Carboxamide Appended Quinoline Moieties as Potential Antiproliferative Agents, Apoptotic Inducers and Pim-1 Kinase Inhibitors

Yousry A. Ammar

Al-Azhar University

Gamil A.M. Elhagali

Helwan University

Moustafa S. Abusaif

Al-Azhar University

Mohamed R. Selim

Al-Azhar University

Medhat A. Zahran

Al-Azhar University

Tamer Naser

Helwan University

Ahmed B.M. Mehany

Al-Azhar University

Eman A. Fayed (✉ alfayed_e@yahoo.com)

Al-Azhar University <https://orcid.org/0000-0002-4579-5180>

Research Article

Keywords: Quinoline, Carboxamides, Anti-proliferative activity, Apoptosis, Pim-1 kinase inhibitors, ADME properties

Posted Date: May 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-477069/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Medicinal Chemistry Research on July 14th, 2021. See the published version at <https://doi.org/10.1007/s00044-021-02765-y>.

Carboxamide Appended Quinoline Moieties as Potential Antiproliferative Agents, Apoptotic Inducers and Pim-1 Kinase Inhibitors

Yousry A. Ammar¹, Gamil A.M. Elhagali², Moustafa S. Abusaif¹, Mohamed R. Selim¹, Medhat A. Zahran¹, Tamer Naser², Ahmed B.M. Mehany³ and Eman A. Fayed⁴

¹Chemistry Department, Faculty of Science, Al-Azhar University, Cairo11884, Egypt.

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University.

³Department of Zoology, Faculty of Science (Boys), Al-Azhar University, Cairo11884, Egypt.

⁴Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, 11754, Egypt.

Abstract

The targeted approach of protein kinases (PKs), as PKs are the main regulators of cell survival and proliferation, has been a promising strategy for treatments for cancer. Here we analyze the potential of quinoline-carboximide derivatives for four cell lines: MCF-7, CACO, HepG-2 and HCT-116 as anticancer agents. **3e**, **4b**, **11b** and **13d** derivatives showed good anti-proliferative activities in comparison to the reference standard Doxorubicin, against the four cell lines tested. They have been chosen for further studies. First of all, the IC₅₀ value surveys were carried out to ensure the protection of our hits and demonstrate that the cytotoxic effect (IC₅₀ > 113 μM) is highly selective on normal human cells (WI-38). Secondly, apoptosis was accomplished by down-regulation of Bcl-2 and up-regulation of BAX and Caspase-3 by these active compounds. Also, the Pim-1 inhibitory activity of the active hybrids was done, which indicate that compound **3e** was the most active with percentage of inhibition 82.27% and IC₅₀ equal 0.11 when compared to SGI-1776 as a reference standard. In addition, the *in silico* assessment of ADME properties, all of the strongest compounds are orally bioavailable without blood brain barrier penetration.

Key words: Quinoline, Carboxamides, Anti-proliferative activity, Apoptosis, Pim-1 kinase inhibitors, ADME properties

✉ Corresponding author: Eman A. Fayed (Ph.D) Tel.: +202 01221330523; E-mail: alfayed_e@azhar.edu.eg & alfayed_e@yahoo.com

✉ Other Corresponding authors; E-mail: yossry@yahoo.com & yossry@azhar.edu.eg (Yousry A. Ammar).

1. Introduction

Cancer remains the world's leading cause of death and consequently new and effective treatments are urgently needed. It differs in a number of biochemical processes, particularly when it comes to cell growth and split control from their normal counterparts.

As novel agents that selectively destroy or inhibit the proliferation of tumor cells without the general toxicity are hard to discover, conventional cancer chemotherapy is still very limited. The search for new, more active, more selective, less toxic compounds remains high in the field of chemotherapeutic drugs, and promising new approaches to anticancer are being tested [1-5].

Cell mortality or apoptosis is mediated through proteolytic enzymes known as caspases, which induce cell death. Apoptotic process activation is initiated by extracellular or intracellular death signals which trigger pro-caspase and caspase activation [6].

In the last decade, certain oncogenic mutations that interrupt apoptosis, causing initiation of tumor, progression or metastasis were thought to be causing cancer initiation. Apoptosis is a good framework for explaining the relationship between cancer genetics and cancer therapy. The clearing up of the genes and pathways that make up the main apoptosis machinery offers new insights into tumor biology and enhances the new therapeutic advantages of cancer therapy [7].

BAX and Bcl-2, are the important members of the Bcl-2 family because they play a powerful role in the development of tumor and prognoses of various human malignancies, have also been strongly attracted in the last couple of years. In response to various cell pressures, BAX enhances cell death. On the other hand, Bcl-2, by inhibiting BAX activity, abandons apoptosis [8].

PKs are key regulator of the pathways to signal transduction, mediating essential functions, including cell proliferation, differentiation and programmed death of cells [9]. Most PKs also play a leading role in carcinogenesis with different types of cancers. The aberrant activation of PKs causes anti-apoptotic symptoms, inducing angiogenesis and metastasis [10,11]. Thus, these oncogenic PK's inhibitors are seen as possible molecules of the anticancer.

Provirus Integration Site for Moloney leukemia virus (Pim-1 kinase) is a constitutively active member of the calcium/calmodulin-regulated serine/threonine kinase family. Pim-1 is constitutively expressed in a few tissues under physiological conditions at a low level [12,13]. Its level increases significantly as it is regulated by JAK-STAT and NF- κ B pathways in response to specific growth factors, mitogens and cytokines [12,14].

Pim-1 is highly expressed in a wide variety of human tumours of both hematopoietic and epithelial origin, including myeloid and lymphoid acute leukemia, large cell lymphoma (LCDL) diffuse, and neoplastic prostate cancer [15].

Various classes of heterocyclic and fused heterocyclic compounds have been identified in recent times in the search for anticancer agents through molecular biology, empirical screening and rational drug development [16,17]. In many alkaloids, for instance camptothecin, the quinoline nucleus occurs naturally.

Therefore, quinoline ring plays a major role in the production of anticancer drugs, as its derivatives have been shown to yield excellent results with various mechanisms of action such as growth inhibitors through cell cycle arrest, apoptosis, angiogenesis inhibition, cell migration interference, and nuclear receptor response modulation [18-22]. The amide is a common backbone of many organic molecules and natural products that are characterized by a wide range of chemical and pharmacological characteristics [22-24]. Amides thus play an important part in essential life processes, because the amide bond is, for example, the key to the production of proteins. In addition, amide bond is commonly used in the structure of 25% of the well-known drugs, according to the Comprehensive Medicinal Chemistry database [25, 26].

More essential for synthesizing polyhetero-aromatic molecules are amides that typically are stable, neutrals and both have the hydrogen-bonds receiver/donor properties. It has now been documented to be effective for cancer therapy [27] and for reduction of undesirable side effects [17] by developing a single molecule with more than one pharmacophore with different action modes.

Figure 1: Structures for Pim-1 inhibitors and the newly designed quinoline hybrids

Based on the above findings, and in continuation to our work on the synthesis of new compounds with biological activities [28-34], we have synthesized a new series of quinolone-3-carboxamide derivatives containing a series of biologically active moieties that are expected to be strongly inhibitory for several human cell lines and PIM-1 inhibitors as a result of our commitment to search for new potential anticancer agents related to heterocyclic [35-42].

2. Results and Discussion

2.1. Chemistry

Schemes 1-4 outline the synthetic pathways used to obtain compounds **1–13**. The starting materials 2-chloro or 2-keto 3-formylquinoline **1** were prepared according to the reported method [43]. The reactivity of **1** towards some active methylene compounds was discussed. Thus, condensation of **1** with some cyanoacetamide derivatives **2a-c** in ethanol containing piperidine as catalyst afforded the corresponding amide derivatives **3a-e**. The structure of **3** has been assigned as a reaction product on the basis of analytical and spectral data. The IR spectrum of **3c** as example displayed absorption bands at 3370 cm^{-1} due to NH function, at 2211 cm^{-1} due to conjugated $\text{C}\equiv\text{N}$ function, at 1694 cm^{-1} due to amidic $\text{C}=\text{O}$ function. The ^1H NMR spectrum ($\text{DMSO}-d_6$) exhibited one sharp singlet signal at δ 4.42 ppm assignable to CH_2 protons, another two multiplet signals at δ 6.31-6.42 ppm due to furan protons and at δ 7.27-8.33 ppm specific for 4 protons of quinoline, in addition to three signals at δ 8.73, 9.10 and 12.29 owing to methine, 4H-quinoline and NH proton. Moreover, its ^{13}C NMR ($\text{DMSO}-d_6$) revealed the most important signals displayed CH_2 at δ 36.91 ppm, two ethylene carbon at δ 107.85, 152.01 ppm, carbon nitrile and carbonyl at δ 115.86 and 160.80 ppm in addition to the aromatic carbons. Heating of compounds **3a,b** with trimethylamine in DMF caused cyclization via elimination of HCl molecule to afford the benzonaphthyridine derivatives **4a, b**. Fortunately, both elemental analysis and spectral data of the isolated product were in assignment with the possible structure.

The IR spectrum of **4a** as example showed the lack of absorption band corresponding to NH which confirm the cyclic structure and presence of bands at 2228 and 1664 cm^{-1} due to nitrile and carbonyl groups respectively. ^1H NMR spectrum exhibited singlet signal at δ 2.45 ppm due to CH_3 in addition to the aromatic protons. In attempt to obtain pyranoquinoline derivative **5** by heating **3d,e** in DMF containing trimethylamine but the attempt was failed which may be due to the presence of 2-hydroxyquinoline in keto form (So we should draw in the form of keton).

Scheme 1: Synthesis of 3-(quinolin-3-yl)acrylamide derivatives **3a-e** and benzo[*b*][1,8]naphthyridine derivatives **4a-b**

5-Acetyl-4-(2-chloroquinolin-3-yl)-6-methyl-2-oxo-1-(aryl)-1,2-dihydropyridine-3-carbonitriles **6** were obtained through the reaction of the aldehyde **1a** with cyanoacetamide and acetylacetone in presence of piperidine as catalyst. The analytical and spectral data are in agreement with the proposed structure. The IR spectrum of **6a** showed the presence of two C=O groups stretching at 1730, 1664 cm⁻¹ and CN group at 2228 cm⁻¹. ¹H NMR spectrum of the reaction product showed three singlet signals at δ 2.42, 2.74 and 2.89 ppm corresponding to three methyl groups. Also, its ¹³C-NMR proved the presence of methyl groups as three signals at δ 11.55, 21.38 ppm and 31.24. On the other hand, when 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one was used as active methylene to react with the aldehyde derivative **1** and cyanoacetanilide, the corresponding tetrahydropyrano[2,3-*c*]pyrazole (**7**) was obtained. The chemical structure of **7** was established on the basis of its elemental analysis and spectral data. The IR spectrum showed the presence of two C=O groups stretching at 1731, 1650 cm⁻¹. Its ¹H NMR spectrum displayed two doublet signals at 5.16 and 5.93 ppm corresponding to the two vicinal pyran protons H-3 and H-4, δ 5.16 (s, 1H, 3H-pyran), 5.93 (s, 1H, 4H-pyran), three singlets at 1.11, 2.23 and 10.20 ppm assignable to two CH₃ and NH groups. The ¹³C NMR spectrum was characterized by signals at δ 13.45, 23.75 ppm assigned to the two methyl carbons, and two signals at δ 56.86, 63.81 ppm due to the two CH

carbon signals at δ 117.33-151.47 ppm assigned to aromatic carbons, and signals at δ 165.83, 168.49 ppm assigned to the two carbonyl carbon atoms.

Scheme 2: Synthesis of 4-(2-chloroquinolin-3-yl)-2-oxopyridine derivatives **6a-b** and 4-(2-chloroquinolin-3-yl)-pyrano[2,3-*c*]pyrazole derivative **7**

N-(4-Aminophenyl)-2-cyanoacetamide (**8**) [44] has two nucleophilic centers which may be to react with the aldehyde function of **1**. Thus, interaction of **1** with compound **8** in acetic acid furnished the corresponding Schiff bases **9a,b**. The chemical structure of **9** was elucidated on the basis of elemental analysis and spectral data. The IR spectrum of **9b** as example indicated the presence of strong absorption bands at 3270, 3157 due to NH groups and two bands at 2212 and 1656 cm⁻¹. Its ¹H NMR spectrum showed a singlet signal at δ 3.90 ppm due to CH₂ protons in addition to the expected signals of aromatic and NH protons. The presence of active methylene in **9** was proved through reaction with salicylaldehyde or the aldehyde derivative to afford the corresponding chromene and the arylidene derivatives **10** and **11a,b** respectively. The IR spectrum of compound **10** as example, showed the complete disappearance of nitrile band which, already present in the parent compound and exhibited two absorption bands at 3340 and 3292 cm⁻¹ due to the NH groups besides carbonyl absorption band at 1679 cm⁻¹. Its ¹H NMR spectrum revealed two D₂O-exchangeable singlets at δ 10.38 and 10.78 ppm due to NH protons, in addition to two singlets at δ 9.07 and 9.26 ppm assignable for H-chromene and 4*H*-quinoline respectively.

Scheme 3: Synthesis of cyanoacetanilide, chromene and quinolin-2(1*H*)-one derivatives tagged with quinoline moieties.

Finally, condensation of the aldehyde derivatives **1** with the Schiff base derivatives **12a, b** through the active methylene to afford the corresponding arylidene derivatives **13a-d**. The IR spectrum of **13a** showed signals at 3339, 2212 and 1678 cm^{-1} corresponding to NH, CN and C=O groups respectively. Moreover, the ^1H NMR spectrum exhibited four singlet signals at 3.88, 8.29, 9.00 and 10.32 ppm due to methoxy, two methine and NH protons in addition to the aromatic protons.

Scheme 4: Synthesis of quinoline derivatives tagged with substituted benzylidene aminophenyl moieties.

2.2. Biological evaluation

2.2.1. *In vitro* cytotoxic activity

All synthesized quinoline derivatives were examined for their *in vitro* cytotoxic activities against four different cancer types, namely the breast cancer (MCF-7), heterogeneous human epithelial colorectal adenocarcinoma (CACO), hepatocellular carcinoma (HepG-2), human colon cancer cell line (HCT-116), in addition to the human amnion-derived (Wish) normal cell line, in comparison with Doxorubicin (DOX) as a reference anticancer drug. The cytotoxic activities expressed as IC₅₀ (μM/mL) value; the dose that reduces cell growth and survival to 50% of our tested compounds which were presented in Table 1.

Regarding, the activity of the quinoline derivatives against the breast cancer (MCF-7) cell line, our results showed that compounds **4b**, **9b**, **10**, **11b** and **13d** showed almost half the potency of DOX (IC₅₀: 5.28, 5.48, 4.57, 4.34 and 5.05 μM/mL vs 2.5 μM/mL; respectively). The antitumor activity of the examined quinolines against MCF-7 cell line had the following descending order: (**11b**>**10**>**13d**>**4b**>**9b**>**9a**>**11a**>**6b**>**4a**>**13b**>**13a**,**13c**>**3e**>**3d**>**3a**,**3b**>**3c**, **6a**>**7**).

It was observed that the highest anticancer activity against MCF-7 was obtained when the quinoline moiety was fused with pyridone derivative (compound **4b**) or tagged with cyanoacetanilide derivative, chromene, second quinoline or benzyldiene aminophenyl moieties (compounds **9b**, **10**, **11b**, **13d**). On the other hand, the weakest activity was observed for quinoline derivatives tagged with acrylamide derivatives, 2-oxo-1-(*p*-tolyl)pyridine moiety or pyrano[2,3-*c*]pyrazole moiety (compounds **3a-e**, **6a**, **7**; respectively).

Moreover, the activity of the quinoline derivatives against the human epithelial colorectal adenocarcinoma (CACO) cell line, the results showed that compound **13d** has the best result and it was almost equipotent to DOX against CACO cell line (IC₅₀: 3.87 μM vs 3.07 μM). Compounds **3e**, **9b**, **10** and **11b** was quite less potent than DOX (IC₅₀: 4.91, 4.12, 4.58, 4.33 μM vs 3.07 μM; respectively). While, compounds **4b**, **6b** showed half the potency of DOX against the same cell line (5.3, 5.62 μM vs 3.07 μM; respectively). The antitumor activity of the examined quinolines against CACO cell line had the following descending order: (**13d**>**9b**,**11b**>**10**,**13c**>**9a**,**3e**>**11a**,**4b**,**13b**>**6b**>**3c**,**3d**>**4a**>**6a**>**13a**,**3b**>**3a**>**7**).

These results revealed that the best activities were obtained when the 2-cyano-acrylamide moieties are directly attached to the quinoline rings (compounds **13d**, **3e** and **11b**). Similarly, good anticancer activity was observed when the quinoline ring is attached to cyanoacetamide moiety through a linker (compound **9b**), chromene ring through a spacer (compound **10**) or fused with 2-pyridone moiety (compound **4b**).

Regarding the activity of the quinoline derivatives against hepatocellular carcinoma (HepG-2) cell line, the results showed that compound **13d** was the most potent and it was more potent than DOX against HepG-2 cell line (IC_{50} : 3.50 μ M vs 4.6 μ M). Compounds **10**, **11a-b** and **13c** were more potent than DOX (IC_{50} : 3.91, 4.04, 3.5 and 3.95 μ M vs 4.6 μ M). Compound **9b** was equipotent to DOX against HepG-2 against DOX (4.56 μ M vs 4.6 μ g) while compound **13b** was slightly less potent than DOX (4.93 μ M vs 4.6 μ M). Compounds **3e**, **4b** and **6b** displayed promising activity against HepG-2 cell line (IC_{50} : 7.01, 6.06 and 6.53 μ M; respectively). The antitumor activity of the examined quinolines against HepG-2 cell line had the following descending order: (**13d**>**10**,**13c**>**11b**> **11a**,**9b**>**13b**>**9a**>**4b**>**4a**,**6b**>**3e**>**13a**>**7**>**3d**>**6a**>**3c**>**3b** >**3a**).

These results revealed that the best activities were obtained when the 2-cyano-acrylamide moieties are directly attached to the quinoline rings (compounds **13d**, **11a**, **11b** and **13c**). Similarly, excellent anticancer activity was observed when the quinoline ring is attached to chromene moiety through a linker (compound **10**) or cyanoacetamide moiety through a spacer (compound **9b**).

Also, the activity of the quinoline derivatives against human colon cancer (**HCT-116**) cell line, the results showed that compound **3e** was the most potent (IC_{50} : 3.41 μ M vs DOX 4.48 μ M). Moreover, compounds **13d**, **13c** were also more potent than DOX against HCT-116 cell line (IC_{50} : 3.92, 4.01 μ M vs 4.48 μ M). Also compound **11b** was almost equipotent to DOX against HCT-116 cell line (IC_{50} : 4.82 μ M vs 4.48 μ M). While, compounds **13b**, **4b**, **10**, **9a** and **11a** were quite less potent than DOX against HCT-116 cell line (IC_{50} : 5.06, 5.16, 5.23, 5.33 and 5.72 μ M vs 4.48 μ M). Compounds **13a**, **9b**, **4a**, **7**, **6b** had almost more than half the potency of DOX against HCT-116 cell line (IC_{50} : 6.55, 6.94, 7.07, 7.72, 8.43 μ M vs 4.48 μ M; respectively). The antitumor activity of the examined quinolones against HCT-116 cell line had the following descending order: (**3e**>**13d**>**13c**>**11b**> **13b**>**4b**>**10**>**9a**>**11a**>**13a**>**9b**>**4a**>**7**>**6b**>**3d**>**3c**>**3b**>**6a**>**3a**).

These results revealed that the best activities were obtained when the 2-cyano-acrylamide moieties are directly attached to the quinoline rings (compounds **3e**, **13d**, **13c**, **13b**, **11b** and **11a**). Similarly, excellent anticancer activity was observed when the quinoline ring is attached to chromene moiety through a linker (compound **10**), cyanoacetamide moiety through a spacer (compound **9a**) or fused with 2-pyridone moiety (compound **4b**).

From the above findings, it was also observed that the weakest anticancer activities against the four tested human cell lines were obtained for quinoline derivatives tagged with acrylamide derivatives (compounds **3a-e**), 2-oxo-1-(substituted)pyridine moieties (compounds **6a-b**) or pyrano[2,3-*c*]pyrazole moiety (compound **7**).

Table 1: *In vitro*^a antitumor activity of the target compounds against MCF-7, CACO, HepG-2, HCT-116 and Wish normal cell line

Cpd.	IC ₅₀ μ M/mL/SI ^b						
No.	R	X	MCF-7	CACO	HepG-2	HCT-116	WI-38
3a		-Cl	12.69 $\pm$ 0.96 (8.99)	10.84 $\pm$ 0.88 (10.53)	22.00 $\pm$ 1.21 (5.19)	16.38 $\pm$ 1.09 (6.97)	114.12
3b		-Cl	12.69 $\pm$ 0.96 (14.93)	8.56 $\pm$ 0.74 (22.13)	14.73 $\pm$ 1.09 (12.86)	12.24 $\pm$ 0.94 (15.47)	189.41
3c		-Cl	16.54 $\pm$ 1.11 (11.44)	5.82 $\pm$ 0.48 (32.50)	11.35 $\pm$ 0.92 (16.67)	11.42 $\pm$ 0.92 (16.56)	189.15
3d		-OH	11.36 $\pm$ 0.93 (16.65)	5.94 $\pm$ 0.49 (31.84)	8.67 $\pm$ 0.77 (21.81)	10.57 $\pm$ 0.85 (17.89)	189.10
3e		-OH	10.93 $\pm$ 0.88 (11.64)	4.91 $\pm$ 0.38 (25.90)	7.01 $\pm$ 0.60 (18.14)	3.41 $\pm$ 0.24 (37.30)	127.18
4a			6.38 $\pm$ 0.53 (27.45)	6.18 $\pm$ 0.51 (28.34)	6.46 $\pm$ 0.54 (27.11)	7.07 $\pm$ 0.61 (24.77)	175.12

4b			5.28 ± 0.47 (21.43)	5.30 ± 0.48 (21.35)	6.06 ± 0.50 (18.67)	5.16 ± 0.40 (21.93)	113.17
6a		-Cl	16.56 ± 1.31 (10.02)	7.87 ± 0.68 (21.09)	9.24 ± 0.83 (17.97)	13.10 ± 0.98 (12.65)	166.00
6b		-Cl	6.19 ± 0.51 (18.28)	5.62 ± 0.45 (20.14)	6.53 ± 0.53 (17.33)	8.43 ± 0.24 (13.42)	113.16
7		-Cl	22.05 ± 1.80 (7.68)	11.94 ± 0.93 (14.19)	7.77 ± 0.66 (21.80)	7.72 ± 0.67 (21.94)	169.38
9a		-Cl	5.70 ± 0.49 (25.65)	4.78 ± 0.36 (30.58)	5.36 ± 0.32 (27.27)	5.33 ± 0.46 (27.43)	146.19
9b		=O	5.48 ± 0.42 (22.33)	4.12 ± 0.30 (29.70)	4.56 ± 0.36 (26.60)	6.94 ± 0.59 (17.63)	122.36
10		-Cl	4.57 ± 0.35 (29.78)	4.58 ± 0.34 (29.72)	3.91 ± 0.28 (34.82)	5.23 ± 0.41 (26.03)	136.13

11a		-Cl	5.90± 0.50 (21.80)	5.21± 0.40 (24.69)	4.47± 0.33 (28.78)	5.72± 0.48 (22.49)	128.63
11b		=O	4.34± 0.34 (17.78)	4.33± 0.34 (17.83)	4.04± 0.30 (19.11)	4.82± 0.37 (16.01)	177.19
13a		-Cl	7.38± 0.63 (16.53)	8.40± 0.73 (14.52)	7.41± 0.64 (16.46)	6.55± 0.55 (18.62)	121.99
13b		-Cl	6.73± 0.57 (17.39)	5.36± 0.41 (21.84)	4.93± 0.38 (23.74)	5.06± 0.40 (23.13)	117.06
13c		=O	7.37± 0.63 (25.77)	4.67± 0.35 (40.67)	3.95± 0.28 (48.08)	4.01± 0.29 (47.36)	189.91
13d		=O	5.05± 0.40 (39.37)	3.87± 0.28 (51.37)	3.50± 0.25 (56.80)	3.92± 0.28 (50.72)	198.81
DOX			2.50 ± 0.02	3.07 ± 0.03	4.60 ± 0.04	4.48 ± 0.04	ND

^a Data represent the mean values of three independent determinations.

^bSI: Selectivity index = (IC₅₀ of WI-38)/(IC₅₀ of cancer cell line).

ND: Not determined.

The cytotoxic effect of the target compounds on Wish normal cell line was evaluated and the results were summarized in table 1. It is clear that most of the target compounds have a selective cytotoxic effect towards cancer cells. The selectivity index was calculated by dividing the IC₅₀ value of each target compound on Wish normal cell line by the IC₅₀ value on cancer cell line (Table 1). Out of the tested nineteen compounds, selectivity index values, only three showed values ranging from 5.19-10.02 (non-selective compounds), while the rest values were greater than 10 (cancer-selective compounds). Compound **13d** was the most selective on MCF-7, COCA, HepG-2 and HCT-116 cell lines with selectivity index value of 39.37, 51.37, 56.80 and 50.72; respectively, followed by compound **10** which was the most selective on MCF-7 and HepG-2 cell lines with SI 29.78 and 34.82 respectively. While compound **13c** was the most selective on CACO cell line with selectivity index equals 40.67. Regarding HCT-116 cell line, compound **13c** displayed the highest selectivity index value of 47.36.

2.2.2. Structure activity relationship (SAR)

The sensitivity of the screened cancer cells to the target compounds were in the following descending order: HCT-116 > HepG-2 > CACO > MCF-7. The anticancer screening of the target compounds showed the importance of both 2-cyanoacrylamide and 2-cyanoacetamide moieties in obtaining good activities. Compounds **13d**, **11b**, **4b**, **3e** were the most promising antiproliferative agents because of their high activity against most of the screened cancer cell lines.

Regarding the activity of the quinoline derivatives against human colon cancer (HCT-116) cell line:

2-Cyano-oxoquinoline-*N*-phenylacrylamide (COQPA) derivatives **13d** and **13c** showed greater activities than their corresponding 2-cyano-chloroquinoline-*N*-phenylacrylamide (CCQPA) analogues **13b**, **13a**; respectively (Figure 2). Moreover, the COQPA derivatives **11a-b** were more potent than CCQPA derivatives. Thus both the NH and the CO moieties of the quinoline ring of COQPA might be involved in hydrogen bond formation with their corresponding target proteins. The smaller the size of the substitution on the para position of the phenyl group of COQPA derivatives the greater the activity (**3e** > **13d** > **13c** > **11b** > **11a**) (Figure 2). 2-Chloroquinolin-3-yl-methyleneaminophenyl (CQMAP) derivatives displayed weaker activity than the 1,2-dihydrobenzo[*b*][1,8]naphthyridine **4b** and CCQPA derivatives. Consequently, **4b** has weaker activity than COQPA. Transforming CQMAP derivatives tagged with cyanoacetamide moiety **11a** to CQMAP derivatives tagged with chromene carboxamide **10** or acrylamide derivative **9a** increased the anticancer activity (Figure 2).

Figure 2: The activity of the quinoline derivatives against human colon cancer (HCT-116) cell line

Regarding the activity of the quinoline derivatives against hepatocellular carcinoma (HepG-2) cell line:

The COQPA derivatives **13c** and **13d** were more potent than their corresponding CCQPA derivatives **13a** and **13b**; respectively (Figure 3). Moreover, the COQPA derivatives **11a** and **11b** were more potent than CCQPA. Lipophilic substitutions on the *para* position of the phenyl moiety resulted in better anticancer activity than hydrophilic substitutions for example **13d** > **13c** and **11a** > **11b** (Figure 3). 2-Oxoquinolin-3-yl-methyleneaminophenyl (OQMAP) derivatives **9b**, **11b** have greater activities than their corresponding 2-chloroquinolin-3-yl-methyleneaminophenyl (CQMAP) derivatives **9a**, **11a**; respectively (Figure 3). Transforming CQMAP and OQMAP tagged with cyanoacetamide moiety **9a**, **9b** to their corresponding CQMAP and OQMAP tagged with acrylamide derivatives **11a** and **11b**; respectively and CQMAP tagged with chromene-3-carboxamide moiety **10** increased the anticancer activity. It was observed that all COQPA derivatives **11a-b** and **13c-d** have better anti-proliferative activities than OQMAP tagged with cyanoacetamide moiety **9b**. Moreover, combining both

chloroquinoline and chromene moieties in one molecule through methyleneaminophenylaminocarbonyl spacer resulted in excellent anticancer activity (compound **10**) (Figure 3).

Figure 3: The activity of the quinoline derivatives against human colon cancer (HepG-2) cell line

Regarding the activity of the quinoline derivatives against CACO cell line:

COQPA derivatives **13d**, **13c** showed greater activities than their corresponding CCQPA derivatives **13b** and, **13a** analogues; respectively. OQMAP derivatives **9b** and **11b** were more potent than their corresponding CQMAP derivatives **9a** and **11a**, respectively. CQMAP tagged with chromene-3-carboxamide moiety, compound **10**, showed promising antiproliferative activity. Transforming the CQMAP and OQMAP tagged with cyanoacetamide derivatives **9a-b** to their corresponding CQMAP and OQMAP derivatives **11a-b**; respectively do not improve their antiproliferative activity (Figure 3).

Figure 3: The activity of the quinoline derivatives against human colon cancer (COCA) cell line

Regarding the activity of the quinoline derivatives against MCF-7 cell line:

The most active compounds were OQMAP tagged with oxoquinoline-3-yl-acrylamide moiety **11b** and CQMAP tagged with chromene-3-carbocamide moiety **10**.

2.2.3. Apoptosis detection studies

2.2.3.1. Effect on the active Caspase-3 level

Stimulation of apoptosis is believed to be the main mechanism of action for most of the known anticancer agents [45]. Elevated level of Caspases is an indication of apoptosis. The effect of the targets quinoline derivatives **3e**, **4b**, **11b** and **13d** on the level of Caspase-3 was evaluated and the obtained results are summarized in table 4. It is clear that the level of Caspase-3 in cancer cells treated with the

target quinoline derivatives was 5 to 6 times more than the level of Caspase-3 in cancer cells, which are not treated with the target quinoline derivatives (Table 2) (Figure 4). This demonstrates that the anti-proliferative activity of the quinoline target compounds is through the stimulation of the apoptotic pathway.

Figure 4: A diagram illustrated the effect of the quinoline derivatives **3e**, **4b**, **11b** and **13d** on the level of Caspase-3 in the examined cancer cells

2.2.3.2. Effect on mitochondrial apoptosis pathway proteins BAX and Bcl-2

It was reported that the over expression of BAX (pro-apoptotic) stimulates apoptosis in cancer cells [46]. The effect of the target quinoline derivatives **3e**, **4**, **b**, **11b** and **13d** on the level of BAX in the examined cancer cells was evaluated and the results are summarized in table 2. The obtained results showed that the target compounds increased the level of BAX in the treated cancer cells from 10 to 13 folds more than the level of BAX in cancer cells which are not treated with the target quinoline compounds. It is possible that Bcl-2 (anti-apoptotic) levels in the mitochondrial membrane must decrease if BAX levels in the mitochondria are to rise. The most active analogs **3e**, **4**, **11b**, and **13d**, were evaluated for their effect on some apoptosis key markers, BAX and Bcl-2 (Table 2).

Table 2: Effect of quinoline derivatives, **3e**, **4**, **11b**, and **13d**, on the gene's expression of some apoptosis key markers

Cpds.	Casp-3		BAX		Bcl-2	
No.	(pg/mL)	FLD	(Pg/mL)	FLD	(Pg/mL)	FLD
3e	357.5	6.40	219.7	12.62	1.241	0.22
4b	321.7	6.20	201.3	10.98	1.631	0.33
11b	281.6	5.22	195.1	10.77	2.214	0.43
13d	276.4	5.04	181.7	9.88	2.411	0.48
Cont. (HepG-2)	56.41	1	18.14	1	5.418	1

2.2.4. Pim-1 -Kinase detection

(Pim-1) is a serine / threonine kinase which regulates multiple cellular functions, such as cell cycle, cell survival, and drug resistance. Aberrant Pim 1 kinase elevation is related to various cancer forms. Two distinct Pim 1 (Pim-1S and Pim-1L) isoforms exhibit distinct cellular functions. Pim-1S is primarily located at the nucleus, and Pim-1L is located at the drug resistance plasma membrane. Latest studies have shown that mitochondrial Pim-1 retains an integrity with ochondrial. Pim-1 has emerged as a target for cancer drugs, particularly in prostate cancer. The active new roles of Pim-1 have recently been identified in immunotherapy, senescence bypass, metastasis and epigenetic dynamics.

Pim-1's inhibition of apoptosis is by phosphorylation in target molecules such as BAD, ASK1 or PRAS 40 that inhibit or activate anti-apoptotic (Bcl-2) and pro-survival (mTOR) functions, for instance, the JNK / p38-Caspa-3 axis. In this way it is possible to minimize apoptosis.

This article aims to update Pim kinase inhibitors with a key role in developing anticancer compounds against Pim kinase targets.

To investigate their function in Pim-1 kinase inhibition with a variety of ten concentrations, compounds **3e**, **4b**, **11b**, and **13d** were chosen, and values for IC₅₀ were measured. The most potent Pim-1 inhibitors

were compounds, **3e**, **4b** and **11b** with IC₅₀ of 0.11-0.92 μ M, and **13d** with less effective activity (IC₅₀ = 3.24 μ M). The positive control with the test SGI-1776 was used for IC₅₀ 0.048 μ M. The profiles showed that Pim-1 is a potential target for those compounds in combination with cell-wide inhibition data for the **3a**, **4b**, **11b** and **13d** compounds described in Table 3.

Table 3: Effect of compounds 3e, 4b, 11b and 13d on Pim-1 Kinase

Compounds	Pim-1 Kinase	
	%inh. (Conc.10 μ g)	IC ₅₀
3e	82.27	0.11
4b	72.52	0.92
11b	75.68	0.81
13d	61.52	3.24
SGI-1776	---	0.048

2.2.5. *In-silico* computational analysis studies

The typical reference medication, Doxorubicin, was also subjected to *in-silico* physicochemical, pharmacokinetic/ADME, and drug likeliness attributes along with all the synthesized compounds. The various physicochemical parameters such as molar refractivity, atomic class count and the number of rotated connections have been reported, lipophilicity and water solubility. A suitable physiochemical vector for the regulation of drug transportation characteristics is the TPSA (Topological Polar Surface Area). It was tried to forecast the absorption rate of all test molecules by the reporting equation (ABS = 109-(0.345 X TPSA) [47].

These physicochemical properties are shown in Table 4. The absorption rate in the chosen compounds varies from 63.82% to 81.78%. Some compounds in water with a good molar refractiveness are moderately soluble and have been shown to be moderately to goodly absorbent with a median of 81.78% and 75.15% of reference and synthesized drugs respectively.

Table 4: Physicochemical properties of the most active compounds 3e, 4b, 11b and 13d

Cpd. No.	Fraction Csp ³ ^a	No. of rotatable bonds	HBA ^b	HBD ^c	iLogP ^d	Molar Refractivity	Log S ^e	TPSA ^f	In-silico % absorption
3e	0.00	4	5	3	2.01	94.27	MS	106.24	72.35
4b	0.00	1	4	1	2.16	91.58	PS	78.91	81.78
11b	0.00	6	5	3	3.27	144.87	PS	130.97	63.82
13d	0.00	6	4	2	3.23	131.76	PS	98.11	75.15
DOX.	0.44	5	12	12	2.50	132.66	S	206.07	37.91

^aThe ratio of sp³ hybridized carbons over the total carbon count of the molecule; ^bnumber of hydrogen bond acceptors; ^cnumber of hydrogen bond donors; ^d lipophilicity; ^e Water solubility (SILICOS-IT [PS= Poorly soluble, MS= Moderately Soluble, S=Soluble]); ^ftopological polar surface area (Å²).

The pharmacokinetic / ADME attributes of the derivatives investigated are given in table 5. All the test compounds with the exception of molecule **11b** have shown that they are easily absorbed by gastrointestinally (GI) and are non-Inhibitors P-gp (p-glycoprotein). Except **4b**, all of the compounds tested were unable to cross the blood-brain barrier (BBB). In addition, Doxorubicin does not inhibit any of the Cytochrome P450 isomers being tested. Many of the compounds being tested inhibit one of the other isomers examined with Cytochrome P450. The coefficient values of the assessed compounds for skin permeability (log Kp; with Kp in cm/s) emerged to be small. (Table 5)

Table 5: Pharmacokinetic/ADME properties of the most active compounds 3e, 4b, 11b and 13d

Cpd. No.	Pharmacokinetic/ADME properties								
	GI abs ^a	BBB permeant ^b	P-gp substrate ^c	CYP1A2 inhibitor ^d	CYP2C19 inhibitor ^e	CYP2C9 inhibitor ^f	CYP2D6 inhibitor ^g	CYP3A4 inhibitor ^h	Log K _p ⁱ
3e	High	No	No	Yes	No	Yes	Yes	No	-5.80
4b	High	Yes	No	Yes	No	Yes	Yes	No	-5.90
11b	Low	No	No	No	Yes	Yes	No	Yes	-6.41
13d	High	No	No	No	Yes	Yes	No	Yes	-5.51
DOX.	Low	No	Yes	No	No	No	No	No	-8.71

^aGastro Intestinal absorption, ^bBlood Brain Barrier permeant, ^cP-glycoprotein substrate, ^dCYP1A2: Cytochrome P450 family 1 subfamily A member 2 (PDB:2HI4), ^eCYP2C19: Cytochrome P450 family 2 subfamily C member 19 (PDB:4GQS), ^fCYP2C9: Cytochrome P450 family 2 subfamily C member 9 (PDB:1OG2), ^gCYP2D6: Cytochrome P450 family 2 subfamily D member 6 (PDB:5TFT), ^hCYP3A4: Cytochrome P450 family 3 subfamily A member 4 (PDB:4K9T), ⁱSkin permeation in cm/s.

The assorted laws regulating drug likeness, i.e. Lipinski [48], Ghose [49], Veber [50], Egan [51], and Muegge [52] had to analyze the molecule as an important candidate for drugs. The number of violations of the rules previously called along with their bioavailability scores are listed in (Table 6). The Lipinski (Pfizer) strain is the rule-of-five (RO5) vogue setter, and according to this law, all the compounds tested are identical to drugs. In terms of the Ghose and Muegge law, the forecasts flaunted that all compounds follow the rule and are drug candidates. Both derivatives displayed drug-like characteristics except derivatives **13d** with 1 violation during the assessment process using the Veber and Egan rules. The bioavailability value of 0.55 was shown in all the molecules examined. The radar of biodisponibility of the compounds tested is shown in (Figure 5).

Table 6: Drug likeness predictions and bioavailability scores of the most active compounds 3e, 4b, 11b and 13d

Cpd.	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability
No.	(violations)	(violations)	(violations)	(violations)	(violations)	Score
3e	Yes (0)	Yes	Yes	Yes	Yes	0.55
4b	Yes (0)	Yes	Yes	Yes	Yes	0.55
11b	Yes (0)	No (2)	Yes	Yes	Yes	0.55
13d	Yes (0)	No (1)	Yes	Yes	No (1)	0.55
DOX.	No (3)	No (2)	No (1)	No (1)	No (3)	0.17

Figure 5: Bioavailability Radar of the selected compounds

The pink surface represents the optimum range for each property (lipophilicity: XLOGP3 between -0.7 and $+5.0$, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 ($\text{\AA}^2$), solubility: log S not greater than 6, saturation: fraction of carbons in the sp³ hybridization not less than 0.25, and flexibility: no more than nine rotatable bonds). In this case, the compound is predicted not orally bioavailable, because too flexible and too polar.

3. Conclusion

In this work, we designed and synthesized new heterocyclic derivatives in constructing innovative, efficacious anti-proliferative agents. The new quinolone-3-carboxamide derivatives have been obtained starting from 2-chloro or 2-keto 3-formylquinoline **1**. The anticancer activity for all new compounds against four different cancer types, MCF-7, CACO, HepG-2 and HCT-116, has been evaluated. Four of the synthesized hybrids, **3e**, **4b**, **11b** and **3d** represented superior anticancer activity in comparison to Doxorubicin as the reference drug. They proved healthy on a representative of normal human cells (WI-38 cell line) with indices of selectivity (5.19-56.80). Furthermore, studies of apoptosis were checked by these active compounds revealing that apoptosis was achieved by down-regulation of Bcl-2 and up-regulation of caspase-3. Additionally, Pim-1 kinase was done to show their mechanism of action for the most promising structures. Finally, the synthesized compounds were tested using the *in-silico* ADME predictions for their physicochemical drug likeliness, pharmacodynamic, and pharmacokinetic properties, respectively. The analyzed compounds are prospective drug-like candidates according to the outcomes presented. In the future work, we can study the *in vivo* physicochemical drug likeliness, pharmacodynamic, and pharmacokinetic properties.

4. Experimental

4.1. Chemistry

Melting points were measured with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrophotometer. ¹H NMR and ¹³C NMR spectra were measured on a Bruker AC 500 (500 MHz) in DMSO-*d*₆ as solvent, using TMS as an internal standard, and chemical shifts are expressed as ppm. Mass spectra were determined on Finnigan Incos 500 (70 eV). Elemental analyses were carried out at the Microanalytical Center of Cairo University. Anticancer activity was carried out in local strain identified in Regional Center for genetic engineering, faculty of Science (Boys), Al-Azhar University and the results were within $\pm 0.4\%$ of calculated value.

3-(2-Substitutedquinolin-3-yl)-2-cyano-N-(aryl)acrylamide (3a-e)

A mixture of 2-substituted-quinoline-3-carbaldehyde **1** (0.01mol) and aryl cyano acetanilides **2a-c** (0.01mol) in ethanol (20 mL), pyridine was added and the mixture was heated under reflux

for 2 h. The solution was allowed to cool down to room temperature and the solid formed was filtered and dried, and recrystallized from ethanol.

3-(2-Chloroquinolin-3-yl)-2-cyano-N-(4-tolyl)acrylamide (3a)

Yellow crystals; Yield (75 %); mp. 230-232 °C; IR (KBr, cm⁻¹): 3325 (NH), 3037 (CH-arom.), 2916 (CH-aliph), 2226 (C≡N), 1680 (C=O), 1600 (C=N); ¹H NMR (DMSO-*d*₆) δ 2.30 (s, 3H, CH₃), 7.21, 7.60 (2d, 4H, *J*=5.5 Hz, 4-tolyl), 7.79, 7.99 (2t, 2H, *J*=5Hz, 6H,7H-quinoline), 8.06, 8.22 (2d, 2H, *J*=5.5Hz, 5H, 8H-quinoline), 8.52 (s, 1H, methine-H), 9.06 (s, 1H, 4H-quinoline), 10.52 (s, 1H, NH; Cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 20.92, 113.61, 113.67, 115.58, 121.50, 121.57, 125.12, 133.42, 134.28, 140.19, 146.53, 147.89, 148.78, 148.89, 158.83, 159.40, 160.00; MS *m/z* (%): 349 (M⁺, 34.68), 347 (M⁺, 1.48), 312 (100.00), 241 (20.60), 180 (0.13), 179 (1.04), 177 (27.60); Anal. Calcd for C₂₀H₁₄ClN₃O (347.80): C, 69.07; H, 4.06; N, 12.08; Found: C, 69.23; H, 3.81; N, 12.01.

3-(2-Chloroquinolin-3-yl)-2-cyano-N-(4-hydroxyphenyl)acrylamide (3b)

Yellow crystals; Yield (68 %); mp. 270-272 °C; IR (KBr cm⁻¹): 3316 (OH-br), 3160 (NH), 3000 (CH-arom.), 2944 (CH-aliph.), 2217 (C≡N), 1658 (C=O), 1555 (CH=N) ; ¹H NMR (DMSO-*d*₆) δ 6.72, 6.92 (2d, 4H, *J*=6Hz, Ar-H), 7.50, 7.56 (2t, 2H, *J*=7Hz, 6H,7H-quinoline), 7.59, 7.97 (2d, 2H, *J*=6Hz, 5H,8H-quinoline), 9.25 (s, 1H, methine-H), 9.68 (s, 1H, 4H-quinoline), 10.09 (s, 1H, NH; Cancelled with D₂O), 11.87 (s, 1H, OH; Cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 114.30, 115.56, 115.96, 122.72, 124.26, 125.24, 125.53, 129.08, 129.56, 130.96, 132.32, 135.61, 145.11, 147.07, 149.63, 153.15, 157.04, 158.90, 163.59; MS *m/z* (%): 351 (M⁺, 0.06), 349 (M⁺, 26.48), 337 (1.61), 315 (2.11), 264 (71.59), 113 (10.84), 107 (100.00); Anal. Calcd for C₁₉H₁₂ClN₃O₂ (349.77): C, 65.24; H, 3.46; N, 12.01; Found: C, 65.23; H, 3.81; N, 12.10.

3-(2-Chloroquinolin-3-yl)-2-cyano-N-(furan-2-ylmethyl)acrylamide (3c)

Orange crystals; Yield (75 %); mp. 250-252 °C; IR (KBr cm⁻¹): 3370 (NH), 3046 (CH-arom.), 2951 (CH-aliph.), 2211 (C≡N), 1694 (C=O); ¹H NMR (DMSO-*d*₆) δ 4.42 (s, *J*=7Hz, 2H, CH₂), 6.31-6.42 (m, 3H, furfuryl), 7.27-8.33 (m, 4H, Ar-H), 8.73 (s, 1H, methine-H), 9.10 (s, 1H, 4H-quinoline), 12.29 (s, 1H, NH; Cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 36.91, 107.85, 108.21, 111.03, 115.86, 118.79, 123.30, 124.63, 130.01, 133.50, 140.22, 141.26, 142.70, 145.43, 152.01, 160.80; Anal. Calcd for C₁₈H₁₂ClN₃O₂ (337.76): C, 64.01; H, 3.58; N, 12.44; Found: C, 64.23; H, 3.81; N, 12.15.

2-Cyano-3-(2-oxo-1,2-dihydroquinolin-3-yl)-N-(4-tolyl)acrylamide (3d)

Yellow crystals; Yield (68 %); mp. 260-262 °C; IR (KBr cm⁻¹): 3333, 3157 (2NH), 3006 (CH, arom.), 2948 (CH, aliph.), 2213 (C≡N), 1680,1659 (2C=O), 1599 (CH=C); ¹H NMR (DMSO-*d*₆) δ 2.29 (s, 3H, CH₃), 7.19, 7.29 (2d, 4H, *J*=5.5 Hz, p-tolyl), 7.56, 7.64 (2t, 2H, *J*=5Hz, 6H,7H-quinoline), 7.82, 7.84 (2d, 2H, *J*=5.5Hz, 5H,8H-quinoline), 8.40 (s, 1H, methine-H), 8.79 (s, 1H, 4H-quinoline), 10.43, 12.35 (2s, 2H, 2NH; Cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 20.99, 109.41, 115.89, 116.31, 118.82, 121.24, 123.29, 124.68, 129.62, 130.07, 133.53, 134.03, 136.07, 140.30, 141.26, 145.10, 160.34, 160.80 ; Anal. Calcd for C₂₀H₁₅N₃O₂ (329.36): C, 72.94; H, 4.59; N, 12.76; Found: C, 72.83; H, 4.81; N, 12.61.

2-Cyano-N-(4-hydroxyphenyl)-3-(2-oxo-1,2-dihydroquinolin-3-yl)acrylamide (3e)

Red crystals, Yield (80 %), mp. 300-302 °C. IR (KBr, cm⁻¹): 3339 (NH), 3302 (br OH), 3162 (NH), 3001 (CH, arom.), 2946 (CH, aliph.), 2215 (C≡N), 1666 (2C=O), 1618 (CH=C); ¹H NMR (DMSO-*d*₆) δ 6.77, 7.36 (2d, 4H, *J*=6Hz, Ar-H), 7.46, 7.47 (2t, 2H, *J*=6Hz, 6H,7H-quinoline), 7.64, 7.82 (2d, 2H, *J*=6Hz, 5H,8H-quinoline), 8.38 (s, 1H, methine-H), 8.77 (s, 1H, 4H-quinoline), 9.41, 10.27 (2s, 2H, 2NH; cancelled with D₂O), 12.33 (s, 1H, OH; Cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 109.42, 115.58, 115.88, 116.39, 118.82, 123.16, 123.26, 124.72, 130.02, 130.04, 133.46, 140.25, 141.12, 144.84, 154.80, 159.97, 160.82, 162.04; Anal. Calcd for C₁₉H₁₃N₃O₃ (331.33): C, 68.88; H, 3.95; N, 12.68; Found: C, 68.53; H, 3.81; N, 12.50.

1-Aryl-2-oxo-1,2-dihydrobenzo[*b*][1,8]naphthyridine-3-carbonitrile (4a, b)

A solution of the carboxamide derivatives **3** (0.01 mol) in DMF (10 mL) containing TEA (0.5 mL) was heated under reflux for 8 h. The reaction mixture was allowed to cool down to room temperature and the solid formed was filtered, washed with ethanol, dried, and recrystallized from DMF.

2-Oxo-1-(4-tolyl)-1,2-dihydrobenzo[*b*][1,8]naphthyridine-3-carbonitrile (4a)

White crystals, Yield (80 %), mp. > 320 °C. IR (KBr, cm⁻¹): 3043 (CH, arom), 2948 (CH, aliph), 2228 (C≡N), 1664 (C=O); ¹H NMR (DMSO-*d*₆) δ 2.45 (s, 3H, CH₃), 7.26, 7.39 (2d, 4H, *J*=5.5 Hz, 4-tolyl), 7.61, 7.83 (2t, 2H, *J*=5.5Hz, 6H,7H-quinoline), 7.66, 8.17 (2d, 2H, *J*=6Hz, 5H,8H-quinoline), 9.00 (s, 1H, 4H-quinoline), 9.10 (s, 1H, 4H-pyridine); ¹³C NMR (DMSO-*d*₆): 21.36,

107.98, 115.22, 115.91, 125.00, 126.47, 128.27, 129.77, 131.00, 132.00, 135.15, 140.28, 147.00, 148.22, 149.08, 160.02; Anal. Calcd for **C₂₀H₁₃N₃O** (311.34): C, 77.16; H, 4.21; N, 13.50; Found: C, 77.25; H, 4.55; N, 13.15.

1-(4-Hydroxyphenyl)-2-oxo-1,2-dihydrobenzo[*b*][1,8]naphthyridine-3-carbonitrile (4b)

Brown crystals, Yield (75 %), mp. 260-262 °C. IR (KBr, cm⁻¹): 3226 (OH-br.), 2198 (C≡N), 1642 (C=O); ¹H NMR (DMSO-*d*₆) δ 6.83, 7.36 (2d, 4H, *J*=5.5Hz, Ar-H), 7.23, 7.56 (2t, 2H, *J*=5Hz, 6H,7H-quinoline), 7.87, 8.61 (2d, 2H, *J*=5.5Hz, 5H,8H-quinoline), 8.79 (s, 1H, 4H-quinoline), 9.63 (s, 1H, 4H-pyridine), 12.12 (s, 1H, OH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 115.58, 115.61, 116.33, 119.47, 122.84, 123.13, 127.25, 129.93, 132.04, 136.99, 139.97, 143.17, 151.94, 157.13, 162.11; MS *m/z* (%): 314 (M⁺, 72.15), 305 (14.98), 285 (2.65), 104 (6.96), 94 (20.06), 44 (100.00) ; Anal. Calcd for **C₁₉H₁₁N₃O₂** (313.32): C, 72.84; H, 3.54; N, 13.41; Found: C, 72.73; H, 3.55; N, 13.35.

5-Acetyl-1-aryl-4-(2-substitutedquinolin-3-yl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6a,b)

To a solution of **3a, c** and acetylacetone (0.01mol) in ethanol (20 mL) containing catalytic amount of piperidine (0.01mol) was added and the reaction mixture was heated under reflux for 8h. The solid formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of EtOH/DMF.

5-Acetyl-4-(2-chloroquinolin-3-yl)-6-methyl-2-oxo-1-(*p*-tolyl)-1,2-dihydropyridine-3-carbonitrile (6a)

Yellow crystals, Yield (60 %), mp. > 320 °C; IR (KBr, cm⁻¹): 3043 (CH-arom.), 2948 (CH-aliph.), 2228 (C≡N), 1730 (CH₃-C=O), 1664 (N-C=O); ¹H NMR (DMSO-*d*₆) δ 2.42, 2.74, 2.89 (3s, 9H, 3CH₃), 7.26, 7.37 (2d, 4H, *J*=5Hz, Ar-H), 7.83, 8.01 (2t, 2H, *J*=5.5Hz, 6H,7H-quinoline), 8.12, 8.23 (2d, 2H, *J*=5.5Hz, 5H,8H-quinoline), 8.74 (s, 1H, 4H-quinoline); ¹³C NMR (DMSO-*d*₆): 11.55, 21.38, 31.24 (3CH₃) 76.73, 89.39, 115.53, 116.27, 126.60, 128.36, 128.48, 128.57, 129.13, 131.46, 131.48, 133.02, 140.09, 146.49, 147.71, 157.55, 157.57, 159.72, 162.80; Anal. Calcd for **C₂₅H₁₈ClN₃O₂** (427.89): C, 70.18; H, 4.24; N, 9.82; Found: C, 70.20; H, 4.51; N, 9.72.

5-Acetyl-4-(2-chloroquinolin-3-yl)-1-(furan-2-ylmethyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6b)

Yellow crystals, Yield (69 %), mp. 280-282 °C; IR (KBr, cm^{-1}): 3039 (CH-arom.), 2229 ($\text{C}\equiv\text{N}$), 1732 ($\text{CH}_3\text{-C=O}$), 1659 (N-C=O); ^1H NMR ($\text{DMSO-}d_6$) δ 2.74, 2.90 (2s, 6H, 2 CH_3), 5.71 (s, 2H, CH_2), 6.37-6.44 (m, 3H, furfuryl), 7.54-8.99 (m, 4H, Ar-H), 9.04 (s, 1H, 4H-quinoline); ^{13}C NMR ($\text{DMSO-}d_6$): 30.36, 36.38, 39.24, 107.37, 109.30, 111.06, 114.94, 125.02, 126.68, 128.34, 129.87, 134.16, 141.71, 142.72, 147.87, 148.46, 148.90, 150.43, 159.27, 162.81, 163.55; Anal. Calcd for $\text{C}_{23}\text{H}_{16}\text{ClN}_3\text{O}_3$ (417.85): C, 66.11; H, 3.86; N, 10.06; Found: C, 66.25; H, 3.50; N, 10.12.

4-(2-Chloroquinolin-3-yl)-3-methyl-6-oxo-1-phenyl-N-(4-tolyl)-1,4,5,6-tetrahydropyrano[2,3-c]pyrazole-5-carboxamide (7)

5-Methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (0.01mol) was added to a solution of **3a** (0.01mol) and piperidine (0.5 mL) in ethanol (20 mL) and the reaction mixture were heated under reflux for 8 h. The solid formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of EtOH/DMF.

Red crystals, Yield (80 %), mp. 185-187 °C. IR (KBr, cm^{-1}): 3182 (NH), 3067 (CH, arom.), 2853 (CH-aliph.), 1731 ($\text{CH}_3\text{-C=O}$), 1650 (N-C=O); ^1H NMR ($\text{DMSO-}d_6$) δ 1.11, 2.23 (2s, 6H, 2 CH_3), 5.16 (s, 1H, 3H-pyran), 5.93 (s, 1H, 4H-pyran), 7.21-8.55 (m, 13H, Ar-H), 9.00 (s, 1H, 4H-quinoline), 10.20 (s, 1H, NH; cancelled with D_2O); ^{13}C NMR ($\text{DMSO-}d_6$): 13.45, 23.75 (2 CH_3), 56.86, 63.81 (2CH), 117.33, 126.88, 127.04, 127.95, 128.00, 128.35, 128.58, 128.65, 128.88, 129.47, 130.44, 132.17, 132.27, 140.54, 146.49, 146.86, 150.26, 151.47 (Ar-C), 165.83, 168.49 (2C=O); MS m/z (%): 522 (M^+ , 1.35), 394 (31.65), 351 (85.64), 323 (10.64), 50 (62.92), 44 (71.32), 42 (100.00); Anal. Calcd for $\text{C}_{30}\text{H}_{23}\text{ClN}_4\text{O}_3$ (522.99): C, 68.90; H, 4.43; N, 10.71; Found: C, 68.85; H, 4.52; N, 10.62.

N-(4-(2-Substitutedquinolin-3-yl)methylene)amino)cynoacetanilide (9a,b)

The aldehyde derivative **1** (0.01 mol) was added to a solution of *p*-amino-cynoacetanilide **8** (0.01mol) in ethanol (20 mL) containing acetic acid (5 mL). The mixture was heated under reflux for 1h and the solid formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of EtOH/DMF.

***N*-(4-(2-Chloroquinolin-3-yl)methylene)amino)cianoacetanilide (9a)**

Brown crystals, Yield (60 %), mp. 270-272 °C; IR (KBr, cm⁻¹): 3280 (NH), 2261 (C≡N), 1658 (N-C=O), 1596 (CH=N); ¹H NMR (DMSO-*d*₆) δ 3.85 (s, 2H, CH₂), 7.48-8.20 (m, 8H, Ar-H), 8.50 (s, 1H, CH=N), 9.03 (s, 1H, 4H-quinoline), 10.29 (s, 1H, NH; cancelled with D₂O); MS *m/z* (%): 350 (M⁺₂, 9.59), 348 (M⁺, 13.01), 340 (5.80), 313 (50.68), 70 (17.37), 65 (16.80), 57 (100.00); Anal. Calcd for C₁₉H₁₃ClN₄O (348.79): C, 65.43; H, 3.76; N, 16.06; Found: C, 65.23; H, 3.55; N, 16.35.

***N*-(4-(2-Oxoquinolin-3-yl)methylene)amino)cianoacetanilide (9b)**

Red crystal; Yield: (71 %); mp. 290-292 °C; IR (KBr, cm⁻¹): 3270, 3157 (2NH), 3007 (CH-arom), 2949 (CH-aliph), 2212 (C≡N), 1656 (2 N-C=O), 1552 (CH=N); ¹H NMR (DMSO-*d*₆) δ 3.90 (s, 2H, CH₂), 7.00-8.20 (m, 8H, Ar-H), 8.50 (s, 1H, CH=N), 9.00 (s, 1H, 4H-quinoline), 10.20, 10.40 (2s, 2H, 2NH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 48.00, 115.88, 118.60, 119.00, 123.15, 126.04, 131.40, 134.18, 141.61, 142.92, 145.00, 148.00, 155.00, 160.00, 161.92; MS *m/z* (%): 330 (M⁺, 2.56), 275 (0.97), 145 (48.64), 117 (85.85), 116 (22.48), 88 (100.00); Anal. Calcd for C₁₉H₁₄N₄O₂ (330.35): C, 69.08; H, 4.27; N, 16.96; Found: C, 69.23; H, 4.44; N, 16.85.

***N*-(4-(((2-Chloroquinolin-3-yl)methylene)amino)phenyl)-2-imino-2*H*-chromene-3-carboxamide (10)**

A mixture of **10a** (0.01mol) and 2-hydroxybenzaldehyde (0.01mol) in ethanol (20 mL) containing catalytic amount of piperidine was heated under reflux for 3 h. The solid formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of EtOH/DMF.

Orange crystal; Yield: (85 %); mp. 250-252 °C; IR (KBr, cm⁻¹): 3340, 3292 (2NH), 3052 (CH, arom.), 2935 (CH, aliph.), 1679 (N-C=O); ¹H NMR (DMSO-*d*₆) δ 6.58-8.98 (m, 13H, Ar-H & CH=N), 9.07 (s, 1H, 4H-chromene), 9.26 (s, 1H, 4H-quinoline), 10.38, 10.78 (2s, 2H, 2NH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 117.03, 117.68, 118.91, 119.61, 119.79, 120.88, 121.18, 122.35, 122.57, 122.72, 128.08, 128.23, 129.68, 130.68, 132.95, 134.38, 136.93, 142.00, 145.00, 146.00, 147.00, 153.00, 154.00, 160.70, 161.18, 192.22; Anal. Calcd for C₂₆H₁₇ClN₄O₂ (452.90): C, 68.95; H, 3.78; N, 12.37; Found: C, 68.84; H, 3.64; N, 12.45.

***N*-(4-(((2-Substitutedquinolin-3-yl)methylene)amino)phenyl)-2-cyano-3-(2-oxo-1,2-dihydroquinolin-3-yl)acrylamide (11)**

A solution of **9a, b** (0.01mol) in ethanol (20 mL) was treated with 2-oxo-1,2-dihydroquinoline-3-carbaldehyde (0.01mol) and catalytic amount of piperidine. The mixture was heated under reflux for 3 h. and the solid formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of EtOH.

***N*-(4-(((2-Chloroquinolin-3-yl)methylene)amino)phenyl)-2-cyano-3-(2-oxo-1,2-dihydroquinolin-3-yl)acrylamide (11a)**

Pale green crystal; yield: (65 %); mp. 260-262 °C; IR (KBr, cm⁻¹); 3317, 3165 (2NH), 3024 (CH, arom.), 2950 (CH, aliph.), 2213 (C≡N), 1659 (2C=O), 1615 (CH=N); ¹H NMR (DMSO-*d*₆) δ 7.23-8.80 (m, 13H, Ar-H & CH=N), 8.83 (s, 1H, methine-H), 9.10 (s, 1H, 4H-quinoline), 9.20 (s, 1H, 4H-quinoline), (10.20, 10.80 (2s, 2H, 2NH; cancelled with D₂O)); ¹³C NMR (DMSO-*d*₆): 109.26, 115.66, 115.89, 116.30, 118.81, 121.98, 122.02, 122.11, 122.91, 123.13, 123.28, 124.63, 128.29, 129.54, 130.14, 131.38, 133.56, 134.15, 137.91, 140.21, 140.32, 141.30, 141.34, 142.90, 145.27, 147.92, 160.80, 161.91, 162.04; Anal. Calcd for C₂₉H₁₈ClN₅O₂ (503.95): C, 69.12; H, 3.60; N, 13.90; Found: C, 69.23; H, 3.44; N, 13.85.

2-Cyano-3-(2-oxo-1,2-dihydroquinolin-3-yl)-*N*-(4-(((2-oxo-1,2-dihydroquinolin-3-yl)methylene)amino)phenyl)acrylamide (11b)

Pale green crystal; Yield: (80 %); mp. > 320 °C; IR (KBr,cm⁻¹); 3346, 3157, 3102 (3NH), 3038 (CH-arom), 2999 (CH-aliph), 2218 (C≡N), 1687 (2C=O), 1649 (C=O), 1534 (CH=N); ¹H NMR (DMSO-*d*₆) δ 7.24-8.77 (m, 13H, Ar-H & CH=N), 8.81 (s, 1H, methine-H), 8.82 (s, 1H, 4H-quinoline), 8.84 (s, 1H, 4H-quinoline), 10.10, 10.25, 10.56 (3s, 3H, 3NH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 109.39, 114.09, 115.90, 118.84, 121.61, 122.00, 122.13, 122.96, 123.32, 130.01, 130.12, 130.18, 131.41, 134.19, 137.96, 140.23, 140.43, 141.40, 142.93, 145.32, 154.84, 160.51, 160.80, 162.05; MS *m/z* (%): 485 (M⁺, 12.37), 477 (10.37), 420 (38.37), 380 (100.00), 355 (40.07), 317 (50.00), 201 (20.22), 145 (31.48), 81 (15.03); Anal. Calcd for C₂₉H₁₉N₅O₃ (485.50): C, 71.74; H, 3.94; N, 14.43; Found: C, 71.63; H, 3.84; N, 14.35.

***N*-(4-((Substitutdbenzylidene)amino)phenyl)-3-(2-substitutdquinolin-3-yl)-2-cyanoacrylamide (13a-d)**

The aldehyde derivatives **1** (0.01mol) was added to Schiff bases cyanoacetanilide **12a, b** (0.01mol) in ethanol (20 mL) in the presence of pyridine. The mixture was heated under reflux for 3h and the solid formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of EtOH/DMF.

3-(2-Chloroquinolin-3-yl)-2-cyano-*N*-(4-((4-methoxybenzylidene)amino)phenyl)-acrylamide (13a)

Brown crystal; Yield (85 %); mp. 245-247 °C; IR (KBr, cm⁻¹): 3339 (NH), 3050 (CH-arom), 2931 (CH-aliph), 2212 (C≡N), 1678 (C=O), 1583 (CH=N); ¹H NMR (DMSO-*d*₆) δ 3.88 (s, 3H, OCH₃), 7.14-8.27 (m, 12H, Ar-H), 8.29, 9.00 (2s, 2H, 2-methine-H), 9.17 (s, 1H, 4H-quinoline), 10.32 (s, 1H, NH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 56.15, 103.98, 104.02, 115.30, 117.30, 121.77, 122.43, 122.85, 124.84, 127.33, 127.77, 128.45, 129.94, 132.31, 132.91, 133.12, 135.04, 138.04, 138.24, 141.89, 146.99, 148.19, 149.93, 150.81, 151.02, 155.03, 161.26; MS *m/z* (%): 468 (M⁺, 5.52), 430 (0.11), 313 (17.29), 312 (4.70), 58 (5.95), 57 (100.00); Anal. Calcd for C₂₇H₁₉Cl N₄O₂ (466.93): C, 69.45; H, 4.10; N, 12.00; Found: C, 69.70; H, 4.60; N, 12.27.

***N*-(4-((4-Chlorobenzylidene)amino)phenyl)-3-(2-chloroquinolin-3-yl)-2-cyanoacrylamide (13b)**

Brown crystal; Yield (50 %); mp. 275-277 °C; IR (KBr, cm⁻¹): 3344 (NH), 3056 (CH-arom), 2214 (C≡N), 1682 (C=O), 1609 (CH=N); ¹H NMR (DMSO-*d*₆) δ 7.32-8.47 (m, 12H, Ar-H), 8.58, 9.02 (2s, 2H, 2-methine-H), 9.05 (s, 1H, 4H-quinoline), 10.21 (s, 1H, NH; cancelled with D₂O); Anal. Calcd for C₂₆H₁₆Cl₂N₄O (471.34): C, 66.25; H, 3.42; N, 11.89; Found: C, 66.70; H, 3.20; N, 11.87.

2-Cyano-*N*-(4-((4-methoxybenzylidene)amino)phenyl)-3-(2-oxo-1,2-dihydroquinolin-3-yl)-acrylamide (13c)

Brown crystal; Yield (80 %); mp. > 320 °C; IR (KBr, cm⁻¹): 3314, 3161 (2NH), 3008 (CH-arom), 2949 (CH-aliph), 2213 (C≡N), 1656 (C=O), 1591 (CH=N); ¹H NMR (DMSO-*d*₆) δ 3.88 (s, 3H, OCH₃), 6.55-8.69 (m, 12H, Ar-H), 8.82, 8.84 (2s, 2H, 2-methine-H), 9.87 (s, 1H, 4H-quinoline),

10.32, 10.41 (2s, 2H, 2NH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 56.16, 115.38, 115.90, 117.32, 118.83, 119.39, 121.76, 122.94, 123.31, 124.85, 126.96, 130.10, 130.85, 131.40, 132.86, 133.58, 134.19, 137.90, 140.33, 141.38, 142.94, 147.72, 150.04, 150.92, 154.69, 160.50, 160.82; Anal. Calcd for C₂₇H₂₀N₄O₃ (448.48): C, 72.31; H, 4.50; N, 12.49; Found: C, 72.70; H, 4.20; N, 12.17.

***N*-(4-((4-Chlorobenzylidene)amino)phenyl)-2-cyano-3-(2-oxo-1,2-dihydroquinolin-3-yl)-acrylamide (13d)**

Brown crystal; Yield (70 %); mp.> 320 °C; IR (KBr, cm⁻¹); 3344, 3320 (2NH), 3060 (CH-arom), 2982 (CH-aliph), 2219 (C≡N), 1675 (C=O), 1645 (C=O) 1591 (CH=N); ¹H NMR (DMSO-*d*₆) δ 7.25-8.79 (m, 12H, Ar-H), 8.79, 8.83 (2s, 2H, 2-methine-H), 8.84 (s, 1H, 4H-quinoline), 10.23, 10.56 (2s, 2H, NH; cancelled with D₂O); ¹³C NMR (DMSO-*d*₆): 115.70, 116.46, 118.83, 121.47, 121.84, 122.15, 122.98, 123.25, 124.60, 129.45, 129.97, 130.07, 130.68, 131.39, 131.67, 132.20, 134.25, 135.24, 139.88, 140.25, 141.53, 143.09, 149.91, 154.86, 159.15, 160.84, 162.00; Anal. Calcd for C₂₆H₁₇ClN₄O₂ (452.90): C, 68.95; H, 3.78; N, 12.37; Found: C, 68.70; H, 3.50; N, 12.27.

4.2. Antitumor activity of compounds against cancer cells

4.2.1. Maintenance of cancer cells in culture

Mammary gland breast cancer cell line (MCF-7), human Intestinal cancer cells (CACO), human hepatocellular carcinoma cell line (Hep G-2), colon carcinoma cell line (HCT116) and human Lung cancer Cells (A 549) were obtained from VACSERA- Cell Culture Unit, Cairo, Egypt. This cell line originally obtained from the American Type Culture Collection (ATCC). The cell line was cultured in RPMI medium supplemented with 10 % inactivated fetal bovine serum (FBS). The reagents RPMI-1640 medium, SRB, DMSO and 5-fluorouracil were purchased from (sigma co., St. Louis, USA). Fetal bovine serum was obtained from (GIBCO, UK). The different cell lines mentioned above were used to determine the inhibitory effects of the tested compounds on cell growth using the SRB assay (SulphoRhodamine-B) [37]. This colorimetric assay is based on the ability of SRB to bind to protein components of cells that have been fixed in tissue culture plates by trichloroacetic acid (TCA).

4.2.1.1. Cytotoxicity screening

The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics were added 100 units/mL penicillin and 100 µg/ml streptomycin at 37 °C in a 5% CO₂ incubator. The cells were seeded in a 96-well plate at a density of 1.0x10⁴ cells/well at 37 °C for 48 h under 5% CO₂. After incubation, the cells were treated with different concentration of compounds and incubated for 24 h, discard the medium. Fixed with 10% trichloroacetic acid (TCA) 150 µL/well for 1 h at 4 °C. Wash by water 3 times (TCA reduce SRB protein binding). Wells will be stained by SRB 70 µL/well for 10 min at room temperature with 0.4%. 70 µL/well [keep in dark place]. Wash with acetic acid 1% to remove unbound dye (end point: colorless drainage). The plates will be air dried 24 h. The dye will be solubilized with 50 µL/well of 10 mM tris base (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well will be measured at 570 nm with an ELISA microplate reader (EXL 800 USA). The relative cell viability in percentage was calculated as (A₅₇₀ of treated samples/A₅₇₀ of untreated sample) X 100 and The IC₅₀ values will be calculated using sigmoidal concentration response curve fitting models (Sigmaplot software) [37].

4.2.2. Apoptosis detection studies

4.2.2.1. Determination of Caspase-3

The PC-3 cells were planted into RPMI 1640 with a 10% fetal serum of bovine serum at 37 °C, were stimulated by Caspase-3 compounds, and lysed with the cell extraction buffer. The lysate was diluted in the regular diluent tank over the test range and tested for human active Caspase-3 content (cells are plated in the density of 1.2-1.8 per 100 µL of cells per well in a 96 wave plate for 48 hours before the enzymes test) [36].

4.2.2.2. Determination of BAX and Bcl-2

Cells PC-3 were grown in RPMI 1640 containing a fetal 10% of fetal serum at a temperature of 37°C, stimulated by the compounds for BAX/Bcl-2 testing and lysed by a buffer for cell removal. This lysate was diluted across the test range and tested for human active BAX / Bcl-2 contents in a Regular Diluent Buffer. (Cells are placed at a density of 1.2 – 1.8 µL per pool in a total growth medium of 10,000 cells / well volume + 100 uL of the compounds tested in a 96-well platform 24 hours before human BAX / Bcl-2 measurement) [37].

4.2.3. PIM1-Kinase detection

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase . The luminescent signal positively correlates with ADP amount and kinase activity. The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling. The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer and then add to the wells of 384 low volume plate: 1 µL of inhibitor or (5% DMSO), 2 µL of the enzyme, 2 µL of substrate/ATP mix, incubate at room temperature for 60 minutes, and add 5 µL of ADP-Glo™ . Incubate at room temperature for 40 minutes, add 10 µL of Kinase Detection Reagent and incubate at room temperature for 30 minutes. Record luminescence (Integration time 0.5-1second) [53].

4.2.4. Method of Computation:

4.2.4.1. In-silico study

In the present study, all the synthesized derivatives as well as the standard reference drug i.e. Doxorubicin were subjected to screening by the Swiss ADMET website (<http://www.sib.swiss>) interface, provoking the *in-silico* ADME characteristics and examining their aptitude to exhibit drug-likeness [54-57].

Notes

The authors declare no competing financial interest.

References

- [1] C. Sawyers, “Targeted cancer therapy,” *Nature*, Vol. 432, 2004, pp. 294-297.
- [2] Q. Li and W. Xu, “Novel anticancer targets and drug discovery in post genomic age,” *Curr. Med. Chem. - Anti-Cancer Agents*, vol. 5, no. 1, pp. 53–63, 2005, doi: 10.2174/1568011053352631.
- [3] S. K. Mencher and L. G. Wang, “Promiscuous drugs compared to selective drugs (promiscuity can be a virtue),” *BMC Clin. Pharmacol.*, vol. 5, pp. 1–7, 2005, doi: 10.1186/1472-6904-5-3.
- [4] A. Jimeno and M. Hidalgo, “Multitargeted therapy: Can promiscuity be praised in an era of political correctness?,” *Crit. Rev. Oncol. Hematol.*, vol. 59, no. 2, pp. 150–158, 2006, doi: 10.1016/j.critrevonc.2006.01.005.
- [5] S. Teimoori, K. Panjamurthy, K. Vinaya, D. S. Prasanna, S. C. Raghavan, and K. S. Rangappa, “Synthesis and Biological Evaluation of Novel Homopiperazine Derivatives as Anticancer Agents,” *J. Cancer Ther.*, vol. 02, no. 04, pp. 507–514, 2011, doi: 10.4236/jct.2011.24069.
- [6] A. G. Porter and R. U. Jänicke, “Emerging roles of caspase-3 in apoptosis,” *Cell Death Differ.*, vol. 6, no. 2, pp. 99–104, 1999, doi: 10.1038/sj.cdd.4400476.
- [7] S. Ghavami *et al.*, “Apoptosis and cancer: Mutations within caspase genes,” *J. Med. Genet.*, vol. 46, no. 8, pp. 497–510, 2009, doi: 10.1136/jmg.2009.066944.
- [8] H. Perlman, X. Zhang, M. W. Chen, K. Walsh, and R. Buttyan, “An elevated bax/bcl-2 ratio corresponds with the onset of prostate epithelial cell apoptosis,” *Cell Death Differ.*, vol. 6, no. 1, pp. 48–54, 1999, doi: 10.1038/sj.cdd.4400453.
- [9] Chang F, SteelmanLS, LeeJT, Shelton JG, Navolanic PM, Blalock WL, “Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: Potential targeting for therapeutic intervention,” *Leukemia*, vol. 17, no. 7, pp. 1263–1293, 2003, doi: 10.1038/sj.leu.2402945.
- [10] Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol*

ExpTher 2005;315:971–9.

- [11] S. Faivre, S. Djelloul, and E. Raymond, “New Paradigms in Anticancer Therapy: Targeting Multiple Signaling Pathways With Kinase Inhibitors,” *Semin. Oncol.*, vol. 33, no. 4, pp. 407–420, 2006, doi: <https://doi.org/10.1053/j.seminoncol.2006.04.005>.
- [12] A. N. Bullock, J. Debreczeni, A. L. Amos, S. Knapp, and B. E. Turk, “Structure and substrate specificity of the Pim-1 kinase,” *J. Biol. Chem.*, vol. 280, no. 50, pp. 41675–41682, 2005, doi: [10.1074/jbc.M510711200](https://doi.org/10.1074/jbc.M510711200).
- [13] N. S. Magnuson, Z. Wang, G. Ding, and R. Reeves, “Why target PIM1 for cancer diagnosis and treatment?,” *Futur. Oncol.*, vol. 6, no. 9, pp. 1461–1478, 2010, doi: [10.2217/fon.10.106](https://doi.org/10.2217/fon.10.106).
- [14] M. C. Nawijn, A. Alendar, and A. Berns, “For better or for worse: The role of Pim oncogenes in tumorigenesis,” *Nat. Rev. Cancer*, vol. 11, no. 1, pp. 23–34, 2011, doi: [10.1038/nrc2986](https://doi.org/10.1038/nrc2986).
- [15] A. Kumar, V. Mandiyan, Y. Suzuki, C. Zhang, J. Rice, J. Tsai, D. R. Artis, P. Ibrahim, R. Bremer, “Crystal Structures v of Proto-oncogene Kinase Pim1: A Target of Aberrant Somatic Hypermutations in Diffuse Large Cell Lymphoma,” *J. Mol. Biol.*, vol. 348, no. 1, pp. 183–193, 2005, doi: <https://doi.org/10.1016/j.jmb.2005.02.039>.
- [16] P. P. Kattimani, R. R. Kamble, M. Y. Kariduraganavar, A. Dorababu, and R. K. Hunnur, “Synthesis, characterization and in vitro anticancer evaluation of novel 1,2,4-triazolin-3-one derivatives,” *Eur. J. Med. Chem.*, vol. 62, pp. 232–240, 2013, doi: [10.1016/j.ejmech.2013.01.004](https://doi.org/10.1016/j.ejmech.2013.01.004).
- [17] A. Husain, M. Rashid, M. Shaharyar, A. A. Siddiqui, and R. Mishra, “Benzimidazole clubbed with triazolo-thiadiazoles and triazolo-thiadiazines: New anticancer agents,” *Eur. J. Med. Chem.*, vol. 62, pp. 785–798, 2013, doi: [10.1016/j.ejmech.2012.07.011](https://doi.org/10.1016/j.ejmech.2012.07.011).
- [18] G. L. Firestone and S. N. Sundar, “Anticancer activities of artemisinin and its bioactive derivatives,” *Expert Rev. Mol. Med.*, vol. 11, p. e32, Oct. 2009, doi: [10.1017/S1462399409001239](https://doi.org/10.1017/S1462399409001239).

- [19] J. J. Lu L. H. Meng, Y. J. Cai, Q. Chen, L. J. Tong, L. P. Lin & J. Ding, "Dihydroartemisinin induces apoptosis in HL-60 leukemia cells dependent of iron and p38 mitogen-activated protein kinase activation but independent of reactive oxygen species," *Cancer Biol. Ther.*, vol. 7, no. 7, pp. 1017–1023, 2008, doi: 10.4161/cbt.7.7.6035.
- [20] E. M. Gedawy, A. E. Kassab, and A. A. El-Malah, "Synthesis and anticancer activity of novel tetrahydroquinoline and tetrahydropyrimidoquinoline derivatives," *Med. Chem. Res.*, vol. 24, no. 9, pp. 3387–3397, 2015, -015-1388 doi: 10.1007/s00044-7.
- [21] K. Li, Y. Li, D. Zhou, Y. Fan, H. Guo, T. Ma, J. Wen "Synthesis and biological evaluation of quinoline derivatives as potential anti-prostate cancer agents and Pim-1 kinase inhibitors," *Bioorganic Med. Chem.*, vol. 24, no. 8, pp. 1889–1897, 2016, doi: 10.1016/j.bmc.2016.03.016.
- [22] R. B. Silverman and M. W. Holladay, "The Organic Chemistry of Drug Design and Drug Action: Third Edition," *Org. Chem. Drug Des. Drug Action Third Ed.*, pp. 1–517, 2015, doi: 10.1016/C2009-0-64537-2.
- [23] Y. A. Ammar, M. A. Salem, E. A. Fayed, M. H. Helal, M. S. A. El-Gaby, and H. K. Thabet, "Naproxen derivatives: Synthesis, reactions, and biological applications," *Synth. Commun.*, vol. 47, no. 15, 2017, doi: 10.1080/00397911.2017.1328066.
- [24] Y. A. Ammar, E. A. Fayed, A. H. Bayoumi and M. A. Saleh, "Synthesis and biological evaluation of new amides pro-drugs containing naproxen moiety as anti-inflammatory and antimicrobial agents," *Der Pharma Chemica*, vol. 8, no.1, pp. 495-508, 20, 2016.
- [25] M. F. El Shehry, M. M. Ghorab, S. Y. Abbas, E. A. Fayed, S. A. Shedid, Y. A. Ammar, *European journal of medicinal chemistry*, vol.143, pp.1463-1473, 2018, doi: <https://doi.org/10.1016/j.ejmech.2017.10.046>.
- [26] S. I. Eissa, A. M. Farrag, S. Y. Abbas, M. F. El Shehry, A. Ragab, E. A. Fayed, Y. A. Ammar, Novel Structural hybrids of quinolone and thiazole moieties: Synthesis and evaluation of antibacterial and antifungal activities with molecular modeling studies, *Bioorganic Chemistry*, 2021, doi: <https://doi.org/10.1016/j.bioorg.2021.104803>

- [27] M. M. Ghorab, M. S. Alsaid, M. S. Al-Dosari, F. A. Ragab, A. A. Al-Mishari, and A. N. Almoqbil, "Novel quinolines carrying pyridine, thienopyridine, isoquinoline, thiazolidine, thiazole and thiophene moieties as potential anticancer agents," *Acta Pharm.*, vol. 66, no. 2, pp. 155–171, 2016, doi: 10.1515/acph-2016-0016.
- [28] E. A. Fayed, R. R. E. Eldin, A. Mehany, A. H. Bayoumi, Y. A. Ammar, "Isatin-Schiff's base and chalcone hybrids as chemically apoptotic inducers and EGFR inhibitors; design, synthesis, anti-proliferative activities and in silico evaluation, " *Journal of Molecular Structure*, 2021, 1234, 130159
- [29] Y. A. Ammar, A. A. Farag, A. M. Ali, E. A. Fayed, D. M. Elsis, A. Ragab, "Antimicrobial evaluation of thiadiazino and thiazolo quinoxaline hybrids as potential DNA gyrase inhibitors; design, synthesis, characterization and morphological studies, " *Bioorganic Chemistry*, 2020, 99, 103841
- [30] E. Yahia, H. Mohammad, T. M. Abdelghany, E. A. Fayed, M. N. Seleem, A. S Mayhoub, "Phenylthiazole antibiotics: A metabolism-guided approach to overcome short duration of action, " *European Journal of Medicinal Chemistry*, 2017, 126, pp. 604–613
- [31] S. A. El-Kalyoubi, E. A. Fayed, A. S. Abdel-Razek, "Erratum: One pot synthesis, antimicrobial and antioxidant activities of fused uracils: pyrimidodiazepines, lumazines, triazolouracil and xanthines," *Chemistry Central Journal*, vol. 11, no. 66, 2017, doi:10.1186/s13065-017-0294-0.
- [32] E. A. Fayed, and H. Y. Ahmed, "Synthesis, characterization and pharmacological evaluation of some new 1,4-diazepine derivatives as anticancer agents, " *Der Pharma Chemica*, 8 no. 13, pp. 77-90, 2016
- [33] B. A. AbdelFattah, M. M. A. Khalifa, H. El-Sehrawi, E. Fayed and A. Bayoumi, "Synthesis and anxiolytic activity of some novel 5-oxo-1, 4-oxazepine derivatives, " *Letters in Drug Design & Discovery*, vol. 8 no. 4, pp. 330-338, 2011, doi: <https://doi.org/10.2174/157018011794839448>.

- [34] S. A. El-Kalyoubi, E. A. Fayed, A. S. Abdel-Razek, "One pot synthesis, antimicrobial and antioxidant activities of fused uracils: pyrimidodiazepines, lumazines, triazolouracil and xanthines," *Chemistry Central Journal*, vol. 11, no. 1, pp. 1-13, 2017, doi:10.1186/s13065-017-0294-0.
- [35] S. Elkalyoubi and E. Fayed, "Synthesis and evaluation of antitumour activities of novel fused tri-And tetracyclic uracil derivatives," *J. Chem. Res.*, vol. 40, no. 12, 2016, doi: 10.3184/174751916X14798125870610.
- [36] E. A. Fayed, S. I. Eissa, A. H. Bayoumi, N. A. Gohar, A. B. M. Mehany, and Y. A. Ammar, "Design, synthesis, cytotoxicity and molecular modeling studies of some novel fluorinated pyrazole-based heterocycles as anticancer and apoptosis-inducing agents," *Mol. Divers.*, vol. 23, no. 1, pp. 165–181, 2019, doi: 10.1007/s11030-018-9865-9.
- [37] E. A. Fayed, Y. A. Ammar, A. Ragab, N. A. Gohar, A. B. M. Mehany, and A. M. Farrag, "In vitro cytotoxic activity of thiazole-indenoquinoxaline hybrids as apoptotic agents, design, synthesis, physicochemical and pharmacokinetic studies," *Bioorg. Chem.*, vol. 100, p. 103951, 2020, doi: 10.1016/j.bioorg.2020.103951.
- [38] Y. A. Ammar, E. A. Fayed, A. H. Bayoumi, R. R. Ezz, M. S. Alsaid, A. M. Soliman, M. M. Ghorab, New chalcones bearing isatin scaffold: synthesis, molecular modeling and biological evaluation as anticancer agents, *Res. Chem. Intermed.* vol. 43, p. 6765–6786, 2017 doi: 10.1007/s11164-017-3019-z.
- [39] E. A. Fayed, R. Sabour, M. F. Harras, and A. B. M. Mehany, "Design, synthesis, biological evaluation and molecular modeling of new coumarin derivatives as potent anticancer agents," *Med. Chem. Res.*, vol. 28, no. 8, pp. 1284–1297, 2019, doi: 10.1007/s00044-019-02373-x.
- [40] M. R. Selim, M .A. Zahran , A. Belal , M. S. Abusaif , S. A. Shedid, A. B. M. Mehany, G. A. M. Elhagali , Y. A. Ammar, "Hybridized Quinoline Derivatives as Anticancer Agents: Design, Synthesis, Biological Evaluation and Molecular Docking," *Anticancer. Agents Med. Chem.*, vol. 19, no. 4, pp. 439–452, 2018, doi: 10.2174/1871520618666181112121058.

- [41] A. R. Al-Marhabi, H. A. S. Abbas, and Y. A. Ammar, "Synthesis, characterization and biological evaluation of some quinoxaline derivatives: A promising and potent new class of antitumor and antimicrobial agents," *Molecules*, vol. 20, no. 11, pp. 19805–19822, 2015, doi: 10.3390/molecules201119655.
- [42] M. M. F. Ismail, K. M. Amin, E. Noaman, D. H. Soliman, and Y. A. Ammar, "New quinoxaline 1, 4-di-N-oxides: Anticancer and hypoxia-selective therapeutic agents," *Eur. J. Med. Chem.*, vol. 45, no. 7, pp. 2733–2738, 2010, doi: 10.1016/j.ejmech.2010.02.052.
- [43] C. R. M. D'Oca *et al.*, "New multicomponent reaction for the direct synthesis of β -aryl- γ -nitroesters promoted by hydrotalcite-derived mixed oxides as heterogeneous catalyst," *J. Braz. Chem. Soc.*, vol. 28, no. 2, pp. 285–298, 2017, doi: 10.5935/0103-5053.20160175.
- [44] J. P. Nirmal, M. P. Patel, and R. G. Patel, "Microwave-assisted synthesis of some new biquinoline compounds catalyzed by DMAP and their biological activities," *Indian J. Chem. - Sect. B Org. Med. Chem.*, vol. 48, no. 5, pp. 712–717, 2009, doi: 10.1002/chin.200938138.
- [45] F. H. Igney and P. H. Krammer, "Death and anti-death: Tumour resistance to apoptosis," *Nat. Rev. Cancer*, vol. 2, no. 4, pp. 277–288, 2002, doi: 10.1038/nrc776.
- [46] J. Y. Zheng, G. S. Yang, W. Z. Wang, J. Li, K.Z. Li, W. X. Guan, W. L. Wang, "Overexpression of Bax induces apoptosis and enhances drug sensitivity of hepatocellular cancer-9204 cells," *World J. Gastroenterol.*, vol. 11, no. 23, pp. 3498–3503, 2005, doi: 10.3748/wjg.v11.i23.3498.
- [47] F. Al-Blewi, N. Rezki, A. adjet, H. Qutb Uddin, S. Al-Sodies, M. Messali, M. R. Aouad, S. Bardaweel, "A Profile of the In Vitro Anti-Tumor Activity and In Silico ADME Predictions of Novel Benzothiazole Amide-Functionalized Imidazolium Ionic Liquids," *Int. J. Mol. Sci.*, vol. 20, no. 12, 2019, doi: 10.3390/ijms20122865.
- [48] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings1PII of original article: S0169-409X(96)00423-1. The article was

- originally published in *Advanced Drug Delivery Reviews* 23 (1997) 3–25.1,” *Adv. Drug Deliv. Rev.*, vol. 46, no. 1, pp. 3–26, 2001, doi: [https://doi.org/10.1016/S0169-409X\(00\)00129-0](https://doi.org/10.1016/S0169-409X(00)00129-0).
- [49] A. K. Ghose, V. N. Viswanadhan, and J. J. Wendoloski, “Prediction of Hydrophobic (Lipophilic) Properties of Small Organic Molecules Using Fragmental Methods: An Analysis of ALOGP and CLOGP Methods,” *J. Phys. Chem. A*, vol. 102, no. 21, pp. 3762–3772, May 1998, doi: 10.1021/jp980230o.
- [50] D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward, and K. D. Kopple, “Molecular Properties That Influence the Oral Bioavailability of Drug Candidates,” *J. Med. Chem.*, vol. 45, no. 12, pp. 2615–2623, Jun. 2002, doi: 10.1021/jm020017n.
- [51] W. J. Egan and G. Lauri, “Prediction of intestinal permeability,” *Adv. Drug Deliv. Rev.*, vol. 54, no. 3, p. 273–289, 2002, doi: 10.1016/s0169-409x(02)00004-2.
- [52] I. Muegge, S. L. Heald, and D. Brittelli, “Simple Selection Criteria for Drug-like Chemical Matter,” *J. Med. Chem.*, vol. 44, no. 12, pp. 1841–1846, Jun. 2001, doi: 10.1021/jm015507e.
- [53] J. Alves, S. A. Goueli, H. Zegzouti, and Promega Corporation, “PIM1 Kinase Assay,” pp. 1–2.
- [54] A. Daina, O. Michielin, and V. Zoete, “SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules,” *Sci. Rep.*, vol. 7, no. January, pp. 1–13, 2017, doi: 10.1038/srep42717.
- [55] E. A. Fayed, A. H. Bayoumi, A. S. Saleh, E. M. Ezz Al-Arab, Y. A. Ammar, “In vivo and in vitro anti-inflammatory, antipyretic and ulcerogenic activities of pyridone and chromenopyridone derivatives, physicochemical and pharmacokinetic studies,” *Bioorganic Chemistry*, vol., 109, p. 104742, 2021, <https://doi.org/10.1016/j.bioorg.2021.104742>.
- [56] E. A. Fayed, R. R. Ezz Eldin, A. Mehany, A. H. Bayoumi, Y. A. Ammar, “Isatin-Schiff's base and chalcone hybrids as chemically apoptotic inducers and EGFR inhibitors; design,

synthesis, anti-proliferative activities and in silico evaluation,” *Journal of Molecular Structure* 1234 (2021), 130159, <https://doi.org/10.1016/j.molstruc.2021.130159>.

- [57] E. A. Fayed, Y. A. Ammar, M. A. Saleh, A. H. Bayoumi, A. Belal, A. B. M. Mehany, A. Ragab, “Design, synthesis, antiproliferative evaluation, and molecular docking study of new quinoxaline derivatives as apoptotic inducers and EGFR inhibitors,” *Journal of Molecular Structure*, 1236, (2021), 130317, <https://doi.org/10.1016/j.molstruc.2021.130317>.

Figures

Figure 1

Structures for Pim-1 inhibitors and the newly designed quinoline hybrids

Figure 2

The activity of the quinoline derivatives against human colon cancer (HCT-116) cell line

Figure 3

The activity of the quinoline derivatives against human colon cancer (HepG-2) cell line. The activity of the quinoline derivatives against human colon cancer (COCA) cell line.

Figure 4

A diagram illustrated the effect of the quinoline derivatives 3e, 4b, 11b and 13d on the level of Caspase-3 in the examined cancer cells

Figure 5

Bioavailability Radar of the selected compounds

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.docx](#)
- [Scheme1.png](#)
- [Scheme2.png](#)
- [Scheme3.png](#)
- [Scheme4.png](#)