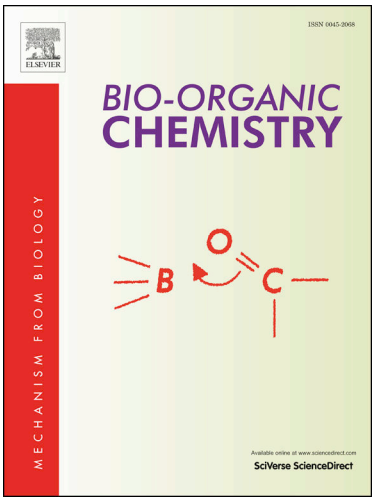
Journal Pre-proofs



Apoptosis: A Target for Anticancer Therapy with Novel Cyanopyridines

Magda M.F. Ismail, Amel M. Farrag, Marwa F. Harras, Mona H. Ibrahim, Ahmed B.M. Mehany

PII: S0045-2068(19)31525-1
DOI: https://doi.org/10.1016/j.bioorg.2019.103481
Reference: YBIOO 103481

To appear in: *Bioorganic Chemistry*

Received Date: 13 September 2019
Revised Date: 23 November 2019
Accepted Date: 25 November 2019

Please cite this article as: M.M.F. Ismail, A.M. Farrag, M.F. Harras, M.H. Ibrahim, A.B.M. Mehany, Apoptosis: A Target for Anticancer Therapy with Novel Cyanopyridines, *Bioorganic Chemistry* (2019), doi: https://doi.org/10.1016/j.bioorg.2019.103481

Apoptosis: A Target for Anticancer Therapy with Novel Cyanopyridines

Magda M.F. Ismail[a], Amel M. Farrag[a*], Marwa F. Harras[a], Mona H. Ibrahim[a] and Ahmed B. M. Mehany[b]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo 11754, Egypt.

[b] Department of Zoology, Faculty of Science (Boys), Al-Azhar university, Cairo11754, Egypt.

***Corresponding author**

Amel M. Farrag
Associate Professor of Pharmaceutical Chemistry
Faculty of Pharmacy, Al-Azhar University
Nasr City, Cairo, Egypt
Mobile: +201006364634
E-mail: amelfarrag@ymail.com
ORCID ID: 0000-0002-8207-0859

Apoptosis: A Target for Anticancer Therapy with Novel Cyanopyridines

Abstract

One of the many methods of treating cancer is to terminate the uncontrolled growth of cancer cells. So, aiming the apoptotic pathway is an exciting approach to finding new anticancer agents. A novel series of cyanopyridines was designed and synthesized for antiproliferative evaluation. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(4-(dimethylamino)phenyl) nicotinonitrile **10f** was the most potent inhibitor against the growth of PC-3, and HepG-2 cancer cell lines with IC_{50} values of 2.04 uM (selectivity index, SI = 78.63, 43, respectively). Also, **10f** was safe against the growth of normal human diploid lung fibroblasts cell line (WI-38) with an IC_{50} value of 160.04 uM. Its analogs, **10b, 10d, 10g,** and **11b**, were also active against the growth of PC-3, and HepG-2 while against MCF-7 cell line, they displayed good cytotoxic activity compared to the reference standard 5-FU. Remarkably, mechanistic studies indicated that compounds **10b, 10d, 10f, 10g,** and **11b** stimulated the level of active caspase 3 and boosted the BAX/BCL2 ratio 20- 95 folds in comparison to the control. Our results have also indicated that **10b, 10d, 10f, 10g,** and **11b** exhibited a very potent inhibitory activity against PIM-1 kinase enzyme, where the IC_{50} values unraveled very potent molecules in the micromolar range (0.47 -1.27 uM). Further investigations have shown that **10f**, the most potent PIM-1 kinase inhibitor, induced a cell cycle arrest at the G2/M phase. Moreover, *in silico* evaluation of ADME properties indicated that all the cyanopyridine compounds are orally bioavailable with no permeation to the blood brain barrier.

Keywords: Synthesis; Cyanopyridine; Anticancer; Apoptosis; PIM-1 kinase; ADME.

1. Introduction

Each minute of a lifetime, millions of cells in our bodies practice a planned form of cell death called apoptosis, or programmed cell death. This altruistic cellular process plays wide-ranging and vital roles in keeping us healthy, not least in protecting us from cancer [1]. So, apoptosis has a critical role in monitoring cell numbers in many developmental and physiological settings. Apoptosis is diminished in many human cancers, portentous that disruption of apoptotic function contributes substantially to the transformation of a normal cell into a tumour cell. The switching apoptosis on and off is determined by the ratio of pro-apoptotic effector proteins (BAX and BAK), and antiapoptotic BCL2 proteins (BCL2, BCL-xL, MCL-1, A1, BCL-B, BCL-w) [2]. Mainly, two main signalling paths for apoptotic cell death have been specified, the first one is the intrinsic mitochondrial apoptotic pathway that is mainly produced by cellular stress, in which mitochondrial permeability plays a crucial role. Whereas; the second path is the extrinsic cytoplasmic pathway that is triggered via pro-apoptotic ligands binding to the cell surface death receptor [1,3-5]. Caspase protease activity is vital for apoptosis; once active, caspases cleave hundreds of different proteins leading to rapid cell death with characteristic biochemical and morphological hallmarks [6-8]. In general terms, caspase activity can be initiated either via the intrinsic pathway of apoptosis or through the extrinsic pathway of apoptosis. Cell death also plays crucial role in the treatment of cancer. Apoptosis deregulation has been extensively documented as a hallmark of cancer throughout cancer pathogenesis. Consequently, the initiation of apoptosis in tumor cells represents an effective tactic for fighting different human malignancies in the current medical age [1,3-5].

On the other hand, the literature survey reveals that 4, 6-diaryl-3-cyano-2-pyridone derivatives have stood out as a promising class of anticancer agents with efficient apoptotic activity. N'-[2-(3-cyano-4,6-bis(3,4-dimethoxyphenyl) pyridin-2-yloxy) acetyl] benzohydrazide **I**, was reported to inhibit the proliferation of MCF-7 cancer cells by inducing apoptosis and arresting the cell cycle at G1 phase via inhibition of CDK2 and CDK4 [9]. 3-Cyano-4,6-diaryl-2-pyridone derivative **II** possess anticancer activity due to its ability to act as survivin inhibitor, which is a unique member of the inhibitors of apoptosis [10].4-Thienyl-6-arylpyridone derivative **III** showed potent antiproliferative activity with high PIM-1 kinase inhibitory activity with IC_{50} value of 0.94 µM, and it was founded to boost the levels of active caspase 3 and BAX and decrease the level of BCL2 [11], **(Fig.1)**. This indicates the potentiality of 4, 6-diaryl-3-cyano-2-substitutedpyridine skeleton as a model for further optimization to get a more potent apoptosis inhibitor.

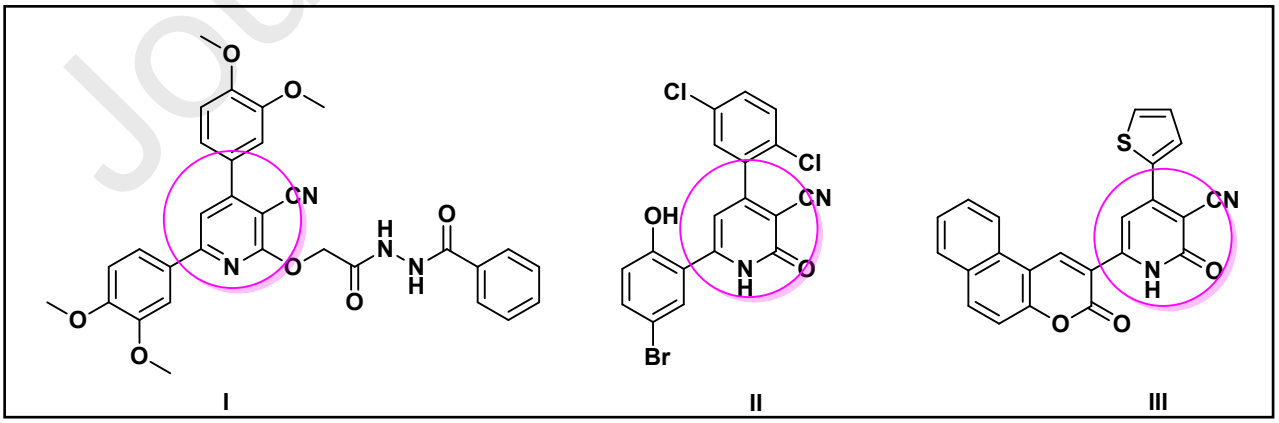


Fig. 1: Chemical structures of reported cyanopyridines endowed with anticancer and apoptosis inducing activities.

Noteworthy, Cheney *et al* study at 2007, proved that some cyanopyridine derivatives also acted as PIM-1 kinase inhibitors e.g., **IV-VI** with IC_{50} of 0.05, 0.43, and 0.99 µM, respectively [12] **(Fig. 2)**

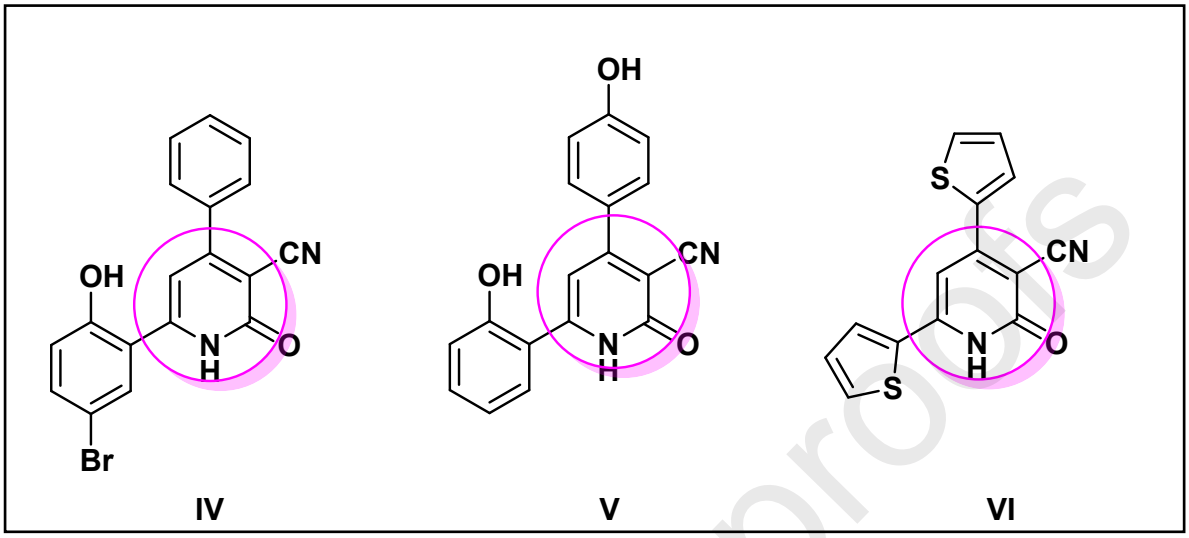


Fig. 2: Chemical structure of reported cyanopyridines as PIM-1 kinase inhibitors.

PIM-1 is an oncogenic constitutively active serine/threonine kinase, a member of a family of proteins containing other homologues, PIM-2 and PIM-3 and is transcriptionally regulated by cytokines, mitogens and numerous growth factors 2-4 [13-15]. PIM-1 expressed in many types of solid and haematological cancers and almost absent in benign lesions, PIM-1 kinase proved to be a successful anti-cancer drug target of low toxicity [16-23]. It appears to contribute to cancer development in three significant ways when it is overexpressed by inhibiting apoptosis, promoting cell proliferation, and promoting genomic instability [24]. PIM-1 kinase phosphorylates and regulates the activity of many proteins involved with many biological processes such as cell cycle, cell proliferation, apoptosis, and drug resistance [25-29]. Through the cell cycle, PIM-1 regulates G1-S phase progression and G2/M checkpoint. [30,31]. Though, in terms of cancer a preponderance of evidence points to PIM-1 playing an important role in the inhibition of apoptosis and the regulation of cell proliferation [32, 33]. *In vitro* studies demonstrated that overexpression of PIM-1 enhances tumour growth and protection from drug-induced apoptosis of cancer cells [34]. Additionally, several specific and potent inhibitors of PIM-1 kinase have also been shown to induce apoptotic death of cancer cells, sensitize cancer cells to chemotherapy and synergize with other anti-tumour agents, thus making it an attractive therapeutic target [24].

Based on the findings obeve, we planned to design and synthesize novel series of cyanopyridine scaffold via substitution at p-4 by aromatic moiety bearing hydrophilic or lipophilic group. Moreover, we introduced at p-6 of cyanopyridine scaffold either; phenyl/ 2-thienyl or the extended flexible side chain (benzyloxyphenyl). What is more, 2-OH group was bioisosterically replaced by 2-NH_2 group; these structural modifications were done to examine their effect on the affinity of PIM-1 and to optimize their antiproliferative activity **(Fig. 3).** All the synthesized hits were evaluated for their *in vitro* antitumor activity against human prostate carcinoma PC-3, human hepatocellular carcinoma HepG-2, and breast adenocarcinoma MCF-7 cell lines using MTT assay. Additionally, cell cycle analysis and apoptosis induction potential of the most active cyanopyridine derivatives

10b, 10d, 10f, 10g, and **11b** were examined in PC-3 cells, in order to attain more mechanistic understandings and to verify and enlighten the antitumor properties of the inspected cyanopyridines. Furthermore, the effect of the most active compounds on the genes expression of some apoptosis key markers was also investigated. Finally, the most active compounds, **10b, 10d, 10f, 10g** and, **11b** were evaluated for inhibition of PIM-1 kinase.

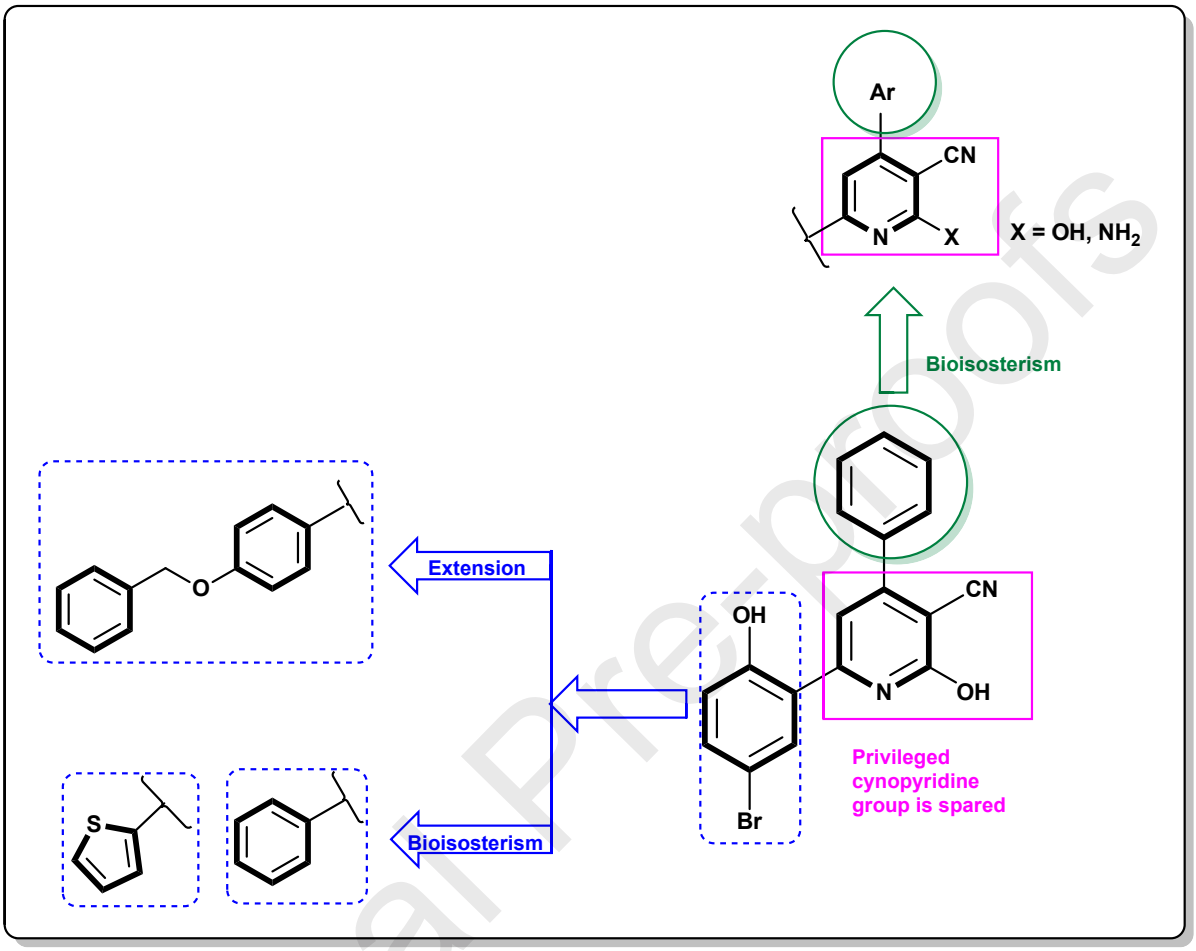
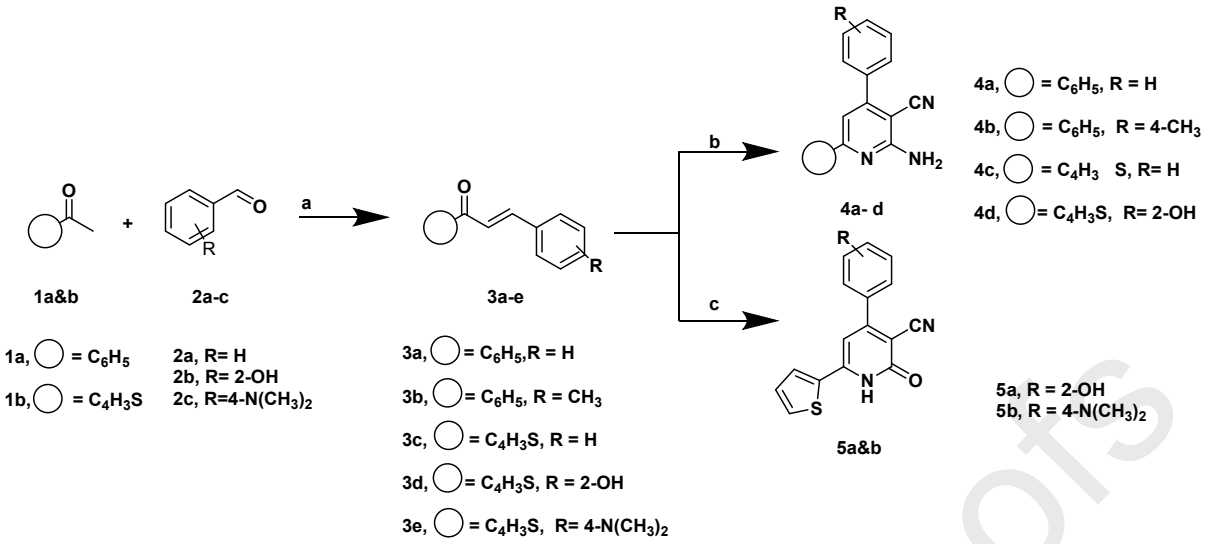


Fig. 3: Design of novel cyanopyridine derivatives as PIM-1 inhibitors and apoptosis inducers.

2. Results and discussion

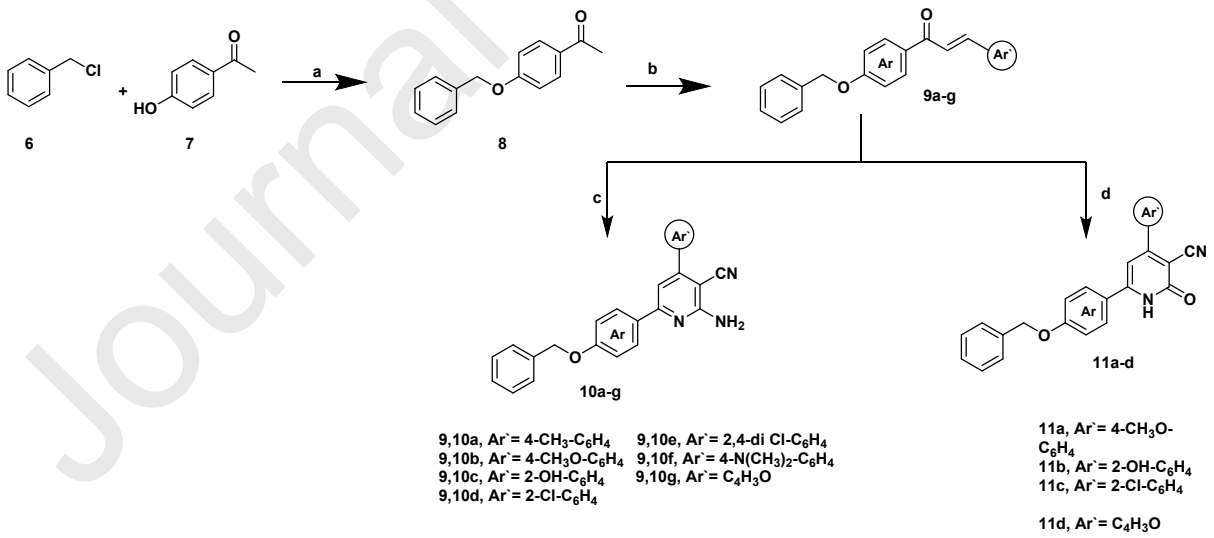
2.1. Chemistry

Synthesis of the new eighteen target compounds and intermediates is depicted in schemes 1 and 2. Initially, Knoevenagel condensation was followed to furnish the reported α,β-unsaturated ketones, **3a-e** by condensation of acetophenone or 2-acetylthiophene with different aromatic aldehydes in 10% aq. NaOH/EtOH. Cyclocondensation of **3a-d** with malononitrile and ammonium acetate afforded 2-amino-4-aryl-6-phenyl/(2-thienyl)-3-cyanopyridines, **4a-d**, while, cyclocondensation of **3d** or **3e** with cyanoacetamide/ pip. yielded 2-oxo-4-aryl-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitriles **5a**, **5b** [35] **(Scheme 1)**.



Scheme 1: Reagents and conditions: a) EtOH, 10% aq. NaOH, stirr, 3h; b) Malononitrile, NH_4OAC, EtOH, reflux, 8h; c) Cyanoacetamide, EtOH, piperidine, reflux, 6h.

Additionally, Knoevenagel condensation of starting material **8** [36] with different aromatic aldehydes/EtOH/KOH, produced the conjugated enones, **9a-g**. Cyclocondensation of the latters with malononitrile/$AcONH_4$/EtOH, yielded the corresponding 2-amino-6-(4-(benzyloxy) phenyl-4-(aryl)nicotinonitriles, **10a-g** [37]. Finally, condensation of **9b-d**, **9g** with ethyl cyanoacetate and ammonium acetate [38] furnished the corresponding 6-(4-(benzyloxy) phenyl)-4-(aryl)-2-oxo-1,2-dihydropyridine-3-carbonitriles **11a-d**, **(Scheme 2)**.



Scheme 2: Reagents and conditions: a) Ethanol, KOH, reflux,3h; b) MeOH, ArCHO, 10% KOH, stirr, 2h; c) EtOH, NH4OAc, malononitrile, reflux, 9 - 12 h; d) EtOH, NH_4OAc, ethylcyano acetate, reflux, 9-12 h.

The infrared (IR) spectra of **4d, 9a,f-g,** and **10a-g** products revealed bands at a stretching frequency (υ) around 3430 cm^{-1} corresponding to the NH_2 group. Moreover, it demonstrated relatively lower values of the carbonyl stretching (υ) around 1650 cm^{-1} than the typical carbonyl stretching, which is usually around 1700 cm^{-1}. This may be due to the single-bond character of the tautomeric enol form, leading to lower absorption frequency, in addition to; the cyano group appeared around 2210 cm^{-1}. Conjugated enones **(9a-c, e)** exhibited absorption bands at υ 1660 cm^{-1} representing CO functionality along with the ^{1}H-NMR spectra, which exhibited two doublets at δ 7.64, 7.74 ppm with characteristic J = 15.6 Hz value for *E* isomers of CH=CH protons. Overall cyanopyridines, **9-11** displayed characteristic multiplet peak distinguished CH_2 protons at the downfield region δ 5.06-5.22 ppm, being flanked between the oxygen atom and a phenyl group. The signals observed in ^{13}C-NMR spectra of all the products are determined to be in accordance with the recommended molecule structures. Mass spectrometry showed molecular ion peaks at M^+, M^++1, M^++2 in addition, to some of important peaks.

2.2. Biological results and discussion

2.2.1. Antiproliferative screening

The antiproliferative profiles of the synthesized compounds were evaluated *in vitro* against human prostate carcinoma PC-3, eleven compounds out of seventeen showed higher cytotoxic activities (IC_{50}'s= 2.04 - 7.5 *u*M), than that of the reference standard 5-FU (IC_{50}= 7.73 uM). 2-Amino-4,6-diphenyl-3-cyanopyridine, **4a** showed better cytotoxicity (IC_{50}'s= 7.01*u*M) than 5-FU. Bio-isosteric replacement with 2-thienyl at p-6 of cyanopyridine, as in analogs, **4b-d** and **5a, b** gave poor anticancer activities. Besides, SAR analysis elucidated that, on introducing benzyloxyphenyl moiety at p-6 of cyanopyridine, a dramatic increase in cytotoxicity of compounds **10 & 11** except for compound **11d**. For human hepatocellular carcinoma HepG-2, seven compounds **10a-d, f, g,** and **11b** elicited cytotoxicity ranging from equipotent to more than double the activity of 5-FU; however breast adenocarcinoma MCF-7 cell line was not sensitive to cyanopyridine hits except for compound **10d** which demonstrated enhanced anticancer effect (IC_{50}= 7.63 uM) than that of the standard. The normal human diploid lung fibroblast cell line WI-38 was also evaluated, in order to evaluate safety of the most active synthesized compounds **10b, 10d, 10f, 10g and 11b** towards the normal cells, cytotoxic activity evaluation of these hits on human diploid lung fibroblasts cell line WI-38 were carried out to investigate the toxicity and selectivity of these active derivatives **(Table 1)**. To our delight, they were found to be inactive against this normal cell (154.39-182.89 μM), revealing that they possessed a large safety margin to selectively target cancerous cells other than normal cells as shown by their specified IC_{50} values. Due to this, **10b, 10d, 10f, 10g,** and **11b** were chosen for further investigations (**Table 1).**

Table 1
In vitro [e] cytotoxic activities of compounds **4a-d, 5a, b, 10a-g** and **11a-d**.

Comp. No.	IC_{50} (µM)[a]/ (SI[b])			
	PC-3[C]	**HEPG-2[C]**	**MCF-7[C]**	**WI-38[C]**
4a	7.01±0.62	13.65±1.20	25.12±1.80	ND
4b	10.14±0.97	13.29±1.20	27.43±1.90	ND
4c	11.02±0.87	15.91±1.61	21.14±1.72	ND
4d	14.06±0.98	21.33±1.70	32.15±2.41	ND
5a	7.38±0.64	16.32±1.30	21.39±0.94	ND
5b	19.65±1.61	20.99±1.60	34.85±2.52	ND
10a	6.24±0.52	8.18±0.64	13.06±0.95	ND
10b	3.14±0.22 (49.17)	4.54±0.28 (34.01)	8.64±0.63 (17.87)	154.39±0.62
10c	5.19±0.52	5.44±0.37	9.46±0.91	ND
10d	2.36±0.16 (77.50)	4.59±0.37 (39.85)	7.63±0.89 (23.97)	182.89±0.39
10e	8.34±0.71	8.52±0.75	18.58±0.67	ND
10f	2.04±0.21 (78.63)	3.73±0.18 (43.00)	8.66±0.73 (18.52)	160.40±0.60
10g	2.99±0.30 (58.47)	4.71±0.22 (37.12)	9.31±0.81 (18.78)	174.82±0.44
11a	7.59±0.67	8.7±0.72	15.93±1.20	ND
11b	3.17±0.28 (56.32)	6.62±0.61 (26.97)	9.44±0.86 (18.91)	178.52±0.34
11c	4.34±0.56	10.46±0.98	12.74±0.96	ND
11d	27.9±1.71	39.7±2.50	68.34±3.11	ND
5 -Fu	7.53 ±0.57	8.15 ±0.61	7.76 ±0.46	

[a] Cytotoxicity, IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay. [b] SI: Selectivity index = (IC_{50} of WI-38)/(IC_{50} of cancer cell line). [C] Cell lines include human prostate carcinoma (PC-3) hepatocellular carcinoma (HepG-2), breast adenocarcinoma (MCF-7), and normal human diploid lung fibroblastscell line (WI-38). [d] ND: Not determined. [e]Data represent the mean values of three independent determinations.

2.2.2. Apoptosis detection studies

2.2.2.1. Effect on the active caspase 3 level

Activation of the caspase 3 pathway is a hallmark of apoptosis and can be used in cellular assays to quantify activators, and inhibitors of the "death cascade." the most active cyanopyridine analogs **10b, 10d, 10f, 10g,** and **11b** were evaluated for their effect on caspase 3. Our investigation results reflected the elevated level of the active caspase 3, which is considered as a marker for apoptosis, **(Table 2).**

2.2.2.2. Effect on mitochondrial apoptosis pathway proteins BAX and BCL2

The mitochondrial apoptotic pathway is harmonized by a family of proteins called BCL2. Among these, BCL2 and BAX finely set this programmed process as BCL2 suppresses apoptosis (anti-apoptotic) whereas BAX induces it (pro-apoptotic). Accordingly, the tuned balance between these two characteristic proteins is judgmental for the cell potential to undergo apoptosis. Several direct BAX activators have been identified to hold promise for cancer therapy with the advantages of specificity and the potential of overcoming chemo- and radio resistance. The most active cyanopyridine analogs **10b, 10d, 10f, 10g,** and **11b were** evaluated for their

effect on some apoptosis key markers, BAX, and BCL2. Our results confirmed the elevated level of BAX/BCL2 ratios that regulate cell death (apoptosis) **(Table 2).**

Table 2
Effect of compounds **10b, 10d, 10f, 10g** and **11b** on the genes expression of some apoptosis key markers.

Comp. No.	Caspase 3 Pg/ml	BAX Pg/ml	BCL2 Pg/ml
10b	277.7	199.1	1.724
10d	266.3	189.4	2.356
10f	363.8	215.9	1.476
10g	285.2	166.4	2.731
11b	398.2	259.4	0.8138
Control	55.3	18.14	5.418

2.2.3. PIM-1 kinase inhibitory activity

PIM-1 is emerging as a cancer drug target, particularly in prostate cancer [39]. PIM-1 inhibitory activity was evaluated for **10b, 10d, 10f, 10g** and **11b**. The obtained results revealed that **10f** was the most active hit as it displayed 80.22% inhibition of PIM-1 expression at 10 µM. **(Table 3).**

Table 3
PIM-1 kinase inhibitory activity of compound **10b, 10d, 10f, 10g** and **11b**.

Comp. No.	% Inhibition (Con.10µM)	IC_{50} (µM)
10b	49.60	11.71
10d	71.79	1.27
10f	80.22	0.47
10g	78.85	0.56
11b	72.99	0.84
Colchicine	64.15	3.98

2.2.4. Cell cycle analysis

To investigate the probable mechanism underlying the antiproliferative effect exerted by the most potent PIM-1 inhibitor compound **10f**, PC-3 cell cycle progression was assessed following treatment with compound **10f** , that also had the marked highest antiproliferative activity (IC_{50} = 2.04 µM), on human prostate carcinoma PC-3 cell line for 24 h using propidium iodide flow cytometry analysis. As seen in **(Fig. 4, Fig. 5, Fig 6 & Table 4)** in comparison with control cells, it was obvious that **10f** induced a significant increase in the percentage of cells at Pre-G1 phase by 18.9 folds, which could be indicative of apoptosis, and increase in G2/M by 4.9 folds. Such an increase was accompanied by a decrease in the percentage of cells at the S-phase of the cell cycle. The Pre-G1 and G2/M phase results indicated that compound **10f** induced apoptosis and arrest the cell cycle at the G2/M phase.

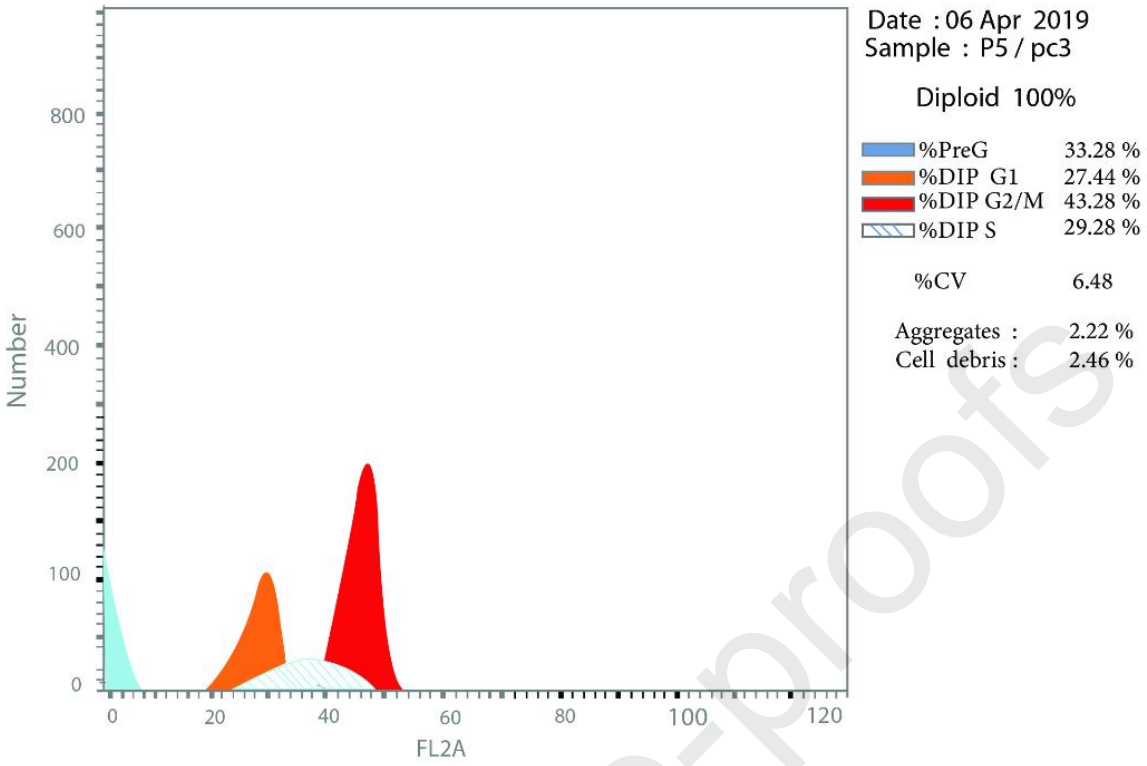


Fig. 4: Cell cycle analysis of PC-3 cells treated with compound **10f** at 0.86 µg/ml concentration.

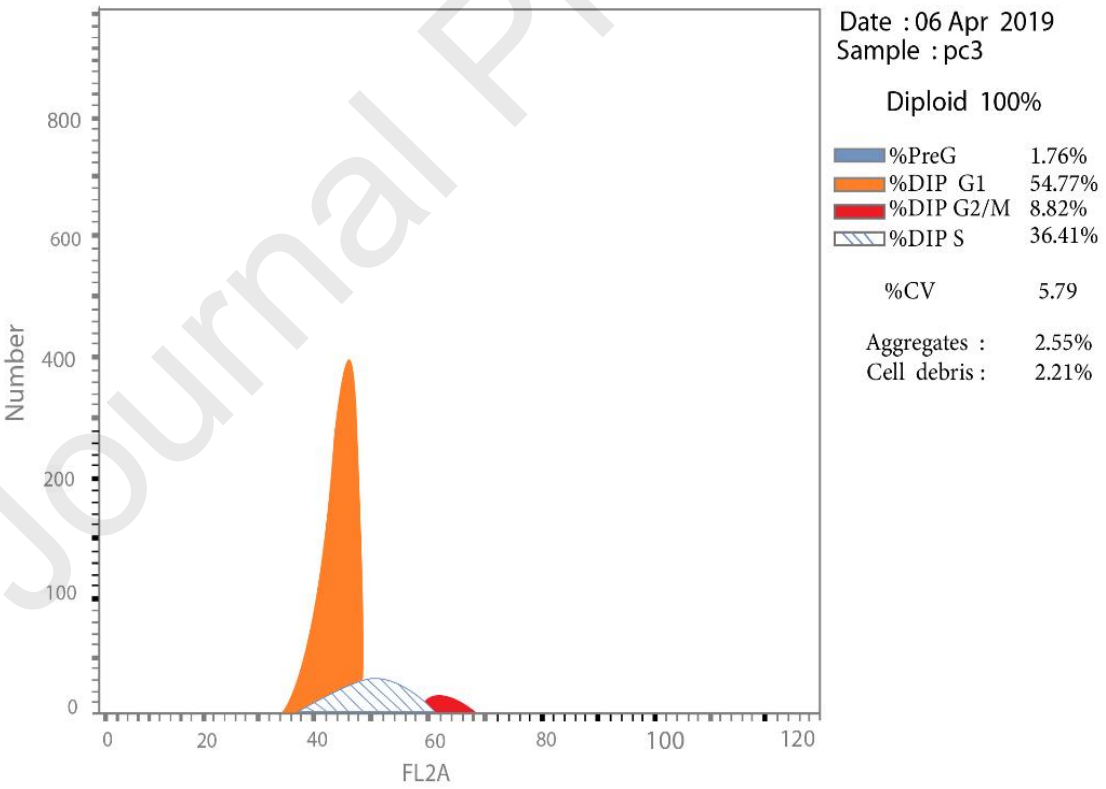


Fig. 5: Cell cycle analysis of PC-3 cells treated with DMSO.

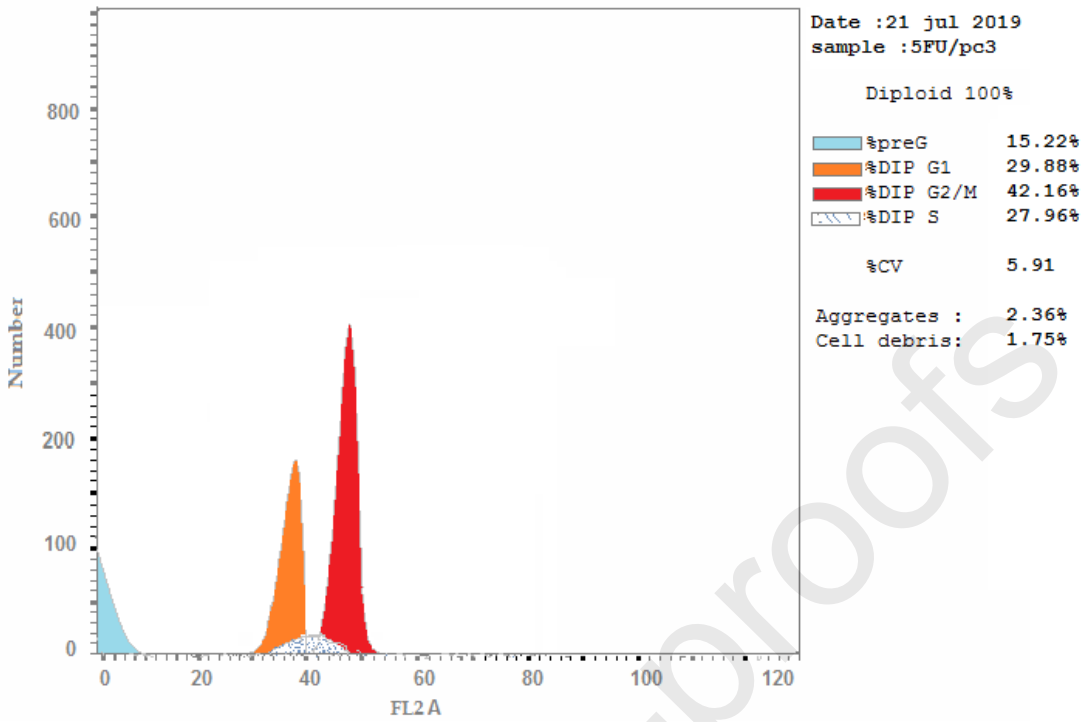


Fig. 6: Cell cycle analysis of PC-3 cells treated with DMSO.

Table 4
Effect of compound **10f**, **DMSO** and **5-FU** on the cell cycle of PC-3 cell line.

Sample	Conc. µM	%G0-G1	%S	%G2/M	%Pre-G1
10f / PC-3	2.04	27.44	29.28	43.28	33.28
DMSO / PC-3 (negative control)	0	54.77	36.41	8.82	1.76
5-FU / PC-3 (positive control)	7.53	29.88	27.96	42.16	15.22

2.2.5. Annexin V-FITC apoptosis assay

One of the earliest cellular changes evident during apoptosis is the translocation of phosphatidylserine (PS) from the inner to the outer side of the plasma membrane. Fluorescently labeled Annexin V, which binds to phosphatidylserine [40], can be used as a sensitive probe for PS in the outer leaflet of the plasma membrane [41]. Also, PI, a fluorescent molecule binding to nucleic acids, but which does not enter live cells, can be used to counterstain dead cells. To differentiate between apoptosis and necrosis, both Annexin V-FITC and PI staining usually performed. Annexin V/PI staining was performed in cells exposed to **10f** for 24h. The results revealed that application of compound **10f** on PC-3 cells for 24 h, increases the early apoptosis ratio (lower right quadrant of the cytogram) from 0.82% to 7.29%, and increases the late apoptosis ratio (higher right quadrant of the cytogram) from 0.43% to 23.56%. This means that compound **10f** induced almost up to 24.68 folds, both early and late, cellular apoptosis when compared with the control. These data suggest that compounds **10f** triggered apoptosis via the programmed cell death pathway rather than a necrotic pathway **(Fig. 6 & Table 5).**

A B

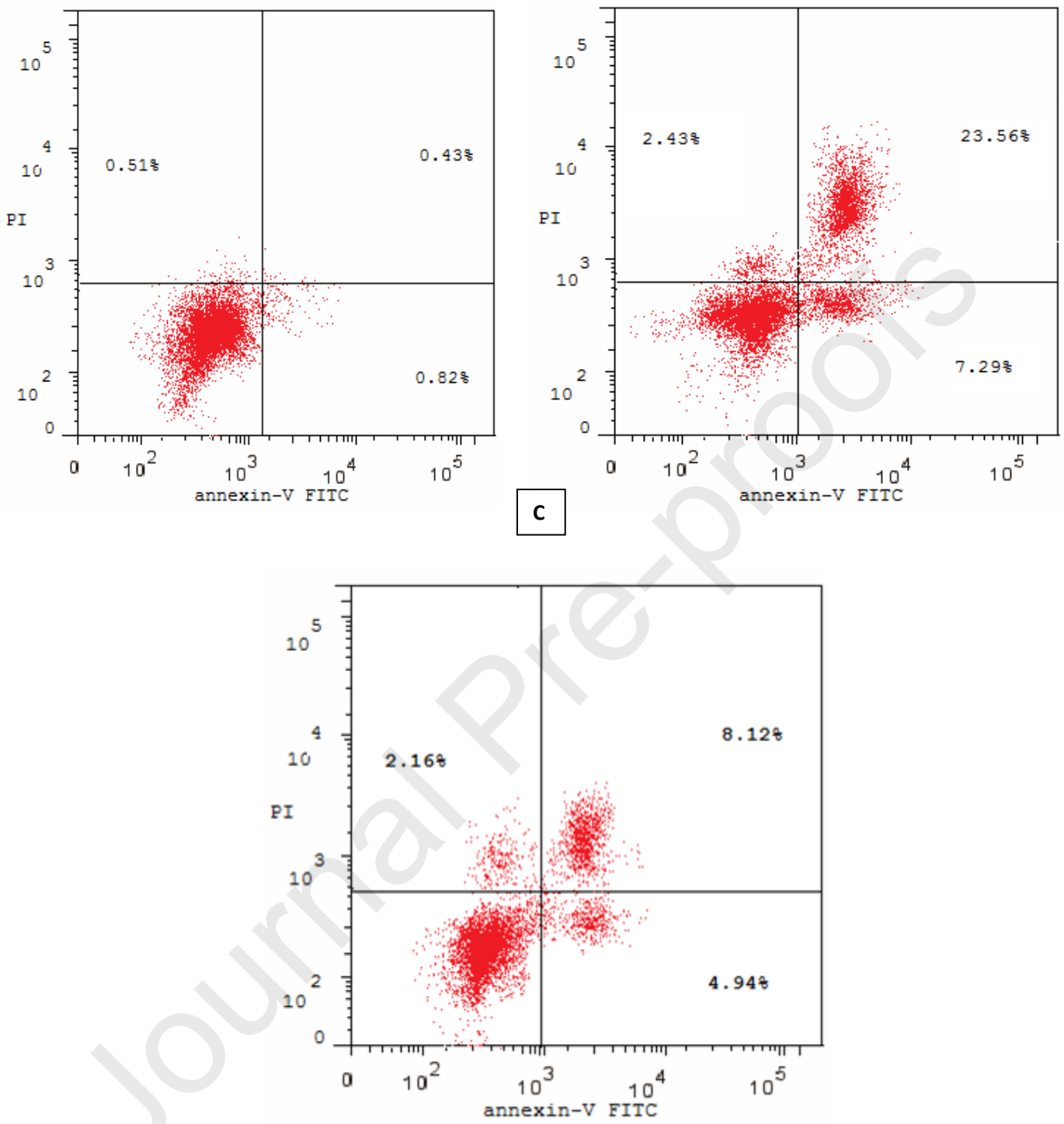


Figure 6: Effect of DMSO (**A**) , **10f** (**B**), and **5-FU** on the percentage of annexin V-FITC-positive staining in PC-3 cells.

Table 5

Effect of **10f, DMSO** and **5-FU** on the percentage of annexin V-FITC-positive staining in PC-3cells.

Sample / cell line	Apoptosis			Necrosis
	Total	Early	Late	
10f / PC-3	33.28	7.29	23.56	2.43
DMSO / PC-3 (negative control)	1.76	0.82	0.43	0.51
5-FU / PC-3 (positive control)	15.22	4.94	8.12	2.16

2.3. *In silico* evaluation of physicochemical and ADME properties

Computational study of the synthesized compounds **4a-d, 5a,b, 10a-g,** and **11a-d** was performed to evaluate physicochemically and ADME properties using SwissADME [42]. With respects to physicochemical properties (**Table 6)**, all the synthesized compounds have zero violation for Lipinski´s rule for oral drugs, except for **10e,** which has only one violation (M log P > 4.15) but it still stays in the orally bioavailable compounds. All the topological polar surface areas (TPSA) are less than 112 $A^{0\,2}$. Additionally, absorption (% ABS) was estimated by using the equation % ABS = 109 – (0.345 x TPSA) [43], found that the calculated % ABS of all these hits ranged between 70.64 % and 87.37 %, demonstrating that these synthesized derivatives may have the required cell membrane permeability and bioavailability. All compounds have rotatable bonds between 2 and 6, which indicating molecular flexibility to their bio target. Regards to pharmacokinetic and medicinal chemistry parameters of the synthesized compounds (**Table S, Supplementary data)**, it was found that, all the derivatives having high gastro-intestinal absorption and most of them have no permeation to the blood brain barrier except for the reported compounds **4a** and **4b**. Thus ensuring that these systemically targeted molecules will have low to no CNS side effects**.** Bioavailability is an index of the amount of drug present in the plasma and is considered as the most crucial factor affecting absorption.

Table 6
Physicochemical properties based on Lipinski´s rule of five, TPSA, % ABS and number of rotatable bonds**.**

Comp. No.	HBD	HBA	MlogP	MW	Lipinski´s Violations	TPSA	% ABS	No. of Rot. bonds
4a	1	2	2.49	271.32	0	62.7	87.37	2
4b	1	2	2.73	285.34	0	62.7	87.37	2
4c	1	2	2.05	277.34	0	90.94	77.63	2
4d	2	3	1.47	293.34	0	111.17	70.65	2
5a	2	3	1.53	294.33	0	105.12	72.73	2
5b	1	2	2.83	320.41	0	84.89	79.71	3
10a	1	3	3.44	391.46	0	71.93	84.18	5
10b	1	4	2.88	407.46	0	81.16	80.99	6
10c	2	4	2.67	393.44	0	92.16	77.20	5
10d	1	3	3.71	411.88	0	71.93	84.18	5
10e	1	3	4.17	446.33	1	71.93	84.18	5
10f	1	3	3.84	419.52	0	71.93	84.18	6
10g	1	4	2.02	367.4	0	85.07	79.65	5
11a	1	4	2.94	408.45	0	75.11	83.09	6
11b	2	4	2.74	394.42	0	86.11	79.29	5
11c	1	3	3.77	412.87	0	65.88	86.27	5
11d	1	4	2.09	368.38	0	79.02	81.74	5
5-FU	2	3	-0.32	130.08	0	65.72	86.33	0

Interestingly, all the synthesized derivatives were found to have high bioavailability scores 0.55. Pan Assay Interference Compounds (PAINS), was performed by SwissADME [42] and PAINS-Remover [44] displayed zero alerts to all the hits. Synthetic accessibility scores of all the analogs were found to be between 2.56 and 3.33, indicating that they can be readily synthesized on a large scale. (**Table S, Supplementary data)**

2. Conclusion

Herein we designed and synthesized a small library of cyanopyridines that resulted in cytotoxic derivatives against prostate carcinoma PC-3, human hepatocellular carcinoma HepG-2, and breast adenocarcinoma MCF-7 cell lines. The most active derivatives **10b, 10d, 10f, 10g,** and **11b** were evaluated against the normal human diploid lung fibroblasts cell line WI-38, indicated that they were inactive against this normal human cell. Apoptosis studies for these hits were conducted to evaluate the pro-apoptotic ability of our compounds. The result showed that they all boosted both the level of active caspase 3 and the BAX/BCL2 ratio in comparison to control. Furthermore, the PIM-1 kinase inhibitory activity for **10b, 10d, 10f, 10g,** and **11b** was evaluated where **10f** showed marked PIM-1 inhibitory activity 80.22%. Additionally, **10f** was found to arrest PC-3 cells at the G2/M and increase the percentage of cells at Pre-G1 phase by 18.9 folds. Moreover, all the derivatives are systemically targeted molecules that may have low to no CNS side effects. Our findings report a well-scalable cyanopyridine scaffold by applying extension tactic at p-6 that may be useful for clinical applications in cancer treatment and may contribute to the design of other molecules to investigate and develop antitumor chemotherapeutics.

4. Experimental section

Reagents and solvents were purchased from usual commercial suppliers and were used without further purification. Yields reported refer to purified products. All reactions were routinely checked by thin-layer chromatography (TLC) on Merck Silica Gel 60 F_{254} (0.25mm thick) and visualization was performed with UV lamp. Melting points were measured in open capillary tubes using Stuart SMP3 apparatus. IR spectra (KBr) were measured on a Shimadzu FT/IR 1650 (Perkin Elmer) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Brüker Advance-400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) in DMSO-d6 Chemical shifts (δ) are reported in ppm relative to TMS as an internal standard, or to the solvent in which the spectrum was recorded. Mass spectra were performed on a Shimadzu GS/MS-QP 2010 plus spectrometer at 70 eV. The intermediates, **3a-e** [45-48], and **9b-d** [49,50] are prepared according to the reported method [51], as well as compounds **4a-c** [52,53] were prepared for cytotoxic screening and SARs study.

4.1. Chemistry

4.1.1. General procedure for the synthesis of 2-amino(un, substituted phenyl)-6-(aryl)-nicotinonitrile derivatives.(4a-d)

A solution of chalcone 3a -d (0.01 mol) with malononitrile (0.66 g, 0.08 mol) and ammonium acetate (6.16 g, 0.08 mol) in ethanol were refluxed for 8 h. The progress of the reaction was monitored by TLC. The precipitate was filtered off and washed with cold methanol. Recrystallization from ethanol yielded pure compounds with 50-70% yields.

4.1.1.1. 2-Amino-4-(2-hydroxyphenyl)-6-(thiophen-2-yl)nicotinonitrile (4d) Yield 60%; m.p. 310 - 312 ^{o}C; IR (KBr, cm^{-1}): 3432, 3348, 3238 (NH_2, OH), 3184 (CH aromatic), 2965 (CH aliphatic), 2197 (C≡N), 1613 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 6.88 – 6.92 (t, H, H_5 Ar-H), 6.96 (d, H, H_3 Ar-H), 7.14 – 7.16 (t, H, H_4 Ar-H), 7.28 – 7.32 (t, H, H_4 thiophene), 7.57 (d, 2H, H_6 Ar-H), 7.71 (d, H, H_3 thiophene), 7.79 (d, H, H_5 thiophene), 8.41 (s, 1H, Pyridine-H), 9.89, 10.10 (2s, 3H, NH_2, OH; exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ (ppm): 85.48, 111.22, 113.54, 116.34, 116.92, 122.88, 127.26, 128.78, 129.96, 143.04, 154.54, 155.99, 156.89, 161.34; MS, m/z: 293 (M^+, 5.01 %), 294 [$(M+1)^+$, 3.82 %]; Anal. Calcd. For $C_{16}H_{11}N_3OS$ (293.06): C, 65.51; H, 3.78; N, 14.32; S, 10.93, Found: C, 65.32; H, 4.12; N, 14.59; S, 11.06.

4.1.2. General procedure for the synthesis of 4-(substituted phenyl)-2-oxo-6-thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile.(5a,b)

A mixture of chalcone **3d** or **3e** (0.01 mol) with 2-cyanoacetamide (0.84 g 0.01 mol) was refluxed for 6 h in ethanol containing drops of piperidine as a catalyst. The progress of the reaction was monitored by TLC. The precipitate formed was filtered off and washed with cold methanol. Recrystallization from ethanol yielded the pure product **5a,b**.

4.1.2.1. 4-(2-Hydroxyphenyl)-2-oxo-6-(thiophen-2-yl)-1,2 dihydropyridine-3-carbonitrile (5a). Yield 67%; m.p. 380-382 ^{o}C; IR (KBr, cm^{-1}): 3440-3200 (broad, NH, OH), 3102 (CH aromatic), 2969 (CH aliphatic), 2205 (C≡N), 1699 (C=O), 1601 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.16 - 7.18 (t, H, H_5 Ar-H), 7.24 (d, H, H_3 Ar -H), 7.29 - 7.33 (t, H, H_4 Ar -H), 7.36 (s, 1H, Pyridon-H) 7.51 – 7.54 (t, H, H_4 thiophene), 7.59 (d, H, H_6 Ar -H), 7.96 (d, H, H_3 thiophene), 8.29 (d, H, H_5 thiophene) 9.79, 10.60 (2s, 2H, NH, OH; exchangeable with D_2O; ^{13}C NMR (DMSO-d_6) δ (ppm): 111.74, 116.94, 117.23, 124.04, 125.01, 126.53, 128.52, 129.16, 131.52, 136.03, 152.78, 156.79, 168.35; MS, m/z: 294 (M^+, 20.44 %), 295 [$(M+1)^+$, 100 %]; Anal. Calcd. For $C_{16}H_{10}N_2O_2S$ (294.05): C, 65.29; H, 3.42; N, 9.52; S, 10.89 Found: C, 65.47 H, 3.68; N, 9.78; S,11.09.

4.1.2.2. 4-(4-(Dimethylamino)phenyl)-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (5b). Yield 70%; m.p. 330-332 ^{o}C; IR (KBr, cm^{-1}): 3433 (NH), 3090 (CH aromatic), 2920 (CH aliphatic), 2212 (C≡N), 1637 (C=O), 1610 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.01 (s, 6H, 2 CH_3), 6.74 (d, 2H, $H_{3,5}$ Ar-H, J = 8.4 Hz), 7.24 – 7.29 (t, H, H_4 thiophene), 7.55 – 7.62 (m, 2H, $H_{3,5}$ thiophene), 7.64 (s, H, NH; exchangeable with D_2O), 7.69 (d, 2H, $H_{2,6}$ Ar-H, J = 8.4 Hz), 8.23 (s, 1H, Pyridon-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 41.02, 111.09, 112.21, 116.37, 122.26, 125.99, 129.18, 131.28, 132.83, 134.86, 144.73, 146.80, 152.51, 161.76; MS, m/z: 321 (M^+, 83.14%); Anal. Calcd. For $C_{18}H_{15}N_3OS$ (321.09): C, 67.27; H, 4.70; N, 13.07; S, 9.98, Found: C, 67.54; H, 4.89; N, 13.35; S, 9.95.

4.1.3. Synthesis of 4`-(Benzyloxy)acetophenone (8)

A mixture of 4-hydroxyacetophenone (1.08 g, 0.01 mol), benzyl chloride (1.26 g, 0.01 mol) and KOH (0.04 g, 0.01 mol) in ethanol (30 ml) was refluxed for 3 h, then poured onto ice/water. The solid formed was collected, washed with water, dried, and recrystallized from ethanol. Yield 98%; m.p. 96-97 ^{o}C as reported [36].

4.1.4. General procedure for the synthesis of chalcon derivatives.(9a-g)

4`-Benzyloxy acetophenone (2.26 g, 0.01 mol) and different aryl aldehydes (0.01 mol) were dissolved in methanol (25 ml) and 5 ml of 10% alc. KOH solution was slowly added to it under stirring at 15-20°C. Stirring

continued for 2 h at the same temperature. The progress of the reaction was monitored by TLC. The precipitate was filtered off and washed with cold methanol. The obtained product was crystallized from methanol to give the chalcone derivatives **9a-g**.

4.1.4.1. (E)-1-(4-(Benzyloxy)phenyl)-3-(p-tolyl)prop-2-en-1-one (9a). Yield 70%; m.p. 166-168 ^{o}C; IR (KBr, cm^{-1}): 3030 (CH aromatic), 2912 (CH aliphatic), 1651 (C=O), 1592 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.33 (s, 3H, CH_3), 5.21 (s, 2H, OCH_2), 7.13 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 Hz), 7.24 (d, 2H, $H_{3,5}$ Ar`-H, *J* = 8 Hz), 7.31-7.47 (m, 5H, phenyl-Hs), 7.64 (d, 1H, olefinic, *J* = 15.6 Hz), 7.74 (d, 2H, $H_{2,6}$ Ar`-H, *J* = 8 Hz), 7.85 (d, 1H, olefinic, *J* = 15.6 Hz), 8.13 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 Hz); MS, m/z: 328 (M^+, 4.58 %); Anal. Calcd. For $C_{23}H_{20}O_2$ (328.15): C, 84.12; H, 6.14; Found: C, 84.38; H, 6.29.

4.1.4.2. (E)-1-(4-(Benzyloxy)phenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (9e). Yield 75%; m.p. 136-138 ^{o}C; IR (KBr, cm^{-1}): 3063 (CH aromatic), 2925 (CH aliphatic), 1663 (C=O), 1595 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.15 (m, 2H, OCH_2), 7.10 (d, 2H, $H_{3,5}$ Ar-H), 7.29 -7.45 (m, 6H, 5H, phenyl-Hs, 1H, olefinic), 7.62 (s, 1H, H_3 Ar`), 7.82 (d, 1H, olefinic), 8.00 - 8.06 (m, 4H, $H_{5,6}$ Ar`, $H_{2,6}$ Ar); MS, m/z: 383 [$(M+1)^+$ 20.59 %]; Anal. Calcd. For $C_{22}H_{16}Cl_2O_2$ (382.05): C, 68.94; H, 4.21, Found: C, 69.12; H, 4.37.

4.1.4.3. (E)-1-(4-(Benzyloxy)phenyl)-3-(4(dimethylamino)phenyl) prop-2-en-1-one (9f). Yield 95%; m.p. 124-126 ^{o}C; IR (KBr, cm^{-1}): 3032 (CH aromatic), 2927 (CH aliphatic), 1672 (C=O), 1594 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.98 (s, 6H, 2 CH_3), 5.20 (s, 2H, OCH_2), 6.71 (d, 2H, $H_{3,5}$ Ar`-H), 7.11 (d, 2H, $H_{3,5}$ Ar-H), 7.33-7.47 (m, 7H, $H_{2,6}$ Ar`-H, phenyl-Hs), 7.66 (d, 1H, olefinic), 8.08 -8.11 (m, 3H, 1H olefinic, $H_{2,6}$ Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 41.77, 69.96, 112.22, 115.14, 116.55, 122.63, 128.28, 128.47, 128.97, 130.97, 131.09, 131.77, 137.06, 144.78, 152.34, 162.32, 187.44; MS, m/z: 357 (M^+, 100 %); Anal. Calcd. For $C_{24}H_{23}NO_2$ (357.17): C, 80.64; H, 6.49, Found: C, 80.91; H, 6.70.

4.1.4.4. (E)-1-(4-(Benzyloxy)phenyl)-3-(furan-2-yl)prop-2-en-1-one (9g). Yield 78 %; m.p. 124-126 ^{o}C; IR (KBr, cm^{-1}): 3037 (CH aromatic), 2930 (CH aliphatic), 1646 (C=O), 1591 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.07 (s, 2H, OCH_2), 6.83-6.87 (m, 1H, H_4 Ar`-H), 7.08 (d, 2H, $H_{3,5}$ Ar-H), 7.30-7.47 (m, 6H, 5H, phenyl-Hs,1H olefinic), 7.57 (d, 1H, $H_3$ Ar`-H), 7.77 (d, 1H, olefinic), 7.89 – 7.91 (m, 3H, $H_{2,6}$ Ar-H, H_5 Ar`-H); MS, m/z: 304 (M^+, 96.46 %); Anal. Calcd. For : $C_{20}H_{16}O_3$ (304.11) : C, 78.93; H, 5.30, Found: C, 79.21; H, 5.44.

4.1.5. General procedure for the synthesis of 2-amino-6-(4-(benzyloxy)phenyl-4-(aryl)nicotinonitrile* derivatives *(10a-g).

A mixture of **9a-g** (2 mmol), ammonium acetate (6.16 g, 16 mmol) and malononitrile (0.132 g, 2 mmol), in ethanol (20 ml) was refluxed with stirring for 9-12 h After completion the reaction mixture was poured into ice cold water; the crude product was filtered, dried and recrystallized from 96% ethanol with 47 - 77% yields.

4.1.5.1. 2-Amino-6-(4-benzloxy)phenyl_-4-tolylnicotinonitrile (10a). Yield 50%; m.p. 200-202 ^{o}C; IR (KBr, cm^{-1}): 3350 , 3220 (NH_2), 3102 (CH aromatic), 2910 (CH aliphatic), 2202 (C≡N), 1610 (C=N), 1595 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.33 (s, 3H, CH_3), 5.06 - 5.21 (m, 2H, OCH_2), 6.86 (s, 2H, NH_2; exchangeable with D_2O), 6.92 (s, H, pyridine- H), 7.06 -7.55 (m, 9H, $H_{3,4,5}$ phenyl-H, Ar`-Hs, $H_{3,5}$ Ar-H), 7.90 (d, 2H, $H_{2.6}$ phenyl-H), 8.07 (d, 2H, $H_{2,6}$ Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 21.40, 69.94, 85.94, 113.73, 115.11, 115.32, 127.19,

128.17, 128.23, 128.39, 128.96, 129.69, 130.94, 136.65, 154.89, 156.99, 159.62, 162.79; MS, m/z: 391 (M^+, 14.41 %), 392 [$(M+1)^+$, 4.54%]; Anal. Calcd. For $C_{26}H_{21}N_3O$ (391.17) C, 79.77; H, 5.41; N, 10.73, Found: C, 80.02; H, 5.63; N, 10.94.

4.1.5.2. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(4-methoxyphenyl) nicotinonitrile (10b). Yield 51 %; m.p. 190 - 192 °C; IR (KBr, cm^{-1}): 3369, 3200 (NH_2), 3095 (CH aromatic), 2919 (CH aliphatic), 2208 (C≡N), 1607 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.80 (s, 3H, OCH_3), 5.15 – 5.20 (m, 2H, OCH_2), 7.03 (d, 2H, $H_{3,5}$ Ar`-H, *J* = 8.8 Hz), 7.12 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 Hz), 7.33 - 7.48 (m, 5H, phenyl-H), 7.70 (d, 2H, $H_{2,6}$ Ar`-H, *J* = 8.8 Hz), 8.06 (s, H, pyridine- H), 8.27 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 Hz), 8.89 (s, 2H, NH_2; exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.07, 69.94, 85.61, 113.55, 114.40, 127.09, 128.99, 129.12 130.68, 135.20, 154.62, 156.99, 157.85,161.22, 161.95; MS, m/z: 407 (M^+, 26.08 %), 408 [$(M+1)^+$, 8.07 %], 409 [$(M+2)^+$, 1.93 %]; Anal. Calcd. For $C_{26}H_{21}N_3O_2$ (407.16): C, 76.64; H, 5.19; N, 10.31, Found: C, 76.88; H, 5.34; N, 10.62.

4.1.5.3. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(2-hydroxyphenyl) nicotinonitrile (10c). Yield 70 %; m.p. 305 -307 °C; IR (KBr, cm^{-1}): 3421, 3290, 3220 (OH, NH_2), 3150 (CH aromatic), 2913 (CH aliphatic), 2201 (C≡N), 1628 (C=N), 1584 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.22 (s, 2H, OCH_2), 7.19 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 Hz), 7.33 – 7.50 (m, 7H, 5H, phenyl-Hs, 2H, $H_{3,5}$Ar`-H), 7.65 – 7.69 (m, 2H, $H_{4,6}$Ar`-H), 8.29 (s, H, pyridine- H), 8.38 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 Hz), 8.88, 11.60 (2s, 3H, NH_2, OH; exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ (ppm): 69.91, 85.09, 111.50, 113.49, 117.88, 121.42, 126.68, 128.34, 128.44, 128.96, 130.85, 133.52, 137.24, 155.98, 156.23, 157.59, 161.14; MS, m/z: 393 (M^+, 5.94 %), 395 [$(M+2)^+$, 4.10 %]; Anal. Calcd. For $C_{25}H_{19}N_3O_2$ (393.14): C, 76.32; H, 4.87; N, 10.68, Found: C, 76.47; H, 4.98; N, 10.89.

4.1.5.4. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(2-chlorophenyl) nicotinonitrile(10d). Yield 66 %; m.p. 115 -117 °C; IR (KBr, cm^{-1}): 3321,3210 (NH_2), 3062 (CH aromatic), 2921 (CH aliphatic), 2210 (C≡N), 1610 (C=N), 1596 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.12 – 5.22 (m, 2H, OCH_2), 6.99 (s, 2H, NH_2; exchangeable with D_2O), 7.08 (d, 2H, $H_{3,5}$ Ar-H, *J*= 8.8 Hz), 7.14 (s, H, pyridine- H), 7.30 -7.73 (m, 9H, 5H, phenyl-Hs, 4H, Ar`-Hs), 8.06 (d, 2H, $H_{2,6}$ Ar-H, *J*= 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ (ppm):70.04, 85.69, 111.55, 114.31, 115.34, 127.76, 128.16, 128.25, 128.40, 128.95, 130.14, 130.53, 130.58, 130.94, 131.90, 132.16, 136.98, 137.28, 156.23, 157.89, 161.54; MS, m/z: 411 (M^+, 10.18 %), 413 [$(M+2)^+$ 3.76 %]; Anal. Calcd. For $C_{25}H_{18}ClN_3O$ (411.11): C, 72.90; H, 4.40; N, 10.20; Found: C, 72.84; H, 4.63; N, 10.46.

4.1.5.5. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(2,4-dichlorophenyl) nicotinonitrile (10e). Yield 50%; m.p. 105 - 107 °C; IR (KBr, cm^{-1}): 3382, 3220 (NH_2), 3032 (CH aromatic), 2915 (CH aliphatic), 2199 (C≡N), 1623 (C=N), 1571 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.01 (s, 2H, NH_2; exchangeable with D_2O) 5.08 – 5.20 (m, 2H, OCH_2), 6.80 (s, H, pyridine- H), 7.06 (d, 2H, $H_{3,5}$ Ar-H, *J*= 8.8 Hz), 7.15 (d, 1H, H_5 Ar`-H), 7.18 - 7.56 (m, 5H, phenyl-Hs), 7.80 (d, 1H, $H_6$ Ar`-H), 7.89 (d, 2H, $H_{2,6}$ Ar-H, *J*= 8.8 Hz), 8.10 (s, 1H, H_3 Ar`-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 69.94, 85.52, 111.98, 113.55, 115.11, 127.16, 128.26, 128.39, 128.47, 128.76, 128.96, 130.94, 131.52, 133.75, 137.30, 156.55, 159.47, 160.83; MS, m/z: 445 (M^+, 12.78%), 447 [$(M+2)^+$, 5.44 %], 449 [$(M+4)^+$, 0.74 %]; Anal. Calcd. For $C_{25}H_{17}Cl_2N_3O$ (445.07): C, 67.28; H, 3.84; N, 9.41; Found: C, 67.50; H, 4.01; N, 9.67.

4.1.5.6. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(4 (dimethylamino)phenyl) nicotinonitrile (10f). Yield 47 %; m.p. 145 -147 °C; IR (KBr, cm^{-1}): 3325 , 3209 (NH_2), 3031 (CH aromatic), 2914 (CH aliphatic), 2204 (C≡N), 1619

(C=N), 1597 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.01 (s, 6H, CH_3), 5.09 (s, 2H, OCH_2), 6.71, (d, 2H, $H_{3,5}$ Ar`-H, *J* = 8.8 Hz), 6.81 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 Hz), 7.33 - 7.46 (m, 5H, phenyl-Hs), 7.72 (d, 2H, $H_{2,6}$ Ar`-H, *J* = 8.8 Hz), 7.78 (s, H, pyridine- H), 7.87 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 Hz), 8.29 (s, 2H, NH_2; exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ (ppm): 41.75, 69.85, 85.66, 112.25, 115.29, 115.42, 116.00, 116.75, 119.21 128.22, 128.41, 128.96, 129.39, 129.53, 130.52, 130.77, 134.06, 137.28, 154.79, 159.30; MS, m/z: 420 (M^+, 6.76 %), 421 [$(M+1)^+$, 3.32 %]; Anal. Calcd. For $C_{27}H_{24}N_4O$ (420.20): C, 77.12; H, 5.75; N, 13.32;, Found: C, 77.34; H, 5.89; N, 13.49.

4.1.5.7. 2-Amino-6-(4-(benzyloxy)phenyl)-4-(furan-2-yl)nicotinonitrile (10g). Yield 52 %; m.p. 182 -184 ^{o}C; IR (KBr, cm^{-1}): 3337 , 3201 (NH_2), 3050 (CH aromatic), 2926 (CH aliphatic), 2207 (C≡N), 1620 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.16 – 5.20 (m, 2H, OCH_2), 6.75 – 6.80, (m , H, H_4 Ar`-H), 6.90 (s, 2H, $NH_2$; exchangeable with $D_2O$), 6.97 (s, H, pyridine- H), 7.10 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 Hz), 7.25 -7.52 (m, 5H, phenyl-Hs), 7.65 (d, H, $H_5$ Ar`-H), 7.83 (d, H, H_3 Ar`-H), 8.06 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ (ppm): 69.92, 84.09, 104.10, 112.24, 113.38, 114.28, 127.99, 128.25, 128.93, 128.97, 129.12 130.47, 137.09, 141.59, 149.30, 158.88, 160.60, 161.60; MS, m/z: 367 (M^+, 100 %), 368 [$(M+1)^+$, 27.77 %], 369 [$(M+2)^+$, 4.25 %]; Anal. Calcd. For $C_{23}H_{17}N_3O_2$ (367.13): C, 75.19; H, 4.66; N, 11.44, Found: C, 75.45; H, 4.80; N, 11.62.

4.1.6. General procedure for the synthesis of 6-(4-(benzyloxy)phenyl)-4-(aryl)-2-oxo-1,2dihydropyridine-3-carbonitrile derivatives.(11a-d)

A mixture of **9b–d**, **9g** (0.01 mol), ammonium acetate (6.16 g, 0.08 mol) and ethylcyanoacetate (1.13g, 0.01 mol) was refluxed in ethanol (20ml) for 9-12 h. After completion of the reaction, it was further cooled and filtered the solid. The solid was purified by ethanol to give derivatives **11a-d**.

4.1.6.1. 6-(4-(Benzyloxy)phenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (11a). Yield 79 %; m.p. 239-241^{o}C; IR (KBr, cm^{-1}): 3240 (NH), 3023 (CH aromatic), 2906 (CH aliphatic), 2216 (C≡N), 1641 (C=O), 1598 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.82 (s, 3H, OCH_3), 5.19 (s, 2H, OCH_2), 6.57 (s, H, NH; exchangeable with D_2O), 6.73 (s, H, pyridone- H), 7.08- 7.15 (m, 4H, $H_{3,5}$ Ar`-H, $H_{3,5}$ Ar-H), 7.32 - 7.45 (m, 5H, phenyl-Hs), 7.69 (d, 2H, $H_{2,6}$ Ar`-H), 7.85 (d, 2H, $H_{2,6}$ Ar-H)); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.91, 69.92, 105.45, 114.65, 115.63, 117.56, 125.06, 127.22, 127.46, 128.68, 128.96, 129.89, 130.47, 137.08, 158.40, 161.18, 161.51, 162.78; MS, m/z: 408 (M^+, 7.18%), 409 [$(M+1)^+$, 2.01 %]; Anal. Calcd. For $C_{26}H_{20}N_2O_3$ (408.15): C, 76.45; H, 4.94; N, 6.86, Found: C, 76.80; H, 4.91; N, 7.12.

4.1.6.2. 6-(4-(Benzyloxy)phenyl)-4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (11b). Yield 85 %; m.p. 290 -292^{o}C; IR (KBr, cm^{-1}): 3375 – 3268 (br. OH, NH), 3032 (CH aromatic), 2929 (CH aliphatic), 2202 (C≡N), 1698 (C=O), 1601 (C=C) ; ^{1}H NMR (DMSO-d_6) δ (ppm): 5.20-5.22 (m, 2H, OCH_2), 6.81 (s, H, NH; exchangeable with D_2O), 7.14 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 HZ), 7.33 – 7.48 (m, 5H, phenyl-Hs), 7.62-7.70 (m, 2H, $H_{3,5}$ Ar`-H), 7.94 (s, H, Pyridone- H), 7.99 (d, H, $H_4$ Ar`-H), 8.26 (d, 2H, $H_{2.6}$ Ar-H, *J* = 8.8 HZ), 8.48 (d, H, H_6 Ar`-H),), 11.02 (s, H, OH; exchangeable with D_2O) ; ^{13}C NMR (DMSO-d_6) δ (ppm): 69.93, 105.02, 115.08, 115.25, 115.57, 116.17, 116.72, 117.48, 117.91, 121.22, 124.70, 126.52, 128.22, 128.96, 129.56, 130.01, 137.29, 158.93, 160.20, 166.75; **MS**, m/z: 394 (M^+, 3.40 %), 395 [$(M+1)^+$, 7.38 %]; Anal. Calcd. For $C_{25}H_{18}N_2O_3$ (394.13): C, 76.13; H, 4.60; N, 7.10, Found: C, 76. 34; H, 4.73; N, 7.36.

4.1.6.3.6-(4-(Benzyloxy)phenyl)-4-(2-chlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (11c). Yield 70 %; m.p. 260 -262 ^{o}C; IR (KBr, cm^{-1}): 3272 (NH), 3030 (CH aromatic), 2904 (CH aliphatic), 2214 (C≡N), 1650 (C=O), 1603 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.18 (s, 2H, OCH_2), 6.77 (s, H, NH; exchangeable with D_2O), 7.11 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 HZ), 7.30 – 7.55 (m, 9H, 5H, phenyl-Hs, 4H, Ar`-Hs), 7.65 (s, H, pyridone- H), 7.85 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 HZ); ^{13}C NMR (DMSO-d_6) δ (ppm): 70.05, 104.72, 114.85, 115.70, 123.33, 127.44 128.24, 128.47, 128.96, 129.93, 131.50, 135.73, 136.99, 158.33, 159.93, 160.87, 166.54; MS, m/z: 412 (M^+, 100 %), 414 [$(M+2)^+$, 35.38%]; Anal. Calcd. For $C_{25}H_{17}ClN_2O_2$ (412.10): C, 72.73; H, 4.15; N, 6.79, Found: C, 72.51; H, 4.38; N, 6.98.

4.1.6.4. 6-(4-(Benzyloxy)phenyl)-4-(furan-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (11d). Yield 85%; m.p. 214 -216 ^{o}C; IR (KBr, cm^{-1}): 3221 (NH), 3050 (CH aromatic), 2926 (CH aliphatic), 2205 (C≡N), 1720 (C=O), 1595 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.16 – 5.20 (m, 2H, OCH_2), 6.81 – 6.83, (m , H, H_4 Ar`-H), 7.14 (d, 2H, $H_{3,5}$ Ar-H, *J* = 8.8 HZ), 7.28 (d, H, $H_5$ Ar`-H), 7.37-7.52 (m, 5H, phenyl-Hs), 7.67 (d, H, H_3 Ar`-H), 7.83 (d, 2H, $H_{2,6}$ Ar-H, *J* = 8.8 HZ), 8.01 (s, H, NH; exchangeable with D_2O), 8.08 (s, H, pyridone- H); ^{13}C NMR (DMSO-d_6) δ (ppm): 69.93, 104.55, 110.57, 114.09, 115.68, 121.29, 123.99, 128.23, 128.47, 129.79, 136.10, 143.65, 149.87, 158.32, 159.42, 160.21, 169.95; MS, m/z: 368 (M^+, 100 %), 369 [$(M+1)^+$, 26.93 %], 370 [$(M+2)^+$, 4.44 %]; Anal. Calcd. For $C_{23}H_{16}N_2O_3$ (368.12): C, 74.99; H, 4.38; N, 7.60, Found: C, 75.16; H, 4.51; N, 7.79.

4.2. Biological evaluation

4.2.1. Methodology: cell culture

Cancer cells from different cancer cell lines; Human prostate carcinoma cell lines (PC-3), hepatocellular carcinoma cell lines (HEPG-2), and human breast adenocarcinoma cell line (MCF-7) were obtained from VACSERA- Cell Culture Unit, Cairo, Egypt. Also, the cytotoxic evaluation of all compounds against normal cells WI-38 was carried out to explore the toxicity and selectivity of the tested compounds. For comparison, 5-FU (5-Fluro uracil) was used as a standard reference drug.

Anti-proliferative activities of the synthesized compounds were carried out based on MTT assay [54]. MTT assay is a laboratory experiment, and a standard colorimetric assay (an assay measures changes in color) for measuring cellular growth, yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole] was reduced to purple formazan in the mitochondria of living cells. Briefly, human cancer cell lines were dropped in 96-well plates at a density of 3–8×10^3 cells/well. Next, the wells were incubated for 12 h in a 5% CO_2 incubator at 37 °C. Then, for each well, the growth medium was exchanged with 0.1 mL of fresh medium containing graded concentrations of the test compounds to be or equal DMSO and incubated for two days. Then 10 µl MTT solution (5 µg/ml) was added to each well, and the cells were incubated for an additional 4 h. The crystals of MTT-formazan were dissolved in 100 µl of DMSO; the absorbance of each well was measured at 490 nm using an automatic ELISA reader system (TECAN, CHE). The IC_{50} values were calculated using the nonlinear regression fitting models (Graph Pad, Prism Version 5). The means of at least three separate experiments gave the reported results. Statistical differences were analyzed according to a one-way ANOVA test wherein the differences were significant at $p < 0.05$.

4.2.2. Cell cycle analysis

PC-3 cells were seeded in a 6-well plate at a concentration of 1 X 10^5 cells per well, then incubated for 24 h. The cells were treated with (0.1% DMSO) vehicle or 2.04 μM of **10f** compound for 24 h. After that, cells were harvested and fixed for 12h using ice-cold 70% ethanol at 4 °C. Removal of ethanol and washing cells with cold PBS was done. Then incubated for 30 min at 37°C in 0.5 mL of PBS containing 1 mg/mL Rnase. The cells were stained for 30 min with propidium iodide in the dark. Then flow cytometer was used to detect DNA contents. [55].

4.2.3. Apoptosis detection studies

4.2.3.1. Annexin V-FITC assay.

PC-3 cells were seeded in a 6-well plate (1 X10^5 cell/well), incubated for 24 h, then treated with vehicle (0.1% DMSO) or 2.04 μM of **10f** compound for 24 h. The cells were then harvested, washed using PBS and stained for 15 min at room temperature in the dark using annexin V-FITC and PI in binding buffer (10mM HEPES, 140mM NaCl, and 2.5mM CaCl2 at pH 7.4), then analyzed by the flow cytometer [56].

2.2.3.2. Determination of the active caspase 3

The active caspase 3 level was measured by using Quantikine-Human active Caspase 3 Immunoassay (R&D Systems, Inc. Minneapolis, USA) according to the manufacturer protocol. Briefly, after washing the cells with PBS, the cells were collected and lysed by adding it to the extraction buffer containing protease inhibitors (1 mL per 1 x 107 cells.) then the lysate was diluted immediately before the assay. At the end of the assay, the optical density of each well was determined within 30 min using a microplate reader set at 450 nm.

4.2.3.3. Determination of the genes expression of some apoptosis key markers (BAX and BCL2)

Cells were obtained from American Type Culture Collection, cells were grown in RPMI 1640 containing 10% fetal bovine serum at 37°C, stimulated with the compounds to be tested for BAX or BCL2 and lysed with Cell Extraction Buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for human active BAX or BCL2 content. (cells are Plated in a density of 1.2 – 1.8 × 10,000 cells/well in a volume of 100μl complete growth medium + 100 ul of the tested compound per well in a 96-well plate for 24 hours before measured for human active BAX or BCL2).

4.2.4. Determination of PIM-1 kinase activity

The effects of the synthesized compounds on the activity of PIM-1 kinase were measured using Human proto-oncogene serine/threonine- protein kinase PIM-1 (PIM-1) ELISA Kit (catalog #MBS701210). The cells were centrifuged for 15 min at 1000x g, 2-8 °C and assayed immediately according to the manufacturer's instructions. Shortly, the assay was performed using 100 mL of the supernatant of the cells in each well, which were incubated for 2 h at 37°C before starting the assay procedure. Finally, the optical density of each well was determined within 5 min using a microplate reader set at 450 nm.

4.3. *In silico* physicochemical and ADME properties prediction

The molecular structures were converted into SMILES database using Chemdraw 12.0. Then, these SMILES were inserted as input in SwissADME website to calculate the physicochemical descriptors, lipophilicty, pharmacokinetics properties, ADME parameters, and medicinal chemistry friendliness.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Declared none.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1- Lopez, J.; Tait, S., W., G. Mitochondrial apoptosis: killing cancer using the enemy within. British Journal of Cancer,2015,85,1-6.
2- Adams, J., M.; Cory, S. The Bcl-2 protein family: arbiters of cell survival. Science, 1998, 281, 1322–26.
3- Wei, H.; John, J., K. Anticancer therapy targeting the apoptotic pathway. The Lancet Oncology, 2003, 4(12), 721–729.
4- Alex, R. D. D.; Stephanie, G.; Andreas, S.; David, L. V. Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies. Nature Reviews Cancer, 2016, 16(2), 99–109.
5- Susan, E. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol., 2007, 35(4), 495–516.
6- Howard, Y., C.; Xiaolu, Y. Proteases for Cell Suicide: Functions and Regulation of Caspases. Microbiology and molecular biology reviews, 2000, 64, 821–884.
7- Taylor, R., C.; Cullen, S., P.; Martin, S., J. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol, 2008, 9(3), 231–241.
8- Xiao-He, Y.; Todd, L. S.; Xuesong, Liu.; Bryn, R., B.; Christopher, J. F.; Ann, D., T. Reconstitution of Caspase 3 Sensitizes MCF-7 Breast Cancer Cells to Doxorubicin- and Etoposide-induced Apoptosis. Cancer Research, 2001, 61, 348-354.
9- Ahmed, M.; Mona, M.; Hassan, A.; Ola, R.; Omaima, S.; Mohamed, E.; Zaki, A., S.; Hayam, A. New 3-Cyano-2-Substituted Pyridines Induce Apoptosis in MCF 7 Breast Cancer Cells. Molecules, 2016, 21(230), 1-25
10- Michael, D., W.; Chaohong, S.,b.; Aaron, K.; Daryl, S.; Kathy, S.; Ethan, H.; Liping, Y.; David, G., N.; Jun, C.; Sha, J.; Kenneth, M., C.; Yihong, F.; Steven, N., A.; Binumol, I.; Edward, T., O.; Philip, J., H.; Saul, H., R.; Steven, W., E. Discovery of a novel small molecule binding site of human surviving. Bioorganic & Medicinal Chemistry Letters 2007, 17, 3122–3129.
11- Khaled, A., M., A.; Ghada, H., A.; Abeer, M., E. Eco-friendly synthesis of novel cyanopyridine derivatives and their anticancer and PIM-1 kinase inhibitory activities. European Journal of Medicinal Chemistry, 2017, 134 ,357-365.
12- Cheney, I., W.; Yan, S.; Appleby, T.; Walker, H.; Vo, T.; Yao, N.; Hamatake, R.; Hong, Z.; Wu, J. Z. Identification and structure-activity relationships of substituted pyridines as inhibitors of PIM-1 kinase. Bioorg. Med. Chem. Lett. 2007, 17, 1679-1683.
13- van der Lught, N., M.; Domen, j.; Verhoeven, E.; Linders, K.; van der Gulden, H.; Allen, J.; Berns, A. Proviral tagging in E mu-myc transgenic mice lacking the PIM-1 proto-oncogene leads to compensatory activation of PIM-2. EMBO J., 1995, 14, 2536-2544.
14- Feldman J., D.; Vician, L; Crispino, M.; Tocco, G.; Marcheselli, V., L.; Bazan, N., G.; Baudry, M.; Herschman, H., R. KID-1, a protein kinase induced by depolarization in brain. J Biol Chem., 1998, 273(26), 16535-43.
15- Wang, Z.; Bhattacharya, N.; Weaver, M.; Petersen, K.; Meyer, M.; Gapter, L.; Magnuson, N., S. PIM-1: a serine/threonine kinase with a role in cell survival, proliferation, differentiation and tumorigenesis. J Vet Sci., 2001, 2(3), 167-79.

16- Tursynbay, Y.; Zhang, J.; Li, Z.; Tokay, T.; Zhumadilov, Z.; Wu, D.; Xie, Y. PIM-1 kinase as cancer drug target: an update (Review). Biomed Rep, 2016, 4, 140–146.

17- Keane, N., A.; Reidy, M.; Natoni, A.; Raab, M., S.; O'Dwyer, M. Targeting the PIM kinases in multiple myeloma. Blood Cancer J, 2015, 5, e325.

18- Foulks, J., M.; Carpenter, K., J.; Luo, B.; Xu, Y.; Senina, A.; Nix, R.; Chan, A.; Clifford, A.; Wilkes, M.; Vollmer, D.; Brenning, B1.; Merx, S.; Lai, S.; McCullar, M., V.; Ho, K., K.; Albertson, D., J.; Call, L., T.; Bearss, J.,J.; Tripp, S., 5.; Liu, T.; Stephens, B., J.; Mollard, A.; Warner, S. L.; Bearss, D., J.; Kanner, S.;B. A small-molecule inhibitor of PIM kinases as a potential treatment for urothelial carcinomas. Neoplasia, 2014, 16, 403–412.

19- Decker, S.; Finter, J.; Forde, A., J.; Kissel, S.; Schwaller, J.; Mack, T.,S., Kuhn, A.; Gray, N.; Follo, M.; Jumaa, H.; Burger, M.; Zirlik, K.; Pfeifer, D.; Miduturu, C., V.; Eibel, H.; Veelken, H.; Dierks, C. PIM kinases are essential for chronic lymphocytic leukemia cell survival (PIM2/3) and CXCR4-mediated microenvironmental interactions (PIM1). Mol Cancer Ther, 2014, 13, 1231–1245.

20- - Lu, J.; Zavorotinskaya, T.; Dai, Y.; Niu, X., H.; Castillo, J.; Sim, J.; Yu, J.; Wang, Y.; Langowski, J., L.; Holash, J.; Shannon, K.; Garcia, P., D. PIM-2 is required for maintaining multiple myeloma cell growth through modulating TSC2 phosphorylation. Blood, 2013, 122, 1610–1620.

21- Drygin, D.; Haddach, M.; Pierre, F.; Ryckman, D., M. Potential use of selective and nonselective PIM kinase inhibitors for cancer therapy. J Med Chem, 2012, 55, 8199–8208.

22- Guo, S.; Mao, X.; Chen, J.; Huang, B.; Jin, C.; Xu, Z.; Qiu, S. Overexpression of PIM-1 in bladder cancer. J Exp Clin Cancer Res, 2010, 29, 161–167.

23- Brault, L.; Gasser, C.; Bracher, F.; Huber, K.; Knapp, S.; Schwaller, J. PIM serine/threonine kinases in the pathogenesis and therapy of hematologic malignancies and solid cancers. Haematologica, 2010, 95, 1004–1015.

24- Nancy, S., M.; Zeping, W.; Gang, D.; Raymond, R. Why target PIM1 for cancer diagnosis and treatment? Future Oncol., 2010, 6(9), 1461–1478.

25- Le, B., T.; Kumarasiri, M.; Adams, J., R., J.; Yu, M.; Milne, R.; Sykes, M., J.; Wang, S. Targeting PIM kinases for cancer treatment: opportunities and challenges. Future Med Chem, 2015, 7, 35–53.

26- Narlik-Grassow, M.; Blanco-Aparicio, C.; Carnero, A. The PIM family of serine/threonine kinases in cancer. Med Res Rev, 2014, 34, 136–159.

27- Nawijn, M., C.; Alendar, A.; Berns, A. For better or for worse: the role of PIM oncogenes in tumorigenesis. Nat Rev Cancer, 2011, 11, 23–34.

28- Cuypers, H., T.; Selten, G.; Quint, W.; Zijlstra, M.; Maandag, E., R.; Boelens, W.; van Wezenbeek, P.; Melief, C.; Berns, A. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. Cell, 1984, 37, 141–150.

29- Wang Z, Bhattacharya N, Weaver M et al.: PIM-1: a serine/threonine kinase with a role in cell survival, proliferation, differentiation and tumorigenesis. J. Vet. Sci. 2, 167–179 (2001).

30- Losman, J.; Chen, X., P.; Jiang, H.; Pan, P., Y.; Kashiwada, M.; Giallourakis, C.; Cowan, S.; Foltenyi, K.; Rothman, P.IL-4 signaling is regulated through the recruitment of phosphatases, kinases, and SOCS proteins to the receptor complex. Cold Spring Harb Symp Quant Biol, 1999, 64, 405-416.

31- Malte, B.; Tarik, M. Molecules in focus the serine/threonine kinase PIM-1. The International Journal of Biochemistry & Cell Biology, 2005, 37, 726–730.

32- Amaravadi, R.; Thompson, C., B. The survival kinases Akt and Pim as potential pharmacological targets. J. Clin. Invest, 2005, 115, 2618–2624.

33- Moroy, T.; Grzeschiczek, A.; Petzold, S.; Hartmann, K., U. Expression of a PIM-1 transgene accelerates lymphoproliferation and inhibits apoptosis in lpr/lpr mice. Proc. Natl Acad. Sci. USA 90, 1993, 10734–10738.

34- Fiskus, W., C.; Buckly, K., M.; Rao, R.; Yongchao, W.; Atul, J.; Daniel, G., C.; Anand, J.; Celalettin, U.; Peter, A.; Cornelia, Q.; Kapil, N., B. Synergistic activity of co-treatment with PIM-1 kinase inhibitor SGI-1776 and histone deacetylase inhibitor panobinostat or heat shock protein 90 inhibitor AUY922 against human CML and myeloproliferative neoplasm (MPN) cells. Presented at: 51st ASH Annual Meeting and Exposition. New Orleans, LA, USA, 5–8 December 2009.

35- Farag, M., A., A. Synthesis and Antimicrobial Evaluation of Some Novel Bis-α, β-Unsaturated Ketones, Nicotinonitrile, 1,2-Dihydropyridine-3-carbonitrile, Fused Thieno[2,3-b] pyridine and Pyrazolo[3,4-b]pyridine Derivatives. Int. J. Mol. Sci., 2013,14, 2967-2979.

36- Xorge, A., D.; Beatriz, G.; Erika, H.; Jorge, S. Studies on bis- (benzylisoquinolines). I. Synthesis of dl-N-(4-hydroxyphenylacetyl)-5-iodo-4-acetoxy-3-methoxyphenyl alanine methyl ester. J. Am. Chem. Soc., 1955, 77, 1288-1290.

37- Siham, A., L.; Faeza, H., A.; Nabilah, A., A. Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues. Chem. Cent. J., 2014, 8, 1-10.

38- Eman, M., F.; Hebat-Allah, S., A.; Randa, E., A.; Wafaa, A., Z. Synthesis and Cytotoxic Effect of Some Novel 1,2-Dihydropyridin-3 carbonitrile and Nicotinonitrile Derivatives. Molecules, 2016, 21, 1-15.

39- Yernar T.; Jinfu Z.; Zhi L.; Tursonjan T.; Zhaxybay Z.; Denglong W.; Yingqiu X. Pim-1 kinase as cancer drug target: An update (Review). Biomedical Reports, 2016, 4, 140-146.

40- Andree, H., A.; Reutelingsperger, C., P.; Hauptmann, R.; Hemker, H., C.; Hermens, W., T.; Willems, G. M. Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers, J. Biol. Chem., 1990, 265, 4923–4928.

41- Vermes, I.; Haanen, C.; Steffens-Nakken, H.; Reutelingsperger, C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V, J. Immunol. Methods,1995, 184, 39–51.

42- SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7, 42717. doi: 10.1038/srep42717.

43- Zhao, Y., H.; Abraham, M., H.; Le, J.; Hersey, A.; Luscombe, C., N.; Beck, G.; Cooper, I. Rate-limited steps of human oral absorption and QSAR studies Pharmacol. Res., 2002, 19, 2002, 1446-1457

44- Jonathan, B B, Georgina A H (2010) New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. J Med Chem 53: 2719–2740. doi: 10.1021/jm901137j.

45- William, M., C.; I., V.; Sherlock, T., W. Systematic organic chemistry, third ed, D. Van Nostrand Company, Inc, 1937, 99-100.

46- Magnus, R.; Teerawut, B.; Hannah, B.; Erli, S. Continuous-flow hydration–condensation reaction: Synthesis of α,β-unsaturated ketones from alkynes and aldehydes by using a heterogeneous, solid acid catalyst. Beilstein. J. Org. Chem., 2011, 7, 1680–1687.

47- Arumugam, T. Regiospecific synthesis and biological evaluation of spirooxindolopyrrolizidines via [3+2] cycloaddition of azomethine ylide. Eur. J. Med. Chem., 2010, 45, 6120- 6126.

48- Thanh-Dao, T.; Thi-Thao-Nhu, N.; Tuong-Ha, D.; Thi-Ngoc-Phuong, H.; Cat-Dong, T.; Khac-Minh, T. Synthesis and Antibacterial Activity of Some Heterocyclic Chalcone Analogues Alone and in Combination with Antibiotics. Molecules, 2012, 17, 6684-6696.

49- Sunniya, I.; Sardraz, K.; Aishah, B.; Safia, M.; Muhammad, A.; Abdel-Hamid, E.; Salim, S.; Xin, G.; Ghayoor, A.C.; Amir, F.; Rahman, S.Z.S. Synthesis and evaluation of modified chalcone based p53 stabilizing agents. Bioorg Med Chem Lett., 2017. 27, 4101-4106.

50- Nemai, C.G.; Sushmita, R.; Pallab, M. Cerium (III)-catalyzed regioselective coupling of 2-hydroxychalcones and polyphenols: an efficient domino approach towards synthesis of novel dibenzo-2,8dioxabicyclo [3.3.1] nonanes. RSC Adv., 2014, 4, 42078–42086.

51- Thekke, V.S.; Badiadka, N.; Hemmige, S.Y. Synthesis and characterization of some chalcones and their cyclohexenone derivatives. Cent. Eur. J. Chem., 2010, 8, 174–181.

52- Hetal, C.S.; Vaishali, H.S.; Nirmal, D.D. A novel strategy for the synthesis of 2-amino-4,6-diarylnicotinonitrile. ARKIVOC, 2009, (ii), 76-87.

53- Monica, M.; Olivier, d.; Jacobus, V.V.; Anikó Go¨blyo¨s, Jacobien, K. von Frijtag Drabbe Ku¨nzel, Thea, M.; Regina, L.; Henk, de V.; Margot, W.B.; Johannes, B.; Adriaan, P.IJ. 2-Amino-6-furan-2-yl-4-substituted Nicotinonitriles as A2A Adenosine Receptor Antagonists, J. Med. Chem., 2008, 51, 4449–4455.

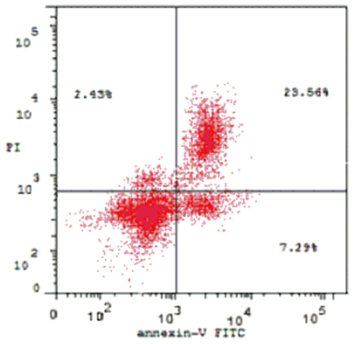
54- van Meerloo, J.; Kaspers; G., J., L.; Cloos, J. Cell Sensitivity Assays: The MTT Assay. In: Cree I. (eds) Cancer Cell Culture. Methods in Molecular Biology (Methods and Protocols). Humana Press, 2011, 731, 237-245.

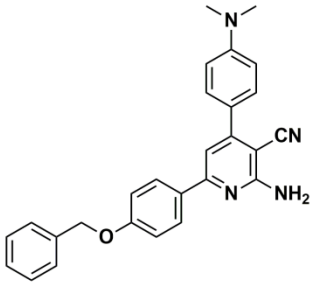
55- Wang, J.; Lenardo, M.J. Roles of caspases in apoptosis, development, and cytokine maturation revealed b,y homozygous gene deficiencies. J. Cell Sci., 2000, 113,753-757.

56- Lo, K., K.; Lee, T., K.; Lau, J., S.; Poon, W., L.; Cheng, S., H. Luminescent biological probes derived from ruthenium (II) estradiol polypyridine complexes, Inorg. Chem. 2008, 47, 200-208.

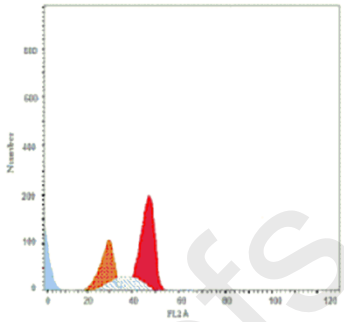
Graphical abstract

10f
Triggered apoptosis via the programed cell death pathway rather than necrotic pathway





10f
Arrest cell cycle at Pre-G1 and G2/M phase



10f
Boosted the level of the pro-apoptotic protein BAX and reduced the level of the antiapoptotic protein Bcl-2.

PIM-1 kinase inhibition IC_{50}= 0.47µM

Highlights

- Synthesis of Novel 14 Cyanopyridines derivatives.
- All the analogues were tested on three different cancer cell lines (PC3, HepG2, MCF-7).
- Assessment of their pro-apoptotic potential.
- Evaluation of PIM-1 kinase inhibitory activity.
- Cell cycle analysis of the most potent PIM-1 kinase inhibitor.
- *In silico* prediction of physicochemical and ADME properties.

Dear Respected Editor,

Conflict of interest for the manuscript entitled:

" Apoptosis: A Target for Anticancer Therapy with Novel Cyanopyridines"

The authors declare no conflict of interest.

Please address all correspondence concerning this manuscript to me and feel free to correspond with me by e-mail (amelfarrag@ymail.com).

Most Sincerely,

On behalf of the authors,

Amel M Farrag

Ass. Prof. of Pharmaceutical Chemistry.
Faculty of Pharmacy (Girls) Al-Azhar University.