New Bis(dihydropyridine-3,5-dicarbonitrile) Derivatives: Green Synthesis and Cytotoxic Activity Evaluation

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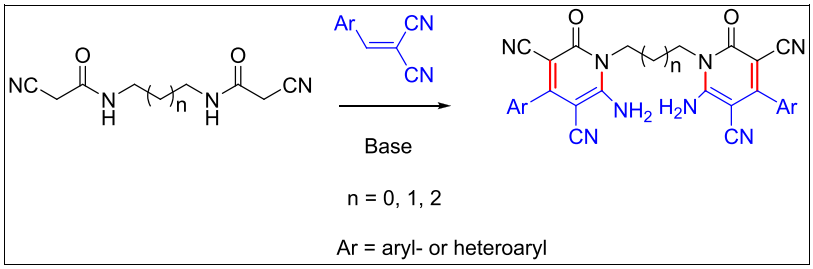


A synthesis of bis(dihydropyridine-3,5-dicarbonitrile) by a three-component reaction of one equivalent of bis-cyanoacetamides with two equivalents of both arylaldehydes and malononitrile in ethanol-containing piperidine is reported. Bis-cyanopyridones could also be obtained by the condensation of bis-cyanoacetamides with substituted arylidenemalononitriles in the presence of piperidine, chitosan, or montmorillonite as basic catalysts. The cytotoxicity of the synthesized products against the heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2) was assessed by WST-1 assay with concentration dependent cellular growth inhibitory effect especially for compounds **5l**, **5h**, and **5d**. The dose response curves indicate that IC50 were 87 ± 3.11 μg/mL, 104 ± 4.78 μg/mL, and 108 ± 5.12 μg/mL, respectively.

INTRODUCTION

Substituted cyanoacetamides are important intermediates in the synthesis of a variety of agrochemicals, dyes, and pharmacologically active compounds [1]. They were also found to exhibit significant biological activities as antifungal and antibacterial agents [2]. In addition, they are versatile and convenient intermediates for the synthesis of wide variety of various nitrogen-containing heterocyclic compounds [3–6].

Moreover, pyridine derivatives have attracted much attention as they exhibit important biological activities including antiviral [7,8], antitumor [9,10], and anti-inflammatory [11].

Furthermore, bis-heterocyclic compounds have been reported to possess interesting biological properties [12–14] including antihypertensive [15,16], antiallergenic [17], and antitumor activities [15,18].

As a part of an ongoing research program on bis-heterocycles [19–29], Michael addition reaction to acrylonitrile derivatives [30–37], as well as the utility of green benign approaches in organic synthesis [32,35], we report the results of our investigations concerning the different reactivity patterns of bis-cyanoacetamides toward cinnamonitrile derivatives.

RESULTS AND DISCUSSION

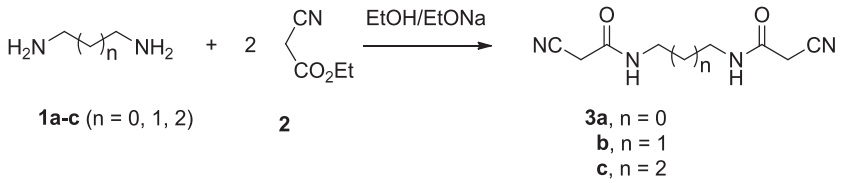
The bis-cyanoacetamides **3** were chosen as key intermediates to a variety of novel bis-cyanopyridone derivatives. They can be prepared by cyanoacetylation of the bisamines **1** with ethyl cyanoacetate **2** according to recently reported procedure (Scheme 1) [38].

To find the optimal reaction conditions for the synthesis of bis-pyridine derivatives **5**, we examined the reaction between the bis-cyanoacetamide **3a** and benzylidenemalononitrile derivative **4a** as a simple model system in the presence of a wide range of bases including piperidine, chitosan, DABCO, DBU, and montmorillonite (Scheme 2). The % yields in all cases are cited in Table 1.

As seen in this table, it was noticed that, although the reaction worked out best in refluxing ethanol in all catalysts, the reactions using piperidine, chitosan, and montmorillonite were found to achieve the best yields.

Encouraged by this success and in a trial to develop the scope of these reactions, a variety of bis-cyanoacetamides **3a**–**c** undergo nucleophilic addition reaction to the double bond of arylidenmalononitrile **4a**–**d** *via* a Michael-type addition reaction under the optimized conditions in the presence of piperidine (Method A), chitosan (Method B),

Scheme 1. Synthesis of bis(2-cyanoacetamide) compounds **3a**–**c**.



Scheme 2. Synthesis of bispyridine compound **5a**.

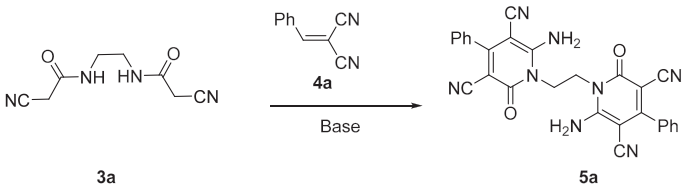


Table 1

Optimizing the yield of compound **5a**.

Entry	Time (h)	Solvent	Catalyst	Yield (%)
1	3	EtOH	Piperidine	92
2	3	EtOH	DABCO	84
3	3	EtOH	Chitosan	90
4	3	EtOH	Montmorillonite	88
5	3	EtOH	DBU	83

and montmorillonite (Method C) as catalysts. The results are summarized in Scheme 3 and Table 2.

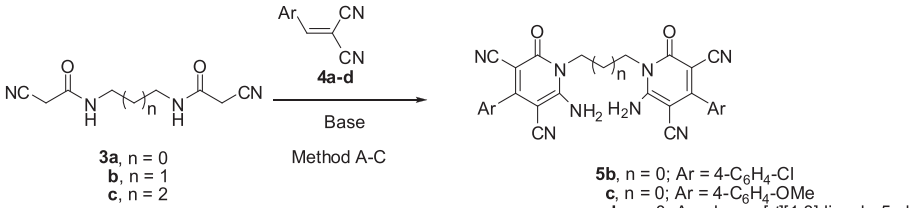
It is honestly to mention that during preparation of this paper we read the articles of Kheder *et al.* [39,40] in which they have also reported the formation of the

Table 2

The % yields of **5b**–**l** obtained using piperidine, chitosan, and montmorillonite.

	Yield (%)		
Compound	Piperidine	Chitosan	Montmorillonite
5b	93	92	90
5c	89	87	90
5d	90	91	89
5e	91	90	89
5f	89	88	90
5g	87	88	85
5h	85	83	85
5i	93	90	86
5j	91	89	85
5k	88	86	87
5l	87	88	85

Scheme 3. Synthesis of bispyridine compounds **5b**–**l**.



bis-pyridine derivatives **5a** upon heating **3a** with **4a** in ethanol at reflux in the presence of piperidine as a catalyst.

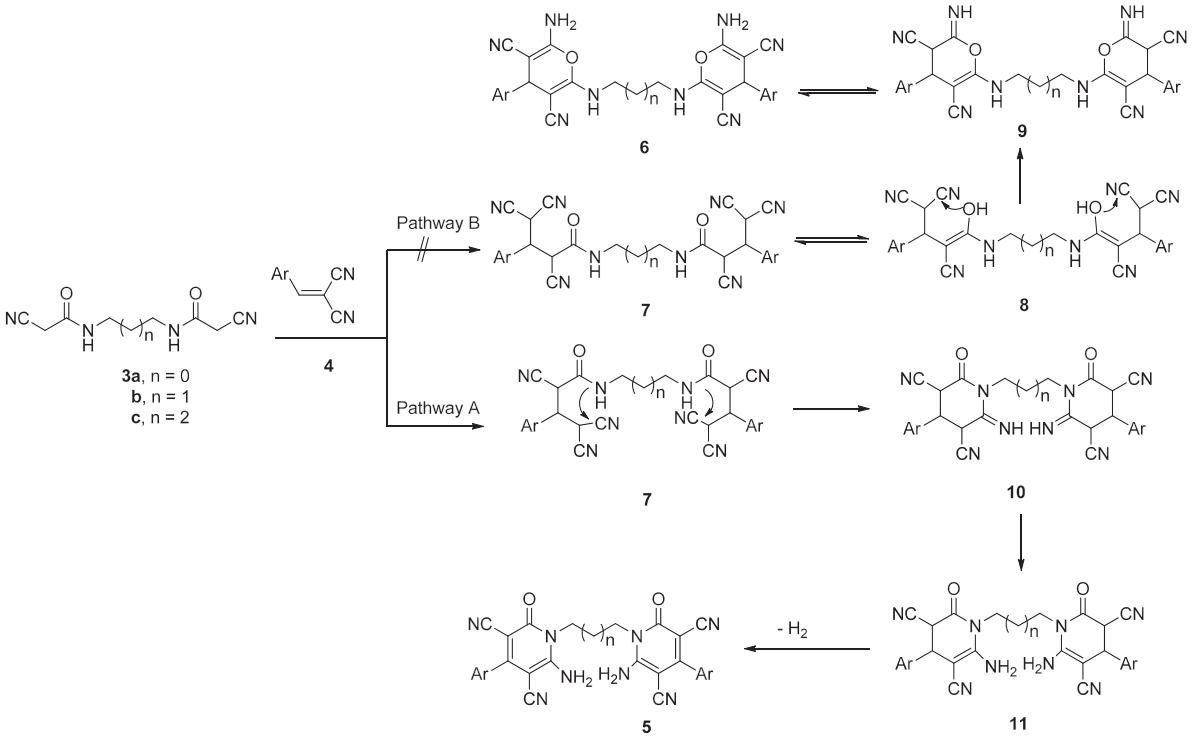
Taking into account the results obtained, one can suggest the following mechanistic pathway. The pyridine structures **5** are formed through the initial addition of the active methylene in the cyanoacetamides **3** to the double bond of cinnamonitriles **4** followed by cyclization involving NH of the amide and then elimination of two molecules of hydrogen (pathway A). On the other hand, the cyclization step that involves the carbonyl group leads to pyran structure **6** (pathway B). Structures **6** were readily excluded because the ^{1}H NMR spectra of the products lacked the pyran H-4 signal, which should appear at approximately $\delta \approx 4$–5 ppm (Scheme 4).

It is worth mentioning that the reaction of malononitrile **14** with a preheated mixture of aldehydes **12** and bis-cyanoacetamides **3** resulted in the formation of the corresponding bis-pyridine derivatives. In support of this viewpoint, we could manage to isolate the Knoevenagel condensation products, *N*,*N*′-(alkane-1,3-diyl)bis(2-cyano-3-arylacrylamide) **13** in some cases. Thus, compounds **13e**–**g** were obtained by Knoevenagel condensation of bis-cyanoacetamides **3b** with the appropriate aromatic aldehyde **12a**–**c** according to reported procedure [41,42]. The bis-pyridine derivatives **5e**–**g** were obtained by the reaction of bis(2-cyano-3-arylacrylamide) **13e**–**g** with malononitrile **14** in refluxing dioxane-containing few drops of piperidine (Scheme 5, Method D).

Compounds **5** were also obtained in good to excellent yields by a three-component reaction of two equivalents of both arylaldehyde **12** and malononitrile **14** with one equivalent of bis-cyanoacetamides **3** in refluxing ethanol with piperidine as a catalyst (Scheme 5, Method E).

Cytotoxic activity against Caco-2 cell line. The cytotoxic effect of synthesized products group 1 [**5a**, **5b**, **5c**, **5d**, **5i**, **5j**] and group 2 [**5k**, **5l**, **5e**, **5f**, **5g**, **5h**] were evaluated against heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2), compared with doxorubicin as a reference drug, using the WST-1 cell proliferation assay for quantification of cytotoxic effect in form of cell proliferation and viability. Basically, WST-1 assay is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. The increase in the number of viable cells results in an increase in the overall mitochondrial dehydrogenases activity. The augmentation in enzyme activity leads to the increase in the amount of formazan dye formed. The formazan dye produced by viable cells can be quantified by a multiwell microplate reader by measuring the absorbance of the dye solution at 450 nm. Tables 3 and 4 represents the cytotoxic effect of the

Scheme 4. Proposed mechanism.



Scheme 5. Synthesis of bis-pyridine compounds **5e–g** through the reaction of cyanoacrylonitriles with malononitrile and through one pot three-component reaction.

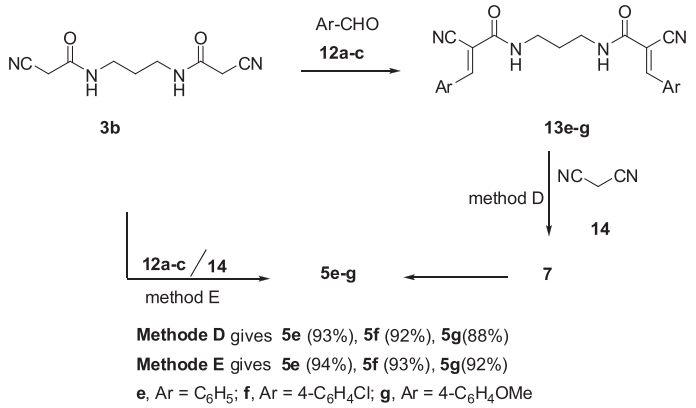


Table 3

Cytotoxic effect (%) of group 1 compounds against Caco-2.

	Cytotoxic effect %					
Conc. (μg/mL)	**5a**	**5b**	**5c**	**5d**	**5i**	**5j**
0	0	0	0	0	0	0
10	8.4218	7.3477	6.86664	9.9268	5.05131	2.56258
20	12.9865	13.5362	9.3097	15.6091	7.67915	5.2207
30	17.0549	16.9272	14.8764	22.4538	10.6434	9.7604
50	25.6566	24.3542	20.5581	32.592	15.7704	17.5443
100	37.8847	40.5667	32.2284	50.8879	27.4308	30.7843
150	57.1156	59.95122	52.6709	65.1504	45.087	46.2823
200	71.3804	75.3305	65.8664	82.5903	57.0844	62.3462

Table 4

Cytotoxic effect (%) of group 2 compounds against Caco-2.

	Cytotoxic effect %						
Conc.(μg/mL)	**5k**	**5l**	**5e**	**5f**	**5g**	**5h**	Reference
0	0	0	0	0	0	0	0
10	1.7654	14.3402	4.9863	6.2387	2.7849	11.638	15.548
20	3.8809	22.5643	8.5497	11.3249	7.4932	18.1895	25.476
30	7.3372	30.5569	12.4887	16.4533	12.4599	23.8392	32.652
50	12.8897	40.5337	20.3385	22.4353	19.5508	34.9765	43.438
100	23.987	60.8975	37.5573	36.0826	30.4326	51.6649	64.622
150	39.7074	79.459	50.3453	51.9768	43.4665	68.4604	82.541
200	53.0448	90.6579	67.1552	65.3709	55.4398	82.3978	97.338

tested compounds in response to their concentration as illustrate in Figures 1 and 2 (data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was estimated).

Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments. The results are represented in Table 5 and Figure 3.

The results revealed that compounds **5l**, **5h**, and **5d** containing benzo[*d*][1,3]dioxole substituent (IC_{50} were

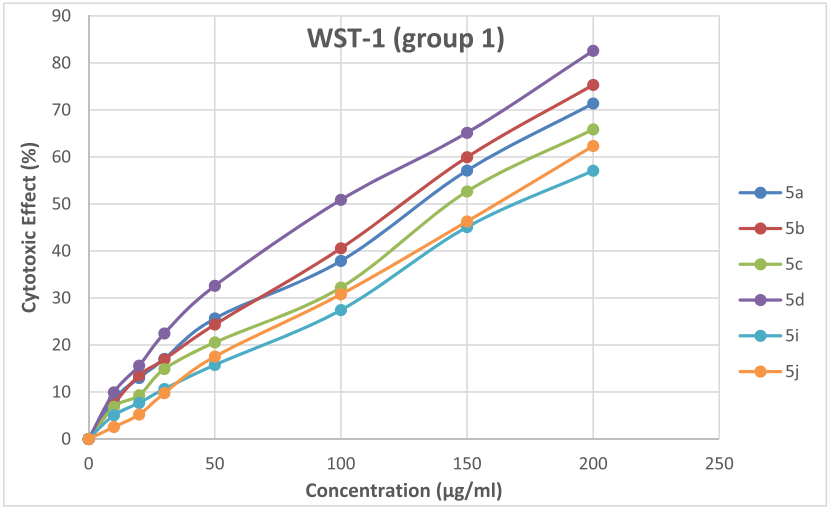


Figure 1. Cytotoxic effect chart of group 1compounds against Caco-2 cell line.

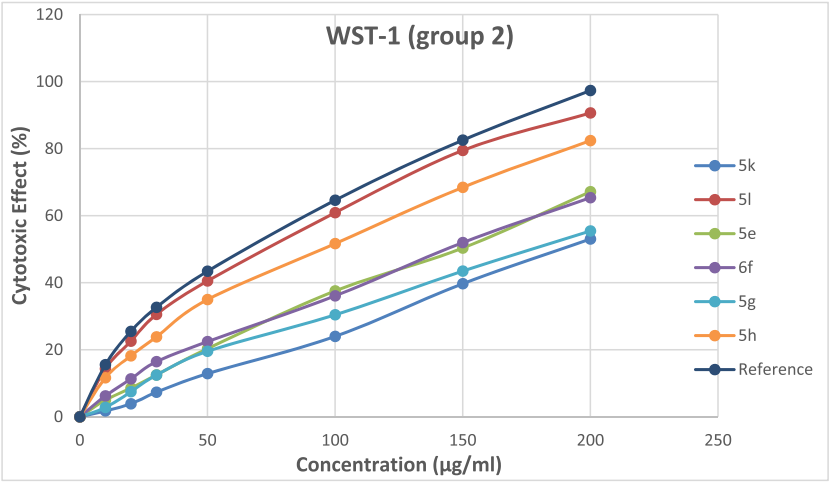


Figure 2. Cytotoxic effect chart of group 2 compounds against Caco-2 cell line.

Table 5

IC_{50} values of tested compounds and ± standard deviation against Caco-2.

Compound No.	IC_{50} (µg/mL)	Compound No.	IC_{50} (µg/mL)
5a	132 ± 6.08	**5 k**	180 ± 9.26
5b	125 ± 5.54	**5 l**	87 ± 3.11
5c	147 ± 6.98	**5e**	148 ± 5.37
5d	108 ± 5.12	**5f**	145 ± 5.79
5i	173 ± 8.67	**5 g**	174 ± 9.17
5j	160 ± 7.84	**5 h**	104 ± 4.78
Reference	80 ± 5.65		

87 ± 3.11 µg/mL, 104 ± 4.78 µg/mL, and 108 ± 5.12 µg/mL, respectively) have the highest cytotoxic effect against heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2), while **5b**, **5a**, **5f**, **5c**, and **5e** have moderate cytotoxic activity (IC_{50} were 125 ± 5.54 µg/mL, 132 ± 6.08 µg/mL, 145 ± 5.79 µg/mL, 147 ± 6.98 µg/mL, and 148 ± 5.37 µg/mL). On the other hand, **5j**, **5i**, **5g**, and **5k** have lower cytotoxic activity against the tested cell line (IC_{50} = 160 ± 7.84 µg/mL, 173 ± 8.67 µg/mL,

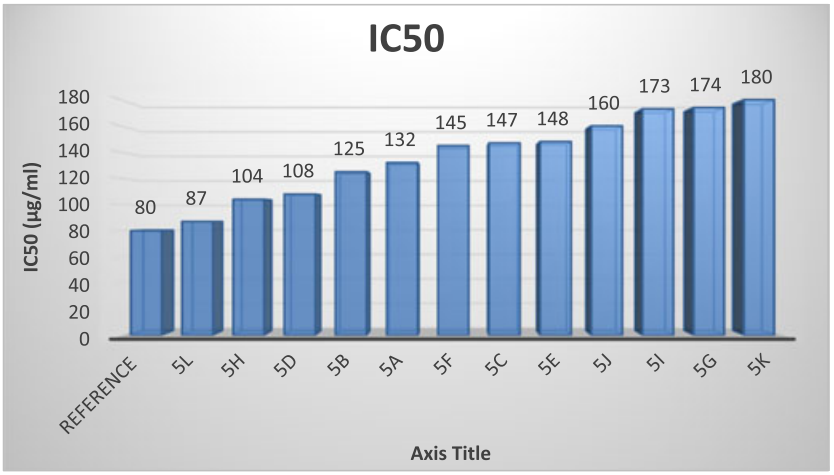


Figure 3. Estimated IC_{50} values of tested compounds against Caco-2 cell line.

174 ± 9.17 µg/mL, and 180 ± 9.26 µg/mL, respectively). IC_{50} of Doxorubicin as a reference drug was found to be 80 ± 5.65 µg/mL, the smaller values of IC_{50} for the higher cytotoxic effect. It is clear that the presence of benzo[*d*][1,3]dioxole substituent has shown to play an important role on the observed growth inhibition. These results are in agreement with that previously reported [43–47].

CONCLUSIONS

The bis-cyanoacetamide moiety, behaving like *C*-nucleophiles, affects simple and facile Michael addition reactions with various arylidenemalononitriles, and regioselectively yielding different 1,2-dihydropyridine-3,5-dicarbonitrile derivatives. Moreover, piperidine, montmorillonite, and chitosan were found to be the best catalysts for this type of reactions. Compounds **5l**, **5h**, and **5d** may have significant and promising growth inhibatory efficiency against heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2). The anticancer effect might be due to such inhibitory effect to the inner mitochondrial membrane dehydrogenase enzyme, which in turn decreases the cellular activity including the rate of cell division.

EXPERIMENTAL

Materials and methods. Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using an FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^{1}H NMR spectra were recorded in DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 400 MHz using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) model. The elemental analyses were performed at the Micro analytical center, Cairo University.

General procedure of synthesis of compound **5a–l** is as follows:

- **Methods A–C**: A mixture of bisamides **3a–c** (1 mmol) and activated cinnamonitriles **4a–d** (2.2 mmol) in absolute ethanol (15 mL) was heated at reflux in the presence of piperidine (0.2 mL, method A), chitosan (0.02 g, method B) or montmorillonite (0.02 g, method C) for 3 h. The crude solid was isolated and recrystallized from the proper solvent whereby chitosan and montmorillonite can be recovered and washed with acetone, dried, and reused in another reaction.
- **Method D**: To a mixture of bis(2-cyanoacrylamides), **13a–l** (1 mmol) and malononitrile **14** (2.2 mmol) in dioxane (10 mL) was added piperidine (0.2 mL), and the mixture was set at reflux for 3 h. The crude solid was isolated and recrystallized from the proper solvent.
- **Method E**: To a mixture of bisamides **3a–c**, aromatic aldehydes **12a–d** (2.2 mmol) and malononitrile **14** (2.2 mmol) in absolute ethanol (15 mL) was added piperidine (0.2 mL) for 3 h, and the mixture was heated at reflux for 3 h. The crude solid was isolated and recrystallized from the proper solvent.

1,1′-(Ethane-1,2-diyl)bis(6-amino-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile) (5a) *[39].* Yellowish white crystals (ethanol/dioxane (1:1)), Mp > 300°C, IR (KBr): ν = 3360, 3282 (br, NH_2), 2218 (CN), 1681 (CO) cm^{-1}, ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.42 (s, 4H, $2CH_2$), 7.42–7.53 (m, 10H, Ar—H), 8.57 (br s, 4H, $2NH_2$) ppm,

MS (EI, 70 eV): m/z (%) = 498 [M^+], *Anal*. Calcd for $C_{28}H_{18}N_8O_2$: C, 67.46; H, 3.64; N, 22.48. Found: C, 67.32; H, 3.55; N, 22.39.

1,1′-(Ethane-1,2-diyl)bis(6-amino-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5b). Pale yellow crystals (ethanol/dioxane (1:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3416, 3362 (br, NH_2), 2215 (CN), 1661, 1630 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 4.39 (s, 4H, $2CH_2$), 7.43–7.64 (dd, 8H, Ar—H), 8.61 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 567 [M^+], *Anal*. Calcd for $C_{28}H_{16}Cl_2N_8O_2$: C, 59.27; H, 2.84; N, 19.75. Found: C, 59.18; H, 2.69; N, 19.59.

1,1′-(Ethane-1,2-diyl)bis(6-amino-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5c). Pale yellow crystals (ethanol/dioxane (1:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3331, 3133 (br, NH_2), 2214 (CN), 1645 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 3.83 (s, 6H, $2OCH_3$), 4.38 (s, 4H, $2CH_2$), 7.06–7.41 (dd, 8H, Ar—H), 8.49 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 558 [M^+], *Anal*. Calcd for $C_{30}H_{22}N_8O_4$: C, 64.51; H, 3.97; N, 20.06. Found: C, 64.46; H, 3.82; N, 20.19.

1,1′-(Ethane-1,2-diyl)bis(6-amino-4-(benzo[d][1,3]dioxol-5-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5d). Yellow crystals (ethanol/dioxane (1:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3309, 3277 (br, NH_2), 2206 (CN), 1646 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 4.16 (s, 4H, $2CH_2$), 6.12 (s, 4H, $2OCH_2O$), 6.93–7.06 (m, 6H, Ar—H), 8.31 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 586 [M^+], *Anal*. Calcd for $C_{30}H_{18}N_8O_6$: C, 61.43; H, 3.09; N, 19.11. Found: C, 61.31; H, 3.15; N, 18.98.

1,1′-(Propane-1,3-diyl)bis(6-amino-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile) (5e). Yellow crystals (ethanol), Mp = 218–220°C, IR (KBr): ν = IR (KBr): ν = 3436, 3348 (br, NH_2), 2216 (CN), 1643 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.56 (m, 2H, CH_2), 4.09 (m, 4H, $2CH_2$), 7.46–7.55 (m, 10H, Ar—H), 8.36 (br s, 4H, $2NH_2$), MS (EI, 70 eV): m/z (%) = 512 [M^+], *Anal*. Calcd for $C_{29}H_{20}N_8O_2$: C, 67.96; H, 3.93; N, 21.86. Found: C, 67.82; H, 3.88; N, 21.79.

1,1′-(Propane-1,3-diyl)bis(6-amino-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5f). Pale yellow crystals (ethanol), Mp = 224–226°C, IR (KBr): ν = IR (KBr): ν = 3429, 3361 (br, NH_2), 2214 (CN), 1645 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.55 (m, 2H, CH_2), 4.07 (m, 4H, $2CH_2$), 7.50–7.63 (dd, 8H, Ar—H), 8.42 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 581 [M^+], *Anal*. Calcd for $C_{29}H_{18}Cl_2N_8O_2$: C, 59.91; H, 3.12; N, 19.27. Found: C, 59.86; H, 3.16; N, 19.19.

1,1′-(Propane-1,3-diyl)bis(6-amino-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5g). Yellow crystals (ethanol), Mp = 220–222°C, IR (KBr): IR (KBr): ν = 3434, 3222 (br, NH_2), 2215 (CN), 1643 (CO) cm^{-1} ^1H NMR (400 MHz, DMSO-d_6): δ = 1.61 (m, 2H, CH_2), 3.82 (s, 6H, 2OCH3), 4.09 (m, 4H, $2CH_2$), 7.05–7.47 (dd, 8H, Ar—H), 8.38 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 572 [M^+], *Anal*. Calcd for $C_{31}H_{24}N_8O_4$: C, 65.03; H, 4.23; N, 19.57. Found: C, 64.91; H, 4.16; N, 19.51.

1,1′-(Propane-1,3-diyl)bis(6-amino-4-(benzo[d][1,3]dioxol-5-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5h). Yellow crystals (ethanol/dioxane (3:1)), Mp = 230–232°C, IR (KBr): IR (KBr): ν = 3343, 3186 (br, NH_2), 2215 (CN), 1648 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.70 (m, 2H, CH_2), 3.99 (m, 4H, $2CH_2$), 6.14 (s, 4H, $2OCH_2O$), 6.97–7.10 (m, 6H, Ar—H), 8.41 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 600 [M^+], *Anal*. Calcd for $C_{31}H_{20}N_8O_6$: C, 62.00; H, 3.36; N, 18.66. Found: C, 61.85; H, 3.19; N, 18.57.

1,1′-(Butane-1,4-diyl)bis(6-amino-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile) (5i). Pale yellow crystals (ethanol/dioxane (3:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3425, 3331 (br, NH_2), 2213 (CN), 1619 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.62 (m, 4H, $2CH_2$), 4.05 (m, 4H, $2CH_2$), 7.48–7.56 (m, 8H, Ar—H), 8.42 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 526 [M^+], *Anal*. Calcd for $C_{30}H_{22}N_8O_2$: C, 68.43; H, 4.21; N, 21.28. Found: C, 68.31; H, 4.14; N, 21.16.

1,1′-(Butane-1,4-diyl)bis(6-amino-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5j). Pale yellow crystals (ethanol/dioxane (3:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3373, 3331, 3212 (br, NH_2), 2217 (CN), 1651 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.62 (m, 4H, $2CH_2$), 4.04 (m, 4H, $2CH_2$), 7.51–7.65 (dd, 8H, Ar—H), 8.45 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 595 [M^+], *Anal*. Calcd for $C_{30}H_{20}Cl_2N_8O_2$: C, 60.51; H, 3.39; N, 18.82. Found: C, 60.44; H, 3.18; N, 18.77.

1,1′-(Butane-1,4-diyl)bis(6-amino-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5k). Pale yellow crystals (ethanol/dioxane (3:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3331, 3168 (br, NH_2), 2212 (CN), 1610 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.62 (m, 4H, $2CH_2$), 3.84 (s, 6H, $2OCH_3$), 4.03 (m, 4H, $2CH_2$), 7.08–7.48 (dd, 8H, Ar—H), 8.34 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 586 [M^+], *Anal*. Calcd for $C_{32}H_{26}N_8O_4$: C, 65.52; H, 4.47; N, 19.10. Found: C, 65.44; H, 4.32; N, 19.03.

1,1′-(Butane-1,4-diyl)bis(6-amino-4-(benzo[d][1,3]dioxol-5-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile) (5l). Yellow crystals (ethanol/dioxane (3:1)), Mp > 300°C, IR (KBr): IR (KBr): ν = 3378, 3215 (br, NH_2), 2214 (CN), 1652 (CO) cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6): δ = 1.61 (m, 4H, $2CH_2$), 4.03 (m, 4H, $2CH_2$), 6.14 (s, 4H, $2OCH_2O$), 6.98–7.09 (m, 6H, Ar—H), 8.38 (br s, 4H, $2NH_2$) ppm, MS (EI, 70 eV): m/z (%) = 614 [M^+], *Anal*. Calcd for $C_{32}H_{22}N_8O_6$: C, 62.54; H, 3.61; N, 18.23. Found: C, 62.49; H, 3.52; N, 18.14.

Cytotoxic activity. The heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2) were cultured and tested at Nanotechnology and Advanced materials central lab, Giza, Egypt. The culture was maintained in DMEM with 10% FBS at 37°C humidified with 5% CO_2. Various concentrations of the compound under test and doxorubicin that was used as a reference drug (0.0, 10, 20, 30, 50, 100, 150, and 200 μg/mL) were dissolved in mixture of 50% DMSO, 40% ethanol, and 10% deionized water, then added to the cell monolayer in triplicate wells individual dose, and its cytotoxicity was tested using a standard WST-1 cell proliferation assay as a fast and sensitive quantification of cell proliferation and viability, in a 96-well microtiter plate for 24 h and measuring the absorbance of the dye solution at 450 nm [48].

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