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SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF SOME NEW HETEROCYCLES ATTACHED TO PYRIDINECARBOXAMIDE MOIETY OF POTENTIAL BIOLOGICAL ACTIVITY

Nabila A. Kheder,[1,2*] Dalia. R. Emam,[3] and Hissana Ather[2]

[1]Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Khalid University, Abha, 61441, Kingdom of Saudi Arabia. [3]Department of Chemistry, Faculty of Science, King Khalid University, Abha, Kingdom of Saudi Arabia; E mail nabila.abdelshafy@gmail.com

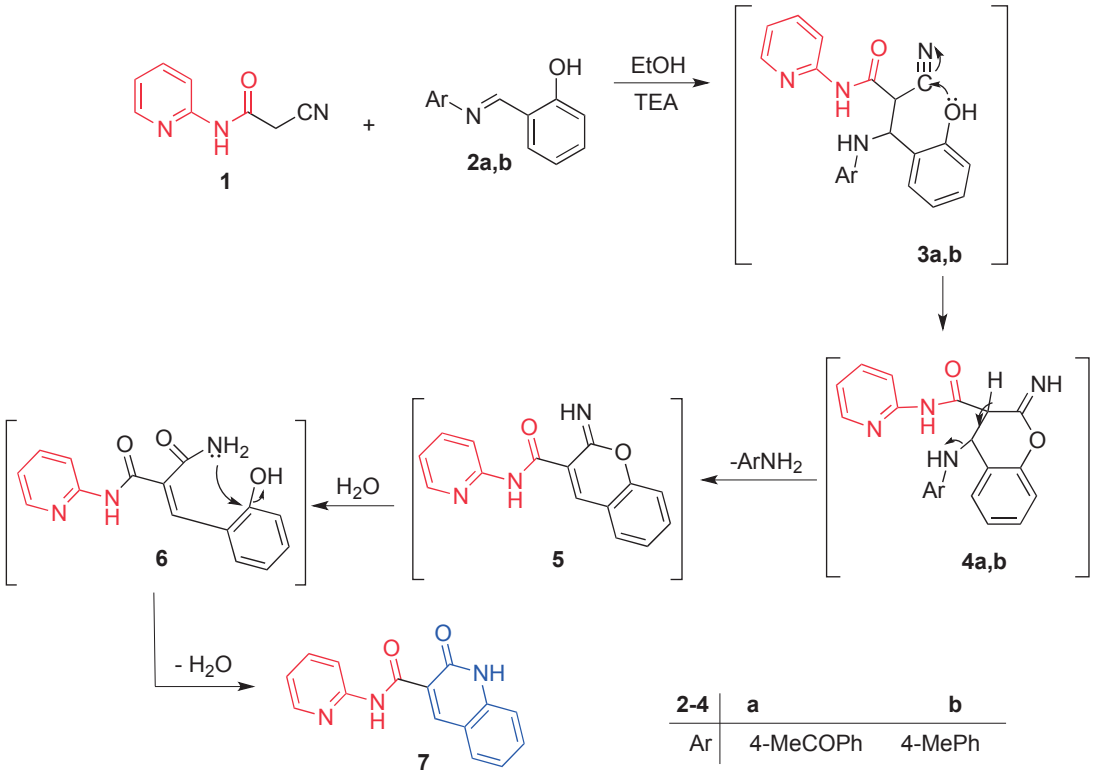
Abstract – A novel series of pyridine, quinoline, coumarin, bipyridine, pyrimidine derivatives attached to pyridinecarboxamide group were prepared by a simple, convenient and one step method using 2-cyano-*N*-(pyrid-2-yl)acetamide or 3-oxo-*N*-(pyridin-2-yl)butanamide. The structures of the novel synthesized compounds were determined by means of IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Antibacterial evaluation of some selected examples was conducted and showed a wide range of activities.

INTRODUCTION

The pyridine nucleus present in numerous natural products such as Vitamin B_6, and pyridoxamine.[1] Also, pyridines possess diverse biological activities like antimalarial, antioxidant, anticonvulsant, anesthetic, antioxidant, antibacterial and antiparasitic properties.[2,3] Moreover, they exhibited fungicidal,[4-6] insecticidal[7,8] and herbicidal activities.[9,10] Also, the pyridine moiety present in many natural alkaloids such as cocaine, opiate, ergot, and quinine alkaloids. Moreover, they are many drugs containing pyridine rings, such as Esomeprazole, Crizotinib, and Loratadine. The high therapeutic properties of the drugs containing pyridine ring have encouraged us to synthesize some novel pyridine derivatives. The carboxamide groups are found in a many biologically important compounds such as peptides and proteins. Also, amide derivatives were reported to have antifungal,[11,12] insecticidal,[13] and herbicidal activities.[14] Motivated by these informations and in continuation of our interest in the synthesis of a variety of heterocyclic carboxamide derivatives of potential biological activities,[15-17] we reported in this article, a simple and efficient methods to obtain the desired pyridinecarboxamide derivatives.

RESULTS AND DISCUSSION

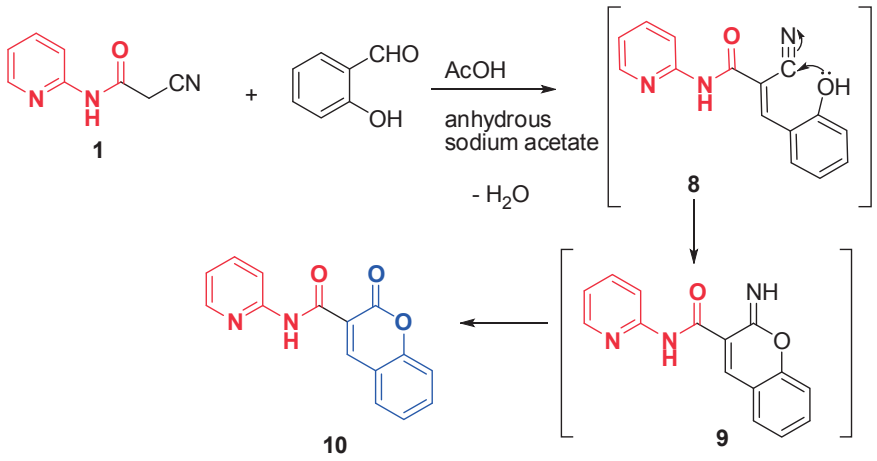
The reaction of 2-cyano-*N*-(pyrid-2-yl)acetamide (**1**)[18] and 1-(4-(2-hydroxybenzylideneamino)-phenyl)ethanone (**2a**) in refluxing ethanol in the presence of catalytic amounts of triethylamine gave the unexpected product **7** as the only reaction product (Scheme 1). The expected product **5** was not detected in the crude reaction mixture. The structure of the product **7** was confirmed based on its spectral data and elemental analyses. For example, its IR spectrum showed two amide carbonyl absorption bands at 1647 and 1682 cm^{-1}. Also, its ^{13}C NMR showed two signals at δ 156.84 and 159.74 due to two amide carbons and absence of any signal at δ 161 corresponding to the reported iminocarbon.[19] Motivated by this result, the reaction was repeated utilizing 2-((*p*-tolylimino)methyl)phenol (**2b**), under the same experimental conditions, which led to the isolation of solid product identical in all respects (mp, mixed mp, IR, NMR and mass spectra) with product **7** (Scheme 1). To account for the unexpected formation of the product **7** we suggested that the studied reactions started with Michael-type addition of the α-carbon atom of cyanoacetamide **1** to imine bond of Schiff base **2**, followed by intramolecular cyclization and elimination of arylamine molecule to afford non-ionizable intermediate **5** which rearranged to the final product **7** in a mechanism similar to that reported in the literature.[20]



Scheme 1. Synthesis of 2-oxo-*N*-(pyridin-2-yl)-1,2-dihydroquinoline-3-carboxamide (**7**)

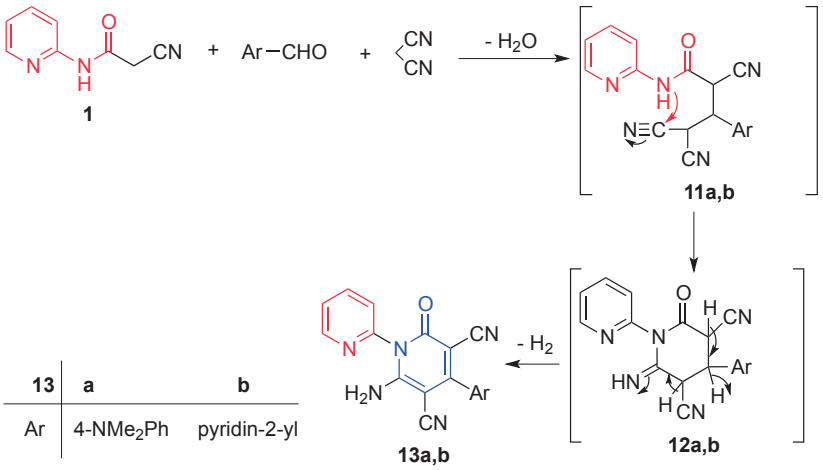
Next, treatment of cyanoacetamide **1** with salicylaldehyde in refluxing glacial acetic acid in the presence of anhydrous sodium acetate afforded 2-oxo-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide (**10**) (Scheme 2).

Formation of compound **10** proceeded via Knoevenagel condensation reaction between cyanoacetamide **1** and salicylaldehyde to afford nonisolable intermediate **8**, which underwent intramolecular cyclization and hydrolysis of the imine function to afford the final product **10**. Its IR spectrum showed absorption bands at 1667, 1708, and 3264 cm^{-1} due to two carbonyl groups and only one NH function. Also, its ^1H NMR spectrum revealed a singlet signal at δ 11.15 due to only one NH proton. The above-mentioned results supported structure **10** and ruled out the other possible product **9** (Scheme 2).



Scheme 2. Synthesis of 2-oxo-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide (**10**)

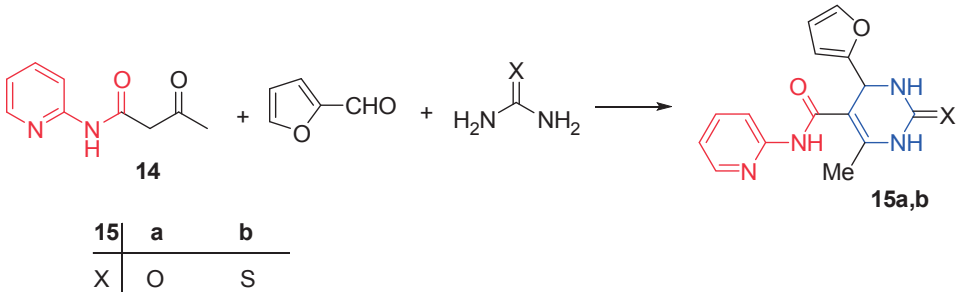
Refluxing cyanoacetamide **1**, malononitrile and the appropriate aldehyde in ethanol and in the presence of a catalytic amount of piperidine afforded a single product in each case as examined by TLC. The structures of the obtained products were assigned as 6-amino-4-(aryl/het)-2-oxo-1-(pyridin-2-yl)-1,2-dihydro-pyridine-3,5-dicarbonitriles **13a,b** (Scheme 3) based on their elemental analyses and spectral data.



Scheme 3. Synthesis of 6-amino-4-(aryl/het)-2-oxo-1-(pyridin-2-yl)-1,2-dihydropyridine-3,5-dicarbonitriles **13a,b**

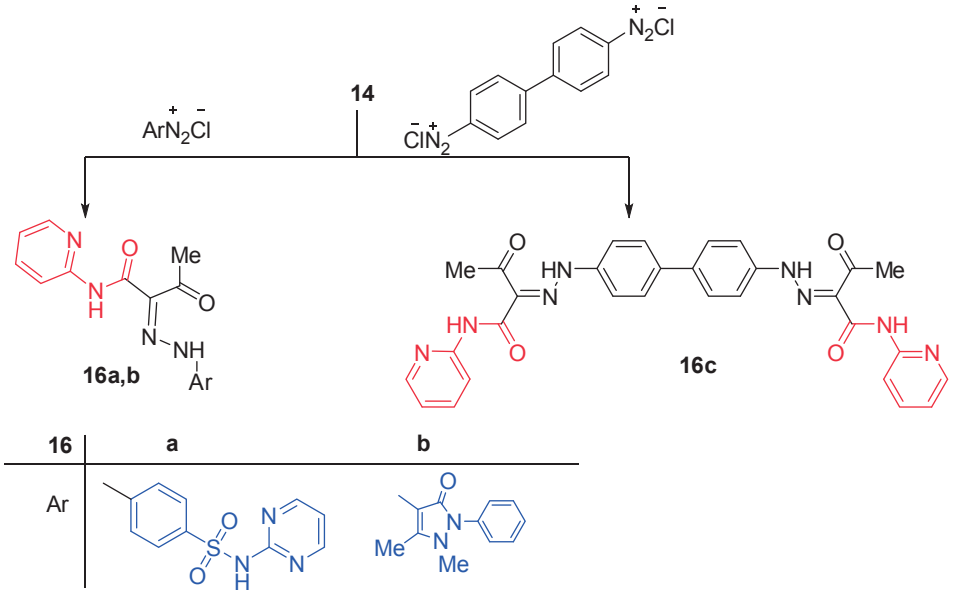
The IR spectrum of **13b** taken as a typical example revealed absorption bands at 1651, 2225, 3332 and 3445 cm^{-1} due to amide carbonyl, cyano and NH_2 groups, respectively. Also its ^1H NMR spectrum afforded signal at δ 8.2 due to amino protons in addition to multiplet peaks at δ 7.63-8.78 due to aromatic protons. Its ^{13}C NMR supported the proposed structure. Moreover, its mass spectrum revealed a molecular ion peak at *m/z* = 314. A plausible mechanism for the synthesis of compound **13** is outlined in Scheme 3.

We also investigated the reactivity of 3-oxo-*N*-(pyridin-2-yl)butanamide (**14**)[18,21] towards furan aldehyde and urea or thiourea in refluxing ethanol in the presence of catalytic amount of HCl. The reaction gave one isolable single product in each case as examined by TLC. The structure of the obtained product was established as 4-(furan-2-yl)-6-methyl-2-(one or thione)-*N*-(pyridin-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide **15a,b**, respectively, on the basis of their elemental analyses and spectral data.



Scheme 4. Synthesis of pyrimidine derivatives **15a,b**

The diazonium salts derived from anilines (sulfadiazine or antipyrine or benzidine) were coupled with butanamide (**14**) in aqueous ethanol, buffered with sodium acetate, to afford the corresponding hydrazones **16a-c** (Scheme 5). The structures of the latter hydrazones were confirmed from their elemental analyses and spectral data.



Scheme 5. Synthesis of hydrazones **16a-c**

Hydrazones **16** can be formulated in three different possible tautomeric structures, as keto-hydrazone tautomer (**A**), azo-enol (**B**), and CH-azo tautomer (**C**) (Figure 1). The ^{1}H NMR spectra of the hydrazones **16A-C** showed the presence of a signal in the region of δ = 13.66–14.16 ppm assignable to hydrazone proton (–CH=N–NH–)[22-24] and absence of a signal at δ = 5.27 ppm,[25] which is corresponding of the CH proton of CH-azo form. Also, IR spectra did not reveal broad signal at 3434 cm^{-1}, which is characteristic for OH of azo-enol tautomer.[26] This spectroscopic analysis enabled us to exclude the azo-enol tautomer (**B**) and CH-azo tautomer (**C**) (Figure 1).

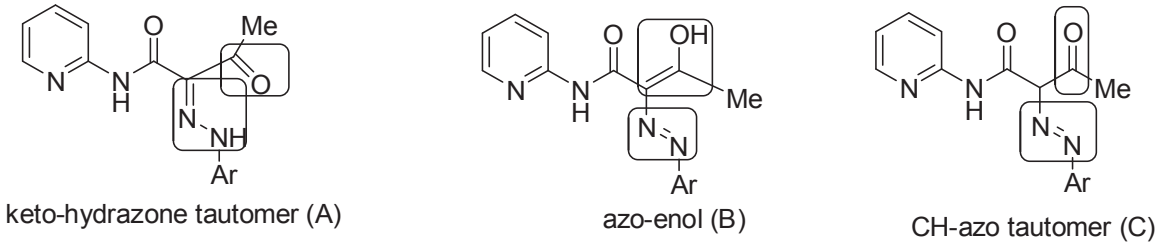


Figure 1. Possible tautomeric structures of hydrazones **16A-C**

Keto-hydrazone tautomer (**A**) can exist in two geometric structures (*E* and *Z*) (Figure 2). The IR spectra of the hydrazones **16a-c** showed a shift of the CO bond stretching to lower wave number due to both conjugation with C=N and formation of a hydrogen bond with the NH group. Moreover, the downfield shift of hydrazone proton signal in ^{1}H NMR is characteristic for the formation of an intramolecular hydrogen bond of the NH proton for tautomer (**A**). These results confirm *E*-form.[27]

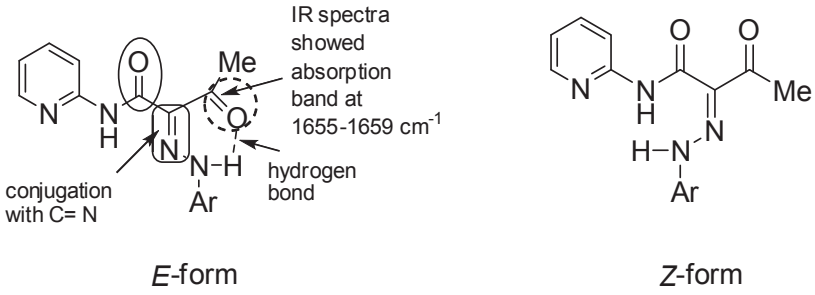


Figure 2. Geometric isomers of keto-hydrazone tautomer (**A**)

Antibacterial Evaluation

The synthesized compounds were subjected to in vitro antibacterial assay using agar diffusion well method[28] against two strains of Gram-positive bacteria (*Streptococcus pneumonia*, *Bacillus subtilis*) and two strains of Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) using inhibition zone diameter (IZD) in mm as a criterion for the antimicrobial activity. The bactericides Ampicillin and Gentamicin were used as reference drugs to evaluate the potency of the tested compounds under the same conditions. The results are shown in Table 1.

Table 1. Antibacterial evaluation of some selected examples

Compound Tested (5 mg/mL)	**Gram-positive Bacteria**		**Gram-negative Bacteria**	
	Streptococcus pneumonia	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***
	Standard (30µg/mL)			
	Ampicillin		**Gentamicin**	
	23.8±0.2	32.4±0.3	17.3±0.1	19.9±0.3
7	12.3±0.5	15.6±0.6	NA	13.4±0. 4
15a	9.6±0.4	12.5±0.5	10.3±0.5	12.9±0.6
15b	NA	8.9±0.2	10.5±0.4	13.6±0.5
16a	10.5±0.4	13.1±0.3	8.3±0.2	10.7±0.3
16c	NA	11.2±0.3	NA	10.5±0.4

Data are expressed as mean ± SD
NA: no activity

Structure-Activity Relationship (SAR):

From the experimental results of the antibacterial activities of the tested compounds, the following structural-activity relationship assumptions can be suggested:

1- The pyrimidinone **15a** had better antimicrobial activity than pyrimidinethione **15b** against *Streptococcus pneumonia*, and *Bacillus subtilis*. While pyrimidinethione **15b** had better antibacterial activity than pyrimidinone **15a** against *Pseudomonas aeruginosa* and *Escherichia coli*.

2- The pyrimidine moiety in compounds **15a,b** is necessary to observe the highest antibacterial activity towards *Pseudomonas aeruginosa*

3- It is worth mentioning that the hydrazone derivative **16a** has the highest antibacterial activities than hydrazone **16c** and this may be due to the presence of sulfonamide in **16a** and low solubility of **16c**.

EXPERIMENTAL

General

All melting points were measured on an Electro thermal IA 9100 apparatus. The infrared spectra were recorded in potassium bromide disks on a Pye Unicam SP 3300 and Shimadzu FT-IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H

NMR (300 MHz) and ^{13}C NMR (75.46 MHz) were run in deuterated dimethyl sulfoxide (DMSO-*d*$_6$). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a GC mass thermo scientific mass spectrometer. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. The biological evaluation of the products was carried out in the Medical Mycology Laboratory of the Regional Center for Mycology and Biotechnology of Al-Azhar University, Cairo, Egypt. The starting materials 2-cyano-*N*-(pyrid-2-yl)acetamide (**1**)[18] and 3-oxo-*N*-(pyrid-2-yl)butanamide (**14**)[18,21] was prepared as previously reported in the literature.

Synthetic methods

Synthesis of 2-oxo-*N*-(pyridin-2-yl)-1,2-dihydroquinoline-3-carboxamide (7).

A mixture of 2-cyano-*N*-(pyridin-2-yl)acetamide (1.61 g, 10 mmol) and the appropriate Schiff base **2a** or **2b** (10 mmol each) was refluxed for 8 h in presence of a catalytic amounts of triethylamine. The precipitated products were filtered off and washed by EtOH. Recrystallization from EtOH afforded shinning yellow crystals of only one product identified as quinoline **7** in yield 77%, mp 219 ºC, IR (KBr) v 3183, 3453 (2NH), 1647, 1682 (2C=O), cm^{-1}, ^{1}H NMR (DMSO-*d*$_6$) δ 5.85 (d, 2H, CH+NH), 6.44 (m, 2H), 7.33 (m, 1H), 7.44 (m, 2H), 7.77 (m, 2H), 7.88 (m, 1H), 8.9 (s, 1H, NH); ^{13}C NMR (DMSO-*d*$_6$) δ 102.1, 107.97, 111.81, 114.56, 116.76, 117.44, 125.45, 129.97, 135.42, 136.91, 147.67, 153.40, 154.02 (Ar-C), 156.84, 159.74(2C=O); MS *m/z* 267 (1.99), 266 (18.65), 265 (M$^+$, 100.0), 264 (16.46), 236 (14.35), 221(5.09), 172 (23.28), 145 (41.18), 121 (14.76), 118 (42.39), 94 (86.18), 93 (2.32), 89 (29.42), 78 (21.80). Anal. Calcd for $C_{15}H_{11}N_3O_2$(265.27): C, 67.92; H, 4.18; N, 15.84. Found: C, 67.78; H, 4.27; N, 15.77.

Synthesis of 2-oxo-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide (10).

To a solution of cyanoacetamide **1** (0.805g, 5 mmol) in acetic acid (25 mL) containing 0.5 g of fused sodium acetate, salicyaldehyde (0.61 g, 0.53 mL, 5 mmol) was added. The mixture was heated under reflux for 4 h. After cooling, the formed solid product was collected by filtration, washed with water and recrystallized from DMF/EtOH mixture to afford Yellow sheets, in 70% yield, mp 254 ºC, IR (KBr) v 3264 (NH), 1667, 1708 (2C=O), 1609 (C=N) cm^{-1}, ^{1}H NMR (DMSO-*d*$_6$) δ 7.21-8.39 (m, 8H), 9.06 (s, 1H, CH), 11.15 (s, 1H, NH); MS *m/z* 266 (M$^+$, not detected), 240 (2.62), 238 (65.08), 237 (55.55), 210 (30.89), 172 (64.66), 165 (12.52), 161 (11.86), 151 (15.14), 137 (17.82), 134.9 (18.8), 121 (43.57), 109 (20.42), 101 (70.93), 89 (1.03), 78 (86.25), 71 (30.05), 69 (50.62), 67 (53.76). Anal. Calcd for $C_{15}H_{10}N_2O_3$ (266.25): C, 67.67; H, 3.79; N, 10.52. Found C, 67.78; H, 3.66; N, 10.45%.

Synthesis of bipyridine derivatives 13a,b.

To a solution of cyanoacetamide (**1**) (0.805g, 5 mmol), appropriate aldehyde (4-*N*,*N*-dimethyl-benzaldehyde or pyridine aldehyde) (5 mmol each), malononitrile (0.33 g, 5 mmol) in EtOH (20 mL), a catalytic amount of piperidine was added. The reaction mixture was heated under reflux for 3 h, then

allowed to cool. The precipitated solid product was filtered off, washed with EtOH and finally recrystallized from DMF to afford the corresponding bipyridine derivatives **13a,b**.

6-Amino-4-(4-(dimethylamino)phenyl)-2-oxo-1-(pyridin-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (13a). yellow crystals, Yield (55%), mp > 300 °C, IR (KBr) ν 3337, 3464 (NH_2), 2203 (C≡N), 1655 (C=O) cm^{-1}, ^1H NMR (DMSO-d_6) δ 3.02 (s, 6H, $2CH_3$), 6.84 (d, 2H, J = 8.7 Hz), 7.45 (d, 2H, J = 8.7 Hz), 7.59 (m, 2H), 7.90 (s, 2H, NH_2), 8.06 (m, 1H), 8.67 (m, 1H). Anal. Calcd for $C_{20}H_{16}N_6O$(356.38): C, 67.40; H, 4.53; N, 23.58. Found: C, 67.52; H, 4.48; N, 23.66%.

6-Amino-2-oxo-1,4-di(pyridin-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (13b). Pinkish white crystals, yield (45%); mp > 300 °C, IR (KBr) ν 3332, 3445 (NH_2), 2225 (C≡N), 1651 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.63 (m,4H), 8.08 (m, 2H), 8.2 (s, 2H, NH_2), 8.69 (m, 1H), 8.78 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 75.56, 88.33, 115.33, 115.90, 123.59, 124.51, 125.71, 130.87, 135.94, 140.05, 147.24, 148.0, 150.73, 151.29, 156.51, 158.65, 159.25; MS *m/z* (%) 316 (2.24), 315 (21.47), 314 (M^+, 100.0), 259 (7.09), 248 (5.75), 236 (1.32), 221 (3.31), 194 (6.58), 158 (8.46), 121 (4.93), 78 (15.57). Anal. Calcd for $C_{17}H_{10}N_6O$ (314.30): C, 64.96; H, 3.21; N, 26.74. Found: C, 64.85; H, 3.32; N, 26.66%.

Synthesis of pyrimidine derivatives 15a,b.

General procedure

To a solution of butanamide **14** (0.89 g, 5 mmol), furan aldehyde (0.480 g, 0.41 mL, 5 mmol), urea or thiourea (5 mmol each), in absolute EtOH (20 mL) were added drops of HCl as a catalyst. The reaction mixture was refluxed for 8 h. The solid product so formed was filtered off, washed with EtOH and dried. Recrystallization from EtOH/DMF or DMF afforded the corresponding tetrahydropyrimidine **15a,b**, respectively.

4-(Furan-2-yl)-6-methyl-2-oxo-*N*-(pyridin-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (15a). Pale yellow crystals (EtOH/DMF), Yield (56%); mp 259 °C; IR (KBr) ν 3252-3117 (br, 3 NH), 1682, 1628 (2C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.13 (s, 3H, CH_3), 5.53 (d, 1H, CH, J = 3.0 Hz), 6.27-8.28 (m, 8H, ArH+NH), 8.91 (s, 1H, NH), 9.93 (s, 1H, NH) ; ^{13}C NMR (DMSO-d_6) δ 17.4 (CH_3), 48.7 (CH), 101.9, 105.9, 110.4, 114.1, 119.2, 137.9, 142.2, 142.5, 147.8, 152.3, 152.7 (Ar-C), 155.7, 165.5 (2C=O); MS *m/z* (%) 299 (3.19), 298 (M^+, 16.35), 205 (7.6), 177 (51.5), 134 (21.14), 122 (59.08), 121 (51.3), 95 (100), 94 (46.8), 93 (10.6), 78 (73.9), 68 (12.68), 67 (48.3). Anal. Calcd for $C_{15}H_{14}N_4O_3$ (298.30): C, 60.40; H, 4.73; N, 18.78. Found: C, 60.29; H, 4.88; N, 18.69%.

4-(Furan-2-yl)-6-methyl-*N*-(pyridin-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (15b). Pale Yellow crystals (EtOH/DMF), yield (48%), mp 244 °C; IR (KBr) ν 3414, 3341, 3298 (3 NH), 1686, 1651 (2C=O), 1243 (C=S) cm^{-1}; ^1H NMR(DMSO-d_6) δ 2.13 (s, 3H, CH_3), 5.52 (d, 1H, CH, J = 3.3 Hz), 6.29-8.29 (m, 7H), 9.5 (s, 1H, NH), 10.08 (s, 1H, NH), 10.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ

16.6 (CH_3), 48.6 (CH), 103.6, 106.7, 110.4, 114.1, 119.3, 137.9, 138.6, 142.9, 147.8, 151.9, 154.3 (Ar-C), 165.1 (C=O), 174.4 (C=S); MS *m/z* (%) 314 (M^+, 25.17), 194 (18.23), 193 (72.89), 192 (28.76), 176 (11.52), 162 (11.13), 152 (8.44), 138 (24.94), 105 (28.09), 95 (63.42), 94 (43.53), 90 (10.48), 78 (97.13), 67 (58.54), 65 (87.59). Anal. Calcd for $C_{15}H_{14}N_4O_2S$ (314.36): C, 57.31; H, 4.49; N, 17.82. Found: C, 57.48; H, 4.56; N, 17.95%.

Synthesis of hydrazone derivatives 16a-c.

General procedure

To a cold solution of compound **14** (1.78 g, 10 mmol) in EtOH (70 mL), buffered with sodium acetate trihydrate (3 g), was added the diazonium chloride [prepared by diazotizing the appropriate arylamine (Sulfadiazine or aminoantipyrine (10 mmol each), or benzidine (5 mmol) dissolved in concentrated hydrochloric acid, with sodium nitrite solution (0.69 g, 10 mmol) in water (4 mL)]. The addition was carried out portion-wise with stirring at 0-5 ºC over a period of 30 min. The reaction mixture was stirred for further 4 h, at room temperature, then kept in an ice chest for 12 h, and finally diluted with water. The precipitated solid was collected by filtration, washed with water, dried and finally recrystallized from DMF/ EtOH to afford the corresponding hydrazones **16a-c**.

3-Oxo-*N*-(pyridin-2-yl)-2-(2-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)hydrazono)butanamide (16a). Yellow crystals (DMF), yield (80%), mp 288 ºC; IR (KBr) ν 3449-3422 (3NH), 1659 (C=O), 1585 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.5(s, 3H, CH_3), 7.05 (m, 1H), 7.17 (m, 1H), 7.68 (d, 1H, *J* = 8.7 Hz), 7.83 (m, 1H), 8.01 (m, 3H), 8.16 (d, 1H, *J* = 8.1 Hz), 8.36 (d, 1H, *J* = 4.5 Hz), 8.51 (d, 2H, *J* = 5.1 Hz), 11.49 (s, 1H, NH), 11.77 (s,1H, NH), 13.66 (s,1H, NH); ^{13}C NMR (DMSO) δ 25.9 (CH_3), 114.1, 115.6, 115.7, 120.4, 128.5, 129.4, 135.4, 138.4, 145.2, 148.5, 150.4, 156.9, 158.3, 158.4, 161.4 (C=O), 198.5 (C=O); MS *m/z* (%) 439 (M^+, 2.78), 379 (21.07), 318 (0.92), 196 (9.22), 176 (8.95), 170 (9.1), 121 (11.25), 111 (32.35), 92 (21.9), 78 (38.74). Anal. Calcd for $C_{19}H_{17}N_7O_4S$ (439.45): C, 51.93; H, 3.90; N, 22.31. Found: C, 51.81; H, 3.78; N, 22.45%.

2-(2-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazono)-3-oxo-*N*-(pyridin-2-yl)-butanamide (16b).

Yellow crystals (DMF), yield (75%), mp 245 ºC; IR (KBr) ν 3453, 3422 (2NH), 1659 (C=O), 1582 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.29 (s, 3H, CH_3), 7.17 (m, 1H), 7.40 (m, 3H), 7.55 (m, 2H), 7.84 (m, 1H), 8.13 (d, 1H, *J* = 8.1 Hz), 8.36 (m, 1H), 11.78 (s, 1H, NH), 14.16 (s, 1H, NH). Anal. Calcd for $C_{20}H_{20}N_6O_3$ (392.41): C, 61.21; H, 5.14; N, 21.42. Found: C, 61.12; H, 5.06; N, 21.51%

2,2'-(2,2'-(Biphenyl-4,4'-diyl)bis(hydrazin-2-yl-1-ylidene))bis(3-oxo-*N*-(pyridin-2-yl)butanamide) (16c).

Red crystals (DMF), yield (90%), mp 293 ºC; IR (KBr) ν 3437, 3414 (2NH), 1655 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5 (s, 6H, 2 CH_3), 7.20 (m, 2H), 7.69 (d, 2H, J = 8.4 Hz), 7.85 (m, 8H), 8.20 (d, 2H, J = 8.4 Hz), 8.4 (d, 2H, J = 4.8 Hz), 11.68 (s, 2H, 2NH), 14.11 (s, 2H, 2NH); MS *m/z* (%) 562 (M^+, 0.95), 313 (4.15), 280 (3.19), 276 (12.0), 262 (3.75), 239 (3.97), 218 (8.45), 193 (2.54), 184 (4.19), 154 (11.12), 113 (10.68), 112 (13.65), 111 (100), 110 (37.10), 81 (45.44), 69 (5.79). Anal. Calcd for $C_{30}H_{26}N_8O_4$ (562.58): C, 64.05; H, 4.66; N, 19.92. Found: C, 64.13; H, 4.48; N, 19.81%.

Agar diffusion well method to determine the antimicrobial activity

The microorganism inoculums were uniformly spread using a sterile cotton swab on a sterile Petri dish Malt Extract Agar (for fungi) and Nutrient agar (for bacteria). Addition of one hundred μL of each sample to each well (10 mm) diameter holes cut in the agar gel, 20 mm apart from one another) was performed, followed by incubation of the systems for one to two days at 37 °C (for bacteria) and at 28 °C (for fungi). Finally, after incubation, the microorganism's growth was noticed. Inhibition of the bacterial and fungal growth was measured in mm. The test was performed three times.[28]

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