Synthesis and Cytotoxic Effect of Some Novel 1,2-Dihydropyridin-3-carbonitrile and Nicotinonitrile Derivatives

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Abstract: 1-(2,4-Dichlorophenyl)-3-(4-fluorophenyl)propen-1-one (1) was prepared and reacted with an active methylene compound (ethyl cyanoacetate) in the presence of ammonium acetate to give the corresponding cyanopyridone 2. Compound 2 reacted with hydrazine hydrate, malononitrile, ethyl bromoacetate and phosphorous oxychloride to afford compounds 4 and 7–11, respectively. The 2-chloropyridine derivative 11 reacted with different primary amines, namely benzyl amine, piperonyl amine, 1-phenylethyl amine, and/or the secondary amines 2-methyl-pipridine and morpholine to give the corresponding derivatives 12–15. Hydrazinolysis of chloropyridine derivative 11 with hydrazine hydrate afforded the corresponding hydrazino derivative 17. Condensation of compound 17 with ethyl acetoacetate, acetylacetone, isatin and different aldehydes gave the corresponding derivatives 18–21. Some of newly synthesized compounds were screened for cytotoxic activity against three tumor cell lines. The results indicated that compounds 8 and 16 showed the best results, exhibiting the highest inhibitory effects towards the three tumor cell lines, which were higher than that of the reference doxorubicin and these compounds were non-cytotoxic towards normal cells (IC_{50} values > 100 µg/mL).

Keywords: nicotinonitrile; 4-fluorophenylpyridine; acetohydrazide; chloropyridine; cytotoxicity

1. Introduction

Cancer is the second leading cause of death in both developing and developed countries [1]. The leading forms were lung cancer, colorectal cancer, liver cancer and breast cancer [2,3]. Cancer treatment has been a major research and development effort in academia and the pharmaceutical industry for numerous years [4,5]. Despite the fact that there is a large amount of information available dealing with the clinical aspects of cancer chemotherapy, we felt that there was a clear requirement for an updated treatment from the point of view of medicinal chemistry and drug design [6]. Another major goal for developing new anticancer agents is to overcome cancer resistance to drug treatment, which has made many of the currently available chemotherapeutic agents ineffective [7].

Chalcones, one of the major classes of natural products with widespread occurrence in vegetables, fruits, spices and soy-based foodstuffs, have been reported to possess several biological activities such as antibacterial [8,9], anti-fungal [10,11], anti-inflammatory [12], and anti-tumor activities [13,14]. An
important feature of chalcones is their ability to act as an intermediate for the synthesis of biologically active heterocyclic compounds such as pyrimidine and pyridine derivatives [15,16]. The pyridine nucleus is an integral part of anti-inflammatory and anticancer agents [17,18]. On the other hand, cyanopyridone and cyanopyridine derivatives have shown to possess promising antimicrobial [19] antioxidant [20,21], antibiotic [22], antiinflammatory [23,24], analgesic, anticonvulsant [25] and anticancer [26–29] properties. 3-Cyano-2-pyridones are analogous to the alkaloid ricinine, the first known alkaloid containing a cyano group. The anticancer activity of 3-cyano-2-pyridone derivatives is of much interest owing to the different types of biological targets they might interfere with, e.g., PDE3, PIM1 kinase, and survivin (Figure 1) [30].

Motivated by the above recent literature observations and our own previous reports [20,21,31–33], herein some new pyridine derivatives were synthesized, leading to interesting heterocyclic scaffolds that are mostly useful for the creation of varied chemical libraries of drug-like molecules for biological screening.

![Figure 1](image_url)

**Figure 1.** Various 3-cyano-2-oxopyridine derivatives with potential growth inhibitory and/or antiangiogenic actions through PDE3 inhibition (a); PIM-1 kinase inhibition (b); or survivin inhibition (c).

2. Results and Discussion

2.1. Chemistry

The synthesis of the designed target compounds was achieved as outlined in Schemes 1–3. During this investigation, the pyridin-3-carbonitrile starting material 2 was prepared by condensation of the corresponding enone 1[34] with ethyl cyanoacetate in the presence of excess ammonium acetate (Scheme 1). Compound 2 can also be obtained in high yield through a four-component modified Hantzch reaction, in a one-step synthesis, by refluxing a mixture of 2,4-dichloro-acetophenone, 4-fluorobenzaldehyde, ethyl cyanoacetate and ammonium acetate in n-butanol. The structure of pyridin-3-carbonitrile 2 was supported by elemental analysis, IR, ($^{1}$H, $^{13}$C) NMR and mass spectral studies. Its IR spectrum showed absorption bands at 3278, 2219, 1632 cm$^{-1}$ indicating the presence of NH, CN and CO groups, respectively. Its $^{1}$H-NMR spectrum displayed a broad D$_2$O exchangeable singlet at $\delta$ 8.10 ppm for the NH proton, while its $^{13}$C-NMR spectrum also revealed signals at $\delta$ 117.6 and 161.8 ppm for CN and CO moieties, respectively. The mass spectrum showed a molecular ion peak at $m/z$ 358 (M$^+$, 98%), which tallies with its molecular formula C$_{18}$H$_9$Cl$_2$FN$_2$O.

Pyridin-3-carbonitrile 2 possesses several reactive sites, *viz.* CN, NH, and CO groups, which can play a great role in the synthesis of heterocyclic derivatives, most of which are interesting from both the chemical and biological point of view. Thus, hydrazinolysis of pyridin-3-carbonitrile 2 with hydrazine hydrate in absolute ethanol for 15 h affords the corresponding pyrazolo[3,4-b]pyridin-3-amine derivative 4 through the elimination of a water molecule from the intermediate 3 (Scheme 1). Pyrazolo[3,4-b]pyridine derivative 4 was identified by the absence of the cyan and carbonyl groups signals in its IR and the presence of an amino group signal at $\delta$ 5.69 ppm and the broad band of the...
NH proton at δ 10.05 ppm in its $^1$H-NMR spectrum. Its mass spectrum showed a molecular ion peak at $m/z$ 372 (M$^+$; 72%), which conforms to its molecular formula C$_{18}$H$_{11}$Cl$_2$FN$_4$.

**Scheme 1.** General methods for the preparation of compounds 2–7. Reagents and conditions: (i) NaOH/EtOH, stirring; (ii) ethyl cyanoacetate/CH$_3$COONH$_4$/EtOH, reflux; (iii) hydrazine hydrate 98% (1 mL)/EtOH, reflux; and (iv) malononitrile/triethylamine (3 mL)/EtOH, reflux.

**Scheme 2.** General methods for the preparation of compounds 8–11. Reagents and conditions: (i) ethyl bromoacetate/anh. K$_2$CO$_3$/dry CH$_3$COCH$_3$, reflux; (ii) hydrazine hydrate 98% (2 mL)/EtOH, reflux; (iii) 4-flurobenzaldehyde/EtOH, reflux; and (iv) phosphorus oxychloride/EtOH, reflux.
Compound 2 was also refluxed with malononitrile to afford 4-amino-7-(2,4-dichlorophenyl)-5-(4-fluorophenyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carbonitrile (7) via the intermediates 5 and 6, as confirmed by elemental analysis, $^1$H- and $^{13}$C-NMR. The R spectrum of compound 7 showed bands at 3312, 3249, 3145 and 1688 cm$^{-1}$ due to NH$_2$, NH and CO groups, respectively; its $^{13}$C-NMR spectrum showed signals at δ 118.1 and 168.8 ppm corresponding to CN and CO groups, respectively. Its mass spectrum showed a molecular ion peak at $m/z$ 424 (M$^+$; 92%), which conforms to its molecular formula C$_{21}$H$_{11}$Cl$_2$FN$_4$O.

Moreover, when pyridin-3-carbonitrile 2 was alkylated with ethyl bromoacetate in acetone using anhydrous potassium carbonate as catalyst, the ester derivatives 8 was produced (Scheme 2).
The $^1$H-NMR spectrum of 8 showed signals at $\delta$ 1.15, 4.13 and 5.11 ppm due to the presence of (CH$_2$-ester), (OCH$_2$-ester) and (O–CH$_2$) respectively; and its $^{13}$C-NMR exhibited signals at $\delta$ 13.5, 43.8, 61.3 and 167.9 ppm due to (CH$_2$), (2CH$_2$) and (CO) groups, respectively.

Ester derivative 8 were condensed with hydrazine hydrate (98%) in ethanol to give 2-[3-cyano-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)pyridin-2-yl]malononitrile (9) (Scheme 2), confirmed by its IR and NMR spectra. Its IR spectrum showed strong peaks at 3314, 3282 and 3116 cm$^{-1}$ indicating the presence of a –NHNH$_2$ group, and the NMR ($^1$H and $^{13}$C) and mass spectra were also in accordance with its structure.

Schiff base 10 can be produced via condensation of acetyloxadiazole 9 with an aromatic aldehyde, namely 4-flourobenzaldehyde, in ethanol (Scheme 2). The structure of Schiff base 10 was elucidated based on the spectral and analytical data. The IR spectrum revealed the absence of the absorption bands of (NH$_2$) group absorption and its $^1$H-NMR spectrum showed a singlet at $\delta$ 8.10 ppm due to the presence of the (CH=N-N) group.

In addition, chlorination of cyanopyridone 2 with phosphorous oxychloride afforded the 2-chloronicotinonitrile derivative 11 (Scheme 2) in good yield, after 8 h. The IR spectrum showed the absence of a characteristic CO group band.

It is known that position 2 in chloronicotinonitrile derivatives shows distinct activities toward nucleophiles, especially nitrogen nucleophiles. Thus, nucleophilic replacement of the chlorine atom of chloronicotinonitrile 11 was performed by refluxing with different primary amines, namely benzyl-amine, piperonylamidine, 1-phenylethylamine and/or secondary amines, namely 2-methyl-piperidine and morpholine in boiling ethanol for 6–12 h to afford the corresponding 2-aminopyridine derivatives 12–15a,b; respectively (Scheme 3). The elemental analysis and spectral data of compounds 12–15a,b were in agreement with the proposed structures. The $^1$H-NMR of compound 15b for example, showed signals at $\delta$ 3.31 and 3.72 ppm due to the presence of (2N–C$_2$–H$_2$) and (2O–C$_1$–H$_2$) respectively; and its $^{13}$C-NMR exhibited signals at $\delta$ 47.9, 49.1, 64.10 and 65.9 ppm due to the presence of (2N–CH$_2$) and (2O–CH$_2$), respectively.

Furthermore, nucleophilic displacement was carried out by heating the chloropyridine derivative 11 with malononitrile in ethanol containing a few drops of triethylamine as a catalyst to give 2-[3-cyano-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)pyridin-2-yl]malononitrile (16). The structure of compound 16 was confirmed by its spectral data; the IR spectrum showed the presence of the CN group at 2218, 2225 cm$^{-1}$. In addition, the NMR ($^1$H and $^{13}$C) and mass spectral data were in accordance with its structure. Hydrazinolysis of the chloropyridine derivative 11 was performed by its reaction with excess hydrazine hydrate in refluxing ethanol to give the hydrazino derivative 17 (Scheme 3). The structure of 17 was confirmed by its spectral data. The IR spectrum exhibited the characteristic absorption bands at 3440, 3320, 3150 cm$^{-1}$ indicating the presence of the –NHNH$_2$ group. Its mass spectrum showed a molecular ion peak at m/z 372 (M$^+$; 39%), which conforms to its molecular formula C$_{16}$H$_{12}$Cl$_2$FN$_4$.

The 2-hydrazinonicotinonitrile 17 is another key compound, which facilitates the synthesis of diverse heterocyclic compounds. Thus, it reacted with different active methylene ($\beta$-diketones), namely: ethyl acetacetate and acetylacetone in glacial acetic acid, and thus the N-pyrazolo derivatives 18 and 19 were produced (Scheme 3). The IR spectrum of compound 19, for example, showed a characteristic band at 2210 cm$^{-1}$ for the CN group and its $^1$H-NMR spectrum revealed singlets at $\delta$ 2.31, 2.45 and 6.15 ppm due to (2CH$_3$) and the (CH-pyrazole) moieties, respectively. The $^{13}$C-NMR data displayed two characteristic signals at $\delta$ 18.4, 19.3 and 117.9 ppm for 2CH$_3$ and CN groups, respectively. Also, on heating compound 17 with isatin in ethanol it afforded 6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-2-(2-oxoindolin-3-ylidene)hydrazinylnicotinonitrile (20) in good yield (Scheme 3). The structure of compound 20 gave correct elemental analyses values and spectral features.

In addition, a new series of Schiff bases expected to be biologically active, heating of 2-hydrazinonicotinonitrile 17 with different aromatic aldehydes, namely 2-bromobenzaldehyde, 4-nitrobenzaldehyde, 2-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and/or 1-naphthaldehyde
in ethanol gave the corresponding Schiff bases 21a–e, respectively. The structure of compounds 21a–e was characterized by the disappearance of the NH$_2$ group. In addition, the ¹H-NMR spectra showed a singlet at around δ 8.31–8.33 due to the presence of the azomethine group (CH=N=). Finally, reaction of 2-hydrazinonicotinonitrile 17 with acetic acid or DMF afforded the corresponding pyrazolo[3,4-b]pyridin-3-amine derivative 4 through intramolecular cyclization via the addition of the NH$_2$ functional group at the CN group.

2.2. In Vitro Anticancer Screening

The in vitro cytotoxic activity the newly synthesized compounds against human breast cell line (MCF7), non-small cell lung cancer NCI-H460, CNS cancer SF-268 and WI 38 (normal fibroblast cells) were evaluated using doxorubicin as the reference drug, according to the method reported by Skehan et al. [35]. The IC$_{50}$ values of the synthesized compounds compared to the reference drug are shown in Table 1.

| Comp. No. | IC$_{50}$ (µg/mL) |
|-----------|-------------------|
|           | MCF-7             | NCI-H460          | SF-268          | WI 38            |
| 4         | 67.04 ± 6.23 $^c$ | 56.75 ± 8.20 $^c$ | 69.05 ± 9.15 $^c$ | 18.62 ± 1.21     |
| 7         | 36.22 ± 2.14 $^c$ | 74.03 ± 3.65 $^c$ | 62.13 ± 3.61 $^c$ | 22.97 ± 8.2      |
| 8         | 0.02 ± 0.002 $^a$ | 0.01 ± 0.002 $^a$ | 0.02 ± 0.045 $^a$ | non-cytotoxic    |
| 9         | 2.41 ± 1.24 $^a$  | 2.30 ± 2.86 $^a$  | 0.46 ± 0.06 $^a$  | 62.19 ± 2.02     |
| 10        | 30.58 ± 1.10 $^b$ | 30.67 ± 1.64 $^b$ | 28.18 ± 8.83 $^b$ | 19.80 ± 2.68     |
| 13        | 16.26 ± 1.87 $^b$ | 18.92 ± 1.03 $^b$ | 23.24 ± 4.12 $^b$ | 20.38 ± 4.99     |
| 15a       | 37.07 ± 7.34 $^c$ | 16.37 ± 2.32 $^c$ | 38.94 ± 2.63 $^c$ | 30.62 ± 6.21     |
| 16        | 0.01 ± 0.002 $^a$ | 0.02 ± 0.001 $^a$ | 0.01 ± 0.003 $^a$ | non-cytotoxic    |
| 17        | 0.61 ± 0.082 $^a$ | 0.86 ± 0.02 $^a$  | 2.19 ± 0.83 $^a$  | 64.11 ± 1.22     |
| 18        | 20.22 ± 2.26 $^b$ | 0.01 ± 0.003 $^a$ | 20.20 ± 3.26 $^b$ | 29.82 ± 4.88     |
| 19        | 75.20 ± 13.86 $^c$| 62.30 ± 10.35 $^c$| 10.39 ± 4.19 $^a$| 50.20 ± 10.22    |
| 20        | 0.66 ± 0.21 $^a$  | 0.90 ± 0.12 $^a$  | 2.34 ± 0.51 $^a$  | 72.45 ± 2.40     |
| 21d       | 66.02 ± 8.25 $^c$ | 44.95 ± 10.46 $^c$| 32.45 ± 6.04 $^b$| non-cytotoxic    |
| DMSO      | 0.04 ± 0.008      | 0.09 ± 0.008      | 0.09 ± 0.007      | non-cytotoxic    |

MCF-7 (breast adenocarcinoma); NCI-H460 (non-small cell lung cancer); SF-268 (CNS cancer); WI 38 (normal fibroblast cells); Doxorubicin (anticancer positive control); DMSO (solvent, negative control); $^a$ highly active; $^b$ moderately active; $^c$ weakly active.

From the results presented in Table 1 and Figure 2, it is evident that some of the compounds were active against the three human cancer cell lines. Compounds 8 and 16 displayed high cytotoxic activity against the tested cell lines (most of the IC$_{50}$ values ranged from 0.01 ± 0.002 to 0.02 ± 0.001 µg/mL) and these compounds were non-cytotoxic on the normal cells (IC$_{50}$ values > 100 µg/mL) and exhibited better cytotoxicity against most of cancer cell lines than doxorubicin as standard drug. Moreover, compounds 9, 17 and 20 exhibited high growth inhibitory activity on the various cancer panel cell lines (IC$_{50}$ values ranged from 0.46 ± 0.006 to 2.43 ± 0.51 µg/mL) with weak cytotoxicity on the normal cells (IC$_{50}$ values ranged from 62.19 ± 2.02 to 72.45 ± 2.40 µg/mL). In addition, other compounds showed moderate to weak cytotoxicity against all cancer cell lines (IC$_{50}$ values ranged from 10.39 ± 4.19 to 75.20 ± 13.86 µg/mL) with cytotoxic effects on the human normal cell (IC$_{50}$ values ranged from non-cytotoxic to 50.20 ± 10.22 µg/mL) in comparison with doxorubicin. The resultant data can be analyzed with respect to the chemical structures of the examined compounds; thus it can be noticed that the derivatives 8 and 16 that bear ester or malononitrile side chains on the parent cyanopyrine nucleus showed the highest potency as growth inhibiting agents against the three human cancer cell
lines, which might be due to their lipophilicity that allows their accumulation inside tumor tissues inducing growth inhibition effects [36].

![Figure 2. Cytotoxic activity of some newly synthesized compounds against human cancer cell lines and normal cells.](image)

3. Experimental Section

3.1. General Information

All melting points are uncorrected and were determined on a Stuart electric melting point apparatus. The microanalyses were within ±0.4% of the theoretical values and were carried out at the Microanalytical Centre, National Research Centre, Cairo, Egypt. IR spectra (KBr) were recorded on a FT-IR 400D infrared spectrometer (Shimadzu-series, Kyoto, Japan) using the OMNIC program and are reported as frequency of absorption in cm$^{-1}$. $^1$H-NMR spectra were recorded on a Bruker (Rheinstetten, Germany) spectrophotometer at 400 MHz using TMS as internal standard and with residual signals of the deuterated solvent $\delta = 7.26$ ppm for CDCl$_3$ and $\delta = 2.51$ ppm for DMSO-$d_6$. $^{13}$C-NMR spectra were recorded on the same spectrometer at 100 MHz and referenced to solvent signals $\delta = 77$ ppm for CDCl$_3$ and $\delta = 39.50$ ppm for DMSO-$d_6$. The mass spectra were recorded on a Shimadzu GCMS-QP-1000 EX mass spectrometer (Kyoto, Japan) at 70 eV using the electron ionization technique. Homogeneity of all compounds synthesized was checked by TLC which was performed on Merck 60 (Munich, Germany) ready-to-use silica gel plates to monitor the reactions and test the purity of the new synthesized compounds. The chemical names given for the prepared compounds are according to the IUPAC system.

3.2. Synthetic Procedures

3.2.1. 6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-oxo-1,2-dihydropyridin-3-carbonitrile (2)

*Method A*: A mixture of 1-(2,4-dichlorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (1, 2.95 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and ammonium acetate (6.16 g, 0.08 mol) in ethanol (40 mL) was refluxed for 10 h. After cooling, the precipitate was filtered, dried and recrystallized from dioxane to give compound 2 (35% yield).
Method B: A mixture of 2,4-dichloroacetophenone (1.88 g, 0.01 mol), 4-fluorobenzaldehyde (1.24 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and ammonium acetate (6.16 g, 0.08 mol) in n-butanol (20 mL) was refluxed for 3 h, to give yellow crystals that were then filtered, washed with water, dried and recrystallized to give the title compound 2 (85% yield).

**Compound 2**: m.p. 276–277 °C; IR (KBr) νmax in cm⁻¹: 3278 (NH2), 2219 (CN), 1632 (C=O);¹H-NMR (DMSO-d₆): 6.61 (s, 1H, pyridine H5), 7.41–7.85 (m, 7H, Ar–H), 8.10 (br. s, 1H, NH, D); MS, m/z 141.54, 147.65, 148.02, 149.53 (18 Ar–C), 158.6 (C=N), 168.9 (C=O); ¹³C-NMR (DMSO-d₆): 117.6 (CN), 119.61, 120.23, 122.00, 125.24, 123.33, 123.59, 133.93, 135.87, 136.12, 142.45, 144.64, 146.21, 146.87, 151.32, 151.98 (16 Ar–C), 161.8 (C=O); MS, m/z (%): 358 [M+] (98), 360 [M + 2]+ (59), 362 (M* + 4; 11%). Anal. Calcd. for C₁₈H₉Cl₂FN₂O (359.18): C, 60.19; H, 2.53; N, 7.80%; found: C, 59.97; H, 2.74; N, 7.94%.

3.2.2. 6-(2,4-Dichlorophenyl)-4-(2,4-fluorophenyl)pyridin-2-yl methanol (3.2 g, 0.01 mol) and malononitrile (0.7 g, 0.01 mol) were added. The reaction mixture was refluxed for 6 h., then left to overnight and then poured into ice/cold water to complete precipitation. The product was filtered, washed with water, dried and recrystallized from benzene to give compound 4 (75% yield).

**Method A**: A mixture of compound 2 (3.6 g, 0.01 mol) and hydrazine hydrate 98% (1 mL, 0.02 mol) in absolute ethanol (30 mL) was refluxed for 15 h. The reaction mixture was left at room temperature overnight and then poured into ice/cold water to complete precipitation. The product was filtered, washed with water, dried and recrystallized from benzene to give compound 4 (75% yield).

**Compound 4**: m.p. 309–311 °C; IR (KBr) νmax in cm⁻¹: 3414, 3335, 3180 (NH2, NH);¹H-NMR (DMSO-d₆): 5.69 (s, 2H, NH2, D₂O exchangeable), 7.12–7.97 (m, 8H, Ar–H + pyridine H5), 10.05 (s, 1H, NH, D₂O exchangeable);¹³C-NMR (DMSO-d₆): 109.76, 111.54, 112.32, 114.29, 115.21, 115.98, 120.54, 121.87, 123.34, 126.30, 132.64, 138.97, 144.54, 145.92, 148.40 (16 Ar–C), 157.1, 158.9 (2C=O); MS, m/z (%): 372 [M+] (72), 374 [M + 2]+ (40), 376 [M + 4]+ (8). Anal. Calcd. for C₁₈H₁₁Cl₂FN₄ (373.21): C, 57.93; H, 2.97; N, 15.01%; found: C, 58.12; H, 2.70; N, 15.23%.

3.2.3. 4-Amino-7-(2,4-dichlorophenyl)-5-(4-fluorophenyl)pyridin-3-carbonitrile (7)

To a solution of compound 2 (3.6 g, 0.01 mol) in absolute ethanol (30 mL), triethylamine (3 mL), and malononitrile (0.7 g, 0.01 mol) were added. The reaction mixture was refluxed for 6 h., then left to cool to room temperature, poured into cold water and neutralized with diluted hydrochloric acid to complete precipitation. The solid obtained was filtered, washed with water, dried and recrystallized from methanol to give compound 7. Yield 70%; m.p. 188–189 °C; IR (KBr) νmax in cm⁻¹: 3312, 3249, 3145 (NH2, NH), 2223 (CN), 1688 (C=O);¹H-NMR (DMSO-d₆): 5.46 (s, 2H, NH2, D₂O exchangeable), 6.89 (s, 1H, pyridine H5), 7.10–7.41 (m, 7H, Ar–H), 8.20 (s, 1H, NH, D₂O exchangeable);¹³C-NMR (DMSO-d₆): 118.1 (CN), 121.32, 122.87, 123.76, 125.32, 128.43, 132.65, 133.87, 134.12, 139.56, 141.54, 147.65, 148.02, 149.53 (18 Ar–C), 158.6 (C=O); MS, m/z (%): 424 [M+] (92), 426 [M + 2]+ (59), 428 [M + 4]+ (10). Anal. Calcd. for C₂₁H₁₁Cl₂FN₄ (425.24): C, 59.31; H, 2.61; N, 13.18%; found: C, 59.12; H, 2.79; N, 13.33%.

3.2.4. Ethyl 2-[3-cyano-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)pyridin-2-yl oxy]acetate (8)

A mixture of compound 2 (3.6 g, 0.01 mol), ethyl bromoacetate (1.2 mL, 0.01 mol) and anhydrous potassium carbonate (2.10 g, 0.015 mol) in dry acetone (50 mL) was refluxed for 24 h. The reaction mixture was cooled and poured onto ice/cold water; the solid that separated out was filtered, dried and recrystallized from dioxane to give compound 8. Yield 75%; m.p. 119–121 °C; IR (KBr) νmax in cm⁻¹: 2220 (CN), 1755 (C=O ester);¹H-NMR (DMSO-d₆): 1.15 (t, J = 7.5 Hz, 3H, CH₃-ester), 4.13 (q, J = 7.5 Hz, 2H, O-CH₂-ester), 5.11 (s, 2H, O-CH₂), 7.00–7.40 (m, 8H, Ar–H + pyridine H5);¹³C-NMR (DMSO-d₆): 135 (CH₃), 44.8, 61.3 (2CH₂), 118.6 (CN), 123.30, 123.54, 127.10, 127.98, 131.34, 133.54, 137.07, 138.76, 141.99, 142.76, 144.65, 126.87, 148.43 (16 Ar–C), 159.1 (C=N), 167.9 (C=O); MS, m/z
3.2.5. 2-[3-Cyano-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)pyridin-2-yl]oxyacetohydrazide (9)

A mixture of compound 8 (4.5 g, 0.01 mol) and hydrazine hydrate 98% (2 mL, 0.04 mol) and absolute ethanol (30 mL) was refluxed for 4 h. The reaction mixture was cooled and the formed solid was filtered, dried and recrystallized from acetic acid to give compound 9. Yield 76%; m.p. 197–199 °C; IR (KBr) ν max in cm−1: 3314, 3282, 3116 (NH2, NH), 2225 (CN), 1696 (C=O ester); 1H-NMR (DMSO-d6): 4.92 (s, 2H, CH2), 7.00–7.40 (m, 8H, Ar–H + pyridine H5), 8.2 (s, 1H, NH, D2O exchangeable), 9.8 (s, 2H, NH, D2O exchangeable); 13C-NMR (DMSO-d6): 62.4 (CH2), 119.7 (CN), 121.65, 124.05, 124.90, 125.71, 128.23, 129.98, 133.76, 137.54, 142.12, 143.45, 145.86 (16 Ar–C), 158.6 (C=Н); MS, m/z (%): 430 [M]+ (31), 432 [M + 2]+ (20), 434 [M + 4]+ (4), 371 [M-CONNH2]+ (8). Anal. Calcd. for C20H13Cl2F2N4O2 (431.25): C, 55.70; H, 3.04; N, 12.99%; found: C, 55.52; H, 3.21; N, 13.05%.

3.2.6. 2-[3-Cyano-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)pyridin-2-yl]oxy-N′-(4-fluorobenzylidene)acetohydrazide (10)

A mixture of compound 9 (4.3 g, 0.01 mol) and 4-fluorobenzaldehyde (1.24 g, 0.01 mol) in ethanol (20 mL) was refluxed for 6 h. The solid formed after cooling was filtered, dried and recrystallized from acetic acid to give compound 10. Yield 79%; m.p. 219–221 °C; IR (KBr) ν max in cm−1: 3218 (NH), 2226 (CN), 1665 (C=O); 1H-NMR (DMSO-d6): 5.1 (s, 2H, O–CH2), 7.32–7.87 (m, 12H, Ar–H + pyridine H5), 8.10 (s, 1H, CH=N (azomethine proton)), 11.08 (s, 1H, NH, D2O exchangeable); 13C-NMR (DMSO-d6): 64.1 (CH2), 117.5 (CN), 120.98, 121.01, 121.48, 123.40, 125.21, 127.83, 128.03, 128.99, 132.06, 133.56, 137.98, 138.43, 141.32, 142.65, 144.64, 146.10, 149.01, 149.97, 150.16, 150.63 (23 Ar–C), 157.2 (C=N), 159.4 (CH=N); MS, m/z (%): 536 [M]+ (97), 538 [M + 2]+ (64), 540 [M + 4]+ (11). Anal. Calcd. for C27H16Cl2F2N4O2 (537.34): C, 60.35; H, 3.00; N, 10.43%; found: C, 60.56; H, 3.21; N, 10.65%.

3.2.7. 2-Chloro-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)nicotinonitrile (11)

A mixture of compound 2 (3.6 g, 0.01 mol) and phosphorus oxychloride (4.6 mL, 0.03 mol) was refluxed for 8 h. The reaction mixture was poured into crushed ice and the separated solid was filtered, dried and recrystallized from dioxane to give compound 11. Yield 62%; m.p. 181–182 °C; IR (KBr) ν max in cm−1: 2223 (CN); 1H-NMR (DMSO-d6): 6.80 (s, 1H, pyridine H5), 7.62–8.01 (m, 7H, Ar–H); 13C-NMR (DMSO-d6): 119.2 (CN), 122.31, 125.86, 127.90, 128.54, 131.98, 133.32, 138.32, 139.09, 144.89, 145.07, 148.48 (16 Ar–C), 158.7 (C=Н); MS, m/z (%): 376 [M]+ (62), 378 [M + 2]+ (60), 380 [M + 4]+ (18); 382 [M + 6]+ (2). Anal. Calcd. for C18H12Cl2F2N2 (377.63): C, 57.25; H, 2.14; N, 7.42%; found: C, 57.10; H, 2.43; N, 7.65%.

3.2.8. General procedure for the synthesis of 2-(benzylamino)-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)nicotinonitrile (12), 2-(benzyl[d]1,3]dioxol-5-ymethylarnine)-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)nicotinonitrile (13), 6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-2-(1-phenylethylamine)nicotinonitrile (14), and 6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-2-(2-substituted-1-yl)nicotinonitriles 15a,b

A mixture of chloropyridine 11 (3.8 g, 0.01 mol) and the appropriate amine, namely benzylamine, piperonylamine, 1-phenylethylamine, 2-methylpyrideridine and/or morpholine (0.01 mol) in absolute ethanol (30 mL) was refluxed for 6–12 h. The reaction mixture was poured onto ice/cold water, filtered, washed with petroleum ether 60–80 and finally crystallized from ethanol to give the desired derivatives 12–15a,b, respectively.
2-(Benzod[b][1,3]dioxol-5-ylmethylamino)-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)nicotinonitrile (13): Yield 59%; m.p. 149–151 °C; IR (KBr) \(\nu_{\text{max}}\) in cm\(^{-1}\): 3229 (NH), 2218 (CN); \(^1\)H-NMR (DMSO-\(d_6\)): 4.85 (s, 2H, CH\(_2\)), 6.21 (s, 2H, CH\(_2\)), 7.02 (br s, 1H, NH, D\(_2\)O exchangeable), 7.68–8.22 (m, 11H, Ar–H + pyridine H5); \(^13\)C-NMR (DMSO-\(d_6\)): 49.5, 88.6 (2CH\(_2\)), 117.2 (CN), 121.72, 122.98, 124.61, 128.03, 131.43, 133.92, 134.98, 137.38, 138.51, 142.26, 143.13, 144.06, 144.97, 148.26, 149.49 (22 Ar–C), 158.7 (C=N); MS, \(m/z\) (%): 491 [M\(^+\)] (19), 493 [M + 2\(^+\)] (13), 495 [M + 4\(^+\)] (2). Anal. Calcd. for C\(_{26}\)H\(_{16}\)Cl\(_2\)FN\(_3\)O (462.35): C, 67.54; H, 3.92; N, 9.09%; found: C, 67.69; H, 3.71; N, 8.89%.

6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-phenylethylamino)nicotinonitrile (14): Yield 32%; m.p. 142–143 °C; IR (KBr) \(\nu_{\text{max}}\) in cm\(^{-1}\): 3203 (NH), 2228 (CN); \(^1\)H-NMR (DMSO-\(d_6\)): 2.93 (m, 2H, CH\(_2\)), 3.39 (m, 2H, CH\(_2\)), 7.32 (br s, 1H, NH, D\(_2\)O exchangeable), 7.76–8.36 (m, 13H, Ar–H + pyridine H5); \(^13\)C-NMR (DMSO-\(d_6\)): 39.1, 46.9 (2CH\(_2\)), 119.1 (CN), 122.01, 122.86, 123.84, 124.62, 127.97, 128.12, 131.82, 133.27, 136.46, 139.65, 141.54, 144.87, 146.32, 147.07, 148.86, 149.41 (22 Ar–C), 159.0 (C=N); MS, \(m/z\) (%): 461 [M\(^+\)] (32), 463 [M + 2\(^+\)] (23), 465 [M + 4\(^+\)] (3). Anal. Calcd. for C\(_{26}\)H\(_{18}\)Cl\(_2\)FN\(_3\)O (462.35): C, 67.54; H, 3.92; N, 9.09%; found: C, 67.69; H, 3.71; N, 8.89%.

6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-(methylpiperidin-1-yl)nicotinonitrile (15a): Yield 47%; m.p. 98–100 °C; IR (KBr) \(\nu_{\text{max}}\) in cm\(^{-1}\): 2219 (CN); \(^1\)H-NMR (DMSO-\(d_6\)): 1.36 (s, 3H, CH\(_3\)), 1.59–1.73 (m, 6H, 3CH\(_2\)-piperidine protons), 2.78 (m, 3H, (CH + CH\(_2\)) piperidine protons), 7.48–8.01 (m, 8H, Ar–H + pyridine H5); \(^13\)C-NMR (DMSO-\(d_6\)): 18.6 (CH\(_3\)), 22.4, 25.2, 36.9, 49.3 (4CH\(_2\)), 56.7 (CH), 119.7 (CN), 120.76, 121.20, 125.85, 128.32, 129.04, 132.85, 133.25, 137.37, 138.13, 139.08, 141.24, 143.79, 143.9 (16 Ar–C), 157.9 (C=N). Anal. Calcd. for C\(_{32}\)H\(_{20}\)Cl\(_2\)FN\(_3\) (440.34): C, 65.46; H, 4.58; N, 9.54%; found: C, 65.70; H, 4.32; N, 9.39%.

6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-morpholinonicotinonitrile (15b): Yield 32%; m.p. 112–114 °C; IR (KBr) \(\nu_{\text{max}}\) in cm\(^{-1}\): 2227 (CN); \(^1\)H-NMR (DMSO-\(d_6\)): 3.31 (m, 4H, 2N-CH\(_2\)), 3.72 (m, 4H, 2O-CH\(_2\)), 7.52–7.78 (m, 8H, Ar–H + pyridine H5); \(^13\)C-NMR (DMSO-\(d_6\)): 47.9, 49.1 (2N-CH\(_2\)), 64.10, 65.9 (2O-CH\(_2\)), 119.1, 120.46, 122.04, 122.83, 126.23, 127.85, 129.27, 131.47, 133.36, 134.26, 138.93, 139.08, 142.86, 144.75, 146.12, 146.91, 147.16 (16 Ar–C), 158.9 (C=N). Anal. Calcd. for C\(_{32}\)H\(_{18}\)Cl\(_2\)FN\(_3\)O (428.29): C, 61.70; H, 3.77; N, 9.81%; found: C, 61.88; H, 3.42; N, 9.69%.

3.2.9. 2-[3-Cyano-6-(2,4-dichlorophenyl)pyridin-2-yl]malononitrile (16)

To a solution of compound 11 (3.8 g, 0.01 mol) in absolute ethanol (30 mL), triethylamine (1 mL), and malononitrile (0.7 g, 0.01 mol) were added. The reaction mixture was refluxed for 6 h, then left to cool to room temperature, poured into cold water and neutralized with dilute hydrochloric acid to complete precipitation. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol to give the hydrazinyl derivative 17. Yield 85%; m.p. 223–224 °C; IR (KBr) \(\nu_{\text{max}}\) in cm\(^{-1}\): 3440, 3320, 3150 (NH\(_2\), NH), 2218 (CN); \(^1\)H-NMR (DMSO-\(d_6\)): 5.40 (s, 2H,
NH₂, D₂O exchangeable), 7.39–8.21 (m, 8H, Ar–H + pyridine H5), 9.40 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR (DMSO-d₆): 119.5 (CN), 121.09, 122.58, 123.71, 125.35, 129.34, 132.98, 134.46, 138.47, 139.07, 142.32, 144.35, 145.07, 147.43, 148.54, 149.76, 150.12 (16 Ar–C), 158.9 (C=N); MS, m/z (%): 372 [M]+ (39), 374 [M + 2]+ (21), 346 [M + 4]+ (4). Anal. Calcd. for C₁₈H₁₁Cl₂F₄N₄ (373.21): C, 57.93; H, 2.97; N, 15.01%; found: C, 58.23; H, 2.80; N, 14.89%.

3.2.11. General procedure for the synthesis of 6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)nicotinonitrile (18) and 6-(2,4-dichlorophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-4-(4-fluorophenyl)nicotinonitrile (19)

A mixture of compound 17 (3.7 g, 0.01 mol) and ethyl acetoacetate or acetylacetone (0.01 mol) in acetic acid (15 mL) was refluxed for 8 h. The solid formed after cooling was filtered, dried and recrystallized from ethanol to give compounds 18, and 19 respectively.

6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)nicotinonitrile (18): Yield 40%; m.p. 246–248 °C; IR (KBr) νmax in cm⁻¹: 2227 (CN), 1701 (C=O); ¹H-NMR (DMSO-d₆): 1.94 (s, 3H, CH₃), 2.26 (s, 2H, CH₂), 7.52–7.78 (m, 8H, Ar–H + pyridine H5); ¹³C-NMR (DMSO-d₆): 19.3 (CH₃), 41.5 (CH₂), 118.3 (CN), 122.62, 123.42, 124.61, 129.17, 131.11, 133.91, 136.23, 137.05, 139.54, 14.13, 142.53, 143.06, 143.94 (16 Ar–C), 158.4, 159.3 (2C=N), 166.5 (C=O); MS, m/z (%): 348 [M]+ (16), 440 [M + 2]+ (11), 442 [M + 4]+ (2). Anal. Calcd. for C₂₂H₁₅Cl₂F₄N₄O (439.24): C, 60.15; H, 2.98; N, 12.75%; found: C, 60.25; H, 3.12; N, 12.54%.

6-(2,4-Dichlorophenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-4-(4-fluorophenyl)nicotinonitrile (19): Yield 32%; m.p. 287–289 °C; IR (KBr) νmax in cm⁻¹: 2210 (CN); ¹H-NMR (DMSO-d₆): 2.31 (s, 3H, CH₃), 2.45 (s, 3H, CH₃), 6.15 (s, 1H, CH-pyrazole), 7.46–8.09 (m, 8H, Ar–H + pyridine H5); ¹³C-NMR (DMSO-d₆): 18.4, 19.3 (2CH₃), 108.4 (CH-pyrazole), 117.9 (CN), 122.94, 123.27, 124.18, 128.97, 129.46, 132.74, 133.24, 136.93, 138.08, 139.50, 144.14, 144.85, 148.23, 148.86 (17 Ar–C), 158.3, 159.5 (2C=N); MS, m/z (%): 436 [M]+ (12), 438 [M + 2]+ (8), 440 [M + 4]+ (1). Anal. Calcd. for C₂₃H₁₅Cl₂F₄N₂O (437.30): C, 63.17; H, 3.46; N, 12.81%; found: C, 62.98; H, 3.17; N, 12.61%.

3.2.12. 6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-[2-(2-oxoindolin-3-ylidene)hydrazinyl]nicotinonitrile (20)

A mixture of the compound 17 (3.7 g, 1 mmol) and isatin (1.5 g, 1 mmol) in ethanol (25 mL) containing 3 drops of acetic acid was refluxed for 2 h, then left overnight at room temperature. The formed precipitate was filtered, dried and recrystallized from benzene to give 20. Yield 79%; m.p. 268–269 °C; IR (KBr) νmax in cm⁻¹: 3299, 3150 (2NH), 1723 (C=O), 2227 (CN); ¹H-NMR (DMSO-d₆): 6.98 (br s, 1H, NH, D₂O exchangeable), 7.22–8.24 (m, 12H, Ar–H + pyridine H5), 10.02 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR (DMSO-d₆): 120.01 (CN), 120.42, 121.07, 122.86, 123.07, 124.52, 129.05, 129.96, 131.46, 133.63, 135.64, 137.93, 139.75, 141.25, 143.86, 144.52, 145.28, 147.94, 148.6, 149.25 (22 Ar–C), 157.9, 158.3 (2C=N), 167.23 (C=O); MS, m/z (%): 501 [M]+ (85), 503 [M + 2]+ (60), 505 [M + 4]+ (9). Anal. Calcd. for C₂₆H₂₃Cl₂F₄N₅O (502.33): C, 62.17; H, 2.81; N, 13.94%; found: C, 61.97; H, 2.60; N, 14.19%.

3.2.13. General procedure for the synthesis of 6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-2-[2-(2-substitutedbenzyldiene)hydrazinyl]nicotinonitriles (1a–e)

A mixture of compound 17 (3.7 g, 0.01 mol), an appropriate aromatic aldehyde namely 2-bromo-benzaldehyde, 4-nitrobenzaldehyde, 2-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and/or 1-naphthaldehyde (0.01 mol) in ethanol (20 mL) containing 3 drops of acetic acid was refluxed for 6–8 h. The precipitate formed after cooling was filtered, dried and recrystallized to give compounds 21a–e, respectively.

2-[2-(2-Bromobenzylidene)hydrazinyl]-6-(2,4-dichlorophenyl)-4-(4-fluorophenyl)nicotinonitrile (21a): Yield 41%; m.p. 226–228 °C; IR (KBr) νmax in cm⁻¹: 3299 (NH), 2217 (CN); ¹H-NMR (DMSO-d₆): 7.26–8.19 (m, 12H, Ar–H + pyridine H5), 8.32 (s, 1H, CH=N azomethine proton), 10.13 (s, 1H, NH, D₂O
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exchangeable); $^{13}$C-NMR (DMSO-$d_6$): 118.6 (CN), 121.83, 122.94, 123.10, 123.86, 127.39, 128.93, 129.27, 132.81, 135.23, 139.38, 141.04, 142.50, 144.52, 148.01, 148.87, 149.08, 150.65, 151.31 (22 Ar–C), 158.3 (C=N), 161.3 (CH=N); MS, $m/z$ (%): 538 [M$^+$] (14), 540 [M + 2]$^+$ (10), 542 [M + 4]$^+$ (1). Anal. Calcd. for $C_{25}H_{14}$BrCl$_2$FN$_4$ (540.21): C, 55.58; H, 2.61; N, 10.37%; found: C, 55.68; H, 2.79; N, 10.51%.

6-(2,4-Dichlorophenyl)-4-(4-fluorophenyl)-2-[2-(3,4-dimethoxybenzylidene)hydrazinyl]nicotinonitrile (21b): Yield 23%; m.p. 281–283 °C; IR (KBr) $\nu_{\text{max}}$ in cm$^{-1}$: 3253 (NH), 2223 (CN); $^1$H-NMR (DMSO-$d_6$): 7.29–8.14 (m, 12H, Ar–H + pyridine H5), 8.31 (s, 1H, CH=N (azomethine proton)), 10.08 (s, 1H, NH, D$_2$O exchangeable); $^{13}$C-NMR (DMSO-$d_6$): 119.0 (CN), 121.52, 122.61, 123.48, 127.85, 128.03, 129.53, 130.21, 133.25, 134.56, 136.87, 137.04, 139.08, 141.15, 144.08, 148.60, 149.31 (22 Ar–C), 158.7 (C=N), 160.8 (CH=N); MS, $m/z$ (%): 505 [M$^+$] (60), 507 [M + 2]$^+$ (39), 509 [M + 4]$^+$ (7). Anal. Calcd. for $C_{25}H_{14}$Cl$_2$FN$_4$O$_2$ (506.32): C, 59.30; H, 2.79; N, 13.83%; found: C, 59.58; H, 2.63; N, 13.51%.

3.3. Anticancer Activity

3.3.1. Cell Cultures

The newly synthesized compounds were evaluated in vitro against three human cancer cell lines; which are MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer), and WI 38 (normal fibroblast cells) were used in this study. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) but NCI-H460, SF-268 and WI 38 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayers routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated fetal bovine serum (FBS), 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 µg/mL), at 37 °C in a humidified atmosphere containing 5% CO$_2$. Exponentially growing cells were obtained by plating 1.5 × 105 cells/mL for MCF-7 and SF-268, and 0.75 × 104 cells/mL for NCI-H460 followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.
3.3.2. Cancer Cell Growth Assay

The effect of compounds on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, Austin, TX, USA) in the “*In vitro* Anticancer Drug Discovery Screen” that uses the protein-binding dye sulforhodamine B (SRB) to assess cell growth [35]. In the assay protocol, all cells were incubated at 37 °C under humidified atmosphere containing 5% CO₂. Briefly, exponentially cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 µg/mL. Following this exposure period, adherent cells were fixed, washed and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a Power Wave XS plate reader (Bio-Tek Instruments Inc., Winston, NC, USA). For each test compound and cell line, a dose response curve was obtained and the inhibitory concentration of 50% (IC₅₀), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere [37]. Doxorubicin was used as a positive control and tested in the same manner.

4. Conclusions

This study focused on the synthesis of a new 1,2-dihydropyridin-3-carbonitrile and nicotinonitrile derivatives as potential anticancer agents. Some of newly synthesized derivatives were examined *in vitro* as cytotoxic agents against three human cancer cell lines. It could be noticed that the ester functionality-bearing derivative 8 and the derivative 16 carrying a malononitrile side chain attached to the parent cyanopyridine nucleus showed the best results, exhibiting the highest inhibitory effects towards the three tumor cell lines, which were higher than that of the reference compound doxorubicin and these compounds were non-cytotoxic towards normal cells (IC₅₀ values >100 µg/mL). In addition, compounds 9, 17 and 20 exhibited high growth inhibitory activity on the various cancer panel cell lines, with weak cytotoxicity on the normal cells.

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**Sample Availability:** Samples of the compounds are available from the authors.

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