Synthesis and antifungal activity of novel polyheterocyclic compounds containing fused 1,2,4-triazine moiety

Magdy A. Ibrahim,* Reda M. Abdel-Rahman, Ali M. Abdel-Halim, Salah S. Ibrahim, and Hassan A. Allimony

Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, 11711, Cairo-Egypt
E-mail: magdy_ahmed1977@yahoo.com

Abstract

3-Amino-4-(4-chlorophenyl)-7-hydrazino-8*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1`,5`-*a*]pyridine-5-carbonitrile (**4**) was synthesized from 4-(4-chlorophenyl)-1,6-diamino-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile (**1**). Reaction of **4** with α,β-bifunctional compounds gave pyrazolotriazinotriazolopyridines (**8-14**). The behavior of **4** towards condensation reactions with indole-2,3-dione in different media gave different products **15-16**. Acetylation of **16** led to different products depending on the reaction conditions. Structures of the products have been deduced from analytical and spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectra). Some of the products were screened for antifungal activity.

Keywords: Synthesis, *o*-diamine, triazolopyridine, pyrazolotriazinotriazolopyridine, fungicidal activity

Introduction

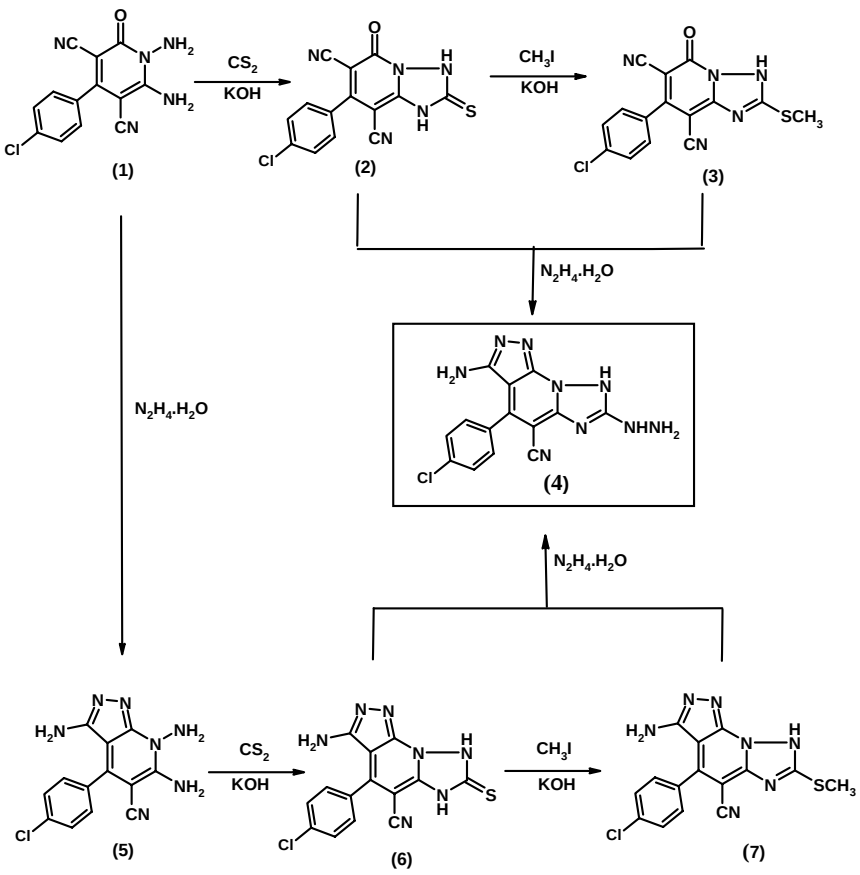
Polyfunctional pyridines are highly reactive reagents that have been used extensively in heterocyclic synthesis[1-3] and that possess biological as well as pharmacological activity.[4-6] Triazolopyridines are also interesting compounds due to their pronounced biological activity, as they can be used as antidepressants.[7-8] Various 1,2,4-triazine derivatives are well known to possess an array of physiological activities, such as anticancer, muscle relaxant, hypnotic, anti-inflammatory, diuretic and antihypertensive activities.[9-12]

In continuation of our work in the area of fused 1,2,4-triazines[13-19] and their heterocyclization via ring closing reactions with α,β-bifunctional reagents,[20-25] the present work aimed at the synthesis of fused heteropolycyclic nitrogen systems containing a fused

1,2,4-triazine moiety starting from 4-(4-chlorophenyl)-1,6-diamino-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (**1)** and evaluation of their antifungal activity.

Results and Discussion

The hydrazine derivative **4**, as starting material for polyfused heterocyclic systems, was obtained from the reaction of 4-(4-chlorophenyl)-1,6-diaminopyridine-3,5-dicarbonitrile (**1**) with CS_2/KOH to give thioxotriazolopyridinone **2** followed by hydrazinolysis. Also, methylation of **2** with MeI/KOH produced the 2-methylthio derivative **3** which on hydrazinolysis afforded **4**.[26-27] On the other hand, compound **4** was also obtained using alternative pathways. Treatment of diamine **1** with hydrazine hydrate afforded 4-(4-chlorophenyl)-3,6,7-triamino-7*H*-pyrazolo [3,4-*b*]pyridine-5-carbonitrile (**5**). Heating an ethanolic solution of **5** with CS_2/KOH at reflux yielded thioxopyrazolotriazolopyridine **6** which on methylation yielded the methylthio derivative **7**. Hydrazinolysis of **6** and/or **7** furnished the hydrazino derivative **4** (Scheme 1). Compound **4** was confirmed by its elemental analysis and spectral data. The IR spectrum revealed the disappearance of the absorption band at 1669 cm^{-1} assigned to the C=O group of the pyridinone ring in compound **1** and a new band was observed at 1637 cm^{-1} corresponding to an azomethine group in addition to the hydrazino group at 3469 and 3308 cm^{-1}. Its ^{13}C NMR spectrum showed one signal at δ 117.1 ppm corresponding to one cyano group. The mass spectrum of **4** showed a molecular ion peak at *m/e* 340 with the base peak at *m/e* 285, presumably due to the higher stability of the fused pyrazolopyridine system.

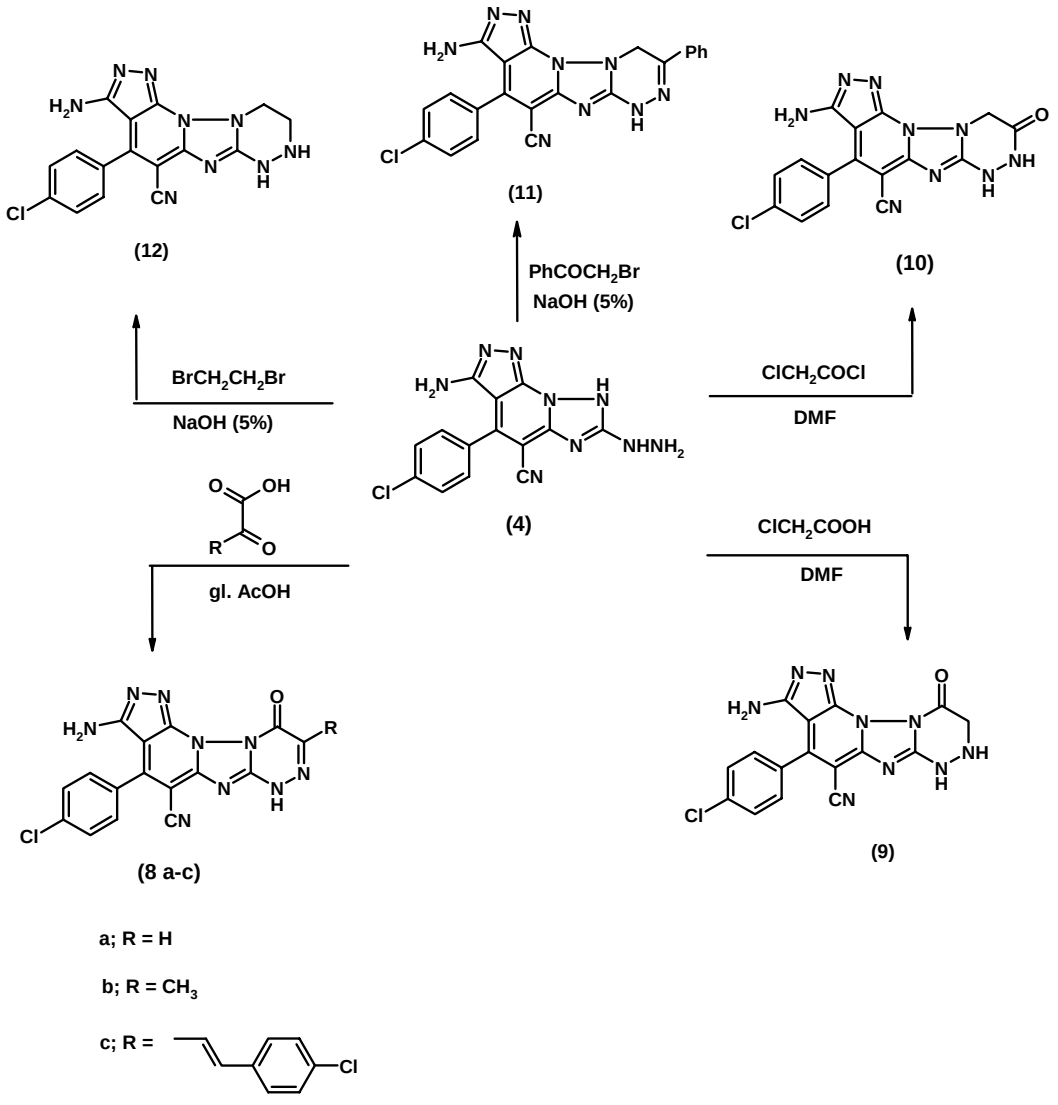


Scheme 1. Synthetic pathway for the preparation of target compound **4**.

The target compound **4** was used for the synthesis of polyfused systems. Thus, fused triazinones **8a-c** were obtained from cyclocondensation of **4** with α-oxoacids, namely; glyoxalic, pyruvic, *p*-chlorostyrylglyoxalic acid in glacial acetic acid[28] (Scheme 2).

The isomeric fused triazinones **9** and **10** were obtained from cyclocondensation of compound **4** with monochloroacetic acid and/or chloroacetyl chloride,[29] respectively (Scheme 2). ^{13}C NMR spectra of compounds **9** and **10** showed characteristic signals at δ 35.7 and 35.9 ppm assigned to CH_2 carbons, respectively (Figure 1).

Perhydro fused 1,2,4-triazinotriazoles **11** and **12** were obtained from boiling compound **4** with phenacyl bromide and 1,2-dibromoethane[30-31] in ethanolic NaOH (5%), respectively (Scheme 2).



Scheme 2. Synthetic pathway for the preparation of compounds **8-12**.

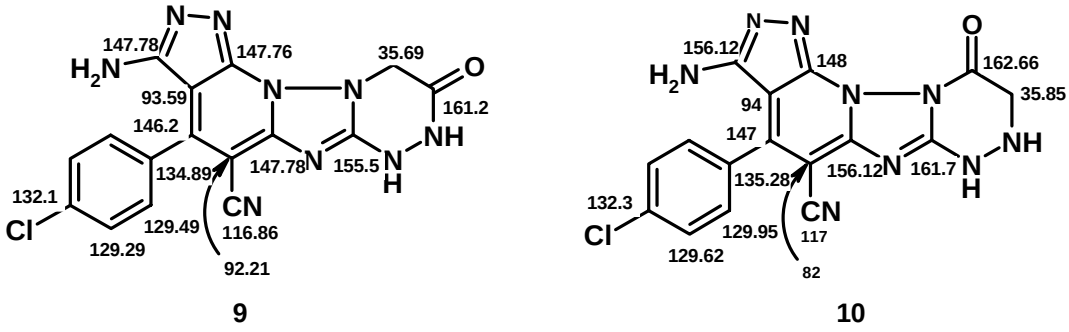


Figure 1. ^{13}C NMR of compounds **9** and **10**.

Reactions of **4** with α,β-dicarbonyl compounds have been investigated. Thus, treatment of **4** with diethyl oxalate[32] in boiling DMF produced the 9,10-dioxo derivative **13**. Also, cyclocondensation of **4** with benzoin in glacial acetic acid yielded the 9,10-diphenyl derivative **14** (Scheme 3). On the other hand, cyclocondensation of compound **4** with indole-2,3-dione in boiling DMF afforded 3-amino-4-(4-chlorophenyl)-7*H*-indolo[2,3-*e*]pyrazolo[3``,4``:6`,5`] pyrido[1`,2`:2,3][1,2,4]triazolo[5,1-*c*][1,2,4]triazine-5-carbonitrile (**15**), while treatment of an equimolar ratio of **4** and indole-2,3-dione in ethanolic NaOH solution afforded 3-amino-9-(2-aminophenyl)-4-(4-chlorophenyl)-10-oxo-12-hydro-7*H*-pyrazolo[4,3-*e*][1,2,4]triazino[3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (**16**) (Scheme 3). Compound **15** was obtained authentically by cyclization of **16** in glacial acetic acid and a few drops of concentrated H_2SO_4, where the absorption band of C=O group disappeared and showed co-identical IR spectra. Acetylation of compound **16** using acetic anhydride furnished the diacetyl derivative **17**. The IR spectrum revealed the presence of new absorption bands at 1720, 1655 cm^{-1} for two C=O groups and at 3422 cm^{-1} for NH group (Scheme 3).

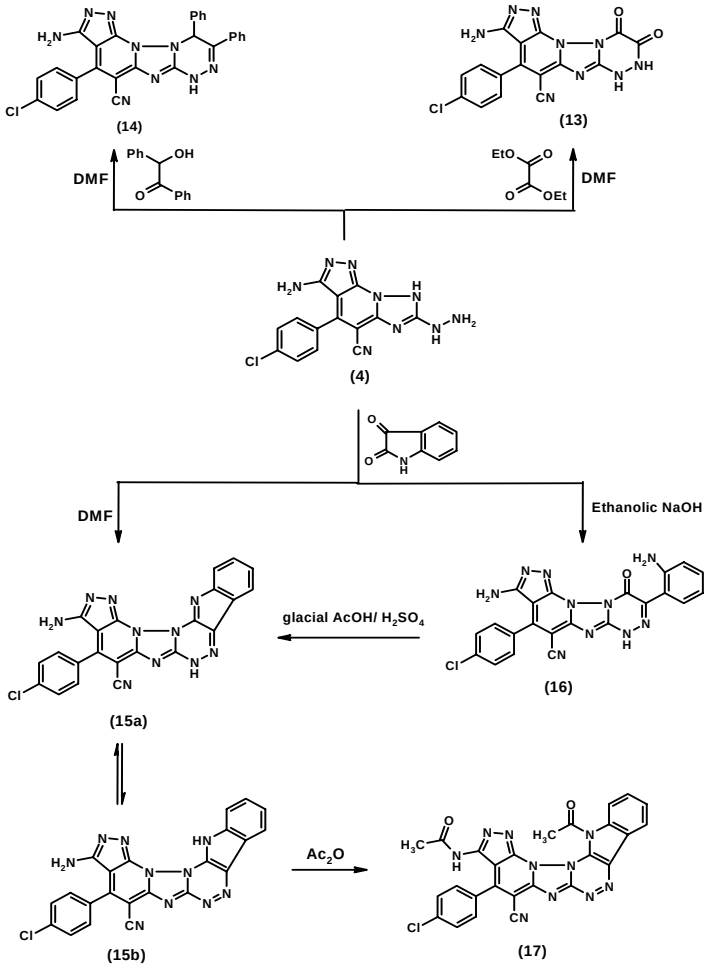
Finally, the behavior of compound **16** towards acetylation reactions has been studied under different conditions. Thus, refluxing **16** with glacial acetic acid afforded the mono-acetyl derivative **18**. However, when the reaction was carried out in boiling glacial acetic acid containing a few drops of acetic anhydride, the diacetyl derivative **19** was isolated (Scheme 4). Structures of mono- and diacetyl derivatives **18** and **19** were established form their elemental analysis and spectral data. IR spectrum of **18** showed an absorption band at 1764 cm^{-1} assigned to one C=O, while **19** showed two absorption bands at 1740 and 1702 cm^{-1} assigned to two C=O functions. ^{1}H NMR of **18** showed a signal at δ 1.91 ppm, characteristic for one $COCH_3$ group, while that of **19** showed two signals at δ 1.56 and 1.92 ppm, characteristic for two $COCH_3$ groups.

Fungicidal activity

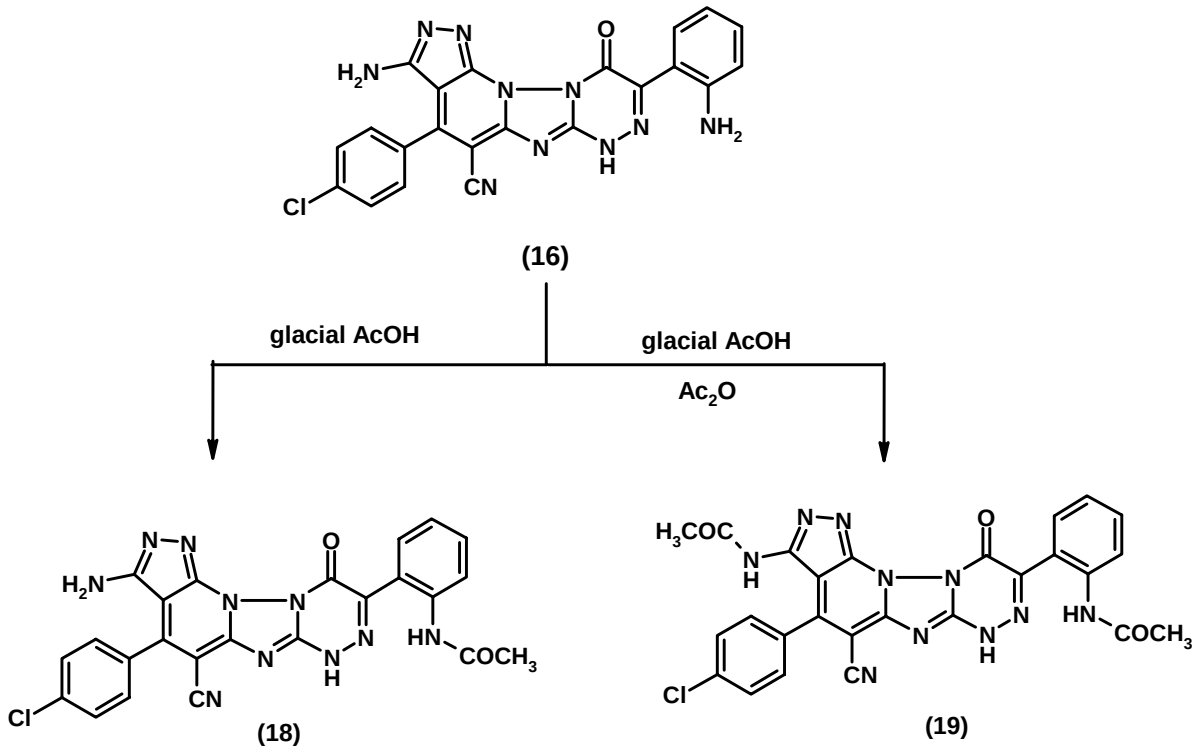
Some new synthesized compounds were screened for their antifungal activities against two fungi, *Alternaria alternata* and *Aspergillus niger* using the disc diffusion method.[33-34] The tested compounds were dissolved in DMF [which has no inhibition activity] to get 1 mg/ml solution. The antibiotic flucanazole was used as standard antifungal reference. The inhibition zones of the microbial growth surrounding the filter paper disc (2.5 mm) were measured in millimeters at the end of an incubation period at 30 ^oC for 3 days (Table 1).

All the tested compounds showed variable activities toward the two species in comparison to the standard flucanazole which revealed that these compounds are biologically active due to the presence of different heterocycles and functional groups.

From the results obtained, it is clear that most of the tested compounds showed moderate activity toward the tested fungi except compound **13** showed higher activity towards *Alternaria alternata* fungi which mainly due to the expected dihydroxy structure (Table 1)



Scheme 3. Synthetic pathway for the preparation of compounds **13-17**.



Scheme 4. Synthetic pathway for the preparation of compounds **18-19**.

Table 1. Fundicidal activity of some of the prepared compounds **2-19**.

Compd. No.	Diameter of inhibition zone (mm)	
	Alternaria alternata	***Aspergillus niger***
4	++	+
5	+	+
6	++	+
8c	++	++
9	+	++
10	+	++
13	+++	+
15	++	++
16	++	+
19	++	++
(Flucanazole)	+++	+++

Lower active = + (inhibition zone 1–10 mm), Moderately active = ++ (inhibition zone 11–25 mm) and High active = +++ (inhibition zone > 25 mm).

Experimental Section

General Procedures. Melting points are uncorrected and were recorded in open capillary tubes on a Stuart SMP3 melting point apparatus. Infrared spectra were recorded on FT-IR Bruker Vector 22 spectrophotometer using KBr wafer technique. UV absorption spectra (DMF) were recorded on a Jasco model (V-550) UV spectrophotometer. ^{1}H NMR spectra were measured on Gemini spectrometer 200 MHz and AC spectrometer 250 MHz using DMSO-d_6 as solvent and TMS (chemical shift in δ ppm) as an internal standard. ^{13}C NMR spectra were measured on AC spectrometer 250 MHz using DMSO as solvent and TMS (chemical shift in δ ppm) as an internal standard. Mass spectra were obtained using gas chromatography GCMS qp 1000 ex Schimadzu instrument mass spectrometer (70 eV). Elemental microanalyses were performed at the Cairo University Microanalytical Center. 4-(4-chlorophenyl)-1,6-diamino-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (**1)** has been prepared according to the reported method.[35]

7-(4-Chlorophenyl)-5-oxo-2-thioxo-1,2,3,5-tetrahydro[1,2,4]triazolo[1,5-*a*]pyridine-6,8-di-carbonitrile (2). A mixture of **1** (2.85 g, 0.01 mol) and carbon disulfide (0.60 mL, 0.01 mol) in ethanolic potassium hydroxide (10%, 100 mL) was refluxed for 4 hours, after cooling the reaction mixture was poured onto ice-HCl. The solid obtained was filtered, washed several times with water and crystallized from methanol to give **2** as yellow crystals, yield 2.13 g (65%), mp > 300 ºC. UV λ_{max} (log ε): 346 (4.18), 275 nm (2.405). IR (KBr, cm^{-1}): 3212, 3184 (2 NH), 2216 (2 C≡N), 1674 (C=O), 1284 (C=S). ^{1}H NMR (δ, DMSO-d_6): 7.64 (d, 2H, Ar-H, J = 8.4 Hz), 7.82 (d, 2H, Ar-H, J = 8.4 Hz), 9.24 (s, 1H, NH exchangeable with D_2O), 10.36 ppm (s, 1H, NH exchangeable with D_2O). Anal. Calcd. for $C_{14}H_6ClN_5OS$ (327.76): C, 51.26; H, 1.83; N, 21.36; S, 9.76. Found C, 51.12; H, 1.80; N, 20.95; S, 9.55.

7-(4-Chlorophenyl)-2-methylthio-5-oxo-,3,5-dihydro[1,2,4]triazolo[1,5-*a*]pyridine-6,8-di-carbonitrile (3). A mixture of **2** (1.64 g, 0.005 mol) and methyl iodide (0.31 mL, 0.005 mol) in ethanolic potassium hydroxide (10%, 100 mL) was refluxed for 4 hours, after cooling the reaction mixture was poured onto ice-HCl. The solid obtained was filtered, wash several times with water and crystallized from aqueous DMF to give **3** as yellow crystals, yield 1.14 g (67%), mp > 300 ºC. IR (KBr, cm^{-1}): 3209 (NH), 2960 (CH_3), 2220 (2 C≡N), 1672 (C=O), 1640 (C=N). ^{1}H NMR (δ, DMSO-d_6): 4.14 (s, 3H, CH_3), 8.31 (d, 2H, Ar-H), 8.42 (d, 2H, Ar-H), 8.61 ppm (s, 1H, NH exchangeable with D_2O).

4-(4-Chlorophenyl)-3,6,7-triamino-7H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (5). A mixture of **1** (2.85 g, 0.01 mol) and hydrazine hydrate (5 mL) was refluxed for 6 hours, after cooling the reaction mixture was poured onto ice. The solid obtained was filtered and crystallized from aqueous DMF to give **5** as yellow crystals, yield 1.44 g (48%), mp > 300 ºC. UV λ_{max} (log ε); 395 (3.00), 350 (4.5), 275 nm (4.3). IR (KBr, cm^{-1}): 3470, 3303, 3188 (3 NH_2), 2214 (C≡N), 1637 (C=N). ^{1}H NMR (δ, DMSO-d_6): 4.59 (bs, 4H, 2NH_2 exchangeable

with D_2O), 5.29 (bs, 1H, NH exchangeable with D_2O), 5.76 (bs, 1H, NH exchangeable with D_2O), 7.56 (d, 2H, Ar-H, J = 8.6 Hz), 7.67 ppm (d, 2H, Ar-H, J = 8.6 Hz). MS (Int.%): 299 (100), 300 (48.05), 284 (22.77), 189 (7.11), 172 (5.49), 111 (23.09). Anal. Calcd. for $C_{13}H_{10}ClN_7$ (299.72): C, 52.05; H, 3.34; N, 32.69. Found C, 51.85; H, 3.42; N, 32.35.

3-Amino-4-(4-chlorophenyl)-7-thioxo-7,8-dihydro-6*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*a*] pyridine-5-carbonitrile (6). A mixture of **5** (2.99 g, 0.01 mol) and carbon disulfide (0.60 mL, 0.01 mol) in ethanolic potassium hydroxide (10%, 100 mL) was refluxed for 4 hours, after cooling the reaction mixture was poured onto ice-HCl. The solid obtained was filtered, washed several times with water and crystallized from aqueous DMF to give **6** as yellow crystals, yield 2.49 g (73 %), mp > 300 ºC. UV λ_{max} (log ε); 410 (3.1), 355 (4.7), 278 nm (4.4). IR (KBr, cm^{-1}): 3469, 3305, 3187 (2NH, NH_2), 2214 (C≡N) 1633 (C=N), 1290 (C=S). Anal. Calcd. for $C_{14}H_8ClN_5S$ (341.78): C, 49.20; H, 2.36; N, 28.69; S, 9.38. Found C, 48.97; H, 2.24; N, 28.75; S, 9.26.

3-Amino-4-(4-chlorophenyl)-7-methylthio-7,8-dihydro-6*H*-pyrazolo[4,3-*e*][1,2,4]triazolo [1,5-*a*]pyridine-5-carbonitrile (7). A mixture of **6** (1.71 g, 0.005 mol) and methyl iodide (0.31 mL, 0.005 mol) ethanolic potassium hydroxide (10%, 50 mL) was refluxed for 6 hours, after cooling the reaction mixture was poured onto ice-HCl. The solid obtained was filtered, washed several times with water and crystallized from aqueous DMF to give **7** as yellow crystals, yield 1.22 g (69 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3469, 3317, 3219 (NH_2, NH), 2925 (CH_3), 2216 (C≡N), 1623 (C=N) and 1459, 1422 (*def*. CH_3) cm^{-1}. ^1H NMR (δ, DMSO-d_6): 3.96 (s, 3H, CH_3), 5.62 (bs, 2H, NH_2 exchangeable with D_2O), 8.21 (d, 2H, Ar-H, J = 8.2 Hz), 8.42 (d, 2H, Ar-H, J = 8.2 Hz), 8.95 ppm (s, 1H, NH exchangeable with D_2O).

Hydrazinolysis of 2, 3, 6 and/or 7. Formation of 4

A mixture of **2, 3, 6** and/or **7** (0.005 mol) and hydrazine hydrate (5 mL) refluxed for 16 hours, after cooling the reaction mixture was poured onto ice-AcOH. The solid obtained was filtered and crystallized from DMF to give **4** as yellow crystals, mp > 300 ºC. UV λ_{max} (log ε): 347(4.22), 271 nm (2.49). IR (KBr, cm^{-1}): 3469, 3308, 3200 ($2NH_2$, 2NH), 2209 (C≡N), 1628 (C=N) cm^{-1}. ^1H NMR (δ, DMSO-d_6): 5.21 (s, 6H, 2NH and $2NH_2$), 7.53 (d, 2H, Ar-H), 7.64 ppm (d, 2H, Ar-H). ^{13}C NMR (δ, DMSO-d_6): 92.76 (C_5-CN), 93.47 (C_{3a}), 117.12 (C≡N), 129.11, 129.57, 132.58, 134.79 (6C of aryl carbons), 146.69 (C_4), 148.64 (C_3), 154.79 (C_{5a}) and 162.34 ppm (C_7 and C_{9a}). MS (Int.%): 340 (0.63), 313 (1.27), 285 (100), 228 (1.88), 113 (5.34) and 56 (3.16). Anal. Calcd. for $C_{14}H_{10}ClN_9$ (339.75): C, 49.49; H, 2.96; N, 37.10. Found C, 48.65; H, 2.62; N, 37.35.

3-Amino-4-(4-chlorophenyl)-9-(un)substituted-10-oxo-12hydro-7*H*-pyrazolo[4,3-*e*][1,2,4] triazino[3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitriles (8a-c). A mixture of **4** (1.70 g, 0.005 mol) and α-oxoacids such as glyoxalic, pyruvic and *p*-chlorostyrylglyoxalic acids (0.005 mol) in glacial acetic acid (40 mL) was refluxed for 4 hours, after cooling the reaction mixture was poured onto ice. The solid obtained was filtered and crystallized to give

8a-c. Compound **8a** crystallized from DMF as yellow crystals, yield 1.02 g (54 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3382, 3199 (NH_2, NH), 2224 (C≡N), 1700 (C=O), 1624 (C=N). MS (Int.%): 284 (3.85), 267 (1.13), 173 (1.35), 149 (7.3), 73 (100), 69 (20.95), 55 (13.62); Anal. Calcd. for $C_{16}H_8ClN_9O$ (377.75): C, 50.87; H, 2.13; N, 33.37. Found C, 50.61; H, 2.03; N, 33.08. Compound **8b** crystallized from DMF as yellow crystals, yield 1.1 g (56 %), mp > 300 ºC. IR (KBr) cm^{-1}: 3462, 3315, 3195 (NH_2, NH), 2215 (C≡N), 1702 (C=O), 1625 (C=N) cm^{-1}. ^{1}H NMR (δ, DMSO-d_6): 2.7 (s, 3H, CH_3), 6.09 (s, 2H, NH_2 exchangeable with D_2O), 8.32 (d, 2H, Ar-H), 8.43 (d, 2H, Ar-H), 12.71 ppm (bs, 1H, NH ⇌ OH exchangeable with D_2O). MS (Int.%): 395 (0.26), 335 (8.68), 285 (100), 270 (4.85) and 193 (4.33) Anal. Calcd. for $C_{17}H_{10}ClN_9O$ (391.78): C, 52.12; H, 2.57; N, 32.18. Found C, 51.88; H, 2.40; N, 32.11.; Compound **8c** crystallized from DMF as yellow crystals, yield 1.68 g (62 %), mp > 300 ºC. UV λ_{max} (log ε): 349 (2.95), 278 nm (1.92). IR (KBr, cm^{-1}): 3467, 3314, 3144 (NH_2, NH), 2217 (C≡N), 1701 (C=O), 1623 (C=N). Anal. Calcd. for $C_{24}H_{13}Cl_2N_9O$ (514.34): C, 56.09; H, 2.55; N, 24.49. Found C, 56.61; H, 2.83; N, 25.01.

3-Amino-4-(4-chlorophenyl)-10-oxo-12-hydro-7*H*,8*H*,9*H*-pyrazolo[4,3-*e*][1,2,4]triazino [3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (9). A mixture of **4** (1.70 g, 0.005 mol) and monochloroacetic acid (0.47 g, 0.005 mol) in DMF (40 mL) was refluxed for 4 hours, after cooling the reaction mixture was poured onto ice. The solid obtained was filtered and crystallized from DMF to give **9** as yellow crystals, yield 1.44 g (76 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3329, 3303 (NH_2, NH), 2926 (CH_2), 2212 (C≡N), 1639 (C=O), 1555 (C=N).

^{1}H NMR (δ, DMSO-d_6): 2.89 (s, 2H, CH_2), 5.19 (s, 2H, NH_2), 7.4 (d, 2H, 2Ar-H), 7.6 (d, 2H, Ar-H), 11.85 ppm (bs, 2H, 2NH). ^{13}C NMR (δ, DMSO-d_6): 35.69 (CH_2), 92.21 (C_5-CN), 93.59 (C_{3a}), 116.86 (C≡N), 129.19, 129.29, 129.49, 132.11 and 134.89 (6C of aryl carbons), 146.22 (C_4), 147.76 (C_{12a}), 147.78 (C_3 and C_{5a}), 155.58 (C_{6a}) and 161.21 ppm (C_{10} as C=O). Anal. Calcd. for $C_{16}H_{10}ClN_9O$ (379.77): C, 50.60; H, 2.65; N, 33.19. Found C, 50.27; H, 2.43; N, 32.82.

3-Amino-4-(4-chlorophenyl)-9-oxo-12-hydro-7H,8H,10*H*-pyrazolo[4,3-*e*][1,2,4]triazino [3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (10). A mixture of **4** (1.70 g, 0.005 mol) and chloroacetyl chloride (0.40 mL, 0.005 mol) in DMF (40 mL) was refluxed for 4 hours, after cooling the reaction mixture was poured onto ice. The solid obtained was filtered and crystallized from DMF to give **10** as yellow crystals, yield 1.23 g (65%), mp > 300 ºC. IR (KBr, cm^{-1}): 3466, 3310, 3210 (NH_2, 2NH), 2945, 2832 (CH_2), 2209 (C≡N), 1700 (C=O), 1632 (C=N), 1493, 1421 (*def.* CH_2). ^{1}H NMR (δ, DMSO-d_6): 2.75 (s, 2H, CH_2), 5.3 (s, 2H, NH_2), 7.5 (d, 2H, 2Ar-H), 7.9 (d, 2H, 2Ar-H) and 11.90 ppm (bs, 2H, 2NH). ^{13}C NMR δ: 35.85 (CH_2), 82 (C_5-CN), 94 (C_{3a}), 117 (C≡N), 129.62, 129.95, 132.39 and 135.28 (6C of aryl carbons), 147 (C_4), 148 (C_{12a}), 156.12 (C_3 and C_{5a}), 161.7 (C_{6a}) and 162.66 ppm (C_{10} as C=O). Anal. Calcd. for $C_{16}H_{10}ClN_9O$ (379.77): C, 50.60; H, 2.65; N, 33.19. Found C, 50.42; H, 2.31; N, 32.94.

3-Amino-4-(4-chlorophenyl)-9-phenyl-12-hydro-7H,10*H*-pyrazolo[4,3-*e*][1,2,4]triazino [3`,4`-5``,1``][1,2,4] triazolo [2``,3``-*a*] pyridine-5-carbonitrile (11). A mixture of **4** (1.70 g, 0.005 mol) and phenacyl bromide (1 g, 0.005 mol) in ethanolic NaOH (5%, 100 mL) was refluxed for 4 hours, after cooling the reaction mixture was acidified with conc. HCl. The solid obtained was filtered and crystallized from DMF to give **11** as yellow crystals, yield 1.12 g (51 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3470, 3315, 3140 (NH_2, NH), 2942, 2831 (CH_2), 2214 (C≡N), 1625 (C=N). Anal. Calcd. for $C_{22}H_{14}ClN_9$ (439.87): C, 60.07; H, 3.21; N, 28.66. Found C, 59.75; H, 3.13; N, 28.28.

3-Amino-4-(4-chlorophenyl)-12-hydro-7*H*,8*H*,9*H*,10*H*-pyrazolo[4,3-*e*][1,2,4]triazino[3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (12). A mixture of **4** (1.70 g, 0.005 mol) and 1,2-dibromoethane (0.43 mL, 0.005 mol) in ethanolic NaOH (5%, 50 mL) was refluxed for 4 hours, after cooling the reaction mixture was acidified with conc. HCl. The solid obtained was filtered and crystallized from DMF to give **12** as yellow crystals, yield 1.04 g (57 %), mp > 300 ºC. UV λ_{max} (log ε): 350 (4.50), 275 (4.25) nm. IR (KBr, cm^{-1}): 3468, 3313, 3207 (NH_2, 2NH), 2924, 2840 (CH_2), 2215 (C≡N), 1625 (C=N). ^{1}H NMR (δ, DMSO-d_6): 3.3 (s, 4H, $2CH_2$), 5.93 (bs, 4H, NH_2 and 2NH exchangeable by D_2O), 8.28 (d, 2H, Ar-H) and 8.53 (d, 2H, Ar-H). Anal. Calcd. for $C_{16}H_{12}ClN_9$ (365.79): C, 52.49; H, 3.28; N, 34.45. Found C, 52.61; H, 3.43; N, 34.15.

3-Amino-4-(4-chlorophenyl)-9,10-dioxo-12hydro-7*H*,8*H*-pyrazolo[4,3-*e*][1,2,4]triazino [3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (13). A mixture of **4** (1.70 g, 0.005 mol) and diethyl oxalate (0.68 mL, 0.005 mol) in DMF (40 mL) was refluxed for 8 hours, after cooling the reaction mixture was poured onto ice. The solid obtained was filtered and crystallized from DMF to give **13** as yellow crystals, yield 1.2 g (61%), mp > 300 ºC. (KBr,
cm^{-1}): 3477, 3321, 3147 (NH_2, 2NH), 2217 (C≡N), 1711 (C=O), 1629 (C=N). ^{1}H NMR (δ, DMSO-d_6): 5.24 (s, 2H, NH_2), 7.52-7.73 (m, 4H, Ar-H) and 11.88 (s, 2H, NH and OH of 1,2,4-triazine). ^{13}C NMR (δ, DMSO-d_6): 92.15 (C_5-CN), 93.52 (C_{3a}), 116.90 (C≡N); 129.19, 129.49, 132.05, 134.87 (6C of aryl carbons), 146.15 (C_4), 146.18 (C_{12a}), 146.22 (C_{5a}), 147.75 (C_3), 155.62 (C_9 and C_{6a}), 161.19 (C_{10} as C=O).

3-Amino-4-(4-chlorophenyl)-9,10-diphenyl-12-hydro-7H,10*H*-pyrazolo[4,3-*e*][1,2,4] triazino[3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (14). A mixture of **4** (1.70 g, 0.005 mol) and benzoin (1.06 g, 0.005 mol) in DMF (40 mL) was refluxed for 8 hours, after cooling the reaction mixture was poured onto ice. The solid obtained was filtered and crystallized from DMF to give **14** as yellow crystals, yield 1.68 g (65 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3470, 3315, 3142 (NH_2, NH), 2922, 2850 (CH_2) 2215 (C≡N), 1626 (C=N). ^{1}H NMR (δ, DMSO-d_6): 5.5 (s, 2H, NH_2), 7.6-7.9 (m, 15H, Ar-H and CH of 1,2,4-triazin-5-yl) and 11.9 ppm (s, 1H, NH). ^{13}C NMR (δ, DMSO-d_6): 92 (C_5-CN), 94 (C_{3a}), 117.98 (C≡N and C_{10}), 130.14-136.41 (18C of aryl carbons), 147 (C_4 and C_{12a}), 148 (C_3), 156 (C_{5a}), 162 (C_{6a}), 195.69 (C_9). MS (Int.%): 461 (2.99), 285 (100), 270 (7.83), 178 (2.35), 174 (6.17), 160

(4.61). Anal. Calcd. for $C_{28}H_{18}ClN_9$ (515.97): C, 65.12; H, 3.49; N, 24.42. Found C, 64.61; H, 3.43; N, 24.35.

3-Amino-4-(4-chlorophenyl)-15-hydro-7*H*-pyrazolo[4,3-*e*]indolo[2,3-5`,6`][1,2,4]triazino [3`,4`-5``,1``][1,2,4]triazolo[2``,3``-*a*]pyridine-5-carbonitrile (15). A mixture of **4** (1.70 g, 0.005 mol) and isatine (0.74 g, 0.005 mol) in DMF (40 mL) was refluxed for 8 hours, the reaction mixture was cooled and poured onto ice. The solid obtained was filtered and crystallized from DMF to give **15** as yellow crystals, yield 1.06 g (47 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3468, 3316, 3179 (NH_2, NH), 2218 (C≡N), 1624 (C=N). ^{1}H NMR (δ, DMSO-d_6): 5.3 (s, 2H, NH_2), 7.5-7.9 (m, 8H, Ar-H) and 11.9 ppm (s, 1H, NH). ^{13}C NMR δ: 92 (C_5-CN), 93 (C_{3a}), 117 (C≡N), 129.36-135.29 (10C of aryl carbons), 146 (C_4), 147 (C_{15a}), 152 (C_3), 156 (C_{5a}), 157 (C_{6a}), 161.67 (C_{13a}), 163 ppm (C_{8a}). Anal. Calcd. for $C_{22}H_{11}ClN_{10}$ (515.97): C, 58.61; H, 2.46; N, 31.07. Found C, 58.82; H, 2.30; N, 30.74.

3-Amino-9-(2-aminophenyl)-4-(4-chlorophenyl)-10-oxo-12-hydro-7*H*-pyrazolo[4,3-*e*][1,2,4] triazino[3`,4`-5``,1``][1,2,4]triazolo [2``,3``-*a*]pyridine-5-carbonitrile (16). A mixture of **4** (1.70 g, 0.005 mol) and isatine (0.74 g, 0.005 mol) in ethanolic NaOH (5%, 100 mL) was refluxed for 4 hours, the reaction mixture was cooled and acidified with diluted AcOH. The solid so formed was filtered and crystallized from DMF to give **16** as pale brown crystals, yield

55 g (66 %), mp > 300 ºC. UV λ_{max} (log ε): 435 (2.6), 352 (4.15), 275 nm (4.2). (KBr, cm^{-1}): 3467, 3309, 3207 ($2NH_2$, NH), 2210 (C≡N), 1726 (C=O), 1628 (C=N). ^{1}H NMR (δ, DMSO-d_6): 3.47 (bs, 2H, NH_2), 5.40 (s, 2H, 2NH), 7.27-7.94 (m, 8H, Ar-H), 12.06 ppm (s, 1H, NH ⇌ OH of 1,2,4-triazinone). MS (Int.%): 469 (0.99), 322 (1.84), 285 (100), 270 (10.68), 211 (4.24), 146 (12.51), 113 (52.33), 93 (16.40), 67 (28.14). Anal. Calcd. for $C_{22}H_{13}ClN_{10}O$ (468.87): C, 56.36; H, 2.80; N, 29.86. Found C, 56.11; H,3.66; N, 29.84

Acetylation of 15- formation of 17. A mixture of **15** (0.45 g, 0.001 mol) and acetic anydride (10 mL) was refluxed for 2 hours. The solid obtained while hot was filtered, washed with ether and crystallized from AcOH to give **17** as yellow crystals, yield 0.3 g (57 %), mp > 300 ºC. IR (KBr, cm^{-1}): 3422 (NH), 2922, 2852 (CH_3), 2217 (C≡N), 1720, 1655 (2 C=O), 1620 (C=N)**.** Anal. Calcd. for $C_{26}H_{15}ClN_{10}O_2$ (534.93): C, 58.38; H, 2.80; N, 26.18. Found C, 57.81; H, 2.56; N, 25.85.

Formation of N-acetyl derivative 18. A mixture of **16** (0.94 g, 0.002 mol) and glacial acetic acid (40 mL) was refluxed for 4 hours, the reaction mixture was cooled and poured onto ice. The solid obtained was filtered and crystallized from AcOH to give **18** as yellow crystals, yield 0.64 g (63 %), m.p. > 300 ºC. UV λ_{max} (log ε): 384 (3.4), 349 (4.6), 278 nm (4.8). IR (KBr, cm^{-1}): 3465, 3407, 3314, 3143 ($2NH_2$, 2NH), 2219 (C≡N), 1764 and 1671 (2 C=O), 1620 (C=N), 1493, 1394 (*def.* CH_3). ^{1}H NMR (δ, DMSO-d_6): 1.91 (s, 3H, CH_3), 5.32 (bs, 2H, NH_2), 7.48-7.94 (m, 8H, Ar-H), 9.63 (s, 1H, -NHCO-), 11.88 (bs, 1H, NH ⇌ OH). Anal.

Calcd. for $C_{24}H_{15}ClN_{10}O_2$ (510.90): C,56.37; H,2.94; N, 27.40. Found C, 55.87; H, 2.75; N, 26.98

Formation of bi-acetyl derivative 19. A mixture of **16** (0.94 g, 0.002 mol) in glacial acetic acid (50 mL) and acetic anhydride (1 mL) was refluxed for 8 hours, the reaction mixture was cooled and poured onto ice. The solid obtained was filtered and crystallized from acetic acid to give **19** as yellow crystals, yield 0.61 g (58%), mp > 300 ºC. IR (KBr, cm^{-1}): 3502, 3397, 33192, (3NH), 2924 (CH_3), 2234 (C≡N), 1740, 1702, 1658 (3 C=O), 1596 (C=N), 1495, 1455 (*def.* CH_3). ^{1}H NMR (δ, DMSO-d_6): 1.56 (s, 3H, CH_3), 1.92 (s, 3H, CH_3), 7.35-7.64 (m, 8H, Ar-H), 9.65 (bs, 1H, NH) and 13.09 (bs, 2H, 2NH). Anal. Calcd. for $C_{26}H_{17}ClN_{10}O_3$ (522.94): C, 59.66; H, 3.25; N, 26.77. Found C, 59.42; H, 3.36; N, 26.74.

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