Synthesis and *in vitro* Antibacterial Activity of Some Novel Fused Pyridopyrimidine Derivatives

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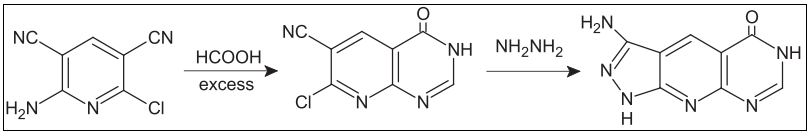

Pyridopyrimidine derivatives **2, 5** and **7–9** were furnished by the reaction of compound **1** with a variety of reagents, namely, formic acid, acetic anhydride, phenyl isothiocyanate, phenyl isocyanate, carbon disulfide, acetamide and thioacetamide, which in turn were treated with hydrazine to give pyrazolopyridopyrimidine derivatives **4, 6** and **11–13**. The potency of the results as antibacterial agents has been evaluated. Most of the tested products showed a highly inhibition zone against the two bacterial strains. All compounds have been characterized based on their IR, ^{1}H-NMR and Mass spectra.

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INTRODUCTION

Pyridine nucleus and their fused heterocyclic systems have attracted a great deal of interest over the years [1]. The pyrazolo[3,4-b]pyridines have promising biological and chemotherapeutic importance. They are known to inhibit VEGFR/PDGFR kinases [2] or glycogen synthesis kinase-3 (GSK-3) [3], which play a key role in chronic inflammatory processes [4], cancer [5] and Alzheimer's disease (AD) [6–9]. These compounds were also reported as antimicrobial agents [10] and potent antitumor agents [11]. Compounds containing a fused pyrimidine ring have significant biological activity, particularly in cancer and virus research [12–14]. In view of the facts mentioned previously and in conjunction with our previous interest in preparing heterocyclic ring systems with wide expected pharmaceutical and biological activities [15–25], we report herein the synthesis and antibacterial evaluation of some like new heterocyclic systems.

RESULTS AND DISCUSSION

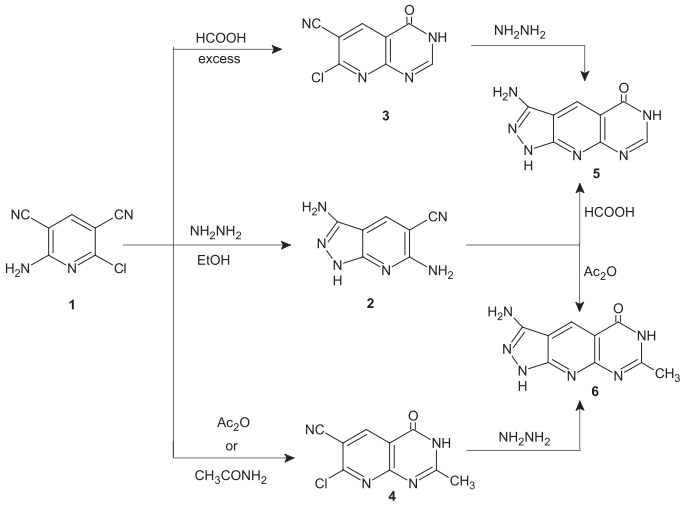
Chemistry. The presence of 2-amino-6-chloropyridine-3,5-dicarbonitrile **1** [1] moiety in our starting precursor allows us to carry out some regioselective attack on the amino- and chloro- groups by variety of reagents in one or two steps.

The reaction of compound **1** with an excess of formic acid, acetic anhydride or acetamide gave compounds **3** and **4**, respectively, which, in turn, were allowed to react with hydrazine hydrate to afford the corresponding pyrazolopyridine derivatives **5** and **6**, respectively. IR spectra of compounds **3** and **4** showed new absorption bands corresponding to C=O at 1658, 1663 cm^{-1}, respectively, and disappearance of absorption bands corresponding to NH_2 groups. ^{1}H-NMR spectra of compounds **3** and **4** showed the following signals at δ 8.11, 8.14 ppm corresponding to NH groups, respectively, to =CH group at 8.21 ppm for compound **3** and at 2.01 ppm CH_3 group of compound **4**. MS of compound **3** gave molecular ion peak at m/z 206.

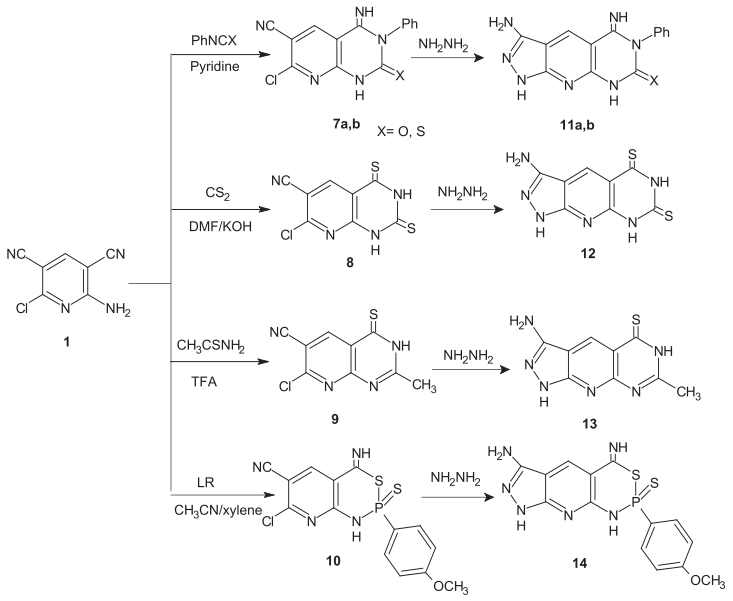
Compounds **5** and **6** were confirmed by the reaction of compound **1** with hydrazine hydrate to afford compound **2** [15] that was used in the next step to produce compounds **5** and **6** via the reaction with formic acid and acetic anhydride. The formation of compounds **5** and **6** was assumed to proceed via the amide formation followed by an intramolecular cyclization through compound **2**, respectively. IR spectra of compounds **5** and **6** showed new absorption bands corresponding to NH_2 groups at 3316–3174 cm^{-1} respectively. Their ^{1}H-NMR spectra showed new signals corresponding to NH_2 groups at 11.33, 11.37 ppm, respectively. MS of compound **5** and **6** gave molecular ion peaks at m/z 202 and 215, respectively (Scheme 1).

Cyclocondensation of compound **1** with phenyl isocyanate, phenyl isothiocyanate, carbon disulfide and thioacetamide afforded the corresponding pridopyrimidine derivatives **7a**,**b**, **8** and **9**, respectively. The treatment of compound **1** with Lawesson's reagent (LR) also gave thiazaphosphinine **10** in a good yield (Scheme 2). IR spectra of compounds **7a**,**b–10** showed disappearance of absorption bands corresponding to NH_2 groups and showed new absorption bands corresponding to NH groups at 3185, 3183, 3186, 3183 and 3179 cm^{-1}, respectively. ^{1}H-NMR spectra of compounds **7a**,**b–10** showed the following signals at δ 8.16, 8.26, 8.10, 8.11 and 8.26 ppm corresponding to NH groups, respectively. MS of compounds **7a**,**b**, **8** and **10** showed molecular ion peaks at m/z 300, 313, 254 and 380, respectively.

Scheme 1. Reaction of 2-amino-6-chloropyridine-3,5-dicarbonitrile with formic acid, acetic anhydride and acetamide.



Scheme 2. Reaction of 2-amino-6-chloropyridine-3,5-dicarbonitrile with phenyl isothiocyanate and other reagents.



The chlorine atom, attached to the pyridine nucleus at **7–10** underwent substitution reaction with hydrazine hydrate to afford the corresponding compounds **11–14**, respectively. The structure of compounds **11–14** was confirmed by IR, ^{1}H-NMR and MS, respectively. IR spectra of compounds **11–14** showed new absorption bands corresponding to NH_2 groups at 3350–31659 cm^{-1}. ^{1}H-NMR spectra of compounds **11–14** showed the following signals at δ 5.60, 5.57, 5.55, 5.52 and 5.28 ppm corresponding to NH_2 groups, respectively. MS of compound **14** showed molecular ion peak at m/z 377 (Scheme 2).

Alkylation of compound **1** with halo compounds (e.g. chloroacetonitrile and chloroacetamide) in DMF as a solvent gave the corresponding pyrrolopyridine derivatives **15** and **16**, respectively, which, in turn, were allowed to react with hydrazine hydrate to give 3,5-diamino-1,7-dihydropyrazolo [3–29]pyrrolo[3,2-*e*]pyridine-6-carbonitrile **18** and 3,5-diamino-1,7-dihydropyrazolo[3–29]pyrrolo[3,2-*e*]pyridine-6-carboxamide **19**, respectively. ^{1}H-NMR spectra of compounds **15** and **16** showed the following signals at δ 8.21 and 8.56 ppm corresponding to NH groups, respectively, and compounds **18** and **19** showed the following signals at δ 5.80 and 5.36 ppm corresponding to NH_2 groups, respectively. MS of compound **16** showed molecular ion peak at m/z 235.

Compound **1** was allowed to react with chloroacetyl chloride to yield pyridopyridine derivative **17**, which in turn was allowed to react with hydrazine to give *N*6,*N*6-dimethyl-3,5-diamino-7-oxo-7,8-dihydro-1*H*-pyrazolo[3–29,1,8]-naphthyridine-6-carboxamide **20**. IR spectra of compounds

17 and **20** showed new absorption bands corresponding to C=O groups at 1662 and 1658 cm^{-1}, respectively. ^{1}H-NMR spectra of compounds **17** and **20** showed the following signals at δ 3.30 and 3.55 ppm corresponding to CH_3 groups, respectively. Also the reaction of compound **1** with ethyl chloroacetate or ethyl bromoacetate afforded the N-substituted derivatives **21a**,**b** which was treated with morpholine to produce the cycled compound 1,8-naphthyridine derivative **22.** IR spectra of compounds **21a**,**b** showed disappearance of absorption bands corresponding to NH_2 groups but showed appeared at 3399–3326 cm^{-1} in compound **22**. ^{1}H-NMR spectra of compounds **21a**,**b** showed the following signals at δ 4.45 and 4.01 ppm corresponding to CH_2 groups, respectively. MS of compound **21a** showed molecular ion peak at m/z 254. ^{1}H-NMR spectra of compound **22** showed the following signal at δ 5.30 ppm corresponding to NH_2 group. MS of compound **22** showed molecular ion peak at m/z 356 (Scheme 3).

Antibacterial activity. Synthesis of new heterocyclic compounds containing pyridine nucleus fused with other heterocyclic rings is of considerable biological importance including antibacterial activity [10].

The synthesized compounds were dissolved in DMSO. In order to ensure that the solvent had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations.

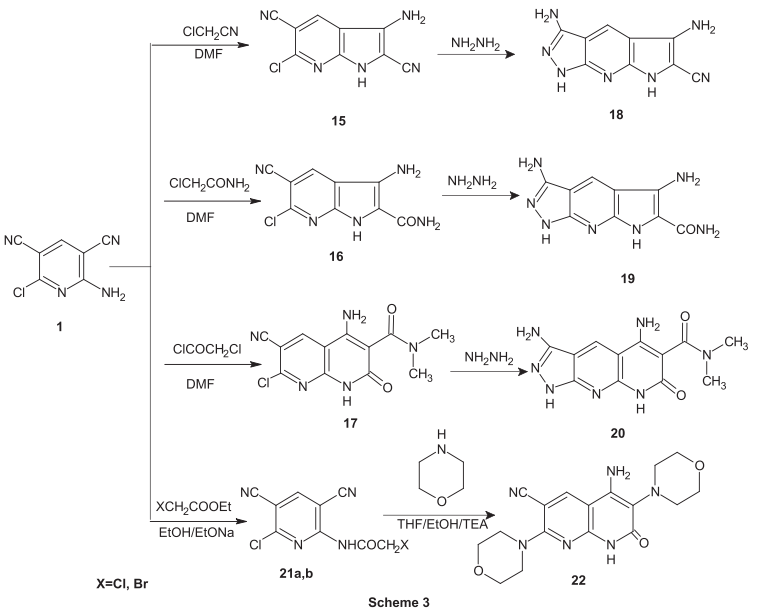
The inhibitory effect of tested compounds **1–19**, **21b** and **22** on the *in vitro* growth of two different types of bacteria *Bacillus cereus*, a gram-positive bacteria (+ve), and *Pseudomonas aureginosa*, a gram-negative bacteria (−ve) was evaluated using agar diffusion method (cup and plate method) [26–29]. All plates were incubated at 37 ± 0.5°C for 24 h. The inhibition zone of active compounds was measured in cm scale. The results shown in Table 1 revealed that compounds **5**, **7a**, **7**, **8**, **9**, **10**, **12**, **13**, **14**, **15** and **17** showed the highest inhibitory effect against the two types of bacteria at the three used concentrations, while compounds **3** and **11a**, showed moderate activity. Compounds **4** and **21** showed high-inhibition zone against a gram-positive bacteria and no activity against a gram-negative bacteria. Compounds **6** and **18** showed high inhibition zone against the two types of bacteria at high concentration, but compound **22** showed high activity at low concentration. On the other hand, compound **19** showed no bactericidal activity against either bacterial strains (Table 1).

EXPERIMENTAL

All melting points were determined on a Kofler melting point apparatus and were uncorrected. IR spectra were obtained on a Shimadzou FTIR spectrometer (Shimadzou Corp., Kyoto, Japan). ^{1}H-NMR spectra were recorded on a Varian Gemini (Varian, Inc., Palo Alto, North Carolina, USA) at 200 MHz using TMS as an internal reference and DMSO-*d*6 as a solvent. MS were obtained on a Shimadzu GCMS-QP1000 mass spectrometer (Shimadzou Corp.) at 70 ev. Purity and monitoring of synthesized compounds were checked by protected TLC plates (E Merck Kieselgel 60 F_{254}). The structure of new products was confirmed by IR, ^{1}HNMR and MS. The precursor product **1** and compound **2** were synthesized by the procedures reported in [1,15].

7-Chloro-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (3). Compound **1** and in all experiments (0.005 mol, 0.89 g), and excess of formic acid (10 mL) was heated under reflux for 10 h; the mixture was left to cool and poured into ice cold water. The obtained solid product was

Scheme 3. Reaction of 2-amino-6-chloropyridine-3,5-dicarbonitrile with halogenated active methylenes.



Scheme 3

Table 1

Antibacterial activity of the tested pyridine derivatives **1–19, 21b,** and **22.**

	Bacillus cereus			*Pseudomonas aureginosa*		
Compound	10 000 ppm	30 000 ppm	50 000 ppm	10 000 ppm	30 000 ppm	50 000 ppm
3	1.1	1.0	—	1.6	1.4	1.1
4	1.5	1.4	—	—	—	—
5	2.0	1.5	1.2	1.8	2.0	2.2
6	—	—	3.5	—	—	3.0
7 a	2.0	1.7	1.6	1.5	1.2	1.1
7 b	1.6	1.9	1.6	2.0	1.8	1.8
8	1.5	1.3	1.2	2.1	1.7	1.7
9	2.0	1.8	1.2	1.0	1.3	1.4
10	1.6	1.2	1.0	1.8	2.0	2.0
11 a	—	1.5	1.7	—	1.3	2.0
12	1.8	2.0	3	1.6	1.8	2.5
13	1.7	1.4	1.9	1.5	1.5	1.7
14	1.6	1.2	1.1	1.2	1.0	1.0
15	1.5	1.3	1.1	2.1	1.8	1.7
16	—	1.1	1.5	0.9	1.1	1.0
17	1.0	1.3	1.8	1.8	1.6	2.0
18	—	—	2.0	—	1.5	1.8
19	—	—	—	—	—	—
21 b	1.5	1.8	1.5	—	—	—
22	1.5	—	—	1.6	—	—

filtered off, washed with water and crystallized from ethanol. Yield (91%), pale yellow, mp 260°C; IR cm^{-1}: 3205 (NH), 2220 (CN), 1658 (CO); ^{1}H-NMR: δ 8.60 (s, 1H, $CH_{pyridine}$), 8.21 (s, 1H, $CH_{prymidine}$), 8.11 (s, 1H, NH); MS: m/z (100%): 206 (5.5%), 188 (53%), 178 (91.8%), 133 (100%); *Anal.* Calcd. for $C_8H_3N_4OCl$ (206.5): C, 46.51; H, 1.46; N, 27.12; Cl, 17.16. Found: C, 46.59; H, 1.43; N, 27.07; Cl, 17.10.

7-Chloro-2-methyl-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (4). **Method A** Compound **1** (0.005 mol, 0.89 g) and excess of acetic anhydride (10 mL) were heated under reflux for 12 h, the mixture was left to cool, and the solvent was evaporated. The obtained solid product was washed with water and crystallized from ethanol.

Method B A mixture of compound **1** (0.005 mol, 0.89 g) acetamide (0.005 mol) in trifluoroacetic acid (TFA) (20 mL) was heated under reflux for 24 h; the solvent was removed under reduced pressure. The obtained solid product was crystallized from ethanol.

Yield (88%), yellow, mp 330°C; IR cm^{-1}: 3453 (NH), 2230 (CN), 1663 (CO); ^{1}H-NMR: δ 8.51 (s, 1H, $CH_{pyridine}$), 8.14 (s, 1H, NH), 2.01 (s, 3H, CH_3). MS: m/z (100%): 221 (30%), 200 (35%), 178 (53%), 149 (100%), 105 (59%); *Anal*. Calcd. for $C_9H_5N_4OCl$ (220.5): C, 49.00; H, 2.28; N, 25.40; Cl, 16.07. Found: C, 49.00; H, 2.23; N, 25.35; Cl, 16.17.

General procedure for the preparation of compounds 5 and **6**. A mixture of the corresponding compounds **3** or **4** (0.004 mol) and hydrazine hydrate (10 mL) was refluxed for 12 h, the solvent was removed under reduced pressure, and the obtained residue was triturated with ice cold water (10 mL). The precipitated solid was filtered off, washed with water, dried and crystallized from ethanol.

3-Amino-2,3,5,6-tetrahydro-1H-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-5-one (5). Yield (67%), dark yellow, mp >360°C; IR cm^{-1}: 3297, 3174 (NH_2), 3140 (NH), 1685 (CO); ^{1}H-NMR: 11.33 (s, 2H, 2NH), 8.61 (s, 1H, $CH_{pyridine}$), 8.22 (s, 1H, $CH_{prymidine}$), 5.59 (br, 2H, NH_2), MS: m/z (100%): 202 (100%), 175 (50%); *Anal*. Calcd. for $C_8H_6N_6O$ (202): C, 47.53; H, 2.99; N, 41.57. Found: C, 47.58; H, 2.99; N, 41.53.

3-Amino-7-methyl-5,6-dihydro-1H-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-5-one (6). Yield (60%), yellow, mp >360°C; IR cm^{-1}: 3316, 3179 (NH_2), 3141 (2NH), 1684 (CO); ^{1}H-NMR: δ 11.37 (s, 2H, 2NH), 8.34 (s, 1H, $CH_{pyridine}$), 5.52 (br, 2H, NH_2), 2.54 (s, 3H, CH3); MS: m/z (100%): 215 (0.8%), 189 (100%); *Anal.* Calcd. for $C_9H_8N_6O$ (216): C, 50.00; H, 3.73; N, 38.87. Found: C, 50.15; H, 3.63; N, 38.80.

General procedure for the preparation of compounds (7a,**b**). A mixture of compound **1** (0.005 mol, 0.89 g) and phenylisocyanate or phenylisothiocyanate (0.005 mol) in a solvent mixture of DMF and ethanol (20 mL) was heated under reflux for 10 h; the mixture was left to cool and poured into ice cold water. The obtained solid product was filtered off, washed with water and crystallized from ethanol.

7-Chloro-4-imino-2-oxo-3-phenyl-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (7a). Yield (77%), browen, mp 230°C; IR cm^{-1}: 3344, 3185 (2NH), 2230 (CN), 1655 (CO); ^{1}H-NMR: δ 8.92 (s, 1H, $CH_{pyridine}$), 8.16 (s, 1H, $1NH_{pyrimidine}$), 7.62–7.46 (m, 5H, phenyl), 5.37 (s, 1H, 1NH); MS: m/z (100%): 300 (0.9%), 119 (11%), 93 (100%); *Anal.* Calcd. for $C_{14}H_8N_5OCl$ (297.5): C, 56.48; H, 2.71; N, 23.58; Cl, 11.21. Found: C, 56.47; H, 2.70; N, 23.50; Cl, 11.21.

7-Chloro-4-imino-3-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (7b). Yield (73%), yellow, mp 263°C; IR cm^{-1}: 3344, 3183 (2NH), 2226 (CN); ^{1}H-NMR: δ 8.79 (s, 1H, $CH_{pyridine}$), 8.26 (s, 1H, $1NH_{pyrimidine}$), 7.64–7.41 (m, 5H, phenyl), 5.71 (s, 1H, 1NH); MS: m/z (100%): 313 (38%), 293 (19%), 185 (23%); *Anal.* Calcd. for $C_{14}H_8N_5SCl$ (313.5): C, 53.59; H, 2.57; N, 22.32; Cl, 11.30; S, 10.22. Found: 53.62; H, 2.50; N, 22.32; Cl, 11.35; S, 10.29.

7-Chloro-2,4-dithioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carbonitrile (8). An equimolar ratio of compound **1** (0.005 mol, 0.89 g), potassium hydroxide (0.005 mol, 0.89 g) and carbon

disulfide (0.005 mol, 0.89 g) in DMF (20 mL) was stirred at room temperature for 48 h, and the reaction mixture was poured into ice cold water. The obtained solid product was filtered off, washed with water and crystallized from ethanol.

Yield (88%), orange, mp 285°C; IR cm^{-1}: 3347, 3186 (2NH), 2230 (CN); ^{1}H-NMR: δ 8.89 (s, 1H, $CH_{pyridine}$), 8.10 (s, 2H, 2NH); MS: m/z (100%): 254 (21%), 217 (10%), 175 (100%); *Anal*. Calcd. for $C_8H_3N_4S_2Cl$ (254.5): C, 37.72; H, 1.19; N, 22.00; Cl, 13.92. Found: C, 37.80; H, 1.15; N, 22.10; Cl, 13.82.

7-Chloro-2-methyl-4-thioxo-3,4-dihydropyrido[2,3-d]pyrimidine-6-carbonitrile (9). A mixture of compound **1** (0.005 mol, 0.89 g) or thioacetamide (0.005 mol) in trifluoroacetic acid (TFA) (20 mL) was heated under reflux for 24 h, and the solvent was removed under reduced pressure. The obtained solid product was crystallized from ethanol.

Yield (85%), red, mp >360°C; IR cm^{-1}: 3183 (NH), 2225 (CN); ^{1}H-NMR: δ 8.66 (s, 1H, $CH_{pyridine}$), 8.11 (s, 1H, 1NH), 2.01 (s, 3H, CH_3); *Anal*. Calcd. for $C_9H_5N_4SCl$ (236.5): C, 45.67; H, 2.13; N, 23.67; Cl, 14.98; S, 13.55. Found: C, 45.60; H, 2.10; N, 23.73; Cl, 14.98; S, 13.59.

7-Chloro-6-cyano-4-imino-2-(4-methoxyphenyl)-1,4,4a,8a-tetrahydro-2H-pyrido-2,3-d][1,3,2]thiazaphosphinine-2-sulfide (10). A mixture of compound **1** (0.005 mol, 0.89 g) and Lawesson's reagent (0.005 mol, 2.1 g) in a mixture of dry p-xylene and acetonitrile (20 mL) was heated under reflux for 10 h, the solvent was removed under reduced pressure, and the obtained residue was triturated with methanol (20 mL). The precipitate was filtered off and crystallized from ethanol.

Yield (65%), bright brown, mp 245°C; IR cm^{-1}: 3340, 3179 (2NH), 2927 $(CH)_{aliph.}$, 2228 (CN); 1H-NMR: δ 8.71 (s, 1H, $CH_{pyridine}$), 8.26 (s, 2H, 2NH), 7.91–7.26 (m, 4H, phenyl), 3.78 (s, 3H, CH_3); MS: m/z (100%): 380 (3.5%), 379 (6.3%), 341 (100%); *Anal*. Calcd. for $C_{14}H_{10}N_4OPS_2Cl$ (380.5): C, 44.16; H, 2.55; N, 14.71; Cl, 9.31; S, 16.84. Found: C, 44.23; H, 2.55; N, 14.70; Cl, 9.39; S, 16.94.

General procedure for the preparation of compounds (11a,b), (12), (13) and (14). A mixture of the corresponding compounds **7a**,**b**, **8**, **9** and **10** (0.004 mol) and hydrazine hydrate (10 mL) was refluxed for 12 h; the solvent was removed under reduced pressure, and the obtained residue was triturated with ice cold water (10 mL). The precipitated solid was filtered off, washed with water, dried and crystallized from ethanol.

3-Amino-5-imino-6-phenyl-5,6,7,8-tetrahydro-1H-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-7-one (11a). Yield (58%), yellow, mp >360°C; IR cm^{-1}: 3321, 3172 (NH_2), 3142 (3NH), 1685 (CO); ^{1}H-NMR: 11.50 (s, 2H, 2NH), 8.32 (s, 1H, $CH_{pyridine}$), 7.62–7.46 (m, 6H, NH + phenyl), 5.60 (br, 2H, NH_2); *Anal*. Calcd. for $C_{14}H_{11}N_7O$ (293): C, 57.33; H, 3.78; N, 33.43. Found: C, 57.23; H, 3.88; N, 33.40.

3-Amino-5-imino-6-phenyl-5,6,7,8-tetrahydro-1H-pyrazolo[4',3':5,6]pyrido-[2,3-d]pyrimidine-7-thione (11b). Yield (58%), yellow, mp >360°C; IR cm^{-1}: 3321, 3199 (NH_2), 3140 (3NH); ^{1}H-NMR: 11.43 (s, 2H, 2NH), 8.35 (s, 1H, $CH_{pyridine}$), 7.60–7.40 (m, 6H, NH + phenyl), 5.57 (br, 2H, NH_2); *Anal*. Calcd. for $C_{14}H_{11}N_7S$ (309): C, 54.36; H, 3.58; N, 31.69; S, 10.37. Found: C, 54.47; H, 3.55; N, 31.59; S, 10.39.

3-Amino-5,6,7,8-tetrahydro-1H-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidine-5,7-dithione (12). Yield (70%), brown, mp 230°C; IR cm^{-1}: 3318, 3199 (NH_2), 3144 (3NH); ^{1}H-NMR: δ 10.97 (s, 2H, 2NH), 8.37 (s, 1H, $CH_{pyridine}$), 8.10 (s, 1H, 1NH), 5.55 (br, 2H, NH_2); *Anal*. Calcd. for $C_8H_6N_6S_2$ (250): C, 38.39; H, 2.42; N, 33.58; S, 25.62. Found: C, 38.30; H, 2.32; N, 33.65; S, 25.74.

3-Amino-7-methyl-5,6-dihydro-1H-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidine-5-thione (13). Yield (65%), red, mp >360°C (dec); IR cm^{-1}: 3350, 3165 (NH_2), 3145 (2NH); ^{1}H-NMR: δ 11.38 (s, 2H, 2NH), 8.43 (s,1H, $CH_{pyridine}$), 5.52 (br, 2H, NH_2), 2.52 (s, 3H, CH_3); *Anal*. Calcd. for $C_9H_8N_6S$ (232): C, 46.54; H, 3.47; N, 36.18; S, 13.81. Found: C, 46.65; H, 3.37; N, 36.08; S, 13.90.

6-Amino-4-imino-2-(4-methoxyphenyl)-1,2,4,4a,8,9a-hexahydropyrazolo[4',3':5,6]-pyrido[2,3-d][1,3,2]thiazaphosphinine-2-sulfide (14). Yield (75%), brown, mp >360°C; IR cm^{-1}: 3321, 3188 (NH_2), 3143 (3NH), 2927 $(CH)_{aliph.}$; ^{1}H-NMR: δ 11.27 (s, 2H, 2NH), 8.81 (s, 1H, $CH_{pyridine}$), 7.87–8.46 (m, 5H, NH + phenyl) 5.28 (br, 2H, NH_2), 3.23 (s, 3H, CH_3); MS: m/z (100%): 377 (3.5%), 379 (6.3%), 341(100%); *Anal*. Calcd. for $C_{14}H_{10}N_4OPS_2Cl$ (376): C, 44.67; H, 3.48; N, 22.33; S, 17.04. Found: C, 44.60; H, 3.45; N, 22.36; S, 17.12.

3-Amino-6-chloro-1H-pyrrolo[2–29]pyridine-2,5-dicarbonitrile (15). A mixture of compound **1** (0.005 mol, 0.89 g) and chloroacetonitrile (0.005 mol, 0.23 mL) in a solution of DMF was heated under reflux for 10 h; the mixture was cooled down and poured into ice cold water. The obtained solid product was filtered off and crystallized from ethanol.

Yield (58%), buff, mp 169–170°C; IR cm^{-1}: 3407, 3343 (NH_2), 3235 (NH), 2211 (CN); ^{1}H-NMR: δ 8.71 (s, 1H, $CH_{pyridine}$), 8.21 (s, 1H, NH), 3.5 (br, 2H, NH_2), m/z (100%): 217 (82%), 198 (100%), 187 (72%), 172 (67%); *Anal*. Calcd. for $C_9H_4N_5Cl$ (217.5): C, 49.67; H, 1.85; N, 32.18; Cl, 16.29. Found: C, 49.47; H, 1.90; N, 32.27; Cl, 16.35.

3-Amino-6-chloro-5-cyano-1H-pyrrolo[2,3-b]pyridine-2-carboxamide (16). A solution of compound **1** (0.005 mol, 0.89 g) in DMF (25 mL) was treated with chloroacetamide (0.005 mol, 0.54 mL). The reaction mixture was refluxed for 5 h and then left to cool. The precipitated product was filtered off, dried and crystallized from ethanol

Yield (70%), brown crystal, mp 220°C; IR cm^{-1}: 3427, 3333 (NH_2), 3229 (NH), 2218 (CN), 1636 (CO); ^{1}H-NMR: δ 8.56 (s, 1H, NH), 8.33 (s, 1H, $CH_{pyridine}$), 3.34 (br, 4H, $2NH_2$); MS: m/z (100%): 235 (4%), 160 (11%), 134 (100%); *Anal*. Calcd. for $C_9H_6N_5OCl$ (234.5): C, 45.88; H, 2.57; N, 29.72; Cl, 15.05. Found: C, 45.90; H, 2.50; N, 29.60; Cl, 15.12.

N3,N3-Dimethyl-4-amino-7-chloro-6-cyano-2-oxo-1,2-dihydro[1,8]naphthyridine-3-carboxamide (17). To a cooled solution of compound **1** (0.005 mol, 0.89 g) in DMF, chloroacetyl chloride was added dropwise with stirring at room temperature for 2 h and then refluxed for 3 h. The reaction mixture was cooled down and poured onto ice cold water. The obtained solid product was filtered off, dried and crystallized from ethanol.

Yield (77%), brown crystal, mp 179°C; IR cm^{-1}: 3398, 3287 (NH_2), 3238 (NH), 2212 (CN), 1662 (CO); ^{1}H-NMR: δ 8.07 (s, 1H, $CH_{pyridine}$), 7.30 (br, 3H, NH, NH_2), 3.30 (s, 6H, $2CH_3$) MS: m/z (100%): 292 (50%), 149 (72%); *Anal*. Calcd. for $C_{12}H_{10}N_5O_2Cl$ (291.5): C, 49.41; H, 3.46; N, 24.01; Cl, 12.15. Found: C, 49.41; H, 3.46; N, 24.01; Cl, 12.15.

General procedure for the preparation of compounds 18, 19 and 20. A mixture of the corresponding compounds **15**, **16** and **17** (0.004 mol) and hydrazine hydrate (10 mL) was refluxed for 12 h; the solvent was removed under reduced pressure, and the obtained residue was triturated with ice cold water (10 mL). The precipitated solid was filtered off, washed with water, dried and crystallized from ethanol.

3,5-Diamino-1,7-dihydropyrazolo[3,4-b]pyrrolo[3,2-e]pyridine-6-carbonitrile (18). Yield (65%), dark yellow, mp >360°C; IR

cm^{-1}: 3350,3241 ($2NH_2$), 3165 (2NH); ^{1}H-NMR: δ 11.12 (s, 2H, 2NH), 8.89 (s, 1H, $CH_{pyridine}$), 5.8 (br, 4H, $2NH_2$); *Anal*. Calcd. for $C_9H_7N_7$ (213): C, 50.70; H, 3.31; N, 45.99. Found: C, 50.75; H, 3.36; N, 45.89.

3,5-Diamino-1,7-dihydropyrazolo[3,4-b]pyrrolo[3,2-e]pyridine-6-carboxamide (19). Yield (68%), yellow, mp >360°C; IR cm^{-1}: 3317, 3210 (3NH2), 3171 (2NH); 1621 (CO); ^{1}H-NMR: δ 11.36 (s, 2H, 2NH), 8.33 (s, 1H, $CH_{pyridine}$), 5.39 (br, 6H, $3NH_2$); *Anal*. Calcd. for $C_9H_9N_7O$ (231): C, 46.75; H, 3.92; N, 42.41. Found: C, 46.85; H, 3.90; N, 42.35.

N6,N6-Dimethyl-3,5-diamino-7-oxo-7,8-dihydro-1H-pyrazolo[3,4-b][1,8]-naphthyridine-6-carboxamide (20). Yield (75%), yellow, mp 280°C; IR cm^{-1}: 3427, 3313 (NH2), 3183 (NH), 1628 (CO); ^{1}H-NMR: δ 11.54 (s, 2H, 2NH), 8.22 (s, 1H, $CH_{pyridine}$), 5.29 (br, 4H, $2NH_2$), 3.55 (s, 6H, $2CH_3$); *Anal*. Calcd. for $C_{12}H_{13}N_7O_2$ (287): C, 50.17; H, 4.56; N, 34.13. Found: C, 50.20; H, 4.45; N, 34.10.

General procedure for the preparation of compounds (21a,b). A solution of compound **1** (0.005 mol, 0.89 g) in ethanol and sodium ethoxide (25 mL) was treated with ethyl chloroacetate and ethyl bromoacetate (0.005 mol), respectively. The reaction mixture was refluxed for 7 h and then left to cool. The precipitated product was filtered off, dried and crystallized from ethanol.

N1-(6-Chloro-3,5-dicyano-2-pyridyl)-2-chloroacetamide (21a). Yield (85%), brown crystal, mp 230°C; IR cm^{-1}: 3183 (NH), 2206 (CN), 1635 (CO); ^{1}H-NMR: δ 9.47 (s, 1H, NH), 8.90 (s, 1H, $CH_{pyridine}$), 4.45 (s, 2H, CH_2); MS: m/z (100%): 254 (84%), 138 (85%), 95 (100%); *Anal*. Calcd. for $C_9H_4N_4OCl_2$ (254): C, 42.38; H, 1.58; N, 21.97; Cl, 27.80. Found: C, 42.28; H, 1.48; N, 21.99; Cl, 27.93.

N1-(6-Chloro-3,5-dicyano-2-pyridyl)-2-bromoacetamide (21b). Yield (88%), yellow, mp 360°C (dec.); IR cm^{-1}: 3185 (NH), 2227 (CN), 1649 (CO); ^{1}H-NMR: δ 9.77 (s, 1H, NH), 8.98 (s, 1H, $CH_{pyridine}$), 4.01 (s, 2H, CH_2); *Anal*. Calcd. for $C_9H_4N_4OCl$ Br (298.5): C, 36.09; H, 1.35; N, 18.71; Cl, 11.84; Br, 26.66. Found: C, 36.19; H, 1.30; N, 18.71; Cl, 11.83; Br, 26.66.

4-Amino-3,7-dimorpholino-1,2,3,4-tetrahydro[1,8]-naphthyridin-6-carbonitrile (22). An equimolar ratio of compound **21a,b** (0.0017 mol, 0.45 g), triethyamine (0.0034 mol, 0.4 mL) and morphline (0.005 mol), was heated under reflux in a mixture of ethanol and THF (20 mL) for 6 h, concentrated and left to cool down. The obtained solid product was filtered off and crystallized from ethanol.

Yield (85%), bright yellow, mp 220°C; IR cm^{-1}: 3399, 3326 (NH_2), 3218 (NH), 2202 (CN), 1644 (CO) ^{1}H-NMR: δ 8.80 (s, 1H, NH), 8.27 (s, 1H, $CH_{pyridine}$), 5.30 (br, 2H, NH_2), 3.75–3.72 (t, 8H, $4CH_2$), 3.68–3.65 (t, 8H, $4CH_2$); MS: m/z (100%): 356 (13%), 323 (10%), 161 (13%), 149 (100%); *Anal*. Calcd. for $C_{17}H_{20}N_6O_3$ (356) C, 59.28; H, 7.02; N, 4.40. Found: C, 59.08; H, 7.08; N, 24.48.

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