

The utilization of 2-aminoprop-1-ene-1,1,3-tricarbonitrile as a precursor to quinoline, furan and thiophene derivatives with antitumor activities

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Abstract

The condensation reaction of 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**1**) with 2-acetyl-furan (**2**) afforded 2-amino-4-(furan-2-yl)penta-1,3-diene-1,1,3-tricarbonitrile (**3**). The latter compound underwent a series of heterocyclization reactions to give quinoline, furan, pyrazole and thiophene derivatives. The antitumor evaluation of the newly synthesized products against three cancer cell lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) were recorded. Three of the synthesized compounds, namely **4**, **5d** and **12** showed high inhibitory effects.

Keywords: antitumor activity; furan; pyrazole; quinoline; thiophene.

Introduction

Nitrogen-containing heterocycles are of substantial interest in organic chemistry as they are integral components of natural products, dyes, agrochemicals and pharmaceuticals. 2-Aminoprop-1-ene-1,1,3-tricarbonitrile (**1**) has proven to be an excellent precursor for the synthesis of pyridines, pyrazoles and thiophenes (Freeman, 1969; Taylor and McKillop, 1970; Fatiadi, 1978). The biological significance of such compounds arises due to their diverse pharmaceutical activities in neurological disorders (Wing et al., 2006), as receptor antagonists (Rozsa et al., 2008; Szarics et al., 2008), tubulin inhibitors (Odlo et al., 2008), kinase inhibitors (Zhao et al., 2006) and for anticancer activity (Muranaka et al., 2008). Moreover, annulated nitrogen heterocycles, bearing pyridine and benzene, constitute a class of biologically active compounds that are potent anti-inflammatory agents (Burguete et al., 2007),

antibacterial agents (Hirokawa et al., 2008), inhibitors of gastric acid secretion (Palmer et al., 2007) and calcium channel blockers (Leon et al., 2008). In light of such important pharmaceutical applications, we have decided to investigate the reaction of 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**1**) with some chemical reagents. All synthesized compounds have been screened for antitumor activity against breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268). Three of the synthesized compounds showed a high inhibitory effect against the three cell lines.

Results and discussion

Chemistry

Recently, we have reported the synthesis of a series of pyridine, pyridazine and thiophene derivatives with antimicrobial activities utilizing 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**1**) as the starting material (Mohareb et al., 2008). Making use of the experience gained from this study, we examined the synthesis of 2-amino-4-(furan-2-yl)penta-1,3-diene-1,1,3-tricarbonitrile (**3**) via condensation of **1** with 2-acetyl-furan (**2**). The structure of compound **3** was confirmed based on analytical and spectral data. Thus, its ¹H-NMR spectrum shows a singlet at δ 3.30 for the CH₃ group, a singlet at δ 3.83 (D₂O-exchangeable) for the NH₂ group, and a multiplet at δ 6.76–8.08 due to the presence of the furyl group. The mass spectrum fragmentations are in agreement with the structure of compound **3** (see Experimental section). Further structure elucidation of compound **3** was obtained through studying its reactivity towards various chemical reagents. Compound **3** underwent cyclization when heated under reflux in an ethanolic NaOEt solution to give the benzene-1,3-dinitrile derivative **4**. Next, we took advantage of the presence of a methyl group activated by the α,β -unsaturated nitrile moiety in compound **3**. This methyl group underwent coupling with aryl diazonium salts, namely benzenediazonium chloride, 4-chlorobenzenediazonium chloride, 4-nitrobenzenediazonium chloride and 4-bromobenzenediazonium chloride, and this reaction afforded the arylhydrazono derivatives **5a–d**. Formation of such arylhydrazone derivatives took place in analogy with the reported literature (Edrees et al., 2010). Reaction of compound **3** with aromatic aldehydes was studied with the aim of forming arylidene derivatives capable of reacting with cyanomethylene reagents forming polyfunctionally substituted pyridine or benzene derivatives of potential biological activities. Thus, condensation of compound **3** with benzaldehyde,

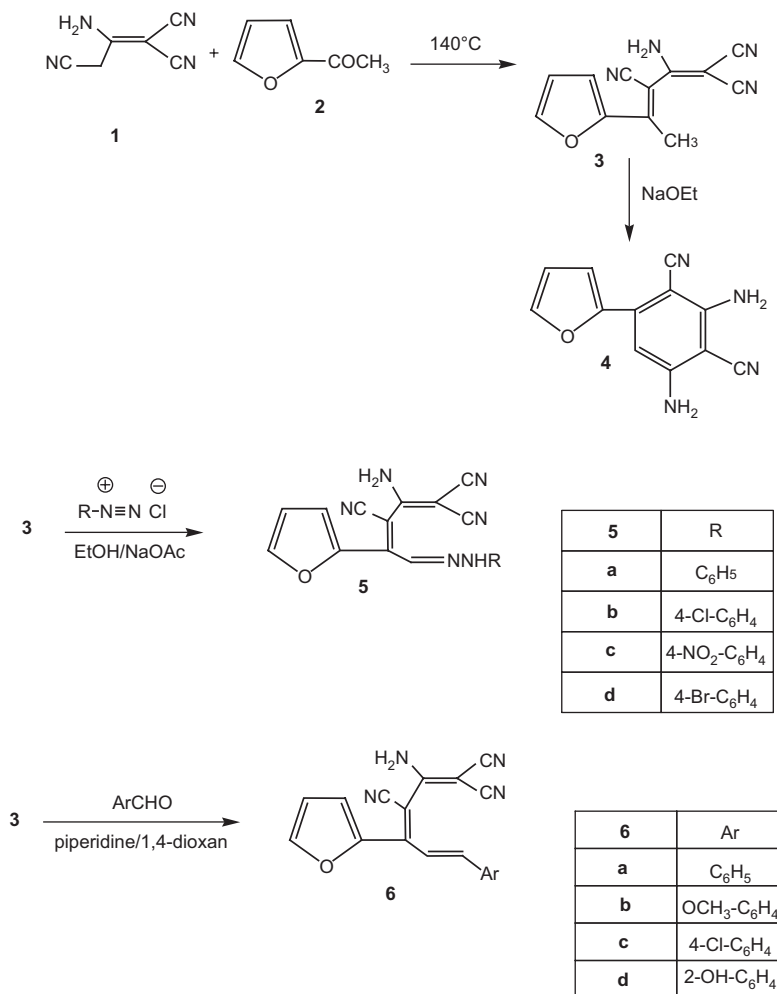
p-methoxybenzaldehyde, *p*-chlorobenzaldehyde and salicylaldehyde in 1,4-dioxane solution containing a catalytic amount of piperidine gave the respective polysubstituted triene derivatives **6a–d** (Scheme 1).

Next, we studied the reactivity of **6a** towards active methylene reagents such as malononitrile (**7a**) and ethyl cyanoacetate (**7b**) in absolute ethanol containing a catalytic amount of triethylamine. The reaction proceeded through the intermediate formation of **8** followed by the loss of hydrogen cyanide to afford the substituted benzene derivatives **9a** and **9b**, respectively. Upon heating **9a** and **9b** in sodium ethoxide solution, quinoline derivatives **10a** and **10b** were produced via the Michael addition of the NH₂ group to the CN group. Structures of compounds **10a,b** were confirmed based on analytical and spectral data. Thus, the ¹H-NMR spectrum of **10a** indicates the presence of two singlets downfield at δ 6.65 and 6.72 belonging to the two NH₂ groups and a multiplet at δ 7.08–7.57 corresponding to one benzene ring CH proton and three protons on the furan ring. Comparing structures **9a** and **10a**, it is evident that the chemical shifts of the amino groups in **9a** differ

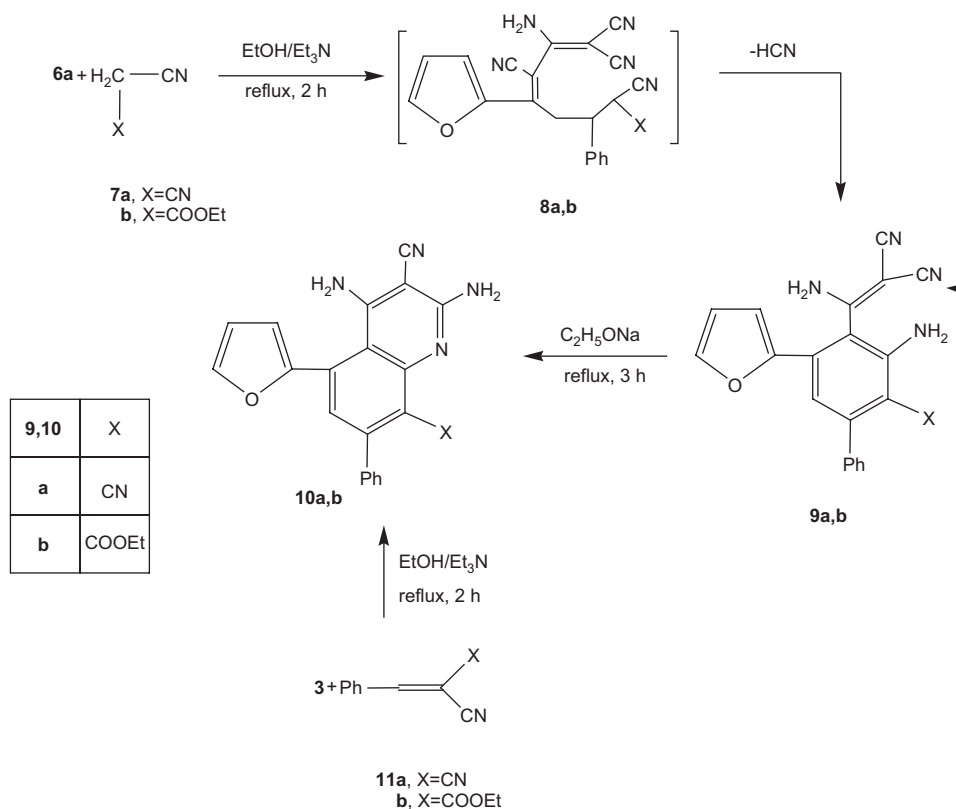
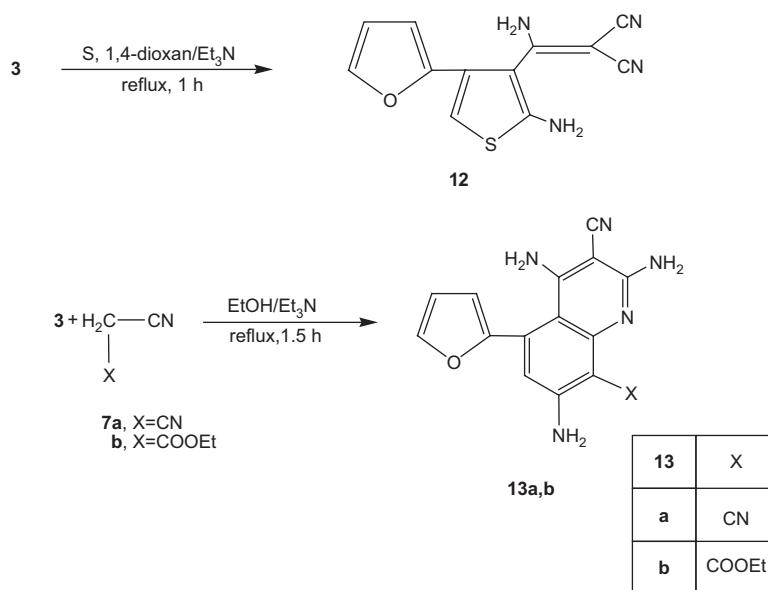
from those of the amino groups in **10a**. This is also consistent with the addition of the amino group to the cyano group to afford the cyclic structures **10a,b**. Further confirmation of the structures of either **10a** or **10b** was obtained through their synthesis using another route. Thus, the reaction of compound **3** with cinnamionitrile derivatives such as α-cyanocinnamionitrile (**11a**) and α-ethoxycarbonyl cinnamionitrile (**11b**) gave the same products **10a** and **10b**, respectively (Scheme 2).

Moreover, the reaction of compound **3** with elemental sulfur in the presence of triethylamine was studied as an application of Gewald's thiophene synthesis (McKibben et al., 1999) to give the 2-aminothiophene derivative **12**. Compound **3** reacted with either malononitrile or ethyl cyanoacetate to give the quinoline derivatives **13a** or **13b**, respectively (Scheme 3). The structures of compounds **13a,b** were based on analytical and spectral data (see Experimental section).

Finally, the reaction of **3** with either hydrazine hydrate or phenylhydrazine was studied. Thus, in the case of the reaction with hydrazine hydrate, 5-(1-(furan-2-yl)ethylidene)-5*H*-pyrazolo[3,4-*b*] pyridine-3,4,6-triamine (**15**) was formed.

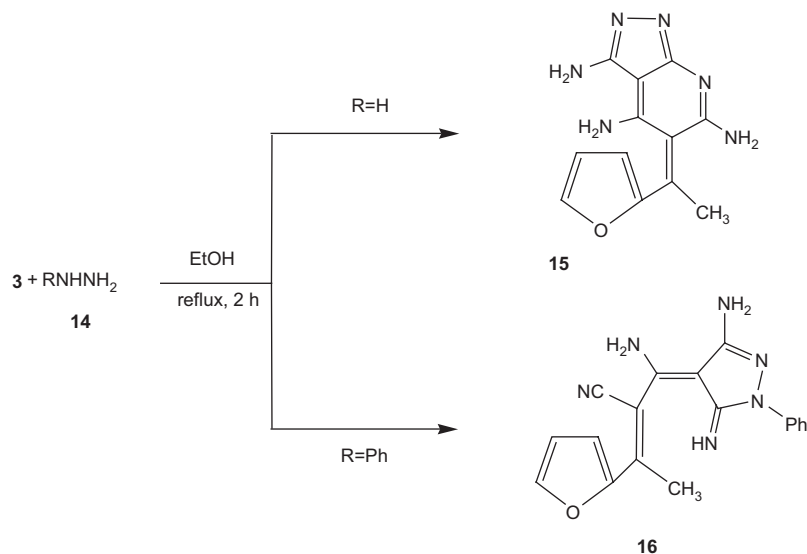


Scheme 1 Synthesis of compounds **3–6a–d**.

Scheme 2 Synthesis of compounds **9a,b** and **10a,b**.Scheme 3 Synthesis of compounds **12** and **13a,b**.

However, the reaction of compound **3** with phenyl hydrazine gave the open-chain product **16**. It is evident that the reaction took place via the addition of hydrazine to compound **3** to afford pyrazoles **15** and **16** (Scheme 4). Structure of **15** was confirmed based on analytical and spectral data. Thus,

the 1H -NMR spectrum shows a singlet at δ 3.42 corresponding to methyl protons, three singlets at δ 3.67, 6.74 and 6.80 corresponding to three NH_2 groups and a multiplet at δ 7.11–8.07 corresponding to the three furan protons. The presence of a singlet at δ 10.25 is consistent with the presence of the



Scheme 4 Synthesis of compounds **15** and **16**.

exocyclic NH group in **16**. Further confirmations for structures **15** and **16** were obtained by analysis of their mass spectra (see Experimental section).

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure for 48 h and show means $\pm$ SEM of three independent experiments performed in duplicate. Doxorubicin was used as positive control.

Effect on the growth of human tumor cell lines

The newly synthesized compounds were evaluated for *in vitro* antitumor activity against three human tumor cell lines representing different tumor types, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) in 48-h drug exposure assays. In this study, the cytotoxicity against non-cancer cell lines was not determined as the preliminary tests showed no significant results. The average GI_{50} values are shown in Table 1. All compounds inhibit the growth of the tested human tumor cell lines in a dose-dependent manner (data not shown). The results shown in Table 1 reveal that compound **4** shows the highest inhibitory effect against two tumor cell lines MCF-7 and NCI-H460 with the respective GI_{50} values of $0.003\pm 0.001\ \mu\text{M}$ and $0.06\pm 0.02\ \mu\text{M}$. The inhibitory effect of compound **4** towards MCF-7 is 10 times greater than that of the reference drug doxorubicin. Compound **3** shows the highest inhibitory effect against SF-268 with a GI_{50} value of $0.06\pm 0.8\ \mu\text{M}$. Close examination of the antitumor activities of compounds **5** reveals that structure **5d** with the bromophenyl moiety is significantly more active than the corresponding chloro and nitro substituted derivatives. By contrast, the hydroxyl, chloro and methoxy functionalities directly attached to the aryl group in compound **6** do not reveal a dramatic enhancement in

the cytotoxic activity compared with unsubstituted derivative **6a**. Comparing structures **9a** and **9b** reveals that **9a** possesses a much higher inhibitory effect than **9b**, possibly owing to the presence of the cyano group in **9a**. This positive effect of the cyano group is in agreement with our previously reported finding (Mohareb et al., 2011). This observation is also emphasized in cyano-substituted derivatives **10a** and **13a**, which possess much higher activity than the carboxyethyl substituted derivatives **10b** and **13**.

Table 1 Effect of newly synthesized products on the growth of three human tumor cell lines.

Compound	GI_{50} (μM)		
	MCF-7	NCI-H460	SF-268
3	2.5 ± 0.4	6.0 ± 0.8	0.06 ± 0.8
4	0.003 ± 0.001	0.06 ± 0.02	0.2 ± 0.1
5a	40.7 ± 16.5	18.2 ± 12.0	44.0 ± 9.01
5b	8.0 ± 0.6	2.0 ± 0.4	3.5 ± 1.0
5c	34.1 ± 0.7	23.2 ± 4.8	18.4 ± 1.8
5d	0.03 ± 0.02	0.6 ± 0.04	0.4 ± 0.06
6a	40.6 ± 12.6	32.6 ± 8.6	60.4 ± 14.8
6b	16.9 ± 0.6	15.1 ± 0.6	20.3 ± 0.5
6c	38.0 ± 1.8	44.0 ± 0.8	20.5 ± 1.1
6d	20.0 ± 0.2	26.6 ± 1.4	26.4 ± 0.6
9a	0.4 ± 0.2	0.1 ± 0.08	0.9 ± 0.08
9b	22.1 ± 0.9	20.2 ± 4.6	16.4 ± 1.8
10a	0.3 ± 0.4	0.5 ± 0.8	0.1 ± 0.2
10b	38.0 ± 1.8	44.0 ± 0.8	20.5 ± 1.1
12	0.01 ± 0.5	0.5 ± 0.6	0.3 ± 0.4
13a	2.6 ± 10.0	4.6 ± 8.6	2.4 ± 0.8
13b	10.8 ± 0.6	12.5 ± 0.8	16.7 ± 1.6
15	14.3 ± 0.7	16.1 ± 4.9	22.5 ± 1.2
16	15.6 ± 14.9	26.9 ± 10.8	10.8 ± 6.6
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Conclusions

In this study, 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**1**) was allowed to react with 2-acetylfuran (**2**) to afford 2-amino-4-(furan-2-yl)penta-1,3-diene-1,1,3-tricarbonitrile (**3**). This compound is a useful starting material for the synthesis of quinoline, pyrazole, furan and thiophene derivatives. The antitumor evaluations of the newly synthesized products were conducted.

Experimental

General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. IR spectra were recorded for KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^1H NMR spectra were measured on a Varian EM-390 at 200 MHz in $\text{DMSO}-d_6$ as solvent using TMS as internal standard. Analytical data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt. Antitumor evaluation for the newly synthesized products was performed by a research group at the National Research Center and the National Cancer Institute at Cairo University. Fetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, NJ, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, MO, USA). Stock solutions of all compounds were prepared in DMSO and kept at -20°C . Appropriate dilutions of the compounds were freshly prepared just prior to assays. Final concentrations of DMSO did not interfere with the cell growth. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt).

Synthesis of 2-amino-4-(furan-2-yl)penta-1,3-diene-1,1,3-tricarbonitrile (**3**)

A mixture of 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**1**) (1.32 g, 0.01 mol), 2-acetylfuran (**2**) (1.10 g, 0.01 mol) and anhydrous ammonium acetate (1.0 g) was heated in an oil bath at 140°C for 30 min. After cooling, the mixture was treated with warm ethanol and poured onto an ice water mixture. The resultant solid product **3** was collected by filtration and crystallized from ethanol; yield 1.50 g (67%); mp 224°C ; IR: ν 3325, 3212 (NH_2), 3150 (CH aromatic), 2885 (CH_3), 2222–2207 (CN), 1647 cm^{-1} (C=C); MS: m/z 224 (M^+ , 51%); ^1H NMR: δ 3.30 (s, 3H, CH_3), 3.83 (s, 2H, NH_2), 6.76–8.08 (m, 3H, furan H). Analysis calculated for $\text{C}_{12}\text{H}_8\text{N}_4\text{O}$: C, 64.28; H, 3.60; N, 24.99. Found: C, 64.15; H, 3.57; N, 24.67.

Synthesis of 2,4-diamino-6-(furan-2-yl)benzene-1,3-dinitrile (**4**)

A suspension of **3** (2.24 g, 0.01 mol) and sodium ethoxide (0.46 g) in 40 ml absolute ethanol (40 ml) was heated in a boiling water bath for 4 h and then left to cool. The solid product formed upon pouring onto ice water containing hydrochloric acid (pH=6) was collected by filtration, dried and crystallized from ethanol; yield: 1.30 g (58%) mp 239°C ; IR: ν 3455–3336 (NH_2), 3250 (CH aromatic), 2229, 2225 cm^{-1} (CN); MS: m/z 225 (M^+ , 88%); ^1H NMR: δ 4.35, 5.21 (2s,

4H, 2NH_2), 6.30–7.40 (m, 4H, benzene H-5 and furan H). Analysis calculated for $\text{C}_{12}\text{H}_8\text{N}_4\text{O}$: C, 64.28; H, 3.60; N, 24.99. Found: C, 64.21; H, 3.59; N, 24.78.

General procedure for synthesis of compounds **5a–d**

A benzenediazonium chloride (0.01 mol) was prepared by addition of sodium nitrite (1.6 g, 0.02 mol) in water (8 ml) to a cold solution of the appropriate aniline in water in the presence of hydrochloric acid. This reagent was added with stirring to a cold solution of **3** (2.24 g, 0.01 mol) and sodium acetate (3.0 g) in ethanol (20 ml). The mixture was stirred for an additional 2 h at 0°C and the formed solid product was collected by filtration, dried and crystallized from ethanol.

Compound 5a Yield 1.95 g (59%); mp 68°C ; IR: ν 3460–3325 (NH_2 , NH), 3150 (CH aromatic), 2885 (CH_3), 2250–2220 (CN), 1657 cm^{-1} (C=N), 1647 cm^{-1} (C=C); MS: m/z 328 (M^+ , 62%); ^1H NMR: δ 3.56 (s, 2H, NH_2), 6.77 (s, 1H, CH=N), 6.23–7.40 (m, 8H, C_6H_5 and furan H), 8.80 (s, 1H, NH). Analysis calculated for $\text{C}_{18}\text{H}_{12}\text{N}_6\text{O}$: C, 65.85; H, 3.68; N, 25.60. Found: C, 65.70; H, 3.59; N, 25.48.

Compound 5b Yield 1.95 g (59%); mp 68°C ; IR: ν 3459–3330 (NH_2 , NH), 3100 (CH aromatic), 2250–2220 (CN), 1657 cm^{-1} (C=N), 1647 cm^{-1} (C=C); MS: m/z 362 (M^+ , 7%); ^1H NMR: δ 3.73 (s, 2H, NH_2), 7.20 (s, 1H, CH=N), 7.15–7.74 (m, 7H, C_6H_4 and furan H), 8.20 (s, 1H, NH). Analysis calculated for $\text{C}_{18}\text{H}_{11}\text{ClN}_6\text{O}$ (362.77): C, 59.59; H, 3.06; N, 23.17%. Found: C, 59.2; H, 3.14; N, 22.9%.

Compound 5c Yield 2.5 g (67%); mp 280°C ; IR: ν 3459–3330 (NH_2 , NH), 3100 (CH aromatic), 2250–2220 (CN), 1657 cm^{-1} (C=N), 1647 cm^{-1} (C=C); MS: m/z 373 (M^+ , 40%); ^1H NMR: δ 3.55 (s, 2H, NH_2), 7.24 (s, 1H, CH=N), 7.61–8.30 (m, 7H, C_6H_4 and furan H), 8.30 (s, 1H, NH). Analysis calculated for $\text{C}_{18}\text{H}_{11}\text{N}_7\text{O}_3$: C, 57.91; H, 2.97; N, 26.26. Found: C, 56.8; H, 2.89; N, 26.19.

Compound 5d Yield 2.7 g (66%); mp 236°C ; IR: ν 3450–3330 (NH_2 , NH), 3110 (CH aromatic), 2250–2220 (CN), 1657 cm^{-1} (C=N), 1647 cm^{-1} (C=C); MS: m/z 406 (M^+ , 21%); ^1H NMR: δ 3.55 (s, 2H, NH_2), 7.24 (s, 1H, CH=N), 7.27–7.89 (m, 7H, C_6H_4 and furan H), 8.19 (s, 1H, NH). Analysis calculated for $\text{C}_{18}\text{H}_{11}\text{BrN}_6\text{O}$: C, 53.09; H, 2.72; N, 20.64. Found: C, 53.1; H, 2.79; N, 20.59.

General procedure for synthesis of compounds **6a–d**

To a solution of compound **3** (2.24 g, 0.01 mol) in 1,4-dioxane (50 ml) containing piperidine (0.5 ml), benzaldehyde (1.08 g, 0.01 mol) or *p*-methoxybenzaldehyde (1.36 g, 0.01 mol) or *p*-chlorobenzaldehyde (1.40 g, 0.01 mol) or salicylaldehyde (1.22 g, 0.01 mol) was added. The mixture was heated under reflux for 2 h and then poured onto ice water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from ethanol.

2-Amino-4-(furan-2-yl)-6-phenylhexa-1,3,5-triene-1,1,3-tricarbonitrile (6a) Yield 2.20 g (70%); mp 238°C ; IR: ν 3460, 3375 (NH_2), 3110 (CH aromatic), 2250–2220 (CN), 1647 cm^{-1} (C=C); MS: m/z 312 (M^+ , 9%); ^1H NMR: δ 3.74 (s, 2H, NH_2), 6.73, 6.74 (2d, 2H, CH=CH), 7.13–7.97 (m, 8H, C_6H_5 and furan H). Analysis calculated for $\text{C}_{19}\text{H}_{12}\text{N}_4\text{O}$: C, 73.07; H, 3.87; N, 17.94. Found: C, 73.23; H, 4.10; N, 17.80.

2-Amino-4-(furan-2-yl)-6-(4-methoxyphenyl)hexa-1,3,5-triene-1,1,3-tricarbonitrile (6b) Yield 2.20 g (70%); mp 242°C; IR: ν 3460, 3375 (NH₂), 3110 (CH aromatic), 2250–2220 (CN), 1647 cm⁻¹ (C=C); MS: m/z 312 (M⁺, 9%); ¹H NMR: δ 3.56 (s, 3H, CH₃), 3.86 (s, 2H, NH₂), 6.65, 6.66 (2d, 2H, CH=CH), 6.31–7.85 (m, 7H, C₆H₄ and furan H). Analysis calculated for C₂₀H₁₄N₄O₂: C, 70.17; H, 4.12; N, 16.37. Found: C, 69.88; H, 4.10; N, 16.11.

2-Amino-6-(4-chlorophenyl)-4-(furan-2-yl)hexa-1,3,5-triene-1,1,3-tricarbonitrile (6c) Yield 2.40 g (69%); mp 221°C; IR: ν 3465, 3380 (NH₂), 3110 (CH aromatic), 2250–2220 (CN), 1647 cm⁻¹ (C=C); MS: m/z 347 (M⁺, 20%); ¹H-NMR: δ 3.56 (s, 2H, NH₂), 6.65, 6.71 (2d, 2H, CH=CH), 7.08–7.50 (m, 7H, C₆H₄ and furan H). Analysis calculated for C₁₉H₁₁ClN₄O: C, 65.81; H, 3.20; N, 16.16. Found: C, 65.62; H, 3.17; N, 15.98.

2-Amino-4-(furan-2-yl)-6-(2-hydroxyphenyl)hexa-1,3,5-triene-1,1,3-tricarbonitrile (6d) Yield 2.50 g (76%); mp 268°C; IR: ν 3550, 3370 (OH, NH₂), 3090 (CH aromatic), 2250–2220 (CN), 1645 cm⁻¹ (C=C); MS: m/z 328 (M⁺, 12%); ¹H NMR: δ 3.86 (s, 2H, NH₂), 6.65, 6.73 (2d, 2H, CH=CH), 7.08–7.80 (m, 7H, C₆H₄ and furan H), 10.30 (s, 1H, OH). Analysis calculated for C₁₉H₁₂N₄O₂: C, 69.51; H, 3.68; N, 17.06. Found: C, 69.36; H, 3.62; N, 17.11.

General procedure for synthesis of compounds 9a and 9b

To a solution of compound **6a** (3.88 g, 0.01 mol) in ethanol (50 ml) containing triethylamine (0.5 ml), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The mixture was heated under reflux for 2 h and then poured onto ice water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from ethanol. Compound **9a** was obtained from malononitrile and compound **9b** was obtained from ethyl cyanoacetate.

Compound 9a Yield 2.27 g (65%); mp>300°C; IR: ν 3450–3340 (2 NH₂), 3100 (CH aromatic), 2250–2220 (CN), 1655 cm⁻¹ (C=C); MS: m/z 351 (M⁺, 14%); ¹H NMR: δ 3.31, 3.56 (2s, 4H, 2NH₂), 7.13–7.51 (m, 9H, C₆H₄, furan H) (m, 7H, C₆H₄ and furan H). Analysis calculated for C₂₁H₁₃N₅O: C, 71.79; H, 3.73; N, 19.93. Found: C, 71.93; H, 3.86; N, 20.03.

Compound 9b Yield 2.35 g (59%); mp 279°C; IR: ν 3460–3340 (2 NH₂), 3090 (CH aromatic), 2880, 2867 (CH₃, CH₂), 2240–2220 (CN), 1680 (C=O), 1675 (C=N), 1635 cm⁻¹ (C=C); MS: m/z 398 (M⁺, 78%); ¹H NMR: δ 1.23 (t, 3H, $J=7.0$ Hz, CH₃), 4.13 (q, 2H, $J=7.0$ Hz, CH₂), 3.32, 3.67 (2s, 4H, 2NH₂), 7.45–8.31 (m, 9H, C₆H₄, furan H). Analysis calculated for C₂₃H₁₈N₄O₃ (398.14): C, 69.34; H, 4.55; N, 14.06. Found: C, 68.97; H, 4.48; N, 14.23.

General procedure for synthesis of compounds 10a and 10b

Method A A suspension of **9a** (3.51 g, 0.01 mol) or **9b** (3.98 g, 0.01 mol) in a sodium ethoxide solution [prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (30 ml)] was heated in a boiling water bath for 3 h, then cooled and poured onto ice water containing a few drops of hydrochloric acid (until pH 6). The formed solid product was collected by filtration and crystallized from ethanol. Compound **10a** was obtained from **9a** and product **10b** was obtained from **9b**.

Method B To a solution of compound **3** (3.24 g, 0.01 mol) in 1,4-dioxane (30 ml) containing triethylamine (0.50 ml) either α -cyanocinnamionitrile (1.54 g, 0.01 mol) or ethyl α -cyanocinnamate (2.01 g, 0.01 mol) was added. The mixture was heated under reflux for 2 h and then left to cool. The solid product formed upon pouring onto ice water was collected by filtration.

2,4-Diamino-5-(furan-2-yl)-7-phenylquinoline-3,8-dicarbonitrile (10a) (Method A) Yield 2.48 g (70%); mp 140°C; IR: ν 3450–3340 (2 NH₂), 3070 (CH aromatic), 2240–2220 (CN), 1635 cm⁻¹ (C=C); MS: m/z 351 (M⁺, 38%); ¹H NMR: δ 6.65, 6.72 (2s, 4H, 2NH₂), 7.08–7.57 (m, 9H, C₆H₄, furan H). Analysis calculated for C₂₁H₁₃N₅O (351.36): C, 71.79; H, 3.73; N, 19.93. Found: C, 71.56; H, 3.82; N, 20.12.

Ethyl 2,4-diamino-3-cyano-5-(furan-2-yl)-7-phenylquinoline-8-carboxylate (10b) (Method A) Yield 2.78 g (70%); mp 159°C; IR: ν 3455–3360 (2 NH₂), 3040 (CH aromatic), 2890, 2867 (CH₃, CH₂), 2240–2220 (CN), 1680 (C=O), 1675 (C=N), 1635 cm⁻¹ (C=C); MS: m/z 398 (M⁺, 80%); ¹H NMR: δ 1.36 (t, 3H, $J=7.0$ Hz, CH₃), 3.10 (q, 2H, $J=7.0$ Hz, CH₂), 3.30, 6.63 (2s, 4H, 2NH₂), 7.08–7.84 (m, 9H, C₆H₄, furan H). Analysis calculated for C₂₃H₁₈N₄O₃: C, 69.34; H, 4.55; N, 14.06. Found: C, 69.57; H, 4.68; N, 14.23.

Synthesis of compound 12

To a solution of compound **3** (2.24 g, 0.01 mol) in 1,4-dioxane (50 ml) containing triethylamine (0.5 ml), elemental sulfur (0.32 g, 0.01 mol) was added. The mixture was heated under reflux for 1 h and then allowed to cool and poured onto an ice water mixture containing a few drops of hydrochloric acid. The mixture was left overnight to settle and the resultant solid product was collected by filtration, dried and crystallized from ethanol; yield: 1.60 g (63%); mp 115°C; IR: ν 3450–3370 (2 NH₂), 3050 (CH aromatic), 2240–2220 (CN), 1645 cm⁻¹ (C=C); MS: m/z 256 (M⁺, 14%); ¹H NMR: δ 3.56, 6.31 (2s, 4H, 2NH₂), 6.84 (s, 1H, thiophene H-5), 7.36–7.84 (m, 3H, furan H). Analysis calculated for C₁₂H₈N₄OS: C, 56.24; H, 3.15; N, 21.86; S, 12.51. Found: C, 56.09; H, 3.23; N, 21.98; S 12.49.

General procedure for synthesis of compounds 13a and 13b

To a solution of compound **3** (2.24 g, 0.01 mol) in ethanol (50 ml) containing triethylamine (0.5 ml), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The mixture was heated under reflux for 1.5 h and then poured onto ice water containing a few drops of hydrochloric acid. The solid product formed was collected by filtration, dried and crystallized from ethanol. Compound **13a** was obtained from malononitrile and compound **13b** was obtained from ethyl cyanoacetate.

2,4,7-Triamino-5-(furan-2-yl)quinoline-3,8-dicarbonitrile (13a) Yield 1.50 g (52%); mp 135°C; IR: ν 3450–3380 (3 NH₂), 3060 (CH aromatic), 2250–2220 (CN), 1660 (C=N), 1645 cm⁻¹ (C=C); MS: m/z 290 (M⁺, 18%); ¹H NMR: δ 3.55, 3.56, 4.1 (3s, 6H, 3NH₂), 7.36–7.84 (m, 4H, C₆H₄, furan H). Analysis calculated for C₁₅H₁₀N₆O: C, 62.06; H, 3.47; N, 28.95. Found: C, 61.98; H, 3.45; N, 29.12.

Ethyl 2,4,7-triamino-3-cyano-5-(furan-2-yl)quinoline-8-carboxylate (13b) Yield 2.50 g (74%); mp 142°C; IR: ν 3450–3380 (3 NH₂), 3060 (CH aromatic), 2987, 2892 (CH₃, CH₂), 2220 (CN), 1685

(C=O), 1660 (C=N), 1645 cm^{-1} (C=C); MS m/z 337 (M^+ , 24.37%); ^1H NMR: δ 1.30 (t, 3H, CH_3), 3.24 (q, 2H, CH_2), 4.32, 4.48, 5.61 (3s, 6H, 3 NH_2), 7.29–8.14 (m, 4H, C_6H , furan H). Analysis calculated for $\text{C}_{17}\text{H}_{15}\text{N}_5\text{O}_3$ (337.33): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.67; H, 4.53; N, 20.83.

Synthesis of 5-(1-(furan-2-yl)ethylidene)-5H-pyrazolo[3,4-b]pyridine-3,4,6-triamine (15)

To a solution of compound **3** (2.24 g, 0.01 mol) in ethanol (50 ml), hydrazine hydrate (0.5 g, 0.01 mol) was added. The mixture was heated under reflux for 2 h and the formed solid product, upon being poured into ice water containing a few drops of hydrochloric acid, was collected by filtration, dried and crystallized from 1,4-dioxane; yield 1.50 g (76%); mp 85°C; IR: ν 3460–3380 (3 NH_2), 3059 (CH aromatic), 2985 (CH_3), 1660 (C=N), 1645 cm^{-1} (C=C); MS: m/z 257 (M^+ , 4%); ^1H NMR: δ 3.42 (s, 3H, CH_3), 3.67, 6.74, 6.80 (3s, 6H, 3 NH_2), 7.11–8.07 (m, 3H, furan H). Analysis calculated for $\text{C}_{12}\text{H}_{12}\text{N}_6\text{O}$ (257.26): C, 56.24; H, 4.72; N, 32.79. Found: C, 56.14; H, 4.69; N, 32.55.

Synthesis of compound 16

To a solution of compound **3** (2.24 g, 0.01 mol) in ethanol (50 ml), phenylhydrazine (1.18 g, 0.01 mol) was added. The mixture was heated under reflux for 2 h and then poured onto ice water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from ethanol; yield 2.60 g (78%); mp 135°C; IR: ν 3450–3360 (2 NH_2), 3079 (CH aromatic), 2985 (CH_3), 2220 (CN), 1660 (exocyclic C=N), 1645 cm^{-1} (C=C); MS: m/z 332 (M^+ , 17%); ^1H NMR: δ 3.30 (s, 3H, CH_3), 6.29, 6.39 (2s, 4H, 2 NH_2), 7.16–7.70 (m, 8H, C_6H_5 , furan), 10.25 (s, 1H, NH). Analysis calculated for $\text{C}_{18}\text{H}_{16}\text{N}_6\text{O}$: C, 65.05; H, 4.85; N, 25.29. Found: C, 65.13; H, 4.83; N, 25.43.

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