Synthesis and antitumour activity of certain pyrido[2,3-*d*] pyrimidine and 1,8-naphthyridine derivatives

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In an effort to establish new candidates with improved anticancer activity, we report here the synthesis of various series of 2,4,5,7-tetrasubstituted pyrido[2,3-*d*]pyrimidines and their related isosteres substituted 1,8-naphthyridines. The cytotoxic activity of the newly synthesised compounds against human breast cancer cell line, MCF7 was investigated. Most of the tested compounds exploited potent to moderate growth inhibitory activity, in particular 7-(4-chlorophenyl)-5-(3-nitrophenyl)pyrido[2,3-*d*]pyrimidin-4-amine exhibited superior potency to the reference drug doxorubicin (IC_{50} = 7.54 and 8.48 μM respectively).

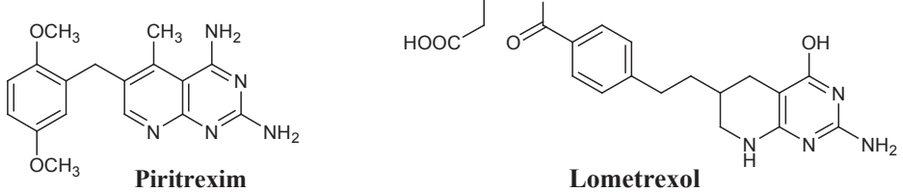
Keywords: synthesis, pyrido[2,3-*d*]pyrimidine, naphthyridine, inhibitor, substituent effect, antitumour

The pyrido[2,3-*d*]pyrimidine ring system are an interesting class of heterocycles because of several biological activities associated with this scaffold such as antibacterial,[1] anti-inflammatory,[2] antihypertensive,[3] diuretic,[4] antihistaminic [5] and more specifically as cytotoxic agents.[6] Their cytotoxic activities might be attributed to inhibition of several enzymes such as tyrosine kinases,[7–9] cyclin-dependant kinase (CDK)[10,11] and dihydrofolate reductase (DHFR).[12] In a clinical study, The second generation dihydrofolate reductase inhibitor piritrexim (PTX), 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine, has been developed as a potent small-molecule inhibitor that is effective against cancer cells resistant to methotrexate.[13] Piritrexim was active against a broad range of tumours, with evidence of high tissue penetration due to its lipophilicity.[14] Also, lometrexol (5,10-dideazatetrahydrofolic acid) was a new antifolate. One of the most promising preclinical features of lometrexol in animal models was its significant activity against a broad panel of solid tumours.[15] (Fig. 1).

On the other hand, naphthyridine derivatives continued to be of great interest due to the wide spectrum of their physiology such as anti-inflammatory,[16] anti-aggressive,[17] and antitumour activities.[18,19] Several synthetic approaches have been developed to synthesise the 1,8-naphthyridine derivatives,[20] but due to their great importance, the development of new synthetic methods remain an active research area.Based on the above finding, coupled with our efforts to synthesise novel fused pyridines acting as antitumour agents,[21] we decided to prepare and evaluate certain new pyrido[2,3-*d*]pyrimidines and their bioisosteric analogue 1,8-naphthyridine derivatives, structurally-related to the aforementioned anticancer agents (Fig. 1). Also the effect of different substituent at positions 2,4,5, and 7 to substantiate the possible influence of such substitution on the *in vitro* antitumour activities against human breast adenocarcinoma MCF-7 cell line.

Results and discussion

The synthetic approaches adopted to obtain the target compounds are outlined in Schemes 1–3. Compounds **2a–d** were synthesised by one-pot reaction of the corresponding cycloalkanones **1a–d**, appropriate aromatic aldehydes, ethyl cyanoacetate and ammonium acetate following a procedure previously reported by Fathalla *et al*.[22] in 55–68% yield. The formation of compounds **2a–d** was confirmed by IR spectra that showed C≡N band at υ 2200 cm^{-1} as well as NH bands at υ 3300–3150 cm^{-1}, in addition to C=O absorption at υ 1660–1650 cm^{-1}. The ^{1}H NMR spectra of compounds **2a–d** showed an exchangeable singlet signal at δ 12.504–12.740 ppm corresponding to the NH protons. Chlorination of 3-cyanopyridones **2a–d** with phosphorus oxychloride/N,N-dimethylaniline mixture gave the corresponding 2-chloro derivatives, **3a–d**, which were confirmed by IR spectra that showed the disappearance of C=O absorption. The latter compounds were reacted with guanidine base, after liberation from its hydrochloride salt, in pyridine to afford the substituted 2,4-diaminopyrido[2,3-*d*]pyrimidines **4a–d**. The structures of compounds **4a–d** were established on the basis of elemental analyses and spectral data. The IR spectra of compounds **4a–d** showed no absorption band for C≡N group, meanwhile revealed characteristic absorption bands at 3400–3180 cm^{-1} indicating the presence of NH_2 functionalities. Furthermore, their ^{1}H NMR spectra revealed the appearance of singlet signals at δ 6.418–7.158 and 11.890–12.026 ppm corresponding to two NH_2 groups. Scheme 2 illustrates the synthesis of several substituted pyrido[2,3-*d*]pyrimidines **6–12** starting from the 2-amino-4,6-disubstituted pyridine-3-carbonitriles **5a**,**b.** Compounds **5a**,**b** were synthesised by applying a one-pot reaction of the respective substituted acetophenone with appropriate aromatic aldehydes and malononitrile in the presence of ammonium



Piritrexim **Lometrexol**

Fig. 1 Some drugs containing pyrido[2,3-*d*]pyrimidine currently in clinical development.

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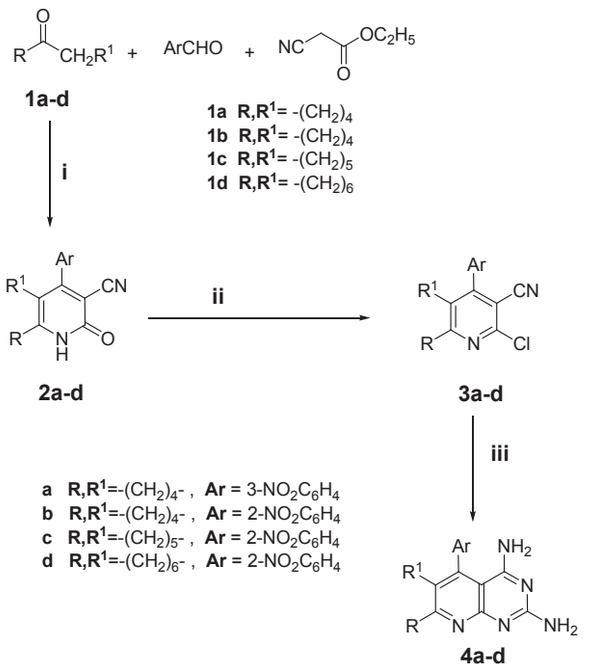
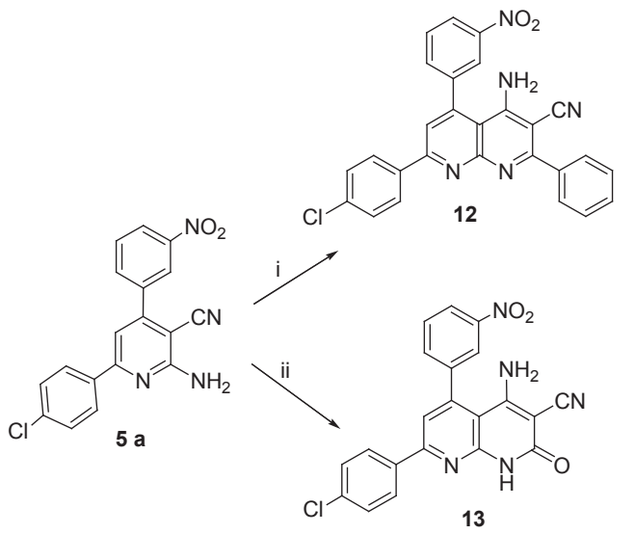
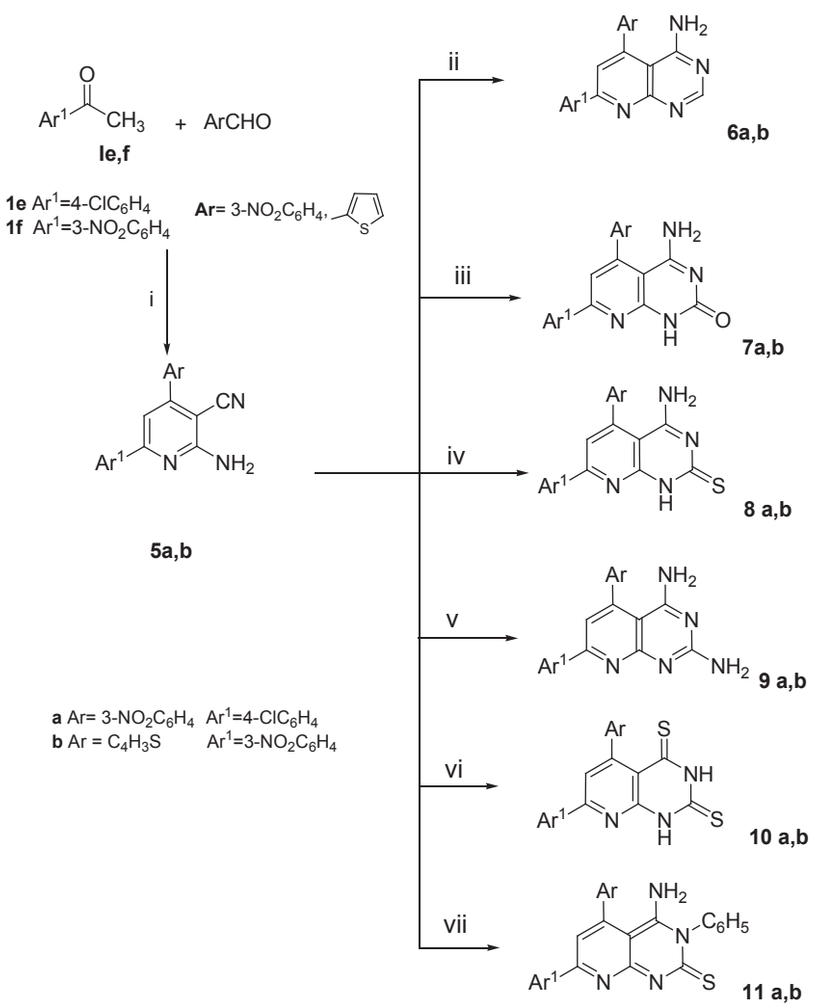


Scheme 1 Reagent and conditions: (i) CH_3COONH_4, n-butanol, reflux 8 h; (ii) $POCL_3$ dimethylaniline, reflux 10 h; (iii) guanidine HCL, $NaOC_2H_5$, pyridine, reflux 5 h.



Scheme 3 Reagent and condition: (i) benzylidenemalononitrile, piperidine, dioxane, reflux 3 h; (ii) ethyl cyanoacetate, triethylamine, ethanol, reflux 3 h.



Scheme 2 Reagent and condition: (i) malononitrile, CH_3COONH_4, n-butanol, reflux 8 h; (ii) formamide, oil bath, reflux 15 h; (iii) urea, oil bath, reflux 4 h; (iv) thiourea, oil bath, reflux 4 h; (v) guanidine HCL methanol, reflux 6 h; (vi) CS_2, pyridine, reflux 15 h; (vii) PhCNS, DMF, TEA, reflux 10 h.

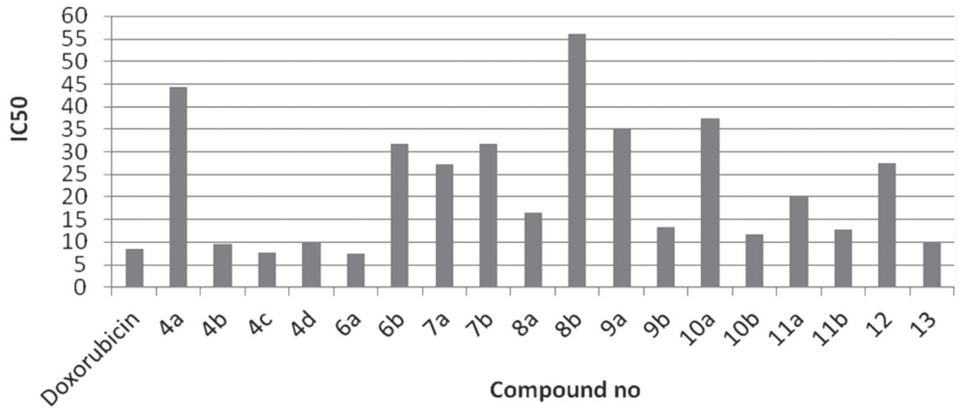


Fig. 2 IC50 in μM of the synthesised compounds and doxorubicin against human breast adenocarcinoma cell line (MCF7).

acetate. The IR spectra of **5a**,**b** revealed the appearance of absorption bands at 3362–3140 cm^{-1} corresponding to NH_2 and absorption bands at 2203 cm^{-1} attributed to CN group. The ^1H NMR spectra was consistent with the proposed structure.

Accordingly, treatment of **5a**,**b** with excess formamide produced 4-amino-5,7-disubstitutedpyrido[2,3-*d*]pyrimidines **6a**,**b** following reported reaction conditions.[23] The IR spectra proved as useful in tracing the disappearance of the C≡N stretching absorption of the parent compound **5a**,**b**. Whereas the ^1H NMR spectra of **6a** and **6b** displayed signals at δ 10.02 and 10.442 ppm respectively corresponding to NH_2 group. Furthermore, the mass spectrum of **6a** displayed molecular ion peaks at *m/z* 377 and 379 corresponding to (M^+) and (M+2) in a ratio 3 : 1. On the other hand, reacting compounds **5a**,**b** with urea or thiourea afforded **7a**,**b** and **8a**,**b** respectively, following the reaction conditions reported for the preparation of related compounds.[23] The structures of compounds **7a**,**b** and **8a**,**b** were confirmed by IR and ^1H NMR spectra. The IR spectra clearly showed the disappearance of the C≡N stretching absorption of the parent compound **5a**,**b** and the appearance of C=O (in compounds **7a**,**b**) or C=S (in compounds **8a**,**b**) stretching signals together with NH_2 absorption band. The ^1H NMR spectra of compounds **7a**,**b** and **8a**,**b** revealed two D_2O exchangeable singlet signals at δ 6.51–7.04 and δ 11.18–11.32 ppm corresponding to NH_2 and NH, respectively. Whereas, 2,4-diamino-5,7-disubstituted pyrido[2,3-*d*] pyrimidines **9a**,**b** were obtained by reacting **5a**,**b** with guanidine hydrochloride in refluxing methanol in the presence of sodium hydroxide. ^1H NMR spectra of compounds **9a**,**b** revealed the appearance of two exchangeable singlet signal at δ 5.02,6.95 and δ 7.23–8.5 ppm corresponding to NH_2 protons. Reacting **5a**,**b** with carbon disulfide in refluxing pyridine afforded the corresponding pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione derivatives **10a**,**b**, which were confirmed by spectral data**.** The IR spectra proved as useful in tracing the disappearance of the C≡N stretching absorption of the parent compound **5a**,**b** and the appearance of a C=S absorption. Finally, reaction of **5a**,**b** with phenyl isothiocyanate in refluxing dimethyl formamide in the presence of triethylamine yielded the corresponding 4-amino-3-phenyl-5,7-disubstituted pyrido[2,3-*d*]pyrimidine-2(3*H*)-thiones (**11a**,**b)** which were confirmed by spectral data. One of the principal objectives of the present work was the synthesis of substituted 1,8-naphthyridine derivatives**12** and **13** (Scheme 3), thereby, reacting **5b** with either benzylidene malononitrile in refluxing dioxane in the presence of piperidine or ethyl cyanoacetate in refluxing ethanol in the presence of triethylamine afforded compounds **12** and **13** respectively. The ^1H NMR spectra of compounds **12** and **13** displayed a singlet signal at δ 6.95 ppm corresponding to C-6 proton of naphthyridine. Both the analytical and spectral data (IR, ^1H NMR ^{13}C NMR and MS) of the newly synthesised compounds were in full agreement with the proposed structures.

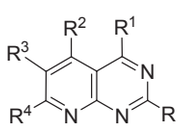
In vitro *anticancer screening*

The newly synthesised compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line, MCF-7. Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The IC_{50} (dose of the compound which caused a 50% reduction of survival values) are shown in Table 1 and represented graphically in Fig. 2. From the results in Table 1, it is evident that all the tested compounds displayed potent to moderate growth inhibitory activity, in particular compounds **4c** and **6a** (IC_{50} = 7.70 and 7.54 μM respectively) were found to be more potent and efficacious than doxorubicin (IC_{50} values 8.48 μM). Further studies are recommended to explore the mechanism of action as well as the effect of substitution on the anticancer effect of these compounds. We hope that the synthesised compounds serve as lead chemical entities for further modification to render them clinically useful drug agents.

Experimental

Melting points are uncorrected and determined in one-end open capillary tubes using Gallenkamp melting point apparatus MFB-595-010M (Gallenkamp, London, UK). Microanalysis was carried out at the Micro-analytical Unit, Faculty of Science, Cairo University and the Regional Centre for Microbiology and Biotechnology, Al-Azhar University. Analyses indicated were within ±0.4% of the theoretical values. Infrared Spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan), and expressed in wavenumber (cm^{-1}), using potassium bromide discs. The NMR spectra were recorded on a Varian Gemini 200 MHz and Varian Mercury VX-300 or Jeol-ECA500, 500 MHz Japan NMR spectrometer. ^1H NMR spectra were run at 300 MHz and ^{13}C NMR spectra were run at 125 MHz in dimethylsulfoxide (DMSO-d_6). Chemical shifts are quoted in δ and were related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu gas chromatograph mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC was carried out using Art. DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were chloroform: benzene: methanol [6.5: 3.5: 0.5] and the spots were visualised at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France). Compound **5a**,[24] benzylidenemalononitrile, [25] were obtained according to reported procedure.

Table 1 *In vitro* cytotoxic activities of the synthesised compounds against MCF-7 cancer cell line



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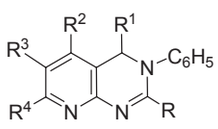

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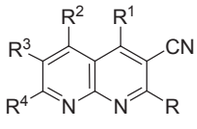

12 and **13**

Compound	R	R^1	R^2	R^3	R^4	IC50/μM[a]
Doxorubicin[b]	–	–	–	–	–	8.48
4a	NH_2	NH_2	3-$NO_2C_6H_4$		$-(CH_2)_4$	44.34
4b	NH_2	NH_2	2-$NO_2C_6H_4$		$-(CH_2)_4$	9.63
4c	NH_2	NH_2	2-$NO_2C_6H_4$		$-(CH_2)_5$	7.70
4d	NH_2	NH_2	2-$NO_2C_6H_4$		$-(CH_2)_6$	9.69
6a	H	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	7.54
6b	H	NH_2	C_4H_3S	H	3-$NO_2C_6H_4$	31.77
7a	=O	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	27.17
7b	=O	NH_2	C_4H_3S	H	3-$NO_2C_6H_4$	31.75
8a	=S	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	16.47
8b	=S	NH_2	C_4H_3S	H	3-$NO_2C_6H_4$	56.15
9a	NH_2	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	34.87
9b	NH_2	NH_2	C_4H_3S	H	3-$NO_2C_6H_4$	13.17
10a	=S	=S	3-$NO_2C_6H_4$	H	4-ClC_6H_4	37.24
10b	=S	=S	C_4H_3S	H	3-$NO_2C_6H_4$	11.66
11a	=S	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	20.32
11b	=S	NH_2	C_4H_3S	H	3-$NO_2C_6H_4$	12.89
12	C_6H_5	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	27.41
13	=O	NH_2	3-$NO_2C_6H_4$	H	4-ClC_6H_4	10.05

[a]The values given are means of three experiments.
[b]Doxorubicin was used as reference drug.

Synthesis of 2-oxo-4,5,6-trisubstituted-1,2-dihydropyridine-3-carbonitriles **2a–d***; general procedure*

A mixture of appropriate cycloalkanones **1a–d** (0.05 mol), appropriate aromatic aldehyde (0.05 mol), ethyl cyanoacetate (5.65 mL, 0.05 mol) and ammonium acetate (30.8 g, 0.4 mol) in butan-1-ol (150 mL) was heated under reflux for 8 h. The separated solid was filtered, washed with ethanol, dried and crystallised from appropriate solvent.

4-(3-Nitrophenyl)-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile **(2a):** Crystallised from butan-1-ol. Yield 63%; m.p. 291–292 °C; IR ν_{max}/cm^{-1}: 3280, 3150 (NH), 3080 (CH aromatic), 2949, 2910, 2866 (CH aliphatic), 2225 (C≡N), 1647(C=O), 1533, 1348 (NO_2); ^{1}H NMR ($CDCl_3$) δ ppm: 1.59–1.72 (m, 4H, 2C***H***$_2$), 2.04–2.12 (m, 2H, C***H***$_2$), 2.62–2.82 (m, 2H, C***H***$_2$), 7.88–8.39 (m, 4H, ArH), 12.56 (s, 1H, N***H***, exch. D_2O). Anal. calcd for $C_{16}H_{13}N_3O_3$ (295.28): C, 65.07; H, 4.43; N, 14.23; found: C, 64.99; H, 4.49; N, 14.13%.

4-(2-Nitrophenyl)-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile **(2b):** Crystallised from ethanol. Yield 55%; m.p. 276–278 °C; IR ν_{max}/cm^{-1}: 3300 (NH),3050 (CH aromatic), 2950, 2850 (CH aliphatic), 2200 (C≡N), 1640(C=O), 1520, 1340 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 1.51–1.66 (m, 4H, 2C***H***$_2$), 1.81–1.99 (m, 2H, C***H***$_2$), 2.63–2.69 (m, 2H, C***H***$_2$), 7.52 (d, 1H, *J*=7.5 Hz, H6 of ArH), 7.84 (t, 1H, *J*=7.5 Hz, H5 of ArH), 7.96 (t, 1H, *J*=7.8 Hz, H4 of ArH), 8.31 (d, 1H, *J*=7.8 Hz, H3 of ArH), 12.51 (s, 1H, N***H***, exch. D_2O). Anal. calcd for $C_{16}H_{13}N_3O_3$ (295.28): C, 65.07; H, 4.43; N, 14.23; found: C, 64.90; H, 4.38; N, 14.45%.

4-(2-Nitrophenyl)-2-oxo-2,5,6,7,8,9-hexahydro-1H-cyclohepta[b]pyridine-3-carbonitrile **(2c):** Crystallised from ethanol. Yield 68%; m.p. 273–275 °C; IR ν_{max}/cm^{-1}: 3300 (NH), 3050 (CH aromatic), 2920, 2820 (CH aliphatic), 2210 (C≡N), 1640 (C=O), 1540, 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 1.26–1.46 (m, 4H, 2C***H***$_2$), 1.62–1.84 (m, 2H, C***H***$_2$), 2.08–2.26 (m, 4H, 2C***H***$_2$), 2.82–2.98 (m, 2H, C***H***$_2$), 7.54–8.32 (m, 4H, ArH), 12.74 (br s, 1H, N***H***, exch. D_2O). Anal. calcd for $C_{17}H_{15}N_3O_3$ (309.30): C, 66.00; H, 4.88; N, 13.58; found: C, 66.14; H, 4.92; N, 13.74%.

4-(2-Nitrophenyl)-2-oxo-1,2,5,6,7,8,9,10-octahydrocycloocta[b]pyridine-3-carbonitrile **(2d):** Crystallised from ethanol. Yield 68%; m.p. >300 °C; IR ν_{max}/cm^{-1}: 3300 (NH), 3050 (CH aromatic), 2920, 2820 (CH aliphatic), 2210 (C≡N), 1640 (C=O), 1540, 1340 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 1.15–1.20 (m, 4H, 2C***H***$_2$), 1.37–1.41 (m, 4H, 2C***H***$_2$), 1.65–1.70 (m, 2H, C***H***$_2$), 2.16–2.24 (m, 4H, 2C***H***$_2$), 2.76–2.80 (m, 2H, C***H***$_2$), 7.56 (d, 1H, *J*=7.8 Hz, H6 of ArH), 7.84 (t, 1H, *J*=8.1 Hz, H5 of ArH), 7.96 (t, 1H, *J*=8.4 Hz, H4 of ArH), 8.33 (d, 1H, *J*=8.1 Hz, H3 of ArH), 12.66 (s, 1H, N***H***, exch. D_2O). Anal. calcd for $C_{18}H_{17}N_3O_3$ (323.32): C, 66.86; H, 5.29; N, 12.99; found: C, 66.79; H, 5.10; N, 13.41%.

Synthesis of 2-chloro-4,5,6-trisubstituted pyridine-3-carbonitriles **(3a–d)***; general procedure*

A mixture of the corresponding pyridones **2a–d** (0.0075 mol), *N, N*-dimethylaniline (10 mL), and phosphorous oxychloride (10 mL) was heated under reflux for 10 h. The reaction mixture was cooled and poured onto crushed ice. The formed solid was filtered, dried and crystallised from ethanol.

2-Chloro-4-(3-nitrophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile **(3a):** Yield 52%; m.p. 200–202 °C; IR ν_{max}/cm^{-1}: 3090 (CH aromatic), 2960, 2935 (CH aliphatic), 2229 (C≡N), 1530, 1340 (NO_2); ^{1}H NMR ($CDCl_3$) δ ppm: 1.76–1.91 (m, 4H, 2C***H***$_2$), 2.33–2.62 (m, 2H, C***H***$_2$), 2.98–3.04 (m, 2H, C***H***$_2$), 7.63–8.38 (m, 4H, ArH). Anal. calcd for $C_{16}H_{12}ClN_3O_2$ (313.73): C, 61.25; H, 3.85; N, 13.39; found: C, 61.45; H, 3.80; N, 13.65%.

2-Chloro-4-(2-nitrophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile **(3b):** Yield 55%; m.p. 154–156 °C; IR ν_{max}/cm^{-1}: 3050(CH aromatic), 2920 (CH aliphatic), 2200 (C≡N), 1520, 1340 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 1.64–1.66 (m, 2H, C***H***$_2$), 1.78–1.80 (m, 2H, C***H***$_2$), 2.10–2.35 (m, 2H, C***H***$_2$), 2.96–2.98 (m, 2H, C***H***$_2$), 7.63 (d, 1H, *J*=7.5 Hz, H6 of ArH**)**, 7.88 (t, 1H, *J*=7.5 Hz, H5 of ArH), 7.98 (t, 1H, *J*=7.5 Hz, H4 of ArH), 8.37 (d, 1H, *J*=8.1 Hz, H3 of ArH); MS *m/z*(%): 316.00 (M+3,12.45), 315(M+2, 5.95), 314(M+1, 25.71) 313(M$^+$,18.67), (C_5H_3,100). Anal. calcd for $C_{16}H_{12}ClN_3O_2$ (313.73): C, 61.25; H, 3.85; N, 13.39; found: C, 60.88; H, 3.80; N, 13.42%.

2-Chloro-4-(2-nitrophenyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b] pyridine-3-carbonitrile **(3c):** Yield 60%; m.p. 165–166 °C; IR ν_{max}/cm^{-1}: 3060 (CH aromatic), 2920 (CH aliphatic), 2200 (C≡N), 1520, 1340 (NO_2); ^{1}H NMR (DMSO–d_6) δ ppm: 1.33–1.46 (m, 2H, C$\boldsymbol{H}_2$), 1.66–1.76 (m, 4H, 2C$\boldsymbol{H}_2$), 2.40–2.43 (m, 2H, C$\boldsymbol{H}_2$), 3.10–3.12 (m, 2H, C$\boldsymbol{H}_2$), 7.60 (d, 1H, *J*=7.5 Hz, H6 of ArH), 7.87 (t, 1H, *J*=7.5 Hz, H5 of ArH), 7.96 (t, 1H, *J*=7.8 Hz, H4 of ArH), 8.36 (d, 1H, *J*=7.8 Hz, H3 of ArH). Anal. calcd for $C_{17}H_{14}ClN_3O_2$ (327.75): C, 62.29; H, 4.30; N, 12.82; found: C, 62.47; H, 4.28; N, 13.01%.

2-Chloro-4-(2-nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[b] pyridine-3-carbonitrile **(3d):** Yield 54%; m.p. 157–158 °C; IR ν_{max}/cm^{-1}: 3064 (CH aromatic), 2937, 2926, 2856 (CH aliphatic), 2210 (C≡N), 1520, 1320 (NO_2); ^{1}H NMR (DMSO–d_6) δ ppm: 1.18–1.51 (m, 6H, 3C$\boldsymbol{H}_2$), 1.71–1.82 (m, 2H, C$\boldsymbol{H}_2$), 2.12–2.14 (m, 2H, C$\boldsymbol{H}_2$), 3.05–3.12 (m, 2H, C$\boldsymbol{H}_2$), 7.71–8.43 (m, 4H, ArH). Anal. calcd for $C_{18}H_{16}ClN_3O_2$ (341.78): C, 63.25; H, 4.71; N, 12.29; found: C, 63.41; H, 4.76; N, 12.42%.

*Synthesis of 2,4-diamino-5,6,7-trisubstituted pyrido[2,3-*d*]pyrimidines* **(4a–d)***; general procedure*

Guanidine hydrochloride (1.84 g, 0.022 mol) was added to a cold solution of sodium (0.506 g, 0.022 mol) in absolute ethanol (50 mL). The separated sodium chloride was filtered off and washed with ethanol, the filtrate was evaporated under reduced pressure. The formed oily mass was diluted with pyridine (5 mL) and finally the corresponding 2-chloro-4,5,6-trisubstituted pyridine-3-carbonitrile **3a–d** (0.0033 mol) was added. The reaction mixture was heated under reflux for 5 h, cooled and finally poured onto water (25 mL). The formed precipitate was filtered, dried and crystallised from DMF/H_2O.

5-(3-Nitrophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline-2,4-diamine **(4a):** Yield 72%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3283, 3180 (NH_2), 3050(CH aromatic), 2950, 2850 (CH aliphatic), 1580, 1380 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 1.22–1.25 (m, 2H, C$\boldsymbol{H}_2$), 1.82–1.90(m, 2H, C$\boldsymbol{H}_2$), 2.73–2.91(m, 4H, 2C$\boldsymbol{H}_2$), 6.58 (br s, 2H, N$\boldsymbol{H}_2$, exch. D_2O), 7.19–8.37 (m, 4H, ArH), 11.89 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O). Anal. calcd for $C_{17}H_{16}N_6O_2$ (336.35): C, 60.71; H, 4.79; N, 24.99; found: C, 60.73; H, 4.79; N, 25.00%.

5-(2-Nitrophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline-2,4-diamine **(4b):** Yield 68%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3400–3200 (NH_2), 3100 (CH aromatic), 2920, 2850 (CH aliphatic), 1580, 1330 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 1.17–1.23 (m, 2H, C$\boldsymbol{H}_2$), 1.71–1.98 (m, 2H, C$\boldsymbol{H}_2$), 2.74–2.89(m, 4H, 2C$\boldsymbol{H}_2$), 7.15 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O), 7.48–7.96 (m, 4H, ArH), 12.02 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O); ^{13}C NMR (DMSO) δ ppm: 22.98, 24.69, 46.19, 52.15.23, 93.91, 110.03, 115.54, 116.28, 130.82, 134.69, 136.13, 138.22, 141.21, 148.49, 155.58, 158.68, 162.23; Anal. calcd for $C_{17}H_{16}N_6O_2$ (336.35): C, 60.71; H, 4.79; N, 24.99; found: C, 60.76; H, 4.69; N, 25.20%.

*5-(2-Nitrophenyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b] pyrido[2,3-*d*]pyrimidine-2,4-diamine* **(4c):** Yield 84%; m.p. 255–258 °C; IR ν_{max}/cm^{-1}: 3480, 3310 (NH_2), 3090 (CH aromatic), 2920 (CH aliphatic) 1580, 1340 (NO_2); ^{1}H NMR (DMSO–d_6) δ ppm: 1.76–1.91 (m, 6H, 3C$\boldsymbol{H}_2$), 3.01–3.21 (m, 4H, 2C$\boldsymbol{H}_2$), 6.46 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O), 7.25–8.35 (m, 4H, ArH), 12.02 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O); ^{13}C NMR (DMSO) δ ppm: 26.09, 29.34, 29.77, 31.23, 38.15, 112.12, 114.41, 118.42, 119.0, 127.89, 132.36, 138.33, 149.74, 155.70, 158.58, 158.88, 167.2, 169.87. Anal. calcd for $C_{18}H_{18}N_6O_2$ (350.37): C, 61.70; H, 5.18; N, 23.99; found: C, 61.80; H, 5.00; N, 24.06%.

*5-(2-Nitrophenyl)-5,6,7,8,9,10-hexahydrocycloocta[b]pyrido[2,3-*d*] pyrimidine-2,4-diamine* **(4d):**Yield 75%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3400–3200 (NH_2), 3050 (CH aromatic), 2920, 2820 (CH aliphatic), 1520, 1340 (NO_2); ^{1}H NMR (DMSO–d_6) δ ppm: 1.36–1.50 (m, 4H, 2C$\boldsymbol{H}_2$), 1.76–1.80 (m, 2H, C$\boldsymbol{H}_2$), 1.85–1.92 (m, 2H, C$\boldsymbol{H}_2$), 2.86–2.88 (m, 2H, C$\boldsymbol{H}_2$), 3.06–3.12 (m, 2H, C$\boldsymbol{H}_2$), 6.41 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O), 7.23 (d, 1H, *J*=6.9 Hz, H6 of ArH), 7.47 (t, 1H, *J*=8.7 Hz, H4 of ArH), 7.52 (t, 1H, *J*=6.9 Hz, H5 of ArH), 8.33 (d, 1H, *J*=8.7 Hz, H3 of ArH), 12.01 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O); ^{13}C NMR (DMSO) δ ppm: 26.41, 26.61, 30.12, 30.57, 35.50, 42.55, 96.91, 112.17, 125.72, 128.49, 131.59, 131.89, 135.32, 146.8, 149.9, 156.28, 156.19, 161.69, 165.37. Anal. calcd for $C_{19}H_{20}N_6O_2$ (364.40): C, 62.62; H, 5.53; N, 23.06; found: C, 62.47; H, 5.51; N, 23.01%.

Synthesis of 2-amino-4,6-diarylpyridine-3-carbonitriles **5a**,**b***; general procedure*

A mixture of substituted acetophenone either **Ie** or **1f** (0.01 mol), appropriate aromatic aldehydes (0.01 mol), malononitrile (5.3 g, 0.08 mol) and ammonium acetate (6.16 g, 0.08 mol) in butan-1-ol (10 mL) was heated under reflux for 8 h then cooled. The obtained precipitate was filtered, washed with ethanol, dried and crystallised from suitable solvent to afford **5a**,**b**.

2-Amino-6-(4-chlorophenyl)-4-(3-nitrophenyl)nicotinonitrile **(5a):** Crystallised from butan-1-ol, yield 73%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3300–3100 (NH_2), 3020(CH aromatic), 2200 (CN), 1550, 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.61 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O), 7.11–8.86 (m, 9H, 8ArH and ***H5***). Anal. calcd for $C_{18}H_{11}ClN_4O_2$ (350.76): C, 61.64; H, 3.16; N, 15.97; found: C, 61.47; H, 3.13; N, 15.64%.

2-Amino-6-(3-nitrophenyl)-4-(thiophen-2-yl)nicotinonitrile **(5b):** Crystallised from ethanol. Yield 80%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3362–3140 (NH_2), 3080(CH aromatic), 2203 (CN), 1537, 1330 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.86 (s, 2H, N$\boldsymbol{H}_2$, exch. D_2O), 7.11–7.86 (m, 8H, 7ArH and ***H5***). Anal. calcd for $C_{16}H_{10}N_4O_2S$ (322.34): C, 59.62; H, 3.13; N, 17.38; found: C, 59.52; H, 3.12; N, 17.35%.

*Synthesis of 4-amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidines* **(6a**,**b)***; general procedure*

A mixture of the appropriate 2-aminopyridine-3-carbonitriles **5a**,**b** (0.01 mol) and excess formamide (15 mL) was refluxed in an oil-bath for 15 h at 210 °C. The mixture was cooled. The separated solid was filtered, dried and crystallised from ethanol.

*7-(4-Chlorophenyl)-5-(3-nitrophenyl)pyrido[2,3-*d*]pyrimidin-4-amine* **(6a):** Yield 58%; m.p. 255–257 °C; IR ν_{max}/cm^{-1}: 3293, 3168 (NH_2), 3050 (CH aromatic), 1559, 1337 (NO_2); ^{1}H NMR (DMSO–d_6) δ ppm: 7.39–8.91 (m, 8H, ArH), 8.02 (s, 1H, ***H***2), 8.53 (s, 1H, ***H***6), 10.44 (s, 2***H***, NH_2 exch.D_2O); ^{13}C NMR (DMSO) δ ppm: 106.88, 121.19, 122.08, 123.99, 129.26, 129.77, 130.62, 130.89, 131.08, 133.24, 135.06, 135.85, 136.56, 139.90, 147.94, 148.60, 155.01, 156.05, 159.66; MS *m/z* (%): 379.22 (M+2, 6.52), 377.70 (M^+, 22.23). Anal. calcd for $C_{19}H_{12}ClN_5O_2$ (377.78): C, 60.41; H, 3.20; N, 18.54; found: C, 60.98; H, 3.45; N, 18.88%.

*7-(3-Nitrophenyl)-5-(thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4-amine* **(6b):** Yield 83%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3327–3193 (NH_2), 3050 (CH aromatic), 1561, 1338 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.85–8.95 (m, 7H, ArH and thienyl ***H***), 7.99 (s, 1H, ***H***2), 8.35 (s,1H, ***H***6), 10.01 (s, 2***H***, NH_2 exch.D_2O). Anal. calcd for $C_{17}H_{11}N_5O_2S$ (349.37): C, 58.44; H, 3.17; N, 20.05; found: C, 58.89; H, 3.09; N, 20.43%.

*Synthesis of 4-amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidin-2(1H)-ones* **(7a**,**b)***; general procedure*

A mixture of the appropriate 2-aminopyridine-3-carbonitrile (**5a**,**b)** (0.01 mol) and urea (1.2 g, 0.02 mol) was heated in an oil bath at 120–130 °C for 2 h. The temperature was then raised to 180 °C for another 2 h. The residue was washed with water then ethanol and finally crystallised from appropriate solvent.

*4-Amino-7-(4-chlorophenyl)-5-(3-nitrophenyl)pyrido[2,3-*d*] pyrimidin-2(1H)-one* **(7a):** Crystallised from butan-1-ol. Yield 55%; m.p. 275–277 °C; IR ν_{max}/cm^{-1}: 3330–3200 (NH, NH_2), 3050 (CH aromatic), 1700 (C=O),1520, 1340 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.93 (s, 1H, ***H6***), 7.01 (s, H, N***H***, exch.D_2O), 7.58–8.13 (m, 3H, ***H4***, ***H5*** and ***H6*** of 3-$NO_2C_6H_4$), 8.85 (d, 2H, *J*=8 Hz, ***H3***, ***H5*** of 4-ClC_6H_4), 8.36 (d, 2H, *J*=7.8 Hz, ***H2***, ***H6*** of 4-ClC_6H_4), 8.47 (s, 1H, ***H2*** of 3-$NO_2C_6H_4$), 11.18 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O); ^{13}C NMR (DMSO) δ ppm: 94.87, 95.32, 116.19, 119.08, 123.99, 124.71, 129.26, 130.87, 131.08, 135.06, 135.85, 136.56, 139.25, 147.96, 148.32, 149.37, 150.46, 154.54; MS *m/z* (%): 393.15 (M^+, 41.18), 104.20 (100). Anal. calcd for $C_{19}H_{12}ClN_5O_3$ (393.78): C, 57.95; H, 3.07; N, 17.78; found: C, 57.69; H, 3.30; N, 17.20%.

*4-Amino-7-(3-nitrophenyl)-5-(thiophen-2-yl)pyrido[2,3-*d*] pyrimidin-2(1H)-one* **(7b):** Crystallised from ethanol. Yield 80%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3419, 3350, 3197 (NH, NH_2), 3100 (CH aromatic), 1676 (C=O),1540, 1368 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.88 (s, 1H, N***H***, exch. D_2O), 7.13–7.92 (m, 8H, 7ArH+***H6***), 11.22 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O); MS *m/z* (%): 365.24 (M^+, 58.59), 359 (100). Anal. calcd for $C_{17}H_{11}N_5O_3S$ (365.37): C, 55.88; H, 3.03; N, 19.17; found: C, 55.83; H, 3.40; N, 19.30%.

*Synthesis of 4-amino-5,7-disubstituted pyrido[2,3-*d*]pyrimidine-2(1H)-thiones* **(8a**,**b)***; general procedure*

A mixture of thiourea (1.52 g, 0.02 mol) and the appropriate 2-aminopyridine-3-carbonitrile **5a**,**b** (0.01 mol) was heated in an oil-bath at 120–130 °C for 2 h then the temperature was raised to 180 °C for additional 2 h. The residue was washed with water then ethanol and finally crystallised from appropriate solvent.

*4-Amino-7-(4-chlorophenyl)-5-(3-nitrophenyl)pyrido[2,3-*d*]pyrimidine-2(1H)-thione* **(8a):** Crystallised from n-butanol. Yield 62%; m.p. 262–264 °C; IR ν_{max}/cm^{-1}: 3450–3300 (NH, NH_2), 3050 (CH aromatic), 1090 (C=S), 1560, 1330 (NO_2); ^{1}H NMR (DMSO–d_6) δ ppm: 6.97 (s, 1H, ***H6***), 7.04 (s, 1H, N$\boldsymbol{H}_2$,exch. D_2O), 7.62–7.90 (m, 3H, ***H4***, ***H5*** and ***H6*** of 3-$NO_2C_6H_4$), 7.88 (d, 2H, *J* = 7.6 Hz, ***H3***, ***H5*** of 4-ClC_6H_4), 8.15 (d, 2H, *J* = 7.6 Hz, ***H2***, ***H6*** of 4-ClC_6H_4), 8.51(s, 2H, N$\boldsymbol{H}_2$,exch. D_2O); MS *m/z* (%): 412 (M + 2, 0.6), 410 (M^+, 1.9), 68 (100). Anal. calcd for $C_{19}H_{12}ClN_5O_2S$ (409.85): C, 55.68; H, 2.95; N, 17.09; found: C, 55.85; H, 2.68; N, 17.10%.

*4-Amino-7-(3-nitrophenyl)-5-(thiophen-2-yl)pyrido[2,3-*d*]pyrimidine-2(1H)-thione* **(8b):** Crystallised from ethanol. Yield 55%; m.p. 178–180 °C; IR ν_{max}/cm^{-1}: 3377, 3277, 3176 (NH, NH_2), 3096 (CH aromatic), 1520, 1340 (NO_2), 1087 (C=S); ^{1}H NMR (DMSO-d_6) δ ppm: 6.93 (s, 1H, N***H***, exch.D_2O), 7.42–8.07 (m, 8H, 7ArH + ***H6***), 11, 32 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O); MS *m/z* (%): 381.762 (M^+, 100). Anal. calcd for $C_{17}H_{11}N_5O_2S_2$ (381.42): C, 53.53; H, 2.91; N, 18.36; found: C, 53.44; H, 3.26; N, 18.08%.

*Synthesis of 2,4-diamino-5,7-disubstituted pyrido[2,3-*d*]pyrimidines* **(9a**,**b)***; general procedure*

Guanidine hydrochloride (2.1 g, 0.025 mol) was added to a solution of sodium (2.6 g, 0.113 mol) in methanol (95 mL), the precipitated sodium chloride was filtered off. The *o*-amino cyano derivative **5a**,**b** (0.015 mol) was added to the filtrate. The reaction mixture was heated under reflux for 6 h. The mixture was cooled and the separated solid was filtered, washed well with ethanol and crystallised from butan-1-ol.

*7-(4-Chlorophenyl)-5-(3-nitrophenyl)pyrido[2,3-*d*]pyrimidine-2,4-diamine* **(9a):** Yield 65%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3410–3200 (NH_2), 3050 (CH aromatic), 1540, 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.95 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O), 7.34–8.51 (m, 11H, 8ArH + ***H6*** + N$\boldsymbol{H}_2$, exch. D_2O); MS *m/z* (%): 395 (M + 3, 45.16), 393 (M + 1, 67.74), 258 (M–Cl + 1, 61.29), 213.65 (100). Anal. calcd for $C_{19}H_{13}ClN_6O_2$ (392.80): C, 58.10; H, 3.34; N, 21.40; found: C, 58.10; H, 3.39; N, 21.73%.

*7-(3-Nitrophenyl)-5-(thiophen-2-yl)pyrido[2,3-*d*]pyrimidine-2,4-diamine* **(9b):** Yield 68%; m.p. > 300 °C; IR ν_{max}/cm^{-1}: 3463, 3349, 3245 (NH_2), 3019 (CH aromatic), 1566, 1380 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 5.02 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O), 7.23–8.28 (m, 10H, 7ArH + ***H6*** + N$\boldsymbol{H}_2$, exch.D_2O); ^{13}C NMR (DMSO) δppm: 94.01, 112.12, 114.17, 114.70, 117.0, 119.0, 120.28, 129.44, 130.07, 133.71, 135.26, 154.02, 154.13, 159.39, 159.70, 160.43. Anal. calcd for $C_{17}H_{12}N_6O_2S$ (364.37): C, 56.03; H, 3.32; N, 23.06; found: C, 56.30; H, 3.29; N, 22.97%.

*Synthesis of 5,7-disubstituted pyrido[2,3-*d*]pyrimidine-2,4(1H,3H)-dithiones* **(10a**,**b)***; general procedure*

Carbon disulfide (3.04 g, 0.04 mol) was added to a solution of 2-aminopyridine-3-carbonitriles **5a**,**b** (0.01 mol) in pyridine (15 mL), the mixture was heated under reflux for 15 h. After cooling excess pyridine was removed by distillation, the precipitated solid was filtered, washed with ethanol and finally crystallised from appropriate solvent.

*7-(4-Chlorophenyl)-5-(3-nitrophenyl)pyrido[2,3-*d*]pyrimidine-2,4(1H,3H)-dithione* **(10a):** Crystallise from DMF/H_2O. Yield 62%; m.p. 266–268 °C; IR ν_{max}/cm^{-1}: 3360, 3200 (NH), 3035 (CH aromatic), 1527, 1346 (NO_2), 1100 (C=S); ^{1}H NMR (DMSO–d_6) δ ppm: 6.95–8.5 (m, 9H, 8ArH + ***H6***), 7.03 (s, 1H, N1***H***, exch.D_2O), 8.5 (s, 1H, N3***H***, exch. D_2O); MS *m/z* (%): 428 (M + 2, 10.4), 426 (M^+,3.7), 374 (100). Anal. calcd for $C_{19}H_{11}ClN_4O_2S_2$ (426.90): C, 53.46; H, 2.60; N, 13.12; found: C, 53.35; H, 2.29; N, 13.28%.

*7-(3-Nitrophenyl)-5-(thiophen-2-yl)pyrido[2,3-*d*]pyrimidine-2,4(1H,3H)-dithione* **(10b):** Crystallise from ethanol, Yield 58%; m.p. 258–260 °C; IR ν_{max}/cm^{-1}: 3377, 3277 (NH), 3095 (CH aromatic), 1521, 1340 (NO_2), 1085 (C=S); ^{1}H NMR (DMSO-d_6) δ ppm: 6.89 (s, 1H, N1***H*** exch. D_2O), 7.06 (s, 1H, N3***H***, exch D_2O), 7.17–8.6 (m, 8H, 7ArH + ***H6***); MS *m/z* (%): 398.30 (M^+, 100). Anal. calcd for $C_{17}H_{10}N_4O_2S_3$ (398.48): C, 51.24; H, 2.53; N, 14.06; found: C, 51.60; H, 2.92; N, 14.28%.

*Synthesis of 4-amino-3-phenyl-5,7-disubstitutedpyrido[2,3-*d*]pyrimidine-2(3H)-thiones* **(11a**,**b)***; general procedure*

A few drops of triethylamine was added to a mixture of the appropriate 2-aminopyridine-3-carbonitrile **5a**,**b** (0.005 mol) and phenyl isothiocyanate (0.67 g, 0.005 mol) in DMF (30 mL). The mixture was heated under reflux for 10 h, then left to cool and finally poured onto cold water (25 mL). The formed solid was filtered, washed with ethanol and crystallised from butan-1-ol.

*4-Amino-7-(4-chlorophenyl)-5-(3-nitrophenyl)-3-phenylpyrido[2,3-*d*]pyrimidine-2(3H)-thione* **(11a):** Yield 62%; m.p. 262–263 °C; IR ν_{max}/cm^{-1}: 3377, 3277 (NH_2), 3095 (CH aromatic), 1521, 1340 (NO_2), 1083 (C=S); ^{1}H NMR (DMSO-d_6) δ ppm: 7.02 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O), 7.02–8.52 (m, 14H, 13ArH + ***H6***); MS *m/z* (%): 487.2(M + 2, 1.2), 485.1(M^+, 3.7), 85.9 (100) Anal. calcd for $C_{25}H_{16}ClN_5O_2S$ (485.94): C, 61.79; H, 3.32; N, 14.41; found: C, 61.75; H, 3.24; N, 14.45%.

*4-Amino-7-(3-nitrophenyl)-3-phenyl-5-(thiophen-2-yl)pyrido[2,3-*d*]pyrimidine-2(3H)-thione* **(11b):** Yield 58%; m.p. 188–190 °C; IR ν_{max}/cm^{-1}: 3350, 3250 (NH_2), 3050 (CH aromatic),1520, 1370 (NO_2) 1040 (C=S); ^{1}H NMR (DMSO-d_6) δ ppm: 6.98 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O), 7.2–7.84 (m, 12H, ArH), 7.99 (s, 1H, ***H***6). Anal. calcd for $C_{23}H_{15}N_5O_2S_2$ (457.51): C, 60.37; H, 3.30; N, 15.30; found: C, 60.69; H, 3.05; N, 15.00%.

4-Amino-7-(4-chlorophenyl)-5-(3-nitrophenyl)-2-phenyl-1,8-naphthyridine-3-carbonitrile **(12):** A mixture of the benzylidenemalononitriles (0.15 g; 0.001 mol), and corresponding 2-aminopyridine-3-carbonitrile derivatives **5a** (0.35 g; 0.001 mol) and piperidine (1 mL) was dissolved in dioxane (10 mL). The mixture was heated under reflux for 3 h. The solvent was removed under reduced pressure. The formed solid was filtered, washed and crystallised from DMF/H_2O. Yield 74%; m.p. 273–274 °C; IR ν_{max}/cm^{-1}: 3300 (NH_2), 3050 (CH aromatic), 2200 (CN), 1520, 1340 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 6.94 (s, 1H, ***H***-6 of naphthridine), 6.96 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O), 7.59–8.47 (m, 13H, ArH).; MS *m/z* (%): 479.75 (M + 3, 9.3), 478 (M + 1, 9.3), 374 (M–C_6H_4CN, 100). Anal. calcd for $C_{27}H_{16}ClN_5O_2$ (477.88): C, 67.85; H, 3.37; N, 14.65; found: C, 68.05; H, 3.70; N, 14.74%.

4-Amino-7-(4-chlorophenyl)-5-(3-nitrophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carbonitrile **(13):** A mixture of 2-amino-6-(4-chlorophenyl)-4-(3-nitrophenyl)nicotinonitrile (**5a**) (3.50 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and triethylamine (2 mL) in ethanol 95% (30 mL) was heated under reflux for 3 h. The formed solid was filtered, washed with ethanol and crystallised from ethanol. Yield 58%; m.p. 270–272 °C; IR ν_{max}/cm^{-1}: 3480, 3360, 3200 (NH_2, NH), 3050 (CH aromatic), 1640 (C=O), 1520, 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ ppm: 7.99 (s, 2H, N$\boldsymbol{H}_2$, exch.D_2O), 6.99–8.4 (m, 9H, 8ArH + ***H6***), 8.51(s, 1H, N***H***, exch.D_2O); MS *m/z* (%): 419.75 (M + 3, 16.67), 418.25 (M + 1, 18.33), 55 (100). Anal. calcd for $C_{21}H_{12}ClN_5O_3$ (417.79): C, 60.36; H, 2.89; N, 16.76; found: C, 60.69; H, 3.19; N, 16.36%.

Cytotoxic activity studies

Anticancer activity studies were done at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

Measurement of potential cytotoxicity: The cytotoxic activity of the newly synthesised compounds was measured *in vitro* on human breast adenocarcinoma cell line (MCF-7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan *et al.* [26] Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 1, 2.5, 5 and 10 mg mL^{-1}) were added to the cell monolayer. Triplicate

wells were prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37 °C in atmosphere of 5% CO_2. After 48 h, cells were fixed with trichloroacetic acid, washed with water and stained for 30 min with 0.4% (wt/vol). Sulforhodamine-B stain dissolved with 1% acetic acid. Excess stain was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and compound concentration was plotted and IC_{50} [the concentration required for 50% inhibition of cell viability] was calculated for each compound and results are given in Table 1.

The authors thank all members of the department of Cancer Biology, Pharmacology Unit, National Cancer Institute, Cairo, Egypt, for carrying out the cytotoxicity testing.

Received 15 November 2013; accepted 9 January 2014
Paper 1302284 doi: 10.3184/174751914X13910886393992
Published online: 7 March 2014

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