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Antiproliferative Activity of Some Newly Synthesized Substituted Pyridine Candidates Using 4-(Aaryl)-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile as Synthon

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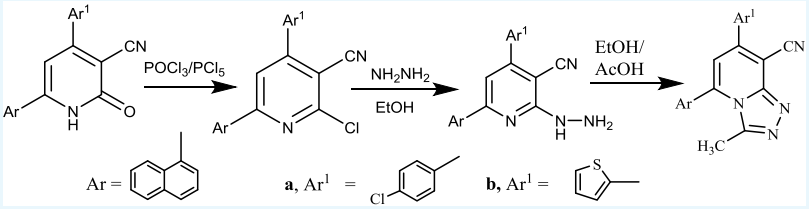

ABSTRACT: Herein, we used nicotinonitrile derivatives **4a,b** as scaffolds to build novel and active antineoplastic agents. The reaction of nicotinonitrile derivatives **4a,b** with $POCl_3/PCl_5$ and/or hydrazine hydrate afforded 2-chloropyridones **6a,b** and 2-hydrazinyl nicotinonitrile derivatives **11a,b**, respectively, as building blocks for various heterocyclic compounds. The structures of all of the synthesized heterocycles were elucidated from their spectral and elemental analyses. The cytotoxic activities of the prepared derivatives were evaluated against different cancer cell lines. Results revealed potential cytotoxic effects of the synthesized compounds against evaluated cell lines, where NCIH 460 and RKOP 27 cell lines were the most affected by the prepared compounds. Derivative **14a** was the most effective against all tested cell lines in terms of the obtained IC_{50} values (25 ± 2.6, 16 ± 2, 127 ± 25, 422 ± 26, and 255 ± 2 nM against NCIH 460, RKOP 27, HeLa, U937, and SKMEL 28 cells, respectively).

1. INTRODUCTION

The recent broad importance of nicotinonitriles,[1] thiophenes,[2] and naphthalenes[3] in synthesis of heterocyclic derivatives and their biological activities has encouraged authors to study these moieties to construct novel series of pharmacologically active nicotinonitrile derivatives.

Cancer is one of the most important health concerns worldwide, being the second-most common cause of death after heart diseases in developed countries, with lung, liver, cervical, and breast cancers being the most devastating malignancies. It has been estimated that more than six million new cases are reported each year across the world.[4]

Pyridine ring is an essential fragment of one of the antitumor and anti-inflammatory mediators.[5,6] Moreover, cyanopyridines (nicotinonitriles) have anti-inflammatory,[7] analgesic,[8] and antihypertensive[9] properties, in addition to being an antitumor tool.[10] Nicotinonitrile derivatives containing the 2-naphthyl moiety **1a,b** showed cytotoxic activity against McF-7 and HEPG2.[11] Nicotinonitrile derivatives containing the pyrazole moiety **2a,b** also showed a significant cytotoxic activity against hepatocellular and cervical carcinomas.[12] Novel fused nicotinonitrile derivatives **3a,b** were constructed and evaluated as anticancer agents against NCI-H460, MCF-7, and SF-268 cell lines[13] (Figure 1).

From this viewpoint and as a continuation of our previous works,[14−18] in heterocyclic synthesis, we have herein synthesized some novel heterocumulenes containing nicotinonitriles, thiophenes, and naphthalenes and examined their antiproliferative activities in comparison to positive controls.

2. RESULTS AND DISCUSSION

2.1. Chemistry. In our increasing interest in ethyl cyanoacetate as well as synthesis of pyridones,[12] we would like to report a mild, cost-effective procedure for the one-pot multicomponent syntheses of 4-(4-chlorophenyl)-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile and 6-(naphthalen-1-yl)-2-oxo-4-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (**4a,b**) by the condensation of aldehydes, namely, 4-chlorobenzaldehydeor thiophene-2-carboxaldehyde, respectively, 1-(naphthalen-1-yl)ethanone and ethyl cyanoacetate, in the presence of a catalytic amount of ammonium acetate and drops of piperidine at ambient temperature in an ethanol

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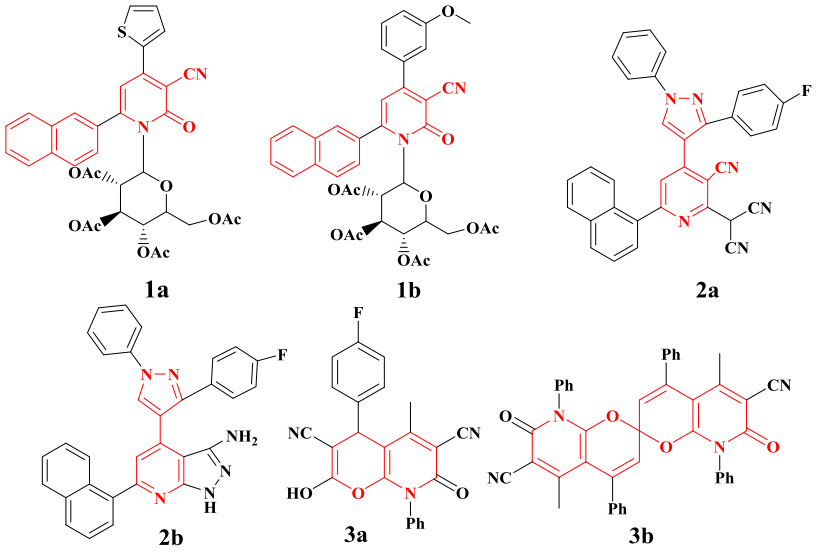
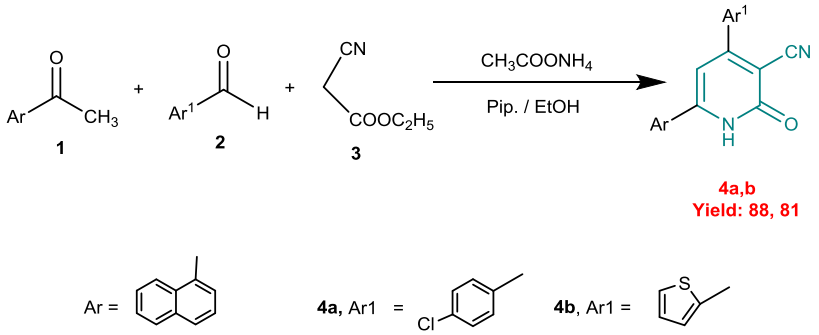


Figure 1. Cytotoxic activity of compounds against McF-7, HEPG2, HeLa cell, NCI-H460, MCF-7, and SF-268.

Scheme 1. Syntheses of Nicotinonitrile Derivatives 4a,b



medium (Scheme 1). The structures of nicotinonitriles **4a,b** were confirmed by their spectral and elemental analyses. Infrared (IR) spectra exhibited 3142 and 3154 cm^{-1} for NH; 2219 and 2220 cm^{-1} for C≡N, and 1649 and 1655 cm^{-1} for C═O. ^{1}H NMR showed 12.92 and 13.08 for NH, which disappeared with D_2O. The ^{13}C NMR spectrum revealed a peak at 161.73 ppm for C═O (compound **4b**).

In an attempt to prepare certain novel heterocycles,[19−21] determine their insecticidal effectiveness,[22−24] and evaluate their pharmaceutical significance,[25−28] compounds **4a,b** were allowed to react with phenylisothiocyanate to yield 4-(4-chlorophenyl)-3-cyano-6-(naphthalen-1-yl)-2-oxo-*N*-phenyl-pyridine-1(2*H*)-carbothioamide and 3-cyano-6-(naphthalen-1-yl)-2-oxo-*N*-phenyl-4-(thiophen-2-yl)pyridine-1(2*H*)-carbothioamide (**5a,b**). Their structures were established from elemental and spectral analyses. Here, the ^{1}H NMR spectrum revealed one peak at 12.92 and 12.83 ppm, respectively, which vanished with D_2O corresponding to one NH proton. The ^{13}C NMR spectrum revealed a peak at 162.14 ppm for C═S (compound **5b**).

On the other hand, chlorination and thiation of compounds **4a,b** afforded nicotinonitrile derivatives **6a,b** and **7a,b**, respectively. The structures of compounds **6** and **7** were substantiated from their elemental and spectral analyses. The formation of compound **6a,b** was evidently expounded by the absence of the stretching band of νC═O in the IR spectrum. Alternatively, the formation of compound **7a,b** was evidently expounded by the absence of the stretching band of νC═O and the presence of the strong band at 1233 and 1238 cm^{-1}, respectively, corresponding to νC═S in the IR spectrum. Also, the ^{1}H NMR spectrum revealed one peak at 8.42 and 8.88 ppm, respectively, which vanished with D_2O corresponding to one NH proton. The structures of compounds **7a,b** were chemically elucidated by the reaction of thiourea with 2-chloropyridine derivatives **6a,b**.

It has been reported that pyridines display significant pharmaceutical importance;[29] consequently, our efforts were devoted to synthesizing and investigating further innovative pyridine analogues with dual functions: anticancer and antimicrobial. In keeping with this scope, some pyridine-3-carbonitriles, bearing biologically active functionalities, were adopted to be synthesized (Scheme 2).

The proclivity of 2-chloropyridine derivatives **6a,b** toward nitrogen nucleophiles is studied in this work (Scheme 2). Upon reaction of 2-chloropyridine derivatives **6a,b** with *n*-octyl amine as the mononucleophilic reagent, two moles of 2-chloropyridine derivatives were reacted, affording 2,2′-(octylazanediyl)bis(4-(4-chlorophenyl)-6-(naphthalen-1-yl)nicotinonitrile) and 2,2′-(octyl-azanediyl)bis(6-(naphthalen-1-yl)-4-(thiophen-2-yl)-nicotinonitrile) (**8a,b**). The structures of compounds **8a,b** were confirmed by their spectral analyses; the IR spectra exhibited no absorption band corresponding to NH, and the acidic NH function did not appear in ^{1}H NMR.

Alternatively, during the reaction of 2-chloropyridine derivatives **6a,b** with 1,4 diaminobenzene, two moles of 2-chloropyridine derivatives were consumed, producing bis nicotinonitrile derivatives **9a,b**. On the contrary, one mole of

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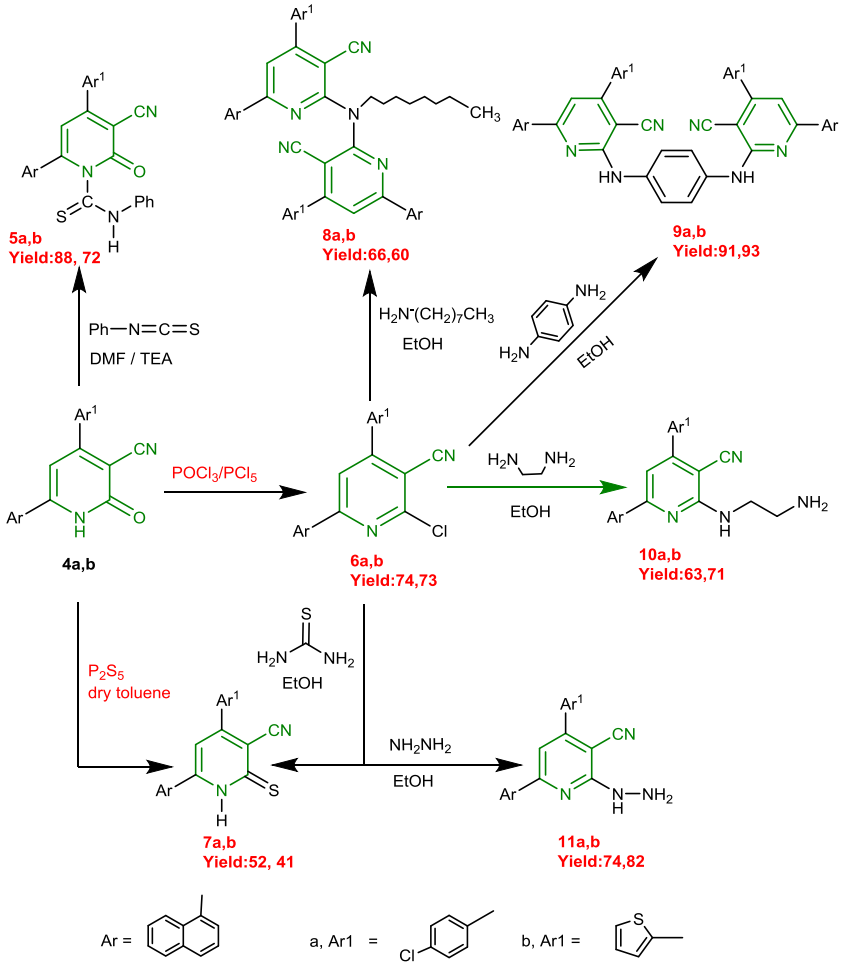
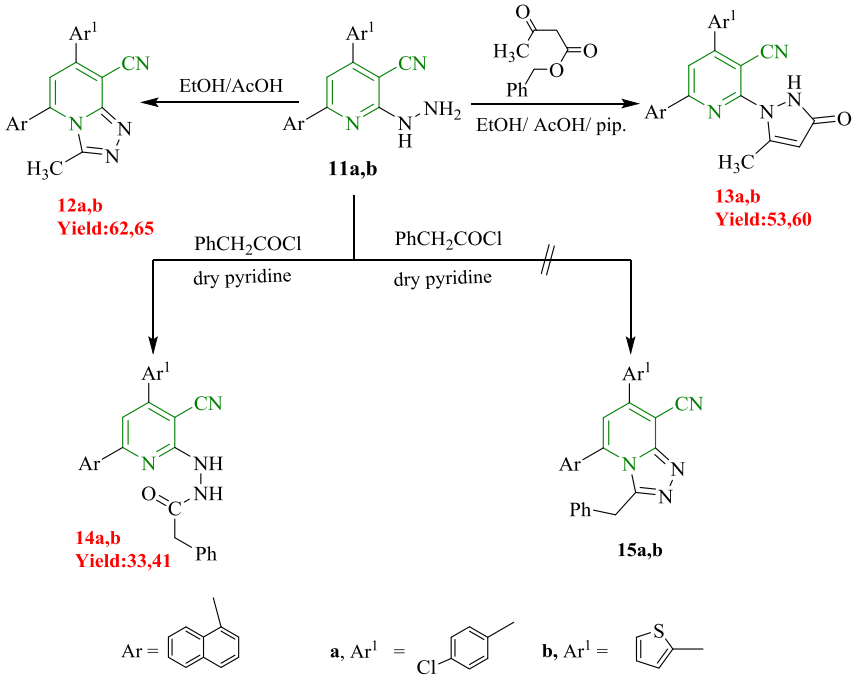
Scheme 2. Synthetic Routes for Compounds 5−11



Scheme 3. Synthetic Routes for Compounds 12−14



2-chloropyridine derivatives was reacted with ethylene diamine and hydrazine hydrate to give (2-aminoethyl)-aminonicotinonitriles **10a,b**, and 2-hydrazinyl nicotinonitriles **11a,b**, respectively.

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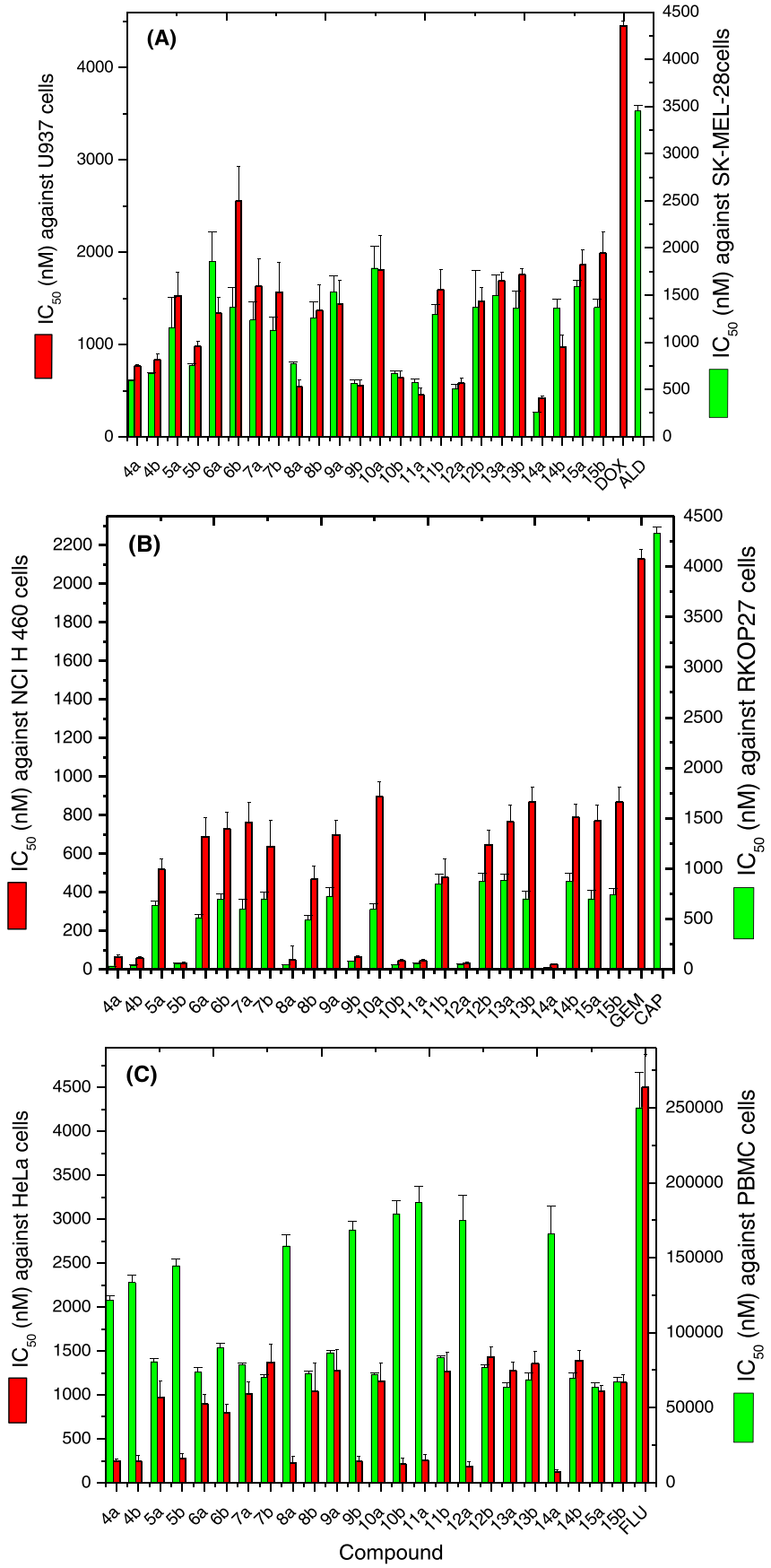

Figure 2. IC_{50} values obtained for the prepared derivatives against different tested cell lines. (A) U937 and SK-MEL-28 cells; (B) NCIH 460 and RKOP 27 cells; and (C) HeLa and PBMC cells.

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Table 1. IC_{50} Values Obtained for Prepared Derivatives against Different Tested Cell Lines

	IC_{50} values (nM)					
comp. no.	U937	SKMEL-28	N CIH 460	RKOP 27	HeLa	PBMC
4a	766 ± 19	590 ± 15	66 ± 9	30 ± 1.5	250 ± 19	121 340 ± 3250
4b	836 ± 69	669 ± 9.8	58 ± 6.9	39 ± 9.8	246 ± 69	133 300 ± 5300
5a	1530 ± 255	1155 ± 318	518 ± 56.6	635 ± 45.8	973 ± 185	80 618 ± 2320
5b	980 ± 55	755 ± 18	32 ± 5.5	56 ± 3.2	277 ± 55	144 560 ± 4600
6a	1341 ± 174	1854 ± 321	689 ± 98	512 ± 32	896 ± 115	73 496 ± 3500
6b	2554 ± 374	1369 ± 215	728 ± 88	695 ± 56	795 ± 96	89 640 ± 2940
7a	1634 ± 298	1235 ± 196	764 ± 102	596 ± 96	1015 ± 136	78 369 ± 1636
7b	1569 ± 321	1129 ± 137	638 ± 138	697 ± 72	1369 ± 213	69 970 ± 2050
8a	544 ± 74	774 ± 21	48 ± 7.4	40 ± 2.1	229 ± 74	157 780 ± 7600
8b	1369 ± 284	1259 ± 168	468 ± 69	486 ± 49	1038 ± 326	72 698 ± 1786
9a	1439 ± 258	1532 ± 176	698 ± 76	725 ± 89	1273 ± 239	86 538 ± 1690
9b	559 ± 63	563 ± 35	66 ± 6.3	77 ± 3.5	244 ± 63	168 540 ± 5690
10a	1812 ± 369	1786 ± 236	896 ± 78	596 ± 59	1159 ± 206	72 136 ± 896
10b	642 ± 69	669 ± 26	46 ± 6.9	46 ± 2.6	211 ± 69	179 250 ± 8690
11a	458 ± 72	572 ± 36	44 ± 7.2	55 ± 3.6	255 ± 72	186 680 ± 10 690
11b	1594 ± 215	1296 ± 106	478 ± 96	846 ± 96	1265 ± 227	83 069 ± 1096
12a	581 ± 51	510 ± 41	33 ± 5.1	48 ± 4.1	185 ± 51	174 900 ± 16 900
12b	1468 ± 151	1369 ± 389	648 ± 76	869 ± 87	1435 ± 108	76 806 ± 2030
13a	1692 ± 89	1496 ± 218	765 ± 89	879 ± 68	1273 ± 103	63 607 ± 3050
13b	1758 ± 68	1359 ± 182	869 ± 76	698 ± 79	1359 ± 139	68 617 ± 4610
14a	422 ± 25	255 ± 2	25 ± 2.6	16 ± 2	127 ± 25.5	165 760 ± 18 500
14b	976 ± 125	1360 ± 98	789 ± 69	869 ± 87	1391 ± 110	69 618 ± 3596
15a	1865 ± 159	1585 ± 76	769 ± 86	698 ± 84	1038 ± 68	63 640 ± 2986
15b	1989 ± 235	1369 ± 92	869 ± 79	736 ± 67	1139 ± 89	67 166 ± 2769
doxorubicin	4450 ± 50					
aldelseukin		3450 ± 64				
gemcitabine			2130 ± 50			
capecitabine				4330 ± 64		

2-Hydrazinyl nicotinonitrile derivatives **11a,b** were allowed to react with electrophilic reagents such as acetic acid and benzyl acetoacetate, affording the triazolopyridines **12a,b** and pyrazolyl nicotinonitriles **13a,b**. However, reaction of the hydrazinyl derivatives **11a,b** with phenylacetyl chloride afforded the nicotinonitrile derivatives **14a,b** instead of the triazolopyridine derivatives **15a,b** (Scheme 3).

2.2. Antiproliferative Activity. The antiproliferative activities of the prepared derivatives were evaluated against different cancer cell lines in comparison with their effect on one normal cell line. Results obtained (Figure 2) showed that all tested derivatives have promising potentials against tested cell lines. Furthermore, it can be seen that different compounds affected different cell lines depending on the cell type. This can be attributed to the fact that cell response to different derivatives depends on the membrane structure and organization as well as the nature of the affecting compound itself.[30−33] From the IC_{50} values calculated, it can also be noticed that NCIH 460 and RKOP 27 cells were the most affected among tested cell lines, where the obtained IC_{50} values ranged from 25 ± 2.6 to 66 ± 9 and 16 ± 2 to 77 ± 4 nM for NCIH 460 and RKOP 27 cells, respectively.

On the other hand, other cell lines required higher concentrations of the prepared compounds to be affected (IC_{50} values: U937 cells ranged from 422 ± 26 to 836 ± 69 nM; SKMEL 28 cells ranged from 255 ± 2 to 774 ± 21 nM; HeLa cells ranged from 127 ± 25 to 277 ± 6 nM). The results for normal PBMC cells showed that cells required high doses to be affected (IC_{50} values ranged from 121 340 ± 3250 to 186 680 ± 10 690 nM), which indicates that the prepared derivatives are less toxic to normal cells tested. The compound−cancer cell activity patterns showed that compound **14a** is the most active against all tested cancer cells, where it recorded the lowest IC_{50} values obtained (25 ± 2.6, 16 ± 2, 127 ± 25, 422 ± 26, and 255 ± 2 nM for NCIH 460, RKOP 27, HeLa, U937, and SKMEL 28 cells, respectively).

Furthermore, it can also be noticed that compounds **5a**, **6a,b**, **7a,b**, **8b**, **9a**, **10a**, **11b**, **12b**, **13a,b**, **14b**, and **15a,b** showed variable degrees of moderate antiproliferative potentials against all studied cell lines. Also, it can be seen that their cytotoxic potentials were higher when compared to the corresponding positive controls. However, they recorded much lower IC_{50} values when tested against normal PBMC cells, reflecting their higher toxicity on normal cells.

Additionally, the results obtained for positive control drugs tested parallel to the prepared compounds revealed that the prepared derivatives are very effective when compared to their corresponding IC_{50} values obtained on their corresponding positive controls (Table 1).

Throughout the present work, a multicomponent reaction approach was used to synthesize compounds **6a,b** and **11a,b**, which were used as scaffolds for synthesizing novel derivatives.

Our results showed that compound **14a** is the most effective against all tested cell lines. This can be attributed to the length of the side chain present in the compound. Furthermore, resonance properties and the 1,3-migration of the hydrogen proton promoted the formation of keto-enol forms, which resulted in forming hydrogen-bonding with the pyridine group.

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3. CONCLUSIONS

In the present study, we synthesized novel scaffolds, namely, 2-chloro-4-(4-chloro-phenyl)-6-(naphthalen-1-yl)nicotinonitrile (**6a**), 2-chloro-6-(naphthalen-1-yl)-4-(thiophen-2-yl)-nicotinonitrile (**6b**), 4-(4-chlorophenyl)-2-hydrazinyl-6-(naphthalen-1-yl)nicotine-nitrile (**11a**), and 2-hydrazinyl-6-(naphthalen-1-yl)-4-(thiophen-2-yl)nicotinonitrile (**11b**), by multicomponent reaction systems. From these compounds, sequences of diverse nicotinonitrile products were synthesized, and their structural and spectral data were determined. Antiproliferative screening studies showed that all synthesized compounds exhibited promising potential antiproliferative effects toward the evaluated cell lines. Additionally, large-cell lung and colon cancer cell lines were the most affected. Furthermore, compound **14a** showed the highest antiproliferative effects among all tested compounds, where it had IC_{50} values of 25 ± 2.6, 16 ± 2, 127 ± 25, 422 ± 26, and 255 ± 2 nM against NCIH 460, RKOP 27, HeLa, U937, and SKMEL 28 cells, respectively. Conclusively, the obtained results suggest further investigation to deduce the possible mechanism of action of the synthesized compounds against cancer cell lines.

4. EXPERIMENTAL SECTION

4.1. Chemistry. All melting points were determined on a Gallenkamp apparatus and are uncorrected. The IR spectra were measured on a Pye-UnicamSP300 instrument in potassium bromide discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury VX (400 MHz) spectrometer (with operating frequencies of 400 MHz for ^{1}H using TMS as an internal standard and 100 MHz for ^{13}C). Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are reported in hertz (Hz). NMR spectra were recorded at a certain temperature for all compounds and were referenced to the residual signals of DMSO-d_6. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the electron impact technique (EI). Elemental analyses were carried out at the Micro Analytical Center of Cairo University, Giza, Egypt.

*4.1.1. Syntheses of Nicotinonitrile Derivatives **4a,b**.* A mixture of 1-acetyl naphthalene (2 mL, 0.01 mol) and aldehydes, namely, *p*-chlorobenzaldehyde and/or 2-thiophencarboxyaldehyde (1.6 gm and/or 1.3 mL, 0.01 mol), ethyl cyanoacetate (1.3 mL, 0.01 mol), ammonium acetate (5.4 gm, 0.07 mol), and drops of piperidine in EtOH (25 mL) was refluxed for 2 h. The precipitate that formed was filtered off, washed with cold water, dried, and recrystallized from ethanol/dioxane to yield the title compounds **4a**,**b**.

*4.1.1.1. 4-(4-Chlorophenyl)-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**4a**).* Yield 88%, mp 184−186 °C. IR, ν, cm^{-1}: 3142 (NH); 2219 (C≡N), 1649 (C═O). ^{1}H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 12.92 (s, 1H, NH, disappeared with D_2O), 8.55−7.31 (m, 12H, Ar H). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 166.21, 154.22, 152.33, 136.00, 135.00, 134.01, 132.77, 131.17, 130.67, 130.31, 129.01, 128.82, 128.71, 128.52, 128.22, 127.66, 126.32, 125.21, 124.51, 121.22, 116.32, 107.21 (22C). MS (EI, 70 eV): m/z (%) = 357 (16) [M]$^+$. Anal. calcd. for $C_{22}H_{13}ClN_2O$ (356.8): C, 74.06; H, 3.67; N, 7.85. Found C, 74.12; H, 3.72; N, 7.90%.

*4.1.1.2. 6-(Naphthalen-1-yl)-2-oxo-4-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (**4b**).* Yield 81%, pale-yellow, mp 262−264 °C. IR, ν, cm^{-1}: 3154 (NH), 2220 (C≡N), 1655 (C═O). ^{1}H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 13.08 (s, 1H, NH, disappeared with D_2O), 8.09−7.57 (m, 11H, Ar H, and thiophene ring). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 161.73, 160.32, 151.24, 134.77, 134.30, 133.59, 132.88, 131.05, 130.59, 130.01, 129.92, 129.34, 128.94, 128.06, 127.67, 127.02, 126.84, 125.82, 125.33, 116.42 (20C). MS (EI, 70 eV): m/z (%) = 328 (32) [M]$^+$. Anal. calcd. for $C_{20}H_{12}N_2OS$ (328.39): C, 73.15; H, 3.68; N, 8.53. Found C, 73.20; H, 3.71; N, 8.56%.

*4.1.2. Reaction of Compounds **4a,b** with Phenyl Isothiocyanates to Afford **5a,b**.* To a solution of compounds **4a**,**b** (0.5 g, 0.0015 mol) in dimethylformamide (DMF) (7 mL), phenylisothiocyanate (0.2 mL, 0.0015 mol) and drops of trimethyl amine (TEA) were added. The reaction mixture was refluxed for 2 h and cooled at room temperature and then poured on ice/water and extracted by diethyl ether. The precipitated solid products were collected and recrystallized from EtOH to afford compounds **5a**,**b**.

*4.1.2.1. 4-(4-Chlorophenyl)-3-cyano-6-(naphthalen-1-yl)-2-oxo-N-phenylpyridine-1(2H)-carbothioamide (**5a**).* Yield 88%, yellow, mp 180−183 °C. IR, ν, cm^{-1}: 3115 (NH); 2218 (C≡N), 1638 (C═O), 1227 (C═S). ^{1}H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 12.92 (s, 1H, NH, disappeared with D_2O), 8.54−7.58 (m, 17H, Ar H). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 167.20, 165.22, 154.32, 138.10, 137.35, 135.62, 135.48, 135.12, 133.60, 133.50, 131.01, 130.76, 129.21, 128.96, 128.00, 127.60, 127.08, 126.22, 125.64, 124.70, 122.25, 116.98, 115.32, 114.50, 104.65 (29C). MS (EI, 70 eV): m/z (%) = 491 (8) [M]$^+$. Anal. calcd. for $C_{29}H_{18}ClN_3OS$ (491.09): C, 70.80; H, 3.69; N, 8.54. Found C, 70.82; H, 3.71; N, 8.55%.

*4.1.2.2. 3-Cyano-6-(naphthalen-1-yl)-2-oxo-N-phenyl-4-(thiophen-2-yl)pyridine-1(2H)-carbothioamide (**5b**).* Yield 72%, pale-yellow, mp 238−240 °C. IR, ν, cm^{-1}: 3110 (NH), 2214 (C≡N), 1648 (C═O), 1240 (C═S). ^{1}H NMR spectrum (DMSO-d_6, 42 °C), δ_H, ppm: 12.83 (s, 1H, NH, disappeared with D_2O), 8.11−7.28 (m, 16H, Ar H, and thiophene ring). ^{13}C NMR spectrum (DMSO-d_6, 42 °C), δ_C, ppm: 169.12, 166.20, 164.22, 138.10, 137.35, 136.60, 135.62, 135.48, 135.12, 131.01, 130.76, 129.21, 128.96, 128.00, 127.60, 127.50, 127.08, 126.22, 125.64, 124.70, 122.25, 116.98, 115.32, 114.50, 104.65 (27C). MS (EI, 70 eV): m/z (%) = 463 (24) [M]$^+$. Anal. calcd. for $C_{27}H_{17}N_3OS_2$ (463.08): C, 69.96; H, 3.70; N, 9.06. Found C, 69.95; H, 3.68; N, 8.99%.

*4.1.3. Synthesis of Chloropyridone (**6a,b**).* A mixture of **4a**,**b** (4.8 g, 0.01 mol) and PCl_5 (3 g, 0.03 mol) in $POCl_3$ (5 mL, 0.03 mol) was refluxed for 7 h; then, it was poured on crushed ice. The formed solid was filtered, dried, and crystallized from EtOH to give compounds **6a**,**b**.

*4.1.3.1. 2-Chloro-4-(4-chlorophenyl)-6-(naphthalen-1-yl)-nicotinonitrile (**6a**).* Yield 74%, yellow, mp 202−204 °C. IR, ν, cm^{-1}: 2224 (C≡N). ^{1}H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.88−7.56 (m, 12H, Ar H); ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 162.01, 155.23, 152.43, 136.07, 135.26, 134.27, 133.86, 131.29, 131.04, 130.41, 129.56, 129.20, 128.45, 127.76, 126.88, 125.32, 124.62, 120.32, 115.63, 107.17 (22C). MS (EI, 70 eV): m/z (%) = 374 (15) [M]$^+$. Anal. calcd. for $C_{22}H_{12}Cl_2N_2$ (374.04): C, 70.42; H, 3.22; N, 7.47. Found C, 70.41; H, 3.20; N, 7.45%.

*4.1.3.2. 2-Chloro-6-(naphthalen-1-yl)-4-(thiophen-2-yl)-nicotinonitrile (**6b**).* Yield 73%, pale-yellow, mp > 300 °C. IR, ν, cm^{-1}: 2232 (C≡N); ^{1}H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.30−7.57 (m, 11H, Ar H, and thiophene ring). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 160.23, 160.01, 150.21, 134.74, 134.52, 133.45, 132.77, 131.25, 130.55, 130.01,

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129.81, 129.33, 128.44, 128.07, 127.77, 127.12, 126.88, 125.82, 125.22, 116.22 (20C). MS (EI, 70 eV): m/z (%) = 346 (18) $[M]^+$. Anal. calcd. for $C_{20}H_{11}ClN_2S$ (346.03): C, 69.26; H, 3.20; N, 8.08. Found C, 69.25; H, 3.19; N, 8.00%.

*4.1.4. Synthesis of 2-Thioxo-pyridine-3-carbonitrile (**7a,b**).* To a solution of cyanopyridones **4a**,**b** (1 g, 0.003 mol) in dry toluene (20 mL), phosphorus pentasulfide (1 g, 0.004 mol) was added. The reaction mixture was refluxed for 1 h; then, it was filtered off while hot. The solid product was crystallized from EtOH to give compounds **7a**,**b**.

Correspondingly, to a solution of chloropyridones **6a**,**b** (1 g, 0.002 mol) in EtOH (20 mL), thiourea (0.5 g, 0.01 mol) was added. The reaction mixture was refluxed for 4 h; then, it was filtered off while hot. The solid product was crystallized from EtOH to give compounds **7a**,**b**.

*4.1.4.1. 4-(4-Chlorophenyl)-6-(naphthalen-1-yl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (**7a**).* Yield 52%, pale-yellow, mp 160−162 °C. IR, ν, cm^{-1}: 3122 (NH), 2224 (C≡N), 1233 (C═S). ^1H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.42 (s, 1H, NH, disappeared with D_2O), 8.16−7.58 (m, 12H, Ar H). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 163.02, 155.22, 152.31, 136.07, 135.22, 134.26, 133.66, 131.28, 131.04, 130.41, 129.44, 129.25, 129.20, 128.99, 128.55, 127.77, 126.76, 125.21, 124.62, 120.22, 115.62, 107.11 (22C). MS (EI, 70 eV): m/z (%) = 373 (25) $[M]^+$. Anal. calcd. for $C_{22}H_{13}ClN_2S$ (372.87): C, 70.87; H, 3.51; N, 7.51. Found C, 70.86; H, 3.49; N, 7.50%.

*4.1.4.2. 6-(Naphthalen-1-yl)-4-(thiophen-2-yl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (**7b**).* Yield 41%, yellow, mp 144−146 °C. IR, ν, cm^{-1}: 3125 (NH), 2221 (C≡N), 1238 (C═S). ^1H NMR spectrum (DMSO-d_6, 46 °C), δ_H, ppm: 8.88 (s, 1H, NH, disappeared with D_2O), 8.44−7.33 (m, 11H, Ar H, and thiophene ring). ^{13}C NMR spectrum (DMSO-d_6, 46 °C), δ_C, ppm: 161.94, 153.44, 146.64, 136.15, 135.33, 133.85, 132.56, 131.86, 130.96, 130.46, 129.40, 129.05, 127.72, 126.90, 125.87, 125.37, 122.99, 118.56, 116.29, 104.35 (20C). MS (EI, 70 eV): m/z (%) = 344 (12) $[M]^+$. Anal. calcd. for $C_{20}H_{12}N_2S_2$ (344.04): C, 69.74; H, 3.51; N, 8.13. Found C, 69.76; H, 3.53; N, 8.14%.

*4.1.5. Reaction of Chloropyridone (**6a,b**) with Diverse Amines: Syntheses of Compounds **8**−**11**.* To a solution of chloropyridones **6a**,**b** (0.5 g, 0.0015 mol) in EtOH (20 mL), amines, namely, *n*-octyl amine (0.2 mL, 0.0014 mol), *p*-phenelyenediamine (0.2 g, 0.0018 mol), ethylene diamine (0.1 mL, 0.0014 mol), and/or hydrazine hydrate (0.15 mL, 0.003 mol), were added. The reaction mixture was refluxed for 2 h and cooled at room temperature. The precipitated solid product was collected and recrystallized from EtOH to afford compounds **8**−**11**.

*4.1.5.1. 2,2′-(Octylazanediyl)bis(4-(4-chlorophenyl)-6-(naphthalen-1-yl)nicotinonitrile) (**8a**).* Yield 66%, white, mp 164−166 °C. IR, ν, cm^{-1}: 2222 (C≡N). ^1H NMR spectrum (DMSO-d_6, 42 °C), δ_H, ppm: 8.85−7.57 (m, 24H, Ar H), 1.62 (t, 2H, CH_2), 1.15−1.22 (m, 12H, $6CH_2$), 0.78 (t, 3H, CH_3). ^{13}C NMR spectrum (DMSO-d_6, 42 °C), δ_C, ppm: 162.02, 155.23, 152.49, 136.11, 135.26, 134.26, 133.88, 131.19, 131.06, 130.42, 129.51, 127.78, 126.91, 125.86, 125.33, 124.71, 115.63, 111.08, 107.15, 85.50, 49.15, 31.65, 29.50, 29.18, 27.34, 27.18, 22.51, 14.40 (52C). MS (EI, 70 eV): m/z (%) = 806 (22) $[M]^+$. Anal. calcd. for $C_{52}H_{41}Cl_2N_5$ (806.84): C, 77.41; H, 5.12; N, 8.68. Found C, 77.35; H, 5.10; N, 8.64%.

*4.1.5.2. 2,2′-(Octylazanediyl)bis(6-(naphthalen-1-yl)-4-(thiophen-2-yl)nicotinonitrile) (**8b**).* Yield 60%, white, mp 138−140 °C. IR, ν, cm^{-1}: 2222 (C≡N). ^1H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.88−7.33 (m, 22H, Ar H, and thiophene ring), 1.66 (t, 2H, CH_2), 1.21−1.24 (m, 12H, $6CH_2$), 1.03 (t, 3H, CH_3). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 160.22, 154.23, 152.44, 136.11, 135.22, 134.22, 133.72, 132.22, 131.21, 130.44, 129.25, 129.19, 127.78, 126.80, 125.66, 125.26, 124.99, 116.71, 110.21, 106.22, 49.10, 31.68, 29.54, 29.20, 27.36, 27.18, 22.52, 14.44 (48C). MS (EI, 70 eV): m/z (%) = 749 (6) $[M]^+$. Anal. calcd. for $C_{48}H_{39}N_5S_2$ (749.26): C, 76.87; H, 5.24; N, 8.55. Found C, 76.88; H, 5.25; N, 8.55%.

*4.1.5.3. 2,2′-(1,4-Phenylenebis(azanediyl))bis(4-(4-chlorophenyl)-6-(naphthalen-1-yl)nicotine Nitrile) (**9a**).* Yield 91%, gray, mp 210−212 °C. IR, ν, cm^{-1}: 3222 (NH), 2220 (C≡N). ^1H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.89−7.58 (m, 28H, Ar H), 5.70 (s, 2H, NH, disappeared with D_2O). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 162.88, 162.08, 161.31, 159.21, 153.44, 148.24, 148.11, 147.21, 132.55, 131.91, 130.99, 129.71, 129.22, 128.88, 127.71, 126.84, 125.81, 125.46, 125.20, 125.00, 122.91, 104.22, 104.01 (50C). MS (EI, 70 eV): m/z (%) = 785 (26) $[M]^+$. Anal. calcd. for $C_{50}H_{30}Cl_2N_6$ (785.73): C, 76.43; H, 3.85; N, 10.70. Found C, 76.40; H, 3.80; N, 10.66%.

*4.1.5.4. 2,2′-(1,4-Phenylenebis(azanediyl))bis(6-(naphthalen-1-yl)-4-(thiophen-2-yl)nicotine Nitrile) (**9b**).* Yield 93%, gray, mp 138−140 °C. IR, ν, cm^{-1}: 3225 (NH), 2224 (C≡N), ^1H NMR spectrum (DMSO-d_6, 47 °C), δ_H, ppm: 8.88−7.15 (m, 26H, Ar H, and thiophene ring), 6.40 (s, 2H, NH, disappeared with D_2O); ^{13}C NMR spectrum (DMSO-d_6, 47 °C), δ_C, ppm: 161.92, 161.40, 159.31, 153.87, 153.44, 148.61, 148.22, 147.22, 132.65, 131.85, 130.97, 129.39, 129.05, 128.99, 128.54, 127.71, 126.89, 125.86, 125.36, 122.98, 104.33, 104.25 (46C). MS (EI, 70 eV): m/z (%) = 728 (10) $[M]^+$. Anal. calcd. for $C_{46}H_{28}N_6S_2$ (728.18): C, 75.80; H, 3.87; N, 11.53. Found C, 75.82; H, 3.87; N, 11.54%.

*4.1.5.5. 2-((2-Aminoethyl)amino)-4-(4-chlorophenyl)-6-(naphthalen-1-yl)nicotinonitrile (**10a**).* Yield 63%, gray, mp 100−102 °C. IR, ν, cm^{-1}: 3379, 3222 (NH_2, NH), 2205 (C≡N). ^1H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.83−7.42 (m, 12H, Ar H), 7.73 (s, 2H, NH_2, disappeared with D_2O), 7.38 (s, H, NH, disappeared with D_2O), 3.67 (t, 2H, CH_2), 3.15 (t, 2H, CH_2). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 161.25, 161.02, 156.21, 136.23, 135.56, 133.88, 133.71, 130.81, 130.61, 129.55, 128.13, 127.53, 127.33, 127.01, 126.71, 126.59, 125.81, 125.30, 116.12, 90.73, 41.40, 37.23 (24C). MS (EI, 70 eV): m/z (%) = 398 (16) $[M]^+$. Anal. calcd. for $C_{24}H_{19}ClN_4$ (398.13): C, 72.27; H, 4.80; N, 14.05. Found C, 72.25; H, 4.79; N, 14.00%.

*4.1.5.6. 2-((2-Aminoethyl)amino)-6-(naphthalen-1-yl)-4-(thiophen-2-yl)nicotinonitrile (**10b**).* Yield 71%, orange, mp 128−131 °C. IR, ν, cm^{-1}: 3374, 3228 (NH_2, NH), 2207 (C≡N). ^1H NMR spectrum (DMSO-d_6, 43 °C), δ_H, ppm: 8.3−7.55 (m, 11H, Ar H, and thiophene ring), 7.74 (s, 2H, NH_2, disappeared with D_2O), 7.38 (s, H, NH, disappeared with D_2O), 3.57 (t, 2H, CH_2), 2.97 (t, 2H, CH_2). ^{13}C NMR spectrum (DMSO-d_6, 43 °C), δ_C, ppm: 160.49, 160.05, 156.45, 137.34, 135.56, 133.99, 133.62, 130.80, 130.06, 129.85, 128.97, 128.23, 127.93, 127.19, 126.82, 126.59, 125.86, 125.30, 116.11, 90.82, 41.26, 37.33 (22C). MS (EI, 70 eV): m/z (%) = 370 (15) $[M]^+$. Anal. calcd. for $C_{22}H_{18}N_4S$ (370.13): C, 71.33; H, 4.90; N, 15.12. Found C, 71.33; H, 4.91; N, 15.14%.

*4.1.5.7. 4-(4-Chlorophenyl)-2-hydrazinyl-6-(naphthalen-1-yl)nicotinonitrile (**11a**).* Yield 74%, yellow, mp 160−162 °C. IR, ν, cm^{-1}: 3323, 3261, 3183 (NH_2, NH), 2220 (C≡N). ^1H NMR spectrum (DMSO-d_6, 45 °C), δ_H, ppm: 8.89−7.53 (m,

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12H, Ar H), 2.65 (s, 2H, NH_2, disappeared with D_2O), 2.30 (s, H, NH, disappeared with D_2O). ^{13}C NMR spectrum (DMSO-d_6, 45 °C), δ_C, ppm: 162.02, 155.24, 152.47, 136.10, 135.26, 134.27, 133.87, 131.36, 131.06, 130.42, 129.45, 129.32, 129.03, 127.76, 126.91, 125.87, 125.33, 124.72, 115.64, 107.35 (22C). MS (EI, 70 eV): m/z (%) = 370 (35) $[M]^+$. Anal. calcd. for $C_{22}H_{15}ClN_4$ (370.10): C, 71.25; H, 4.08; N, 15.11. Found C, 71.26; H, 4.10; N, 15.13%.

*4.1.5.8. 2-Hydrazinyl-6-(naphthalen-1-yl)-4-(thiophen-2-yl)nicotinonitrile (**11b**).* Yield 82%, dark-yellow, mp 140−142 °C. IR, ν, cm^{-1}: 3315, 3265, 3189 (NH_2, NH), 2223 (C≡N). ^1H NMR spectrum (DMSO-d_6, 48 °C), δ_H, ppm: 8.87−7.25 (m, 11H, Ar H, and thiophene ring), 2.60 (s, 2H, NH_2, disappeared with D_2O), 2.25 (s, H, NH, disappeared with D_2O). ^{13}C NMR spectrum (DMSO-d_6, 48 °C), δ_C, ppm: 161.94, 158.00, 153.64, 148.25, 138.46, 136.27, 135.33, 133.84, 132.66, 131.90, 129.40, 129.24, 127.72, 126.90, 125.87, 126.00, 116.49, 166.24, 104.36, 101.46 (20C). MS (EI, 70 eV): m/z (%) = 342 (24) $[M]^+$. Anal. calcd. for $C_{20}H_{14}N_4S$ (342.09): C, 70.15; H, 4.12; N, 16.36. Found C, 70.17; H, 4.13; N, 16.37%.

*4.1.6. Synthesis of [1,2,4]Triazolo[4,3-a]pyridine 8-Carbonitrile (**12a,b**).* A solution of hydrazides **11a,b** (1 g) in EtOH (10 mL) and drops of glacial acetic acid was refluxed for 2 h and cooled at room temperature. The precipitated solid product was collected and recrystallized from EtOH to afford compounds **12a,b**.

*4.1.6.1. 7-(4-Chlorophenyl)-3-methyl-5-(naphthalen-1-yl)-[1,2,4]triazolo[4,3-a]pyridine-8-carbonitrile (**12a**).* Yield 62%, yellow, mp 174−176 °C. IR, ν, cm^{-1}: 2220 (C≡N), 1577 (C═N). ^1H NMR spectrum (DMSO-d_6, 41 °C), δ_H, ppm: 8.89−7.58 (m, 12H, Ar H), 2.48 (s, 3H, CH_3). ^{13}C NMR spectrum (DMSO-d_6, 41 °C), δ_C, ppm: 162.04, 159.49, 155.61, 155.27, 152.94, 152.47, 134.29, 131.50, 131.24, 131.08, 129.52, 129.45, 129.32, 129.03, 127.79, 126.92, 125.88, 125.35, 124.74, 107.14, 106.97, 39.58 (24C). MS (EI, 70 eV): m/z (%) = 394 (42) $[M]^+$. Anal. calcd. for $C_{24}H_{15}ClN_4$ (394.10): C, 73.00; H, 3.83; N, 14.19. Found C, 73.02; H, 3.86; N, 14.20%.

*4.1.6.2. 3-Methyl-5-(naphthalen-1-yl)-7-(thiophen-2-yl)-[1,2,4]triazolo[4,3-a]pyridine-8-carbonitrile (**12b**).* Yield 65%, pale-brown, mp 110−112 °C. IR, ν, cm^{-1}: 2223 (C≡N), 1572 (C═N). ^1H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 8.87−7.18 (m, 11H, Ar H, and thiophene ring), 2.45 (s, 3H, CH_3). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 165.04, 162.49, 159.49, 155.51, 155.23, 152.84, 152.41, 134.29, 131.32, 131.01, 130.02, 129.55, 129.45, 129.13, 129.03, 128.80, 127.77, 125.71, 125.35, 107.11, 106.96, 39.55 (22C). MS (EI, 70 eV): m/z (%) = 366 (17) $[M]^+$. Anal. calcd. for $C_{22}H_{14}N_4S$ (366.09): C, 72.11; H, 3.85; N, 15.29. Found C, 72.09; H, 3.83; N, 15.26%.

*4.1.7. Synthesis of 2-(5-Methyl-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)nicotinonitrile (**13a,b**).* To a solution of hydrazides **11a,b** (0.5 g, 0.001 mol) in EtOH (20 mL), drops of glacial acetic acid and two drops of pipridine as well as benzyl acetoacetate (0.2 mL, 0.001 mol) were added. The reaction mixture was refluxed for 2 h and cooled at room temperature. The precipitated solid product was collected and recrystallized from EtOH to afford compounds **13a,b**.

*4.1.7.1. 4-(4-Chlorophenyl)-2-(5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)-6-(naphthalen-1-yl)nicotinonitrile (**13a**).* Yield 53%, pale-green, mp 234−236 °C. IR, ν, cm^{-1}: 3152 (NH), 2223 (C≡N), 1671 (C═O). ^1H NMR spectrum (DMSO-d_6, 44 °C), δ_H, ppm: 10.25 (s, H, NH, disappeared with D_2O), 8.92−7.51 (m, 12H, Ar H), 2.61 (s, 3H, CH_3), 1.24 (s, 1H, CH). ^{13}C NMR spectrum (DMSO-d_6, 44 °C), δ_C, ppm: 162.05, 159.50, 155.65, 155.28, 152.94, 152.46, 134.64, 131.33, 131.08, 129.60, 129.46, 129.25, 129.03, 128.74, 128.16, 127.80, 127.47, 126.92, 125.88, 125.36, 124.75, 124.65, 120.35, 40.07 (26C). MS (EI, 70 eV): m/z (%) = 436 (8) $[M]^+$. Anal. calcd. for $C_{26}H_{17}ClN_4O$ (436.11): C, 71.48; H, 3.92; N, 12.82. Found C, 71.51; H, 3.95; N, 12.83%.

*4.1.7.2. 2-(5-Methyl-3-oxo-2,3-dihydro-1H-pyrazol-1-yl)-6-(naphthalen-1-yl)-4-(thiophen-2-yl)nicotinonitrile (**13b**).* Yield 60%, off-white, mp 141−143 °C. IR, ν, cm^{-1}: 3169 (NH), 2220 (C≡N), 1676 (C═O). ^1H NMR spectrum (DMSO-d_6, 45 °C), δ_H, ppm: 10.11 (s, H, NH, disappeared with D_2O), 8.88−7.43 (m, 11H, Ar H, and thiophene ring), 2.58 (s, 3H, CH_3); 1.26 (s, 1H, CH). ^{13}C NMR spectrum (DMSO-d_6, 45 °C), δ_C, ppm: 163.05, 159.52, 155.55, 155.21, 152.92, 152.46, 134.64, 131.23, 131.08, 129.61, 129.41, 129.15, 129.03, 128.77, 128.11, 127.70, 127.47, 126.42, 125.81, 125.36, 124.75, 124.61, 120.31, 40.05 (24C). MS (EI, 70 eV): m/z (%) = 408 (18) $[M]^+$. Anal. calcd. for $C_{24}H_{16}N_4OS$ (408.10): C, 70.57; H, 3.95; N, 13.72. Found C, 70.60; H, 3.98; N, 13.76%.

*4.1.8. Synthesis of 3-Cyano-pyridin-2-yl-2-phenylacetohydrazide (**14a,b**).* To a solution of hydrazides (**11a,b**) (0.5 g, 0.001 mol) in dry pyridine (10 mL), phenylacetyl chloride (0.2 mL, 0.001 mol) was added. The reaction mixture was stirred for half an hour in an ice bath. Then, the reaction mixture was poured on ice/water and acidified by conc. HCl. The precipitated solid product was collected and recrystallized from EtOH to afford compounds **14a,b**.

*4.1.8.1. N′-(4-(4-Chlorophenyl)-3-cyano-6-(naphthalen-1-yl)pyridin-2-yl)-2-phenylaceto-hydrazide (**14a**).* Yield 33%, yellow, mp 182−184 °C. IR, ν, cm^{-1}: 3199 (NH), 2222 (C≡N), 1670 (C═O). ^1H NMR spectrum (DMSO-d_6, 40 °C), δ_H, ppm: 10.07 (s, 2H, NH, disappeared with D_2O), 8.89−7.58 (m, 17H, Ar H), 3.49 (s, 2H, CH_2). ^{13}C NMR spectrum (DMSO-d_6, 40 °C), δ_C, ppm: 169.45, 162.05, 159.50, 155.65, 155.28, 152.95, 152.47, 134.29, 131.31, 131.08, 129.52, 129.46, 129.32, 129.03, 128.15, 127.80, 126.92, 126.80, 125.88, 125.34, 124.73, 107.19, 106.96, 85.60, 34.86, 40.04 (30C). MS (EI, 70 eV): m/z (%) = 488 (26) $[M]^+$. Anal. calcd. for $C_{30}H_{21}ClN_4O$ (488.14): C, 73.69; H, 4.33; N, 11.46. Found C, 73.65; H, 4.29; N, 11.43%.

*4.1.8.2. N′-(3-Cyano-6-(naphthalen-1-yl)-4-(thiophen-2-yl)pyridin-2-yl)-2-phenylacetohydrazide (**14b**).* Yield 41%, white, mp 178−180 °C. IR, ν, cm^{-1}: 3198 (NH), 2224 (C≡N), 1679 (C═O). ^1H NMR spectrum (DMSO-d_6, 25 °C), δ_H, ppm: 10.00 (s, 2H, NH, disappeared with D_2O), 8.12−7.18 (m, 16H, Ar H, and thiophene ring), 3.39 (s, 2H, CH_2). ^{13}C NMR spectrum (DMSO-d_6, 25 °C), δ_C, ppm: 169.55, 161.99, 159.41, 155.61, 155.21, 152.99, 152.44, 134.21, 132.22, 132.11, 131.52, 129.66, 129.32, 128.88, 127.81, 126.80, 126.71, 125.61, 125.42, 107.19, 106.11, 86.04, 36.32, 40.01 (28C). MS (EI, 70 eV): m/z (%) = 460 (15) $[M]^+$. Anal. calcd. for $C_{28}H_{20}N_4OS$ (460.14): C, 73.02; H, 4.38; N, 12.17. Found C, 73.10; H, 4.41; N, 12.19%.

4.2. Screening of Antiproliferative Activities. The newly prepared derivatives were screened for their potential antiproliferative activities against a battery of cancer cell lines, including leukemia (U937), melanoma (SKMEL-28), and large-cell lung (NCIH 460), colon (RKOP 27), and cervical (HeLa) cancer cell lines. Additionally, normal primary peripheral blood mononuclear (PBMC) cells were used as normal cells to assess the toxicity of the prepared derivatives against normal cells. The screening depended mainly on a standard MTT assay.[30,31] Cancer cells were obtained from ATCC culture collection, while PBMC cells were isolated from whole blood. The whole blood

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was diluted with phosphate-buffered saline (PBS) and then gently layered over an equal volume of Ficoll in a Falcon tube and centrifuged for 30−40 min at 400−500 g without a break. This resulted in the formation of four layers, each containing different cell types—the uppermost layer contained plasma, which can be removed by pipetting.

Briefly, cells were seeded in 96-well culture plates using an RPMI 1640 medium at a final concentration of 20 000 cells/mL and incubated at standard incubation conditions for 24 h until cells adhered. Afterward, different serial dilutions of the synthesized compounds (0−1 μM/DMSO) were added to each well plate, and the plates were further incubated for 72 h. Accordingly, 20 μL (MTT, 5 mg/(mL PBS)) was pipetted and further incubation was allowed for 4 h. The medium was washed out and DMSO (100 μL/well) was added. The developed formazan color was read with the help of a microplate reader at 570 nm.[32,33] IC_{50} values were calculated from the linear regression of the dose−response curve obtained using Origin 6.1 software (OriginLab Corporation, Northampton, MA). All experiments were run three times, and data are shown as mean ± standard deviation (SD). Doxorubicin, aldelseukin, gemcitabine, capecitabine, and fluorouracil were used as positive controls for comparison. Control compounds were prepared and diluted as the prepared derivatives.

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Author Contributions

The listed authors contributed to this work as follows: A.A.E.-S. developed the concept of the work, interoperated the results and the experimental part, and prepared the manuscript; A.E.-G.E.A. and E.A.E. helped in the preparation of the manuscript and performed the revision before submission; and E.A.E. performed the anticancer studies and received financial support for the work. All authors read and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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