55	-	H-5`	16	198.37	-	-

[a] δ ppm in DMSO-*d6,* 500 MHz for ^{13}C, 500 MHz for ^{1}H

Table 2: ^{1}H, ^{13}C and COSY NMR assignments for compound 5a



5a	$^{13}C(\delta_C)$	$^{1}H\ (\delta_H)^a$	COSY		$^{13}C(\delta_C)$	$^{1}H\ (\delta_H)^a$	COSY
1	-	-	-	8	132.08	8.05	H-7
1`	127.61	8.84	H-3`	9	129.31	-	-
2	150.66	-	-	10	128.25	8.35	-
2`	129.01	-	-	11	128.72	8.05	H-10, H-12
3	124.53	-	-	12	129.31	7.73	H-11, H-13
3`	129.31	7.73	H-1`, H-4`	13	128.72	8.05	H-14, H-12
4	148.25	-	-	14	128.25	8.35	-
4`	127.61	8.84	H-3`	15	132.85	-	-
5	127.61	7.32	-	16	128.10	8.61	-
5`	132.08	8.05	H-6	17	124.82	9.04	-
6	132.85	-	H-5`	18	132.85	-	-

6`	128.25	7.65	-	19	124.82	9.04	-
7	128.25	7.65	H-8	20	128.10	8.61	-

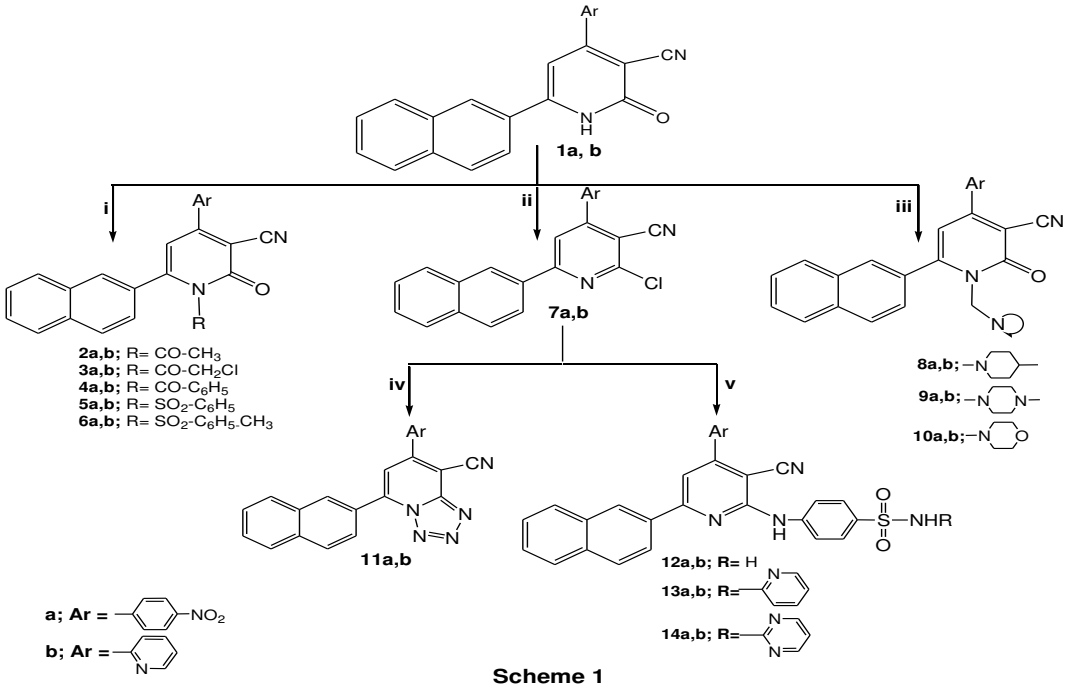
[a] δ ppm in DMSO-*d6*, 500 MHz for ^{13}C, 500 MHz for ^{1}H

Mass spectra of the compounds **2a,b-6a,b** displayed the molecular ion peaks that are assignable to the exact molecular formulae of the derivatives.

Upon refluxing the starting naphthalene-pyridine derivatives **1a,b** with a mixture of phosphorus oxychloride/phosphorus pentachloride, they were converted to the 2-chloro derivatives **7a,b** which in turn were condensed with sodium azide in glacial acetic acid to yield the corresponding fused tetrazolo-pyridine analogues **11a,b**. IR spectra of **7a,b** derivatives represented the disappearance of the characteristic C=O bands of the parent molecules. Mass spectra of **11a,b** represented the molecular ion peaks at m/e 392 and 348 referring to the corresponding molecular formulae of the tetrazolo derivatives. Further condensation of the chloro derivatives **7a,b** with various sulfa drugs namely; sulfanilamide, sulfapyridine and sulfapyrimidine in absolute ethanol containing catalytic amounts of triethylamine as a basic medium afforded the corresponding sulfonamide derivatives **12a,b-14a,b**. IR spectra of the latter compounds exhibited different characteristic vibrational bands at 3460-3145 cm^{-1}, 2220-2200 cm^{-1}, 1345-1160 cm^{-1} assigned to NH_2, NH, CN and SO_2 groups respectively. Mass spectra of the obtained sulfonamide compounds represented their molecular ion peaks that confirmed their molecular formulae.

Mannich reaction provides a suitable method to introduce an aminoalkyl substituent into a molecule. In several instances, the Mannich derivatives exhibit better activity than the corresponding parent analogues. Moreover, the presence of Mannich side chain increases the solubility and hence the bioavailability of the drug molecule [41]. Thus, the compounds **1a,b** were allowed to react with *p*-formaldehyde and different secondary amines such as 4-methylpiperidine, N-methylpiperazine, and morpholine to get the corresponding Mannich bases **8a,b-10a,b**. IR spectra revealed the disappearance of NH bands of the parent molecules. Also, ^{1}H NMR spectra were in agreement with the proposed structures of the gained products. For example, ^{1}H NMR spectrum of the morpholino derivative **10a** displayed the methylene protons of N-$(CH_2)_2$ and O-$(CH_2)_2$ of the morpholine ring as two singlets at δ 2.85, 3.75 ppm, respectively, while the methylene protons of the aminoalkyl linkage appeared as a third singlet at δ 4.31ppm, in addition to the expected signals of the parent compound. At the same time, ^{13}C NMR spectrum of **10b** represented signals at δ 53.27,

66.50 ppm referring to the 4 CH_2 carbons of the morpholine ring and at δ 64.27 ppm due to the methylene carbon of the aminoalkyl linkage, while C≡N, aromatic and C=O carbons appeared as signals at δ 107.12, 116.57-157.04, 162.63 ppm, respectively. (**Scheme-1**).



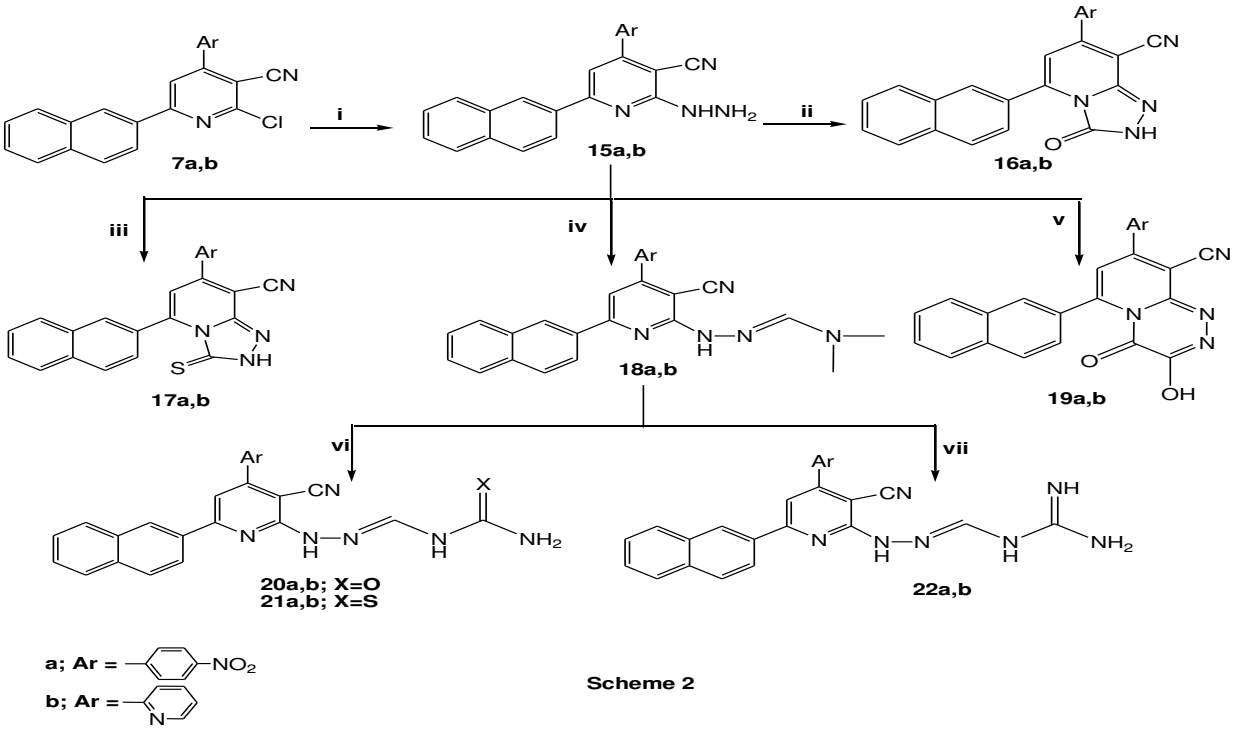
Scheme 1

Reaction conditions: **i**) different acid chloride derivatives, pyridine, reflux for 6h. **ii**) $PCl_5/POCl_3$, reflux for 2h. **iii**) p-formaldehyde, the appropriate amines, ethanol, reflux for 8h. **iv**) sodium azide, glacial acetic acid, reflux for 6 h. **v**) different sulfa drugs, ethanol, TEA, reflux for 8h.

Also, in this work amination of the 2-chloro derivatives **7a,b** was carried out by their treating with hydrazine hydrate in absolute ethanol and reflux for 8h to produce the hydrazinyl derivatives **15a,b** which were used as intermediate precursors for synthesis of new different heterocyclic functionalities attached the main naphthalene-pyridine core. Thus, when they were reacted with ethyl chloroformate in refluxing pyridine, cyclization took place to furnish the triazolo[4,3-*a*]pyridine analogues **16a,b**. Meanwhile, refluxing the hydrazinyl derivatives **15a,b** with carbon disulfide and diethyl oxalate in absolute ethanol led to the formation of the corresponding 3- mercapto-triazolo[4,3-*a*]pyridine and 3,4-dioxo-pyrido[2,1-*c*]triazine compounds **17a,b** and **19a,b**, respectively. IR spectra of **16a,b** derivatives exhibited absorption bands at 3305, 2200-2213, 1710-1670 cm^{-1} due to NH, C≡N and C=O functional groups, respectively. Regarding to the derivatives **17a,b**, their IR spectra displayed C=S groups as vibrational bands at 1195, 1199 cm^{-1}, while those of **19a,b** derivatives exhibited two vibrational bands at 3426-3423, 1650-1646 cm^{-1} corresponding to OH and C=O groups, in addition to the other bands related to the other functionalities of the parent molecules. Mass spectra of the analogues **16a,b**, **17a,b** and **19a,b** showed the molecular ion peaks at the intense molecular formulae of the compounds.

Moreover, the preparation of different N,N-dimethylformamidine derivatives is one of our synthetic strategy in this study, thus the hydrazinyl derivatives **15a,b** were allowed to react with N,N-dimethylformamide dimethyl acetal (DMF-DMA) in toluene to produce the corresponding compounds **18a,b**. ^{1}H-NMR spectra of the resulting derivatives **18a,b** displayed five singlets recognizable as arising from the two methyls at δ 2.91, 3.12 ppm, CH=N- at δ 6.23, 6.45 ppm, CH of the pyrimidine ring at δ 7.05, 6.65 ppm, NH group at δ 9.31, 9.52 ppm, in addition to the multiplet signals referring to the aromatic protons. The Structures were further confirmed by mass spectra, which gave their intense molecular ion peaks at m/z 436 and 392.

Further nucleophilic reactions were carried out by condensation of the compounds **18a,b** with urea, thiourea and guanidine with elimination of dimethylamine group to furnish the corresponding formamidine analogues **20a,b-22a,b**. ^{1}H-NMR spectra revealed the absence of the signals corresponding to the two CH_3 of N,N-dimethylaminoformamidine group, instead they showed two singlets at δ 3.70 and4.32-6.58 ppm corresponding to NH and NH_2 groups. Also, ^{13}C-NMR of the derivative **22a** showed different signals at δ 110.70, 124.32-157.90 ppm referring to C≡N and aromatic carbons, while, the imino carbons of (-N=CH) and (NH=C=NH_2) appeared as two signals at δ 158.41, 163.13 ppm. (**Scheme 2**)



Scheme 2

Reaction conditions: **i**) hydrazine hydrate (99%), absolute ethanol, reflux for 8h. **ii**) ethylchloroformate, pyridine, reflux for 6h. **iii**) KOH, CS_2, ethanol, reflux for 8h. **iv**) N,N-dimethylformamide dimethylacetal, dry toluene, reflux for 6h **v**) diethyl oxalate, absolute ethanol, reflux for 8h. **vi**) urea or thiourea, DMF, HCl, heat with stirring at 60°C for 7h. **vii**) Guanidine sulfate, absolute ethanol, anhydrous K_2CO_3, reflux for 10h.

Antiviral screening

The *in vitro* antiviral activity of the new derivatives against influenza virus H5N1strainswas evaluated. The obtained results investigated that the tested compounds produced moderate to weak activity. It has been noticed that the best potency was obtained by 1-(2-chloroacetyl)-1,2-dihydro-6-(naphthalen-2-yl)-4-(4-nitrophenyl)-2-oxopyridine-3-carbonitrile (**3a**) (TC_{50}; 0.06 μM uL^{-1}). A noticeable decease in the activity has occurred by the bicyclic pyrido[2,1-*c*][1,2,4]triazine derivative **19a** and theguanidine-formamidine derivative **22b** (TC_{50}; 0.14, 0.16 μM uL^{-1}). Further drop in the potency was observed by the compounds carrying the bicyclic ring systems; tetrazolo[1,5-*a*]pyridine and triazolo[4,3-*a*]pyridine **11a**, **16b**, respectively (TC_{50}; 0.22, 0.23 μM uL^{-1}). Approximate equipotency was also obtained by the parent chloro intermediate **7b**, the morpholino-mannich base **10a**, the sulfonamide derivative **12b** and the 3-mercapto-triazolo[4,3-*a*]pyridine derivative **17a** (TC_{50}; 0.27- 0.29 μM uL^{-1}). On the other hand, the hydrazinolysis of the chloro compound **7a** to give the hydrazine analogue **15a**, the pyridine sulfonamide **13b**, the morpholino-mannich base **10b**,the acetyl derivative **2b**, the sulfonamide derivative **12a** and the hydrazine analogue **15b** exhibited antiviral activityof TC_{50} ranging 0.30-0.35 μM uL^{-1}. Unfortunately, other different variations in the structures of the side chains or the fused heterocyclic rings in conjugation with parent moieties 6-(2-naphthyl)-2-oxo-4-(4-nitrophenyl/2-pyridinyl)-1,2-dihydropyridine-3-carbonitrile didn't enhance the anti-avian influenza virus (H5N1). (**Table-3**).

Table 3: Antiviral activity against H5N1 virus of the tested compounds.

Compound code	ConcuMol uL^{-1}	Initial viral count	Viral count (PFU mL^{-1})	Growth inhibition (%)	TC_{50} uM uL^{-1}
2a	0.05	1.15	0.92	20.0	0.43
	0.10		0.85	26.1	
2b	0.05	1.15	0.83	27.8	0.32
	0.10		0.80	30.4	
3a	0.05	0.80	0.67	16.3	0.06

	0.10		0.63	21.3	
3b	0.05	0.80	0.70	12.5	0.55
	0.10		0.70	12.5	
4a	0.05	1.40	1.37	2.1	1.07
	0.10		1.30	7.1	
4b	0.05	1.40	1.34	4.2	0.46
	0.10		1.32	5.7	
5a	0.05	0.80	0.78	2.5	0.40
	0.10		0.70	12.5	
5b	0.05	0.80	0.75	6.2	0.91
	0.10		0.64	20.0	
6a	0.05	0.75	0.73	2.6	0.32
	0.10		0.70	6.6	
6b	0.05	0.75	0.75	0.0	0.36
	0.10		0.73	2.6	
7a	0.05	1.50	1.21	19.3	0.45
	0.10		0.92	38.6	
7b	0.05	1.05	1.05	0	0.29
	0.10		0.83	20.9	

9a	0.05	1.35	1.35	0	0.42
	0.10		1.35	0	
9b	0.05	1.45	1.13	22.0	0.52
	0.10		1.05	27.5	
10a	0.05	1.35	1.13	16.3	0.28
	0.10		1.06	21.4	
10b	0.05	1.45	1.35	6.9	0.31
	0.10		1.00	31.0	
11a	0.05	1.40	1.24	11.4	0.22
	0.10		1.11	20.7	
11b	0.05	1.40	1.05	25.0	0.56
	0.10		1.05	25.0	
12a	0.05	0.80	0.80	0	0.35
	0.10		0.77	3.7	
12b	0.05	0.80	0.70	12.5	0.29
	0.10		0.70	12.5	
13a	0.05	1.10	0.80	27.2	0.44
	0.10		0.80	27.2	
13b	0.05	1.10	0.80	27.2	0.31

	0.10		0.77	30.0	
15a	0.05	1.05	1.05	0	0.30
	0.10		0.82	21.9	
15b	0.05	1.05	0.76	27.6	0.35
	0.10		0.76	27.6	
16a	0.05	1.30	1.25	3.8	0.43
	0.10		1.15	11.5	
16b	0.05	1.30	1.05	19.2	0.23
	0.10		1.05	19.2	
17a	0.05	0.70	0.70	0	0.27
	0.10		0.70	0	
17b	0.05	0.70	0.70	0	0.31
	0.10		0.56	20.0	
18a	0.05	0.90	0.90	0	0.38
	0.10		0.90	0	
19a	0.05	1.25	1.00	20.0	0.14
	0.10		1.00	20.0	
19b	0.05	1.25	1.09	12.8	0.51
	0.10		1.09	12.8	

20a	0.05	0.93	0.82	11.8	0.46
	0.10		0.80	13.9	
20b	0.05	0.93	0.87	6.4	0.26
	0.10		0.87	6.4	
21a	0.05	0.90	0.90	0	0.57
	0.10		0.85	5.5	
21b	0.05	0.90	0.86	4.4	0.31
	0.10		0.78	13.3	
22a	0.05	0.95	0.90	5.2	2.92
	0.10		0.80	15.7	
22b	0.05	1.45	1.30	10.3	0.16
	0.10		1.30	10.3	

Antimicrobial evaluation

The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of the bacterial or fungal growth around the discs measured in mm (**Table 4**) as well as the minimum inhibitory concentration for the compounds using the two-fold serial dilution method **(Table 5)**. In general according to **Table 5**, the tested compounds revealed better antibacterial activity against the tested gram positive bacteria *S. aureus* rather than the gram negative *S. typhimurium*bacterial strain, but less than that exhibited by the reference drug ciprofloxacin (MIC= 5.7 x10^{-4} uM mL^{-1}). It has been noted that the conjugation of the parent 6-naphthalen-4-(pyridin-2-yl)pyridine core with sulfapyrimidine side-chain at position-2 (compound **14b**) exhibited the most potent activity against both gam positive and gram negative bacteria ($MIC_{S.\ aureus}$ = 0.01 uM mL^{-1}, $MIC_{S.\ typhimurium}$= 0.04 uM mL^{-1}). The susceptibility of *S. aureus* slightly decreased against the bicyclic 3-mercapto-triazolo[4,3-*a*]pyridine derivative (compound **17b**) ($MIC_{S.\ aureus}$ = 0.02 uM mL^{-1}), while

the same derivative appeared to be inactive against *S. typhimurium*. Further reduction in the potency was observed upon replacement of the sulfapyrimidine side-chain of **14b** with sulfa pyridine moiety as compound **13b** of $MIC_{S.\ aureus}$ = 0.05 uM mL^{-1}, $MIC_{S.\ typhimurium}$= 0.05 uM mL^{-1}. The sensitivity of *S. aureus* was retained against the phenylsulfonyl derivatives **5b**, **6a** ($MIC_{S.\ aureus}$ = 0.05 uM mL^{-1}) and the tetrazolo[1,5-*a*]pyridine derivative **11a**($MIC_{S.\ aureus}$ = 0.06uM mL^{-1}), while complete drop in the potency was noticed by the latter derivatives against *S. typhimurium* bacteria. The remaining compounds were found either weak or inactive.

Table 4 Agar well diffusion method showing antimicrobial activities of the tested compounds compared with reference drugs, results given in (mm).

Sample no	*S.aureus* ATCC 13565 Enterotoxigenic	*Salmonella* Typhimurium ATCC 13311	*C. albicans* EMCC 105	*Asp. flavus*
Plate1				
1a	**12**	**10**	**25**	**12**
2a	**9**	**-ve**	**14**	**12**
5a	**10**	**-ve**	**26**	**29**
9a	**10**	**10**	**14**	**15**
11a	**16**	**9**	**25**	**18**
19a	**-ve**	**8**	**16**	**20**
DMSO	**-ve**	**-ve**	**-ve**	**-ve**
Plate2				
4a	**10**	**-ve**	**20**	**23**
6a	**16**	**-ve**	**12**	**25**
12a	**-ve**	**-ve**	**20**	**26**
13a	**11**	**-ve**	**18**	**23**
18a	**10**	**10**	**26**	**22**
20a	**12**	**10**	**20**	**23**

21a	**12**	**-ve**	**24**	**23**
Plate3				
1b	**10**	**-ve**	**17**	**12**
5b	**16**	**-ve**	**26**	**17**
9b	**10**	**-ve**	**20**	**15**
11b	**12**	**-ve**	**16**	**18**
16b	**12**	**-ve**	**20**	**16**
17b	**21**	**-ve**	**20**	**20**
Ciprofloxacin 5µg	**20**	**25**	**-**	**-**
Amphotericin 30µg	**-**	**-**	**26**	**35**
Plate 4				
3b	**10**	**-ve**	**17**	**18**
6b	**12**	**-ve**	**20**	**16**
12b	**10**	**-ve**	**10**	**17**
13b	**17**	**16**	**17**	**12**
14b	**25**	**16**	**20**	**-ve**
22b	**10**	**-ve**	**17**	**12**

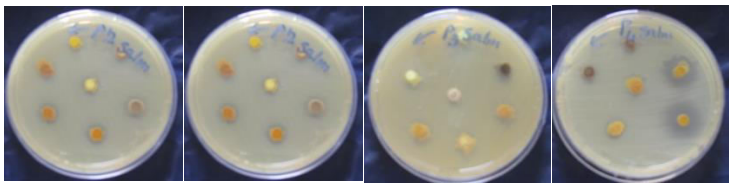
Table 5: Minimum inhibitory concentrations of the tested compounds compared with the reference drugs in uM. mL^{-1}

Compound code	*S. aureus*	*S. typhimurium*	*C. albicans*	*A. flavus*
1a	0.14	0.27	0.01	0.14
1b	0.31	-	0.08	0.15
2a	0.24	-	0.12	0.12

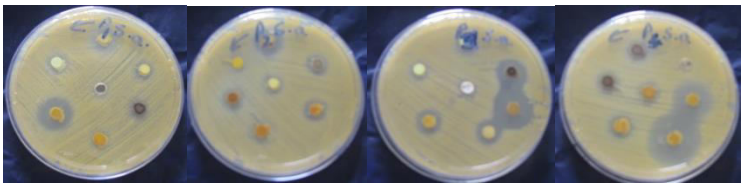
3b	0.25	-	0.06	0.06
4a	0.21	-	0.03	0.01
5a	0.19	-	0.01	0.03
5b	0.05	-	0.01	0.05
6a	0.05	-	0.10	0.01
6b	0.10	-	0.02	0.05
9a	0.21	0.21	0.10	0.05
9b	0.23	-	0.03	0.06
11a	0.06	-	0.01	0.03
11b	0.14	-	0.07	0.03
12a	-	-	0.02	0.01
12b	0.21	-	0.21	0.05
13a	0.17	-	0.02	0.01
13b	0.05	0.05	0.05	0.09
14b	0.01	0.04	0.02	-
16b	0.14	-	0.03	0.07
17b	0.02	-	0.03	0.03
18a	0.23	0.23	0.01	0.01
19a	-	-	0.06	0.03
20a	0.11	0.22	0.03	0.01
21a	0.11	-	0.01	0.01
22b	0.25	-	0.06	0.12
Ciprofloxacin	5.7×10^{-4}	2.9×10^{-4}	-	-
Amphotericin	-	-	0.003	4.22×10^{-4}
DMSO	-	-	-	-

The tested derivatives appeared to be more potent as antifungals than antibacterials. The tested fungal strain *C. albicans* exhibited sensitivity towards the examined derivatives of MIC values ranging 0.12-0.01 uM mL^{-1} comparing to the reference antifungal drug amphotericin (MIC; 0.003 uM mL^{-1}). At the same time, less susceptibility was exhibited by the fungal strain *A. flavus* against the tested compounds (MIC; 0.15-0.01 uM mL^{-1}) when comparing to the reference drug amphotericin (MIC; $4.22x10^{-4}$ uM mL^{-1}).

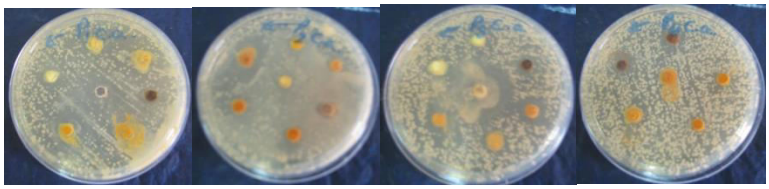
In general, it could be clearly recognized that potential antibacterial activity was encountered with phenylsulfonyl derivatives **5b**, **6a**,the pyridine/pyrimidine sulfonamide derivatives **13b**, **14b** and the bicyclic derivatives tetrazolo[1,5-*a*]pyridine **11a**and3-mercapto-triazolo[4,3-*a*]pyridine**17b**. Meanwhile, the potential antifungal activity was gained by the whole tested derivatives. Further modification in the molecular structures of the compounds should be carried out to gain novel antimicrobials of high potency and lower toxicity to overcome the microbial resistance problem (**Fig. 1**).



***Salmonella Typhimurium* ATCC13311**



Enterotoxigenic*S.aureus* ATCC 13565



***C. albicans* EMCC 105**

Fig. 1 Agar well diffusion method showing antimicrobial activities of the tested compounds.

Conclusion

In this study synthesis a new series of naphthalene-pyridine hybrid compounds was carried out for anti-Avian influenza virus (H5N1) and antimicrobial evaluation utilizing 6-(naphthalen-2-yl) 4-(4-nitrophenyl/-2-pyridinyl)-2-oxo-1,2-dihydropridine-3-carbonitrile **1a,b** as the key starting compounds. Antiviral evaluation exhibited mild activity against influenza virus H5N1strain. Meanwhile, the antimicrobial evaluation revealed that the newly synthesized compounds exhibited potential antibacterial activity against *S. aureus* bacterial strain specially compounds **13b, 14b** and **17b**.Also, potent fungal growth inhibitory effect was obtained specially on *C. albicans*. Further structural modification and optimization are required to get new compounds of potent anti-Avian influenza virus (H5N1) and antimicrobial activity that can be considered as basic nuclei for drug discovery and synthesis.

Acknowledgments

This study was supported by the National Research Centre, Dokki, Cairo, Egypt.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

References

1. Eccles R., Understanding the symptoms of the common cold and influenza. The Lancet Infectious Dis., 2005,**5**(11), 718-725.
2. Yong, Jp. andAisa, H. A., Chemical modification of Rupestonic acid and preliminarily in vitro antiviral activity against influenza A3 and B virus, Bull. Korean Chem. Soc., 2011, **32**(4), 1293-1297.
3. Inamdar, P., Bhandari, S.,Sonawane, B., HoleA. and. Jadhav C, Structure optimization of neuraminidase inhibitors as potential anti-influenza (H1N1inhibitors) agents using qsar and molecular docking studies. Iran J Pharm Res., 2014,**13(1)**, 49-65.
4. Reece, P. A.,Neuraminidase inhibitor resistance in influenza viruses. J Med Virol. , 2007, **79**, 1577–1586.
5. Forero, J. E.,Avila, L.,Taborda, N.,Tabares, P.,López, A., Torres, F.,Quiñones, W., Bucio,Mora-Pérez, Y., Rugeles, M. T., Joseph-Nathan, P. andEcheverri, F.,In vitro anti-influenza screening of several euphorbiaceae species:

structure of a bioactive cyanoglucoside from codiaeumvariegatum, Phytochemistry., 2008,**69**(16), 2815–2819.

6. Y. Li, B. Zhou and R. Wang, Rational design of Tamiflu derivatives targeting at the open conformation of neuraminidase subtype 1.Journal of Molecular Graphics and Modelling. , 2009, **28**, 203–219.
7. T. Gonec, J. Kos, I. Zadrazilova, M. Pesko, R. Govender, S. Keltosova, B. Chambel, D. Pereira, P. Kollar, A. Imramovsky, J. O'Mahony, A. Coffey, A. Cizek, K. Karlova andJ. Jampilek, Antibacterial and herbicidal activity of ring-substituted 2-hydroxynaphthalene-1-carboxanilides. Molecules., 2013,**18,** 9397-9419.
8. G. Turan-Zitouni, Z. A. Kaplancikli, M. T. Yildiz, P. Chevallet and D. Kaya, Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives. Eur J Med Chem., 2005,40(6), 607-613.
9. A. J. Kallen, Y. Mu, S. Bulens, A. Reingold, S. Petit, K. Gershman, S. M. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, W. Schaffner, P. R. Patel andS. K. Fridkin, Health care-associated invasive MRSA infections, 2005–2008. JAMA., 2010, **304**,641–647.
10. B. Özçelik, M. Kartal andI. Orhan, Cytotoxicity, Antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharm Biol., 2011, **49**(4), 396–402.
11. C. Liu, A. Bayer, S. E. Cosgrove,R. S. Daum, S. K. Fridkin, R. J. Gorwitz, S. L. Kaplan, A. W. Karchmer, D. P. Levine, B. E. Murray, M. J. Rybak, D. A. Talan andH. F. Chambers, Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant staphylococcus aureus infections in adults and children: executive summary.Clin. Infect. Dis., 2011,**52**, 285–292.
12. A. E. Rashad, A. H. Shamroukh, M. A. El-Hashash, A. F. El-Farargy, N. M. YousifandM. A. Salama, Synthesis and anti-avian influenza virus (H5N1) evaluation of some novel nicotino-nitriles and their n-acylic nucleosides.Journal of Heterocyclic Chemistry., 2012, **49**(5), 1130–1135.
13. V. Klimešová, M. Svoboda, K. Waisser, M. Pour andJ. Kaustová, New pyridine derivatives as potent antimicrobial agents.IlFarmaco., 1999,**54**(10), 666-672.
14. A. Altundas, S. Ayvaz, and E. Logoglu, Synthesis and evaluation of a series aminocyano-pyridines as antimicrobial agents.Med Chem Res., 2011, **20**,1–8

15. E. R. Kotb, M. M. Anwar, H. S. Abbass and S. Abd El-moez.A concise synthesis and antimicrobial activity of a novel series of naaphthylpyridine-3-carbonitrile compounds. ActaPoloniae Pharmaceutica-Drug Research., 2013, **70**(4), 667-679.

16. W. A. El-Sayed, M. A. Metwally, D. S. Nada, A. A. Mohamed andA. A. H. Abdel-Rahman, Synthesis and antimicrobial activity of new substituted 5-(pyridine-3-yl)-1,3,4-thiadiazoles and their sugar derivatives.Journal of Heterocyclic Chemistry., 2013, **50**(2), 194–201.

17. A. Urzúa, J. Echeverría, M. C. Rezende andM. Wilkens, Antibacterial Properties of 3 *H*-Spiro[1-benzofuran-2,1'-cyclohexane] Derivatives from *Heliotropiumfilifolium.,*Molecules., 2008,**13**(10), 2385-2393.

18. Y. B. Rokade and R. Z. Sayyed, Naphthalene Derivatives: Naphthalene derivatives: A new range of antimicrobial agents with high therapeutic value. Rasayan. J. Chem., 2009,**2**(4), 972-980

19. M. J. Pawar, A. B. Burungale and B. K. Karale, Synthesis and antimicrobial activity of spiro(chromeno-[4,3-d][1,2,3]-thiadiazole-4,1´-cyclohexanes),spiro(chromeno-[4,3-d][1,2,3]-selenadiazole-4,1´-cyclohexanes) and (spiro-chroman-2,1´-cyclohexan-4-one)-5-spiro-4-acetyl-2-(acetylamino)-δ2-1,3,4 -thiadiazoline compounds. Arkivoc. **xiii**,2009, 97–107.

20. N. C..Desai, P. N. Shihora, K. M. Rajpara, V. V. Joshi, H. V. Vaghani, H. M. Satodiya and A. M. Dodiya, Synthesis, characterization, and antimicrobial evaluation of novel naphthalene-based 1,2,4-triazoles. Med Chem Res., 2012,**21**(10), 2981-2989.

21. C. Robert and Jr. Moellering, Discovering new antimicrobial agents. International Journal of Antimicrobial Agents. 2011, **37**(1),2–9.

22. J. Pradhan and A. Goyal, Pyridine Containing Azoles: possible promising antimicrobial molecules. IJPRAS1., 2012,**1**(3), 35-42.

23. M. K. Yun, Y. Wu, Z. Li, Y. Zhao, M. B. Waddell, A. M. Ferreira, R. E. Lee, D and Bashford S. W. White, Catalysis and Sulfa Drug Resistance in Dihydropteroate Synthase.
Science, 2012, **335**(6072), 1110.

24. C. T. Supuran, A. Innocenti, A. Mastrolorenzo andA. Scozzafava. Antiviral sulfonamide derivatives. Mini Rev Med

Chem., 2004, **4**(2), 189-200.

25. M. R. Sayed,Enaminones as Building Blocks for the Synthesis of Substituted Pyrazoles with Antitumor and Antimicrobial Activities. Molecules, 2011,**16**(2), 1834-1853.
26. X. Jia-Yu, X. Zhu-Ping, S. Lei, T. Shu-Hua, L. Huan-Qiu andZ. Hai-Liang. Synthesis and Structure–Activity Relationship Analysis of Enamines as Potential Antibacterial Agents.
Australian Journal Of Chemistry., 2007,**60**(12), 957–962.
27. V. A. Ostrovskii, R. E. Trifonov andE. A. Popova, Medicinal chemistry of tetrazoles. Russian Chemical Bulletin., 2012, **61**(4), 768-780.
28. D. Lingling, C. Shengfeng,,D. V. L. Guri and Z. Chenghe, Recent Advances in the Synthesis and Application of Tetrazoles. Chin. J. Org. Chem., 2013, **33**(2),224:244.
29. H. Bayrak, A. Demirbas, N. Demirbas andS. A. Karaoglu, Synthesis of some new 1,2,4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities. Eur. J. Med. Chem., 2009, **44**, 4362:4366.
30. B. Shivarama Holla, P. M. Akberali and M. K. Shivananda,Studies on nitrophenylfuran derivatives ☆ Part XII. Synthesis, characterization, antibacterial and antiviral activities of some nitrophenylfurfurylidene-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines.Il Farmaco, 2001, **56** (12), 919:927.
31. S. Tabassum, M. Parveen, A. Ali, M. Alam, A. Ahmad, A. U. Khan andR. A. Khan. Synthesis of Aryl-1,2,4,5-tetrazinane-3-thiones, in vitro DNA binding studies, nuclease activity and its antimicrobial activity. Journal of Molecular Structure., 2012,**1020** (8), 33:40.
32. M. J. Di Grandi, K. J. Curran, E. Z. Baum , G. Bebernitz, G. A. Ellestad, W. Ding, S. A. Lang, M. Rossi andJ. D. Bloom. Pyrimido[1,2-b]-1,2,4,5-tetrazin-6-ones as HCMV protease inhibitors: a new class of heterocycles with flavin-like redox properties. Bioorg. Med. Chem. Lett.,2003,**13**(20), 3483-3486.
33. T. Mossman, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol Methods., 1983,**65** (1-2); 55-63.
34. F. G. Hayden, K. M. Cote and R. G. Douglas. Plaque inhibition assay for drug susceptibility testing of influenza

viruses. Antimicrob Agents Chemother., 1980,. **17**, 865–870.

35. D. Sgouras, P. Maragkoudakis, K. Petraki, B. Martinez-Gonzalez, E. Eriotou, S. Michopoulos, G. Kalantzopoulos, E. Tsakalidou and A. Mentis. In vitro and in vivo inhibition of helicobacter pylori by lactobacillus casei strain shirota. Appl Environ Microbiol. 2004, **70**(1), 518-526

36. C. Wiart, Goniothalamus Species: A source of drugs for the treatment of cancers and bacterial infections? Evid Based Complement Alternat Med., 2007,**4**(3), 299–311.

37. J. H. Jorgensen, J. D.Turnidge and J. A. Washington, Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods. In: Murray PR, Barron EJ, Praller MA, Tenover FC, Yolken RH, Eds. Manual of Clinical Microbiology. Washington, D.C., ASM Press. 1999; 1526-1562.

38. E. R. Kotb, M. M. Anwar, M. S. Soliman and M. A. Salama, Synthesis and Reactions of Some Novel Quinoxalines for Anticancer Evaluation. ActaPoloniae Pharmaceutica-Drug Research., 2007,**182**, 1119–1130.

39. T. I. Chaban, R. R. Panchuk, O. V. Klenina, N. R. Skorokhyd, V. V. Ogurtsov andI. G. Chaban, Synthesis and evaluation of antitumor activity of some thiazolo[4,5-b]pyridines. Biopolymers and Cell., 2012,**28**(5), 389:396.

40. S. V. Deshmukh, B. S. Ranea, M. G. Ghagarea, V. B. Gaikwada, A. D. Bholeb andM. N. Jachaka, Synthesis of N-alkylated pyrazolopyridines and study of their antimicrobial activities. J. Chem. Pharm. Res., 2014, 6(1), 540-543.

41. S. G. Supramaniapillai, Mannich reaction: A versatile and convenient approach to bioactive skeletons. J.Chem Sci., 2013,**125** (3),467–482.

Corresponding Author:

Yasmin M Syam*

Email: *yasmin_syam@yahoo.com*;