2, 6-Bis [3-N,N-dimethylamino-1-oxopropen-1-yl]pyridine as a building block in heterocyclic synthesis: synthesis of 2,2':6',2"-terpyridines and 2,6-bis[pyrazolyl, isoxazolyl, diazepinyl, pyrazolo[5,1-a]pyrimidinyl and pyrazolo-[4,3-d]pyridazinyl]pyridines

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A convenient synthesis is reported of some new 2, 2':6', 2"-terpyridines, and polycyclic pyridino-2,6-bis-[heterocycles and fused heterocycles] via reaction of 2,6-bis[3-N,N-dimethylamino-1-oxopropen-1-yl]pyridine (**2**) with various type of nucleophilic reagents.. The biological screening showed that many of the prepared compounds have good antibacterial and antifungal activities comparable to Ampicillin and clotrimazoles.

Keywords: pyridine, enaminone, nitrilimines, terpyridines, diazepines, antimicrobial

Enaminone derivatives are highly reactive intermediates extensively used for synthesis of heterocyclic compounds. On the other hand, a great deal of interest has been focused on the synthesis of the functionalisd pyridine derivatives owing to their biological activities.[1,2] Some 2-pyridine derivatives are considered as cardiotonic agents such as milrinone[3] and as potential HIV-1 specific transcriptase inhibitors[3,4] (see Fig. 1A). We have recently reported the synthesis of similar structures,[5] (see Fig. 1B). A literature survey revealed that although 2,6-bis(3-N,N-dimethylaminoprop-2-en-1-one)pyridine **(2)** was synthesised,[10,11] no reports described its use for the synthesis of terpyridines and 2,6-bis-[fused pyrimidinyl, or pyridazinyl] pyridines. In view of our interest in developing efficient syntheses of polyfunctionally substituted heteroaromatics by utilising the enaminones as starting materials[5-9] we report several approaches to the synthesis of pyridine derivatives using compound **2** as a precursor.

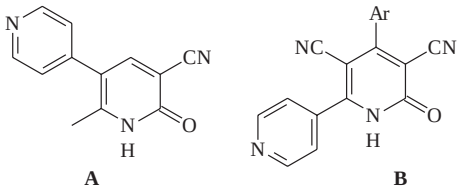
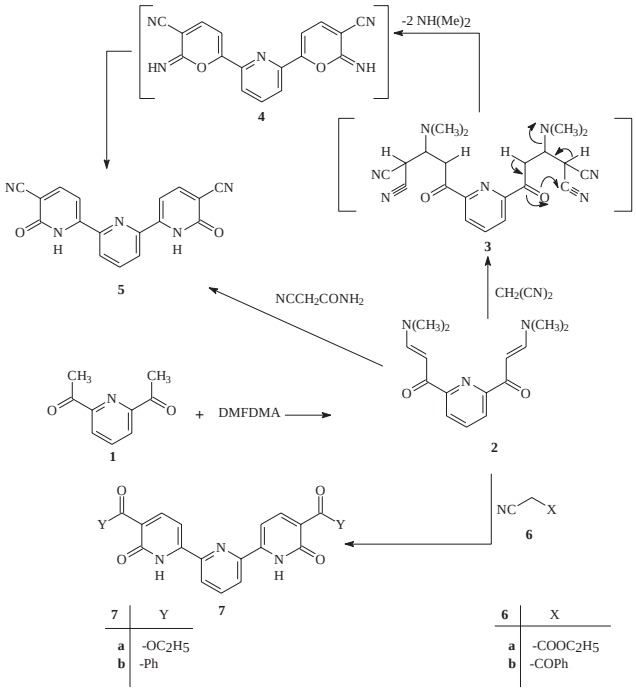


Fig. 1

Results and discussion

The required enaminone **2**[10,11] can be easily prepared in excellent yield by the treatment of 2,6-diacetyl pyridine **(1)**[12] with dimethylformamidedimethylacetal [DMFDMA] in refluxing xylene.

2,2':6',2"-Terpyridines are useful ligands in coordination chemistry.[13] Thus, the reaction of **2** with different types of active methylene compounds under different conditions gave rise to 1:2 adducts formulated as 2,2':6',2"-terpyridine derivatives **5** and **7** in 35–40% yield. The key step of the reaction of **2** with malononitrile is a Michael addition to yield **3** followed by cyclisation and elimination of dimethylamine to give the iminopyran **4** as an intermediate which isomerises to the corresponding **5** *via* Dimroth type rearrangement. The structure of compound **5** was confirmed by analytical and spectroscopic data. Thus, the IR spectrum showed the presence of NH and C≡N groups stretching at 3310 and 2221cm^{-1}, respectively. The ^{1}H NMR spectrum revealed the presence of a triplet at δ =7.78 corresponding to H-4 pyridine, doublet signals at δ =8.13 (J = 9 Hz) which is assigned for H-3, H-5 pyridine, and a singlet at 9.01 related to NH. The mass spectrum was in accordance with the proposed structure, which showed a molecular ion peak at *m/z*, 315 (68.%) corresponding to the molecular formula $C_{17}H_9N_5O_2$. The same product was also isolated from reaction of **2** with cyanoacetamide in toluene under reflux and in the presence of piperidine. The formation of compounds **7a**,**b** occurs in a similar manner from reaction of **2** with ethyl cyanoacetate and benzoyl acetonitrile respectively (Scheme 1).



Scheme 1 Formation of 6,6"-dioxo-2,2':6',2"-terpyridines.

The terpyridines could also be prepared *via* another route. Thus, compound **2** reacted readily with acetylacetone in refluxing acetic acid in the presence of ammonium acetate to yield **9**, via initial addition of the active methylene compound to the double bond in **2** to yield the Michael adduct **8** which in turn cyclised in the presence of ammonium acetate into **9**. The structure of **9a** was established based on its ^{1}H NMR spectra, which revealed two singlets at δ 2.61 and 2.86 for the methyl and acetyl protons and two doublets at δ 8.14 and 8.75 ppm (J = 9.0 Hz) for pyridine H-4' and H-3',3" pyridine, respectively besides the expected signals. Similarly compounds **9b** and **11** were obtained by reaction of **2** with ethyl acetoacetate and benzoyl acetonitrile respectively under the same experimental conditions (Scheme 2).

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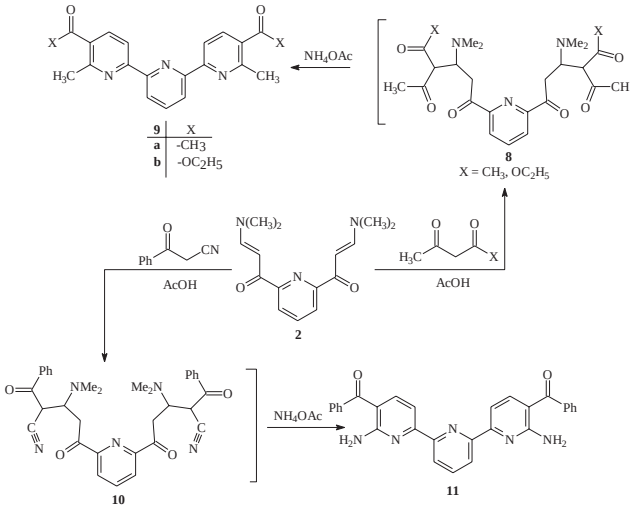


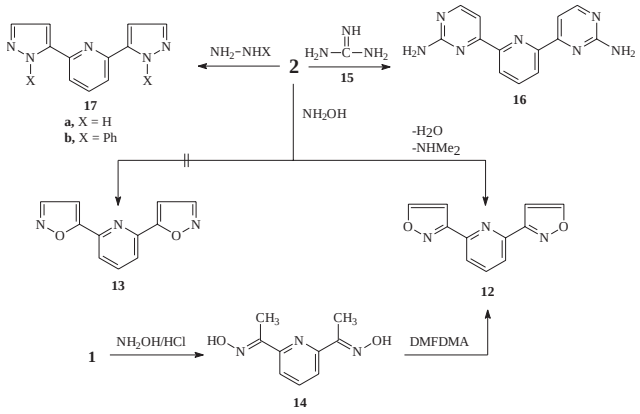
Scheme 2 Synthesis of 2,2':6',2"-terpyridines.

The behaviour of **2** towards some nitrogen nucleophiles was also investigated. Thus, the enaminone **2** reacted with hydroxylamine hydrochloride[14] in the presence sodium acetate in refluxing EtOH to yield the 2,6-bis (isoxazolyl) pyridine **12** rather than **13.** Structure **12** was established based on the ^{1}H NMR spectrum, which showed a resonance at δ 9.21 ppm corresponding to the H-5 of an isoxazole . The alternative product **13** was ruled out as the H-3 protons in **13,** would be expected to resonate at higher field, at around δ 8.3 ppm.[15] Moreover, compound **12** is identical with the product obtained from the reaction of oxime **14** with DMFDMA in refluxing xylene. It is assumed that compound **12** is formed *via* initial addition of hydroxylamine to the carbonyl of enaminone **2** followed by intramolecular cyclisation. Compound **2** also reacted with guanidine hydrochloride, hydrazine hydrate as previously described and phenyl hydrazine to yield **16** [10,11] and **17 a,b**[16] respectively.

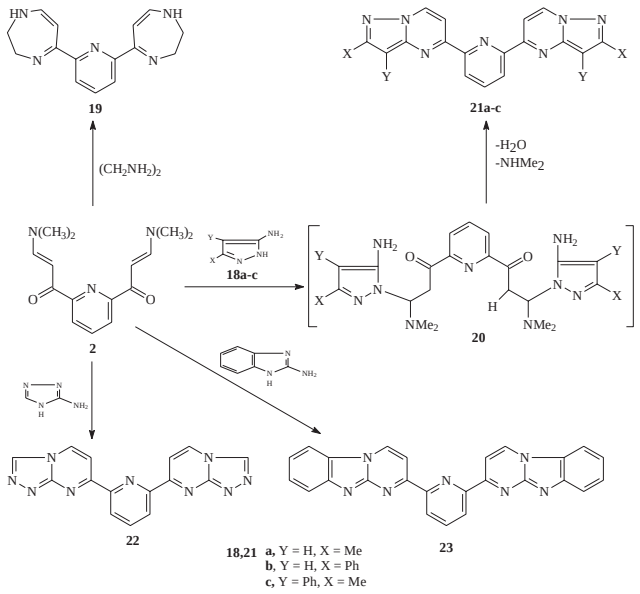
Similarly compound **2** reacted with hydrazine hydrate[16] and phenylhydrazine to yield **17a,b**. The structure of **17b** was established based on the elemental analysis and spectroscopic studies. Thus, the IR spectrum (KBr) revealed the absence of a C=O function in the 1647cm^{-1} region, Also, the ^{1}H NMR spectrum showed, in addition to the expected signals, a doublet at δ 8.21 corresponding to H-3 of a pyrazole (Scheme 3).

The work was further extended to study the behaviour of **2** towards the action of some aliphatic and heterocyclic amines. Thus, compound **2** condensed readily with ethylenediamine and 5-aminopyrazoles **18a–c** yielding the corresponding products that were assigned as **19** and the 2,6-bis[pyrazolo [1,5-a]pyrimidin-7-yl]pyridine derivatives **21a–c** respectively. The elemental analyses and spectral data were in accord with the proposed structure. Similar cyclisation reactions to form pyrazolo[1,5-a]pyrimidine derivatives have been previously reported.[17]

Formation of **21** is assumed to proceed *via* a Michael type addition of the most basic ring-N in **18**[18] followed by intramolecular cyclodehydration and dimethylamine elimination under the reaction conditions. Similarly, compound **2** reacted with both 5-aminotriazole and 2-aminobenzimidazole to afford **22** and **23** respectively, in an acceptable yield. The structure of compounds **22,23** were confirmed based on their elemental analyses and spectroscopic studies. Thus, the IR of the latter products revealed the absence of the C=O function, their ^{1}H NMR showed the absence of N-$(CH_3)_2$ signals beside the presence of the expected signals. Also, the mass spectrum of **22** revealed a molecular ion peak at



Scheme 3 Formation of 2,6 bis(heterocycle)pyridines **12,16** and **17**.

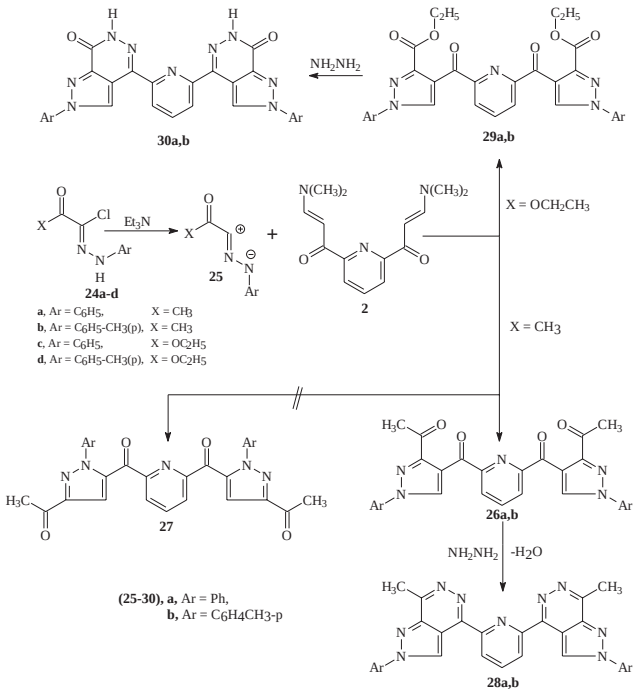


Scheme 4 Formation of 2,6- bis (diazipine, fused pyrimidinyl)pyridines.

m/z =315 (M^+, 100%) corresponding to the molecular formula $C_{15}H_9N_9$ (Scheme 4).

1,3-Dipolar cycloaddition of nitrilimines with alkenes is well documented. We studied the regioselectivity in 1,3-dipolar cycloaddition of nitrilimines with the enaminone **2**. Thus, hydrazonyl chlorides **24a–d**[19] in dry benzene in the presence of triethylamine generated the corresponding nitrilimines **25a–d** which in turn reacted with compound 2 to give a single product in each case, based on TLC.

The expected product **26,** not **27,** was assigned on the basis of the spectroscopic evidence and further transformations. Thus, The IR spectra of compounds **26a,b** showed carbonyl absorption at 1697 and 1651 cm^{-1} and the ^{1}H NMR revealed two singlets at δ 2.84 and 9.12 ppm due to the protons of the acetyl CH_3 and H-5 pyrazole respectively in addition to the expected signals. The formation of **26** is assumed to take place *via* regioselective 1,3-cycloaddition of the nitrilimine intermediate **25** to the enaminone **2** followed by elimination of dimethylamine under the reaction conditions. A further confirmation of the structure of compound **26** came from its reaction with hydrazine hydrate to afford a high yield of a product which was identified as 2, 6-bis[pyrazolo(3,4-d)pyridazin-6-yl] pyridine **(28).** Prompted by these results the enaminone **2** was reacted with the nitrilamine **25c,d** under the same experimental conditions to afford **29**.



Scheme 5 Synthesis of 2, 6- bis (fused pyridazinyl) pyridines.

The latter products underwent cyclocondensation upon treatment with hydrazine hydrate in refluxing ethanol to give 2,6-bis[3-oxo-1-phenylpyrazolo[3,4-d]pyridazin-3-yl)pyridine **30a,b** (Scheme 5).

Antimicrobial activity

All tested compound showed anti-infective (antibacterial and yeast killing activity) and antifungal activities except compound **9a**. The most potent activity was for compounds **16, 5, 23** and **19**. Data obtained were reported in Table 1.

Experimental

Melting points are uncorrected. IR spectra were recorded with FTIR-8201 PC spectrophotometer Shimadzu. ^{1}H NMR spectra were obtained on a Varian Germini 200 MHz spectrometer in DMSO-d_6 as solvent and TMS as an internal reference. Mass spectra were performed on a Shimadzu GCMS-QP-1000 EX using the direct inlet system and EI + QI MSLMRUPLR. Microanalyses were performed by the Microanalytical Unit at Cairo University. Thin layer chromatography was carried out on plates (5 × 20 cm coated with silica gel GF 254 type 60, mesh size 50–250).

2, 6-Bis[(3-dimethylamino)prop-2-one-1-ene]pyridine (**2**):[10,11] A mixture of 2,6-diacetylpyridine (**1**) (3.26g, 0.02 mol) and DMFDMA (5.36g, 0.045 mol) in dry xylene (20 ml) was refluxed for 6 h., then left to cool to room temperature. The yellowish precipitate was filtered off, washed with petroleum ether dried and recrystallised from ethanol to give bright yellow crystals (90%), m.p. 230–235 °C.(Lit. [10-12] m.p. 228–230 °C)

1H,1"H 6,6"-Dioxo-2,2':6',2"-terpyridine-5,5"-dicarbonitrile **(5)**:

Method A: To a solution of **2** (1.36g, 0.005 mol) in ethanol (30 ml), malononitrile (0.66g, 0.02 mol) and few drops of piperidine were added. The reaction mixture was heated on water bath at 30–40°C for 24 h, then left to cool. The solid so formed was collected by filtration, washed by ethanol and recrystallised from DMF to give pale yellow crystals in (0.55g, 35% yield, m.p. 215–18 °C

Method B: Compound (**2**) .(1.36g, 0.005 mol) and cyanoacetamide (0.77g, 0.022 mol) were refluxed in dry toluene (20ml) for 12 h. The solid formed after cooling was collected by filtration and recrystallised from DMF to yield pale yellow crystals in (1.14g, 65% yield. The identify of the samples obtained in methods A and B were confirmed by m.p., mixed m.p. and by comparative spectral data as pale yellow crystals m.p. 215–18 °C, IR (KBr) : ν_{max}/cm^{-1} 3310 (NH), 2221 (C≡N), 1658 (C=O); ^{1}H NMR (300 MHz,DMSO-d_6): δ ppm 7.78 (t, 1H, H-4 pyridine), 7.98 (d, 2H, *J* = 9.0 Hz, H-4 pyridine), 8.13 (d,2H, *J* =9 Hz,H-3, pyridine) 8.31(d,2H, *J* = 9.0 Hz, H-3, 5-pyridine) and 9.01 (s, 2H, NH); ^{13}C(300 MHz,DMSO-d_6) 163.97, 160.93, 148.61, 148.24, 147.98, 139.74, 124.07 , 116.50, 116.17 MS: *m/z* 315 (M^+) 68%.Anal: $C_{17}H_9N_5O_2$ requires C 64.76, H 2.85, N 22.20, found C 64.90, H 2.90, N, 22.20%.

5, 5"-Diethoxycarbonyl-1, 1"-dihydro-6, 6"-dioxo-2,2':6', 2"-terpyridine (**7a**): A toluene solution (30 ml) of **2** (1.36g, 0.005 mol) and ethyl cyanoacetate (1.15g, 0.011 mol) with a few drops of piperidine was refluxed for 6 h. and the solvent was removed under vacuum. The resulting oil solidified after cooling, was purified by column chromatography on silica gel using $CHCl_3$ as eluent, and crystallised from chloroform/petroleum ether to give pale yellow crystals,(0.75g, 35%); m.p. 191–195 °C; IR (KBr): ν_{max}/cm^{-1} 3400 (OH), 1739 (C=O, ester), 1659 (C=O). ^{1}H NMR (300 mhz DMSO-d_6) δ ppm 1.33–1.35 (t, 6H, *J* = 3.3 Hz, 2 CH_3), 4.33–4.36 (q, 4H, *J* = 3.37 Hz, 2 CH_2), 7.83 (d, 2H, *J* = 9Hz, H-4 pyridine), 7.98 (t, 1H, H-4 pyridine), 8.08 (m, 6H, H-3,5 pyridines) and 13.1 (s, 2H, OH). MS: *m/z* 409 (M^+) 28%.Anal: $C_{21}H_{19}N_3O_6$ requires C 61.61., H 4.67, N, 10.26; found C 61.60, H 4.40, N, 9.90%.

Table 1 Antimicrobial activity of the compounds

Compd. no.	Inhibition zone (mm)			
	G +ve bacteria *Bacillus subtilis*	G –ve bacteria *Nisseria gonorrhea*	Fungi *Penicillium notatum*	Yeast *Candida utilis*
2	0.0	11.0 [b]	9.0 [b]	6.0 [a]
5	12.0[b]	14.0 [b]	16.0 [b]	17.0 [b]
7b	14.0 [b]	5.0 [a]	7.0 [a]	13.0 [b]
9a	0.0	0.0	0.0	0.0
9b	13.0 [b]	7.0 [a]	7.0 [a]	15.0 [b]
11	7.0[a]	16.0 [b]	8.0 [b]	9.0 [b]
12	8.0 [b]	0.0	7.0 [a]	16.0 [b]
16	16.0 [b]	14.0 [b]	13.0 [b]	12.0 [b]
17a	8.0 [b]	17.0 [b]	0.0	0.0
19	13.0 [b]	10.0 [b]	14.0 [b]	6.0 [a]
21a	8.0 [b]	6.0 [a]	11.0 [b]	11.0 [b]
21c	9.0 [b]	11.0 [b]	9.0 [b]	11.0 [b]
22	14.0 [b]	5.0 [a]	10.0 [b]	12.0 [b]
23	16.0 [b]	15.0 [b]	10.0 [b]	9.0 [b]
26a	12.0 [b]	13.0 [b]	0.0	0.0
26b	0.0	0.0	7.0 [a]	10.0 [b]
29a	14.0 [b]	7.0 [a]	6.0 [a]	18.0 [b]
clotrimazoles	0.0	0.0	26.0	27.0
Ampicillin	26.0	8.0	0.0	0.0

[a]Moderately active; [b]highly active

5,5''-Dibenzoyl-1,1''-dihydro-2,2'':6',2''-terpyridine-6,6''-dione (**7b**): The same experimental procedure described for preparation of **7a** was followed except for the use of benzoyl acetonitrile instead of ethyl acetoacetate and the solid obtained was crystallised from dioxane as a pale brown powder (1.10g,40%), m.p. > 300°C. IR (KBr) :ν_{max}/ 3310 cm^{-1} (NH), 1678, 1645 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.65–8.03 (m, 15H, H-arom + H-pyridines), 8.31 (d, 2H, *J* = 9.0 Hz, H-3,3' pyridine), 9.56 (br, 2H, NH).Anal: $C_{29}H_{19}N_3O_4$ requires C 73.57, H 4.01, N 8.87; found C 73.50, H 4.00, N 8.6,0%.

Preparation of 2,2':6',2''-terpyridines **9** *and* **11***: General procedures:* To a solution of bis enaminone **2** (1.36g, 0.005 mole) and ammonium acetate (1 g) in acetic acid (10 ml), the appropriate active methylenes (0.012 mole) were added. The reaction mixture was heated with stirring under reflux for 3–4 hours. The solvent was evaporated under reduced pressure and the residual solid was crystallised from ethanol.

5,5''-Diacetyl-6,6''-dimethyl-2,2':6',2''-terpyridine (**9a**): was obtained as a pale green powder (0.95g, 55%), m.p. 203–299°C. IR (KBr): ν_{max}/1660 cm^{-1} (C=O). ^{1}H NMR (300 MHz $CDCl_3$): δ ppm 2.61 (s, 6H, 2 CH_3), 2.86 (s, 6H, 2$COCH_3$), 7.98 (m, 1H, H-4 pyridine), 8.14 (d, 2H, *J* = 9 Hz, H-4 pyridines), 8.54 (d, 2H, *J* = 9 Hz, H3,5-pyridine), 8.75 (d, 2H, *J* = 9 Hz, H-3,3''-pyridine). ^{13}C(300 MHz,DMSO-d_6) 201.13, 157.34, 156.43, 154.74, 139.18, 133.24, 122.42, 118.55, 30.19, 25.24; MS: *m/z* 345 (M^+) 65%. Anal: $C_{21}H_{19}N_3O_2$ requires C 73.04, H 5.54, N 12.16; found C 73.80,H 5.30,N 12.30%.

5,5''-Diethoxycarbonyl-6,6''-dimethyl-2,2':6',2''-terpyridine (**9b**) was obtained as a buff powder (1.21g,60%), m.p. 171–173 °C. IR (KBr):ν_{max}/2983 cm^{-1} (C-H, aliph), 1713 (CO, ester).^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.91 (t, 6H, *J* = 3 Hz, 2 CH_3), 2.45 (s, 6H, 2CH_3), 3.89 (q, 4H, *J* = 3 Hz, 2CH_2), 7.96 (m, 1H, H-4 pyridine), 8.13 (d, 2H, *J* = 9 Hz, H-3,5 pyridine), 8.43 (d, 2H, *J* = 9 Hz, H-3,3''-pyridine), 8.65 (d, 2H, *J* = 9 Hz, H-4,4''-pyridine). MS: *m/z* 405 (M^+) 58%. Anal: $C_{23}H_{23}N_3O_4$ requires C 68.14, H 5.71, N10.37; found C 68.20, H 5.80,N 10.30%.

6,6''-Diamino-5,5''-dibenzoyl-2,2':6',2''-terpyridine (**11**) was formed as a deep brown powder (1.48g, 63%), m.p. 296–298 °C. IR (KBr): ν_{max}/ 3415 cm^{-1} (NH_2), 1648 cm^{-1} (C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.62 (m, 14H, H-arom + 2 NH_2), 7.68 (m, 1H, H-4'-pyridine), 8.01 (d, 2H, *J* = 9 Hz, H-3',5' pyridine), 8.3 (d, 2H, *J* = 9 Hz, H-3,3''-pyridine), 8.53 (d, 2H, *J* = 9 Hz, H-4,4''-pyridine). MS: *m/z* 471 (M^+) 36%. Anal: $C_{29}H_{21}N_5O_2$ requires C 73.88, H 4.48, N14.86; found C 73.80, H 4.60, N 14.90%.

2,6-Bis(isoxazol-5-yl)pyridine (**12**): A mixture of 2 (1.36g, 0.005 mol), hydroxylamine hydrochloride (0.6g, 0.012 mol) and sodium acetate anhydrous (0.025 mol) in absolute ethanol (30 ml) was refluxed for 5h, then left to cool. Dilution with water gave a solid, which was collected by filtration and recrystallised from ethanol to yield colourless crystals (0.56g, 52%), m.p. 155–159 °C. IR (KBr): ν_{max}/cm^{-1} 3030 (CH-arom), 1630 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 5.95 (d, 2H, *J* = 8.0 Hz, H-4 isoxazole), 7.81–8.21 (m, 3H, H-3,4,5 pyridine), 9.21 (d, 2H, *J* = 8.0 Hz, H-5 isoxazole).MS: *m/z* 213 (M^+) 100%. Anal: $C_{11}H_7N_3O_2$ requires C 61.97,H 3.28,N19.71; found C 62.10, H 3.40, N 19.90%.

2,6-Bis(2-aminopyrimidin-4-yl)pyridine (**16**): To a mixture of 2 (1.36g 0.005 mol) and guanidine hydrochloride (1.0g, 0.011 mol) in absolute ethanol (20 ml), potassium carbonate anhydrous (0.08 mol) was added. The mixture was refluxed for 10 h, allowed to cool to room temperature and then diluted with water (10 ml). The solid product so formed was filtered off, washed with water dried and recrystallised from DMF to give a colourless powder (1.20g, 90%), m.p. >320 °C (Lit.[10-11] m.p.>350 °C)

Preparation of **17a,b**: *General procedures*: A mixture of the appropriate hydrazine (0.023 mol) and compound **2** (1.36g, 0.005mol) in absolute ethanol (30 ml) was refluxed for 1–2 h, then left to cool to room temperature. The colourless precipitate was filtered, washed with ethanol and recrystallised from DMF.

2,6-Bis(pyrazol-3-yl)pyridine (**17a**) was isolated as colourless crystals (0.63g, 60%). m.p. 210–211°C.(Lit.[16] m.p. 211 °C)

2,6-Bis(1-phenylpyrazol-3-yl)pyridine (**17b**) was obtained as colourless crystals (1.31g, 72%). m.p. 280–283 °C. IR (KBr): ν_{max}/cm^{-1} 3051 (CH-arom), ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 6.53(d, 2H, *J*=3.3 Hz,H-4 pyrazole), 7.3–7.98 (m, 13H, H-Ar + H-pyridine),8.31,(d,2H,*J*=3.3 Hz,H-3 pyrazole). MS: *m/z* 363 (M^+, 81%).Anal: $C_{23}H_{17}N_5$ requires C 76.03, H 4.68, N 19.28; found C 76.20,H 4.80, N 19.30%.

2,6-Bis(1,2,3-trihydro[1,4]diazepin-5-yl)pyridine (**19**): A mixture of enaminone **2** (1.36g, 0.005 mol) and ethylenediamine (0.65g, 0.011 mol) in absolute ethanol (20 ml) was refluxed for 3 h. The solvent was removed under vacuum and the residue was treated with petroleum ether. The solid so formed was collected by filtration and recrystallised from chloroform/petroleum ether to give a pale brown powder (0.7g, 53%), m.p. 160–163 °C. IR (KBr): ν_{max}/cm^{-1} 3350 (NH), 2983 (CH-aliphatic), 21630 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.88 (br, 8H, 4 CH_2), 6.21 (br, 2H, H-6 diazepine), 7.79–8.28 (m, 5H,H-4, H-3, H-5 pyridine, H-6 diazepine), 10.83 (br, 2H, 2 NH). MS: *m/z* 267 (M^+) 25%.Anal : $C_{15}H_{17}N_5$ requires C 67.39, H 6.41, N 26.19; found C 68.10, H 6.80, N 26.30%.

Preparation of compounds **21–23** :*General procedure*: A mixture of enaminone **2** (1.36g, 0.005 mol) and 5-aminopyrazole derivatives **18a–c** or 5-aminotriazole or 2-aminobenzimidazole (0.011 mol) in dry dioxane (20 ml) was refluxed for 8–12 h., then left to cool to room temperature. The solid so formed was filtered off, washed with ethanol, dried and recrystallisation from DMF afforded the corresponding products.

2,6-Bis(2-methylpyrazolo[1,5-a]pyrimidine-7-yl)pyridine (**21a**) was isolated as pale brown crystals (0.89g, 52%), m.p. 293–295 °C. IR (KBr): ν_{max}/cm^{-1} 2985 (CH_3), 1630 (C=N). ^{1}H NMR (300 MHz,DMSO-d_6): δ ppm 2.63 (s, 6H, 2CH_3) 7.61 (s, 2H, H-4 pyrazole), 7.81(t, 1H, H-4 pyridine), 7.98 (d, 2H, *J* = 9 Hz, H-5), 8.01(d, 2H, *J* = 6 Hz, H-3,5-pyridine), 8.51 (d, 2H, *J* = 9 Hz, H-6). MS: *m/z* 341 (M^+, 28%). Anal: $C_{19}H_{15}N_7$ requires C 66.79, H 4.39, N 28.73; found C 66.80, H 4.30,N 28.70%.

2,6-Bis(2-phenylpyrazolo[1,5-a]pyrimidine-7-yl)pyridine (**21b**) was obtained as pale orange crystals (1.2g, 52%), m.p. 296–299 °C . IR (KBr): ν_{max}/cm^{-1} 3050 (CH-arom), 1625 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.51 (s, 2H, H-4 pyrazole), 7.53–7.93 (m, 11H, H-Ar, H-4 pyridine), 8.03 (d, 2H,*J*=6 Hz, H-3,5 pyridine), 8.29 (d, 2H,*J*=6 Hz, H-5), 9.29 (d, 2H, *J* = 6.0 Hz H-6). MS: *m/z* 465 (M^+,52 %.Anal: $C_{29}H_{19}N_7$ requires C 74.83, H 4.11, N 21.07; found C 74.80, H 4.10, N 21.20%.

2,6-Bis(2-methyl-1-phenylpyrazolo[1,5-a]pyrimidine-7-yl)pyridine (**21c**) was isolated as orange crystals (1.18g,48%), m.p. 271–275 °C. IR (KBr): ν_{max}/cm^{-1} 2993 (CH-aliphatic), 1625 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.35 (s, 6H, 2CH_3), 7.69–7.81 (m, 11H, H-Ar, H4-pyridine), 8.03 (d, 2H,*J*=6 Hz, H-3,5 pyridine), 8.65 (d, 2H,*J*=6 Hz, H-5), 9.25 (d, 2H,*J*=6 Hz, H-6). MS: *m/z* 493 (M^+, 22%. Anal : $C_{31}H_{23}N_7$ requires C 75.45, H 4.66, N 19.87; found C 75.20, H 4.60, N 19.70%.

2,6-Bis(triazolo[3,4-a]pyrimidin-7-yl)pyridine (**22**) was obtained as a pale brown powder (0.95g, 60%), m.p. 308–310 °C. IR (KBr): ν_{max}/ cm^{-1} 1630 (C=N).^{1}H NMR (300 MHz,DMSO-d_6): δ ppm 7.81(t, 1H, H-4 pyridine), 7.91 (d, 2H, *J* = 9.0 Hz, H-5), 8.20 (d, 2H, *J* = 6.0 Hz, H-3,5 pyridine), 8.8 (s, 2H, H-3 triazole), 9.1 (d, 2H, *J* = 9.0 Hz, H-6). ^{13}C(300 MHz,DMSO-d_6) 168.13, 153.13, 148.90, 147.10, 142.10, 139.3, 138.40, 122.10; MS: *m/z* 315 (M^+) 100%. Anal: $C_{15}H_9N_9$ requires C 57.14, H 2.85, N 39.97; found C 57.20, H 2.90,N 39.80%.

2,6-Bis(benzimidazo[1,2-a]pyrimidin-8-yl)pyridine (**23**) was isolated as a brown powder, (1.13g, 55 %), m.p. 313–315 °C. IR (KBr): ν_{max}/cm^{-1} 3050 (CH-Arom), 1625 (C=N). ^{1}H NMR (300 MHz,DMSO-d_6): δ ppm 7.15–8.02 (m, 13H, H-Ar, H-5, H- pyridine), 8.53 (d, 2H, *J* = 6.0 Hz, H-6), MS: *m/z* 413 (M^+) 100%.Anal: $C_{25}H_{15}N_7$ requires C 72.63, H 3.63, N 23.72 ; found C 72.80,H3.60, N 23.50%.

2,6-Bis[3-acetyl-1-arylpyrazole-4-carbonyl]pyridine (**26a,b**) *and* **29a,b**: *General procedure*: To a stirred solution of the appropriate hydrazonyl halides **24a,b** or **24c,d** (0.011 mol) and the enaminone **2** (1.36g,0.005 mol) in dry benzene (20 ml) were added triethylamine (0.4 ml) in portions with stirring at room temperature for 18 h. The precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure. The residue was triturated with ethanol. The solid product, so formed in each case was collected by filtration and recrystallised from ethanol.

2,6-Bis(3-acetyl-1-phenylpyrazole-4-carbonyl)pyridine (**26a**) was obtained as a pale yellow powder (1.55g, 62%), m.p. 215–218 °C. IR (KBr): ν_{max}/cm^{-1} 1697, 1651 (2 C=O), 1627 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.84 (s, 6H, 2 $COCH_3$), 7.31–7.96 (m, 11H, H-Ar, H-4 pyridine), 8.15 (d, 2H,*J*=6 Hz, H-3,5 pyridine), 9.12 (s, 2H, H-5 pyrazole), ^{13}C(300 MHz,DMSO-d_6) 193.70, 188.27, 152.73, 152.24, 139.62, 139.07, 133.26, 130.31, 128.61, 126.16, 121.82, 120.00, 27.19 ;MS: *m/z* 503 (M^+) 11.7%, 460 (100%). Anal: $C_{29}H_{21}N_5O_4$ requires C 69.18, H 4.20, N 13.91 ;found C 69.30, H 4.10, N 14.10%.

2,6-Bis(3-acetyl-1-(p-tolyl)-pyrazole-4-carbonyl)pyridine (**26b**) was isolated as a yellow powder (1.72g, 65%), m.p. 210–213 °C. IR (KBr): ν_{max}/cm^{-1}2953 (CH-aliphatic), 1695, 1652 (2 C=O), 1558 (C=N).^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.02 (s, 6H, 2 CH_3), 2.24 (s, 6H, $COCH_3$), 7.29–7.71 (m, 8H, H-Ar), 8.1(t, 1H, H-5 pyridine), 8.3(d, 2H, *J* = 6 Hz, H-3, H-5 pyridine), 9.01 (s, 2H, H-4

pyrazole). MS: *m/z* 531 (M^+) 16%. Anal: $C_{31}H_{25}N_5O_4$ requires C 70.05, H 4.70,N 13.18 ; found C 70.20, H 4.60, N 13.40%.

2,6-Bis(3-ethoxycarbonyl-1-phenylpyrazole-4-carbonyl) pyridine (**29a**) was obtained as a yellow powder (1.5g, 53%), m.p. 196–198 °C. IR (KBr): ν_{max}/cm^{-1} 2980 (CH-aliphatic), 1715 (CO, ester), 1692, 1652 (2 C=O).^{1}H NMR (300 MHz,DMSO-d_6): δ ppm 0.96 (t, 6H, 2CH_3), 3.88 (q, 4H, 2CH_2), 7.41–7.76 (m, 10H, H-Ar), 7.98 (t, 1H, H-4 pyridine), 8.32 (d, 2H, *J* = 6 Hz, H-3,5 pyridine), 9.00 (s, 2H, H-5 pyrazole). Anal: $C_{31}H_{25}N_5O_6$ requires C 66.07, H 4.44, N12.43 ; found C 66.30, H 4.40, N 12.20%.

2,6-Bis(3-ethoxycarbonyl-1-(p-tolyl)pyrazole-4-carbonyl) pyridine (**29b**) was isolated as pale yellow crystals (1.53g, 52%), m.p. 202–205 °C. IR (KBr): ν_{max}/cm^{-1} 1710 (CO, ester), 1695, 1654 (2 C=O). ^{1}H NMR (300 MHz,DMSO-d_6):δ ppm 0.98 (t, 6H, 2CH_3), 2.23 (s, 6H, 2CH_3), 4.10 (q, 4H, 2CH_2), 7.41–7.71 (m, 8H, H-Ar), 7.98 (t, 1H, H-4 pyridine), 8.41 (d, 2H, *J* = 6 Hz, H-3,5 pyridine), 9.21 (s, 2H, H-5 pyrazole). MS: *m/z* = 591 (M^+, 32%). Anal: $C_{33}H_{29}N_5O_6$, requires C 67.00,H 4.90, N11.84; found C 67.10, H 4.70, N 12.00%.

Preparation of **28** *and* **30**.*General procedure*: A mixture of the appropriate **26a,b** or **29a,b** (0.002 mol) and hydrazine hydrate 80%, (0.5 ml) in absolute ethanol (20 ml) was refluxed for 2–3 h. then left to cool to room temperature. The formed pale orange precipitate was filtered off, washed with ethanol and recrystallised from dimethylformamide to afford the corresponding product.

2,6-Bis(3-methyl-1-phenylpyrazolo[3,4-d]pyridazin-6-yl)pyridine (**28a**) was obtained as a pale yellow powder (0.43g, 43%), m.p. 293–298 °C. IR (KBr): ν_{max}/cm^{-1} 2983 (CH-aliphatic), 1625 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.24 (s, 6H, 2 CH_3), 7.40-7.74 (m, 10H, H-Ar), 8.01(t, 1H, H-4 pyridine), 8.13 (d, 2H, *J* = 6 Hz, H-3,5 pyridine), 9.31 (s, 2H, H-7). ^{13}C(300 MHz,DMSO-d_6) 154.78, 153.73, 152.88, 152.34, 139.82, 139.73, 133.26, 130.33, 128.68, 127.31, 122.93, 121.21, 29.31 ; MS: *m/z* 495 (M^+, 35%). Anal: $C_{29}H_{21}N_9$ requires C 70.30, H 4.24, N 25.45 found C 70.50, H 4.40, N 25.30%.

2,6-Bis(3-methyl-1-(p-tolyl)pyrazolo[3,4-d]pyridazine-6-yl]pyridine (**28b**) was isolated as a yellow powder (0.5g, 47%), m.p. 310-313 °C. IR (KBr): ν_{max}/cm^{-1} 3988 (CH-aliphatic), 1589 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.31 (s, 6H, 2 CH_3), 3.53 (s, 6H, 2 CH_3), 7.45–7.81 (m, 8H, H-Ar), 8.1(t, 1H, H-4 pyridine), 8.21(d, 2H, *J* = 6 Hz, H-3,5 pyridine), 9.41 (s, 2H, H-7). MS: *m/z* 523 (M^+) 38%. Anal: $C_{31}H_{25}N_9$ requires C 71.12, H 4.78, N 24.09, found C 71.30, H 7.80, N 24.30%.

2,6-Bis(3-oxo-1-phenylpyrazolo[3,4-d]pyridazin-6-yl)pyridine (**30a**) was obtained as a yellowish green powder (0.4g, 40%), m.p.320–325 °C. IR (KBr): ν_{max}/cm^{-1} 3981 (CH-aliphatic), 1682, 1605 (2 C=N).^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.4–7.78 (m, 10H, H-Ar), 8.01 (t, 1H, H-4pyridine), 8.13 (d, 2H, *J* = 6 Hz, H-3,5 pyridine), 9.31 (s, 2H, H-7) 9.95(br,2H,2NH). MS: *m/z* = 499 (M^+, 13%). Anal: $C_{27}H_{17}N_9O_2$ requires C 64.92, H 3.40, N 25.25; found C 64.80, H 3.50, N 25.50%.

2,6-Bis(3-oxo-1-(p-tolyl)pyrazolo[3,4-d]pyridazine-6-yl)pyridine (**30b**) was isolated as a yellowish green powder (0.4g, 38%), m.p. 334–348 °C. IR (KBr): ν_{max}/cm^{-1} 2983 (CH-aliphatic), 1668 (C=O).^{1}HNMR (300 MHz, DMSO-d_6): δ ppm 3.61 (s, 6H, 2CH_3), 7.41–7.79 (m, 8H, H-Ar), 8.01 (t, 1H, H-4 pyridine), 8.21 (d, 2H, *J* = 6 Hz, H-3,5 pyridine), 9.31 (s, 2H, H-7) 9.98 (br,2H,2NH) . MS: *m/z* = 527 (M^+) (13%). Anal: $C_{29}H_{21}N_9O_2$ requires C 66.03, H 4.05, N 23.90; found C 66.30, H 3.98, N 24.10%.

Antimicrobial assay method

Procedure: The media were equally dispensed in sterile Petri dishes and cups were made in each solidified plate. The tested micro-organisms were inoculated to the surface of the solidified media. The compound tested (2500 μg) was introduced to each cup. Incubation was conducted for 24 h and 48 h at 30°C for bacterial and fungal cultures respectively. The main diameter of the zone of inhibition of every tested micro-organism was measured in millimeters and was considered as a criterion for the evaluation of the antimicrobial activity of every experimental compound.

Media composition

(1) Nutrient agar (gm/100 ml): Beef extract 0.30, peptone 0.5, agar 2.0
(2) Czapek's agar medium (gm/100 ml): sucrose 3.00, $NaNO_2$ 0.20, KH_2PO_4 0.1, $MgSO_4$ 0.05, KCl 0.05, $FeSO_4$ 0.001, agar 2.00.
(3) Glucose yeast peptone agar (gm/100 ml): malt yeast 0.30, yeast extract 0.30, peptone 0.5, glucose 1.00, agar 2.00.

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