Carboxamide appended quinoline moieties as potential anti-proliferative agents, apoptotic inducers and Pim-1 kinase inhibitors

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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 cancer treatments. Here we analyse the potential of quinoline-carboxamide 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 indicates that compound 3e was the most active with the percentage of inhibition 82.27% and IC50 equals 0.11 when compared to SGI-1776 as a reference standard. In addition, by in silico assessment of ADME properties, all of the strongest compounds are orally bioavailable without blood–brain barrier penetration.

Graphical Abstract

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Introduction

Cancer remains the world’s leading cause of death and consequently, new and effective treatments are urgently needed. It differs in many 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 tumour 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 the tumour, 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 tumour 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 tumour, 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].

Protein kinases (PKs) are a 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 activity.

Proviral integration site for Moloney leukaemia virus (Pim kinase) is a consistently active member of the calcium/calmodulin-regulated serine/threonine kinase family. The Pim family is made up of three genes: pim-1, pim-2 and pim3, all of which have a high degree of similarity. The Pim-1 kinase gene encodes two isoforms: Pim-1L (44 kDa) and Pim-1S (33 kDa). The most significant difference between the two isoforms was their length, with Pim-1L being longer than Pim-1S. The 44 kDa isoform is primarily found on the plasma membrane, while the 33 kDa isoform can be found in both the cytosol and the nucleus [12].

Pim-1 is constitutively expressed in a few tissues under physiological conditions at a low level. Its level increases significantly as it is regulated by JAK-STAT and NF-κB 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 leukaemia, large cell lymphoma (LCLD) 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, the 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 inhibition through cell cycle arrest, apoptosis, angiogenesis inhibition, cell migration interference and nuclear receptor response modulation [18–22]. For example, 1-(2-(1,2,4-triazol-3-yl)quinolin-8-yl)piperidin-4-amine (A) and quinoline derivatives B [21, 23] (Fig. 1).

In addition, 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, 25]. 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, an amide bond is commonly used in the structure of 25% of the well-known drugs, according to the Comprehensive Medicinal Chemistry database [26, 27]. More essential for synthesizing poly hetero-aromatic molecules are amides that typically are stable, neutral and have both the hydrogen-bonds receiver/donator properties. It has now been documented to be effective for cancer therapy and for the reduction of undesirable side effects [17] by developing a single molecule with more than one pharmacophore with different action modes.

Based on the above findings, which indicate that most of the cancer drugs are not selective and also can cause damage to the normal human cells, and in continuation to our work on the synthesis of new compounds with biological activities [28–33], we have synthesized new selective
and safe hybrids 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 [34–41] (Fig. 1).

**Results and discussion**

**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 [39, 42]. The reactivity of 1 towards some active methylene compounds was discussed. Thus, condensation of 1 with some cyano acetamide 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 based on analytical and spectral data. The IR spectrum of 3c as an example displayed absorption bands at 3370 cm⁻¹ due to NH function, at 2211 cm⁻¹ due to conjugated C≡N function, and at 1694 cm⁻¹ due to amide C=O function. The ¹H NMR spectrum (DMSO–d₆) exhibited one sharp singlet signal at δ 4.42 ppm assignable to CH₂ 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 ¹³C NMR (DMSO-d₆) revealed the most important signals displayed CH₂ 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. The heating of compounds 3a,b with trimethylamine in DMF caused cyclization via elimination of HCl molecule to afford the benzo- naphthyridine 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 an 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 CH₃ in addition to the aromatic protons. In an attempt to obtain pyranoquinoline derivative 5 by heating 3d,e in DMF containing trimethylamine, 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 ketone).
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 a 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 1731, 1650 cm$^{-1}$. It's $^1$H NMR spectrum displayed two doublet signals at 5.16 and 5.93 ppm corresponding to the two vicinal pyrane 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 CH$_3$ and NH groups. The $^{13}$C 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$_2$ 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.

5-(4-Aminophenyl)-2-cyanoacetamide (8) [43] has two nucleophilic centres which may be to react with the aldehyde function of 1. Thus, the interaction of 1 with compound 8 in acetic acid furnished the corresponding Schiff bases 9a,b. The chemical structure of 9 was elucidated based on elemental analysis and spectral data. The IR spectrum showed the presence of two C=O groups stretching at 1731, 1650 cm$^{-1}$. It's $^1$H NMR spectrum displayed two doublet signals at 5.16 and 5.93 ppm corresponding to the two vicinal pyrane 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 CH$_3$ and NH groups. The $^{13}$C 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$_2$ protons 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.

N-(4-Aminophenyl)-2-cyanoacetamide (8) [43] has two nucleophilic centres which may be to react with the aldehyde function of 1. Thus, the interaction of 1 with compound 8 in acetic acid furnished the corresponding Schiff bases 9a,b. The chemical structure of 9 was elucidated based on elemental analysis and spectral data. The IR spectrum of 9b as an example indicated the presence of strong absorption bands at 3270 and 3157 cm$^{-1}$ due to NH groups and two bands at 2212 and 1656 cm$^{-1}$. It's $^1$H NMR spectrum showed a singlet signal at δ 3.90 ppm due to CH$_2$ 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 aryldene derivatives 10 and 11a,b, respectively. The IR spectrum of compound 10 as an example showed the complete disappearance of the 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$^{-1}$. It's $^1$H NMR spectrum revealed two D$_2$O-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 4H-quinoline, respectively.

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 \(^1\)H 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.

**Biological evaluation**

**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-2), hepatocellular carcinoma (HepG-2) and 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 were expressed as IC\(_{50}\) (µM/mL) value; the dose that reduces cell growth and survival to 50% of our tested compounds 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 (IC\(_{50}\): 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
| Cpd. no. | R | X | IC<sub>50</sub> µM/mL/SI<sup>b</sup> | MCF-7 | CACO | HepG-2 | HCT-116 | WI-38 |
|----------|---|---|-------------------------------|-------|------|--------|---------|-------|
| 3a       | -Cl | 12.69 ± 0.96 (8.99) | 10.84 ± 0.88 (10.53) | 22.00 ± 1.21 (5.19) | 16.38 ± 1.09 (6.97) | 114.12 |
| 3b       | -Cl | 12.69 ± 0.96 (14.93) | 8.56 ± 0.74 (22.13) | 14.73 ± 1.09 (12.86) | 12.24 ± 0.94 (15.47) | 189.41 |
| 3c       | -Cl | 16.54 ± 1.11 (11.44) | 5.82 ± 0.48 (32.50) | 11.35 ± 0.92 (16.67) | 11.42 ± 0.92 (16.56) | 89.151 |
| 3d       | -OH | 11.36 ± 0.93 (16.65) | 5.94 ± 0.49 (31.84) | 8.67 ± 0.77 (21.81) | 10.57 ± 0.85 (17.89) | 189.10 |
| 3e       | -OH | 10.93 ± 0.88 (11.64) | 4.91 ± 0.38 (25.90) | 7.01 ± 0.60 (18.14) | 3.41 ± 0.24 (37.30) | 27.181 |
| 4a       | -Cl | 6.38 ± 0.53 (27.45) | 6.18 ± 0.51 (28.34) | 6.46 ± 0.54 (27.11) | 7.07 ± 0.61 (24.77) | 175.12 |
| 4b       | -Cl | 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) | 13.161 |
| 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) | 28.631 |
| 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) | 77.191 |
| 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 |
was fused with pyridine derivative (compound 4b) or tagged with cyanoacetamide derivative, chromene, second quinoline or benzylidene 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 CACO cell line, the results showed that compound 13d has the best result and it was almost equipotent to DOX against CACO cell line (IC_{50}: 3.87 μM vs 3.07 μM). Compounds 3e, 9b, 10 and 11b was quite less potent than DOX (IC_{50}: 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 was attached to cyanoacetamide moiety through a linker (compound 9b), chromene ring through a spacer (compound 10) or fused with 2-pyridine 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 μM vs 4.6 μM). Compounds 10, 11a-b and 13c were more potent than DOX (IC_{50}: 3.91, 4.04, 3.5 and 3.95 μM vs 4.6 μM). Compound 9b was equipotent to DOX against HepG-2 against DOX (4.56 μM vs 4.6 μg) while compound 13b was slightly less potent than DOX (4.93 μM vs 4.6 μM). Compounds 3e, 4b and 6b displayed promising activity against HepG-2 cell line (IC_{50}: 7.01, 6.06 and 6.53 μM, respectively). The antitumor activity of the examined quinolines against HepG-2 cell line had the following descending order: (13d > 10, 13c > 11b > 11a, 9b, 6b > 13b > 9a > 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 μM vs DOX 4.48 μM). Moreover, compounds 13d, 13e were also more potent than DOX against HCT-116 cell line (IC_{50}: 3.92, 4.01 μM vs 4.48 μM). Also, compound 11b was almost equipotent to DOX against HCT-116 cell line (IC_{50}: 4.82 μM vs 4.48 μM). 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 μM vs 4.48 μM). 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 μM vs 4.48 μM, 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), cyano acetamide 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).

The cytotoxic effect of the target compounds on the Wish normal cell line was evaluated and the results were summarized in Table 1. Most of the target compounds have a selective cytotoxic effect on cancer cells. The selectivity index was calculated by dividing the IC50 value of each target compound on the Wish normal cell line by the IC50 value on the cancer cell line (Table 1). Out of the tested nineteen compounds, selectivity index values, only three showed values ranging from 5.19 to 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 a 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 selectivity index value of 29.78 and 34.82, respectively. While compound 13c was the most selective on CACO cell line with selectivity index equals 40.67. Regarding the HCT-116 cell line, compound 13c displayed the highest selectivity index value of 47.36.

Structure–activity relationship (SAR)

The sensitivity of the screened cancer cells to the target compounds was 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 anti-proliferative 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 (Fig. 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) (Fig. 2). 2-Chloroquinolinol-3-yl-methyleneaminophenyl (COQPA) 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 cyano acetamide moiety 11a to CQMAP derivatives tagged with chromene carboxamide 10 or acrylamide derivative 9a increased the anticancer activity (Fig. 2).

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 (Fig. 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 (Fig. 3a). 2-Oxoquinolinol-3-yl-methyleneaminophenyl (OQMAP) derivatives 9b, 11b have greater activities than their corresponding CQMAP derivatives 9a, 11a, respectively (Fig. 3a). Transforming CQMAP and OQMAP tagged with cyano acetamide moiety 9a, 9b to their corresponding CQMAP and OQMAP tagged with acrylamide derivatives 11a and 11b, respectively, and OQMAP tagged with chromene-3-carboxamide moiety 10 increased the anticancer activity. It was observed that all COQPA derivatives 11a-b and 13a-d have better anti-proliferative activities than OQMAP tagged with cyano acetamide moiety 9b. Moreover, combining both chloroquinoline and chromene moieties in one molecule through methyleneaminophenylaminocarbonyl spacer resulted in excellent anticancer activity (compound 10) (Fig. 3a).

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 anti-proliferative activity. Transforming the CQMAP and OQMAP tagged with cyano acetamide derivatives 9a-b to their corresponding CQMAP and OQMAP derivatives 11a-b, respectively, do not improve their anti-proliferative activity (Fig. 3a).

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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.

Apoptosis detection studies

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

Effect on mitochondrial apoptosis pathway proteins BAX and Bcl-2  It was reported that the overexpression of BAX (pro-apoptotic) stimulates apoptosis in cancer cells [45]. The effect of the target quinoline derivatives 3e, 4b, 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 that are not treated with the target quinoline compounds. The Bcl-2 (anti-apoptotic) levels in the mitochondrial membrane must decrease if BAX levels in the mitochondria are to rise. The most active analogues 3e, 4, 11b and 13d, were evaluated for their effect on some apoptosis key markers, BAX and Bcl-2 (Table 2).

Pim-1-kinase detection

(Pim-1) is a serine/threonine kinase that 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 integrity with mitochondrial. 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 [27, 46].

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-Casp-3 axis. In this way, it is possible to minimize apoptosis [46].

This article aims to update Pim-1 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 IC\(_{50}\) were measured. The most potent Pim-1 inhibitors were compounds \(3e\), \(4b\) and \(11b\) with IC\(_{50}\) of 0.11–0.92 \(\mu\)M, and \(13d\) was with less effective activity (IC\(_{50}\) = 3.24 \(\mu\)M). The positive control...
The absorption rate in the chosen compounds varies from 63.82% to 81.78%. Some compounds in water with a good lipophilicity and water solubility. A suitable physiochemical 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, 48].

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 refractivity are moderately soluble and are moderate to goodly absorbent with a median of 81.78% and 75.15% of reference and synthesized drugs, respectively.

The pharmacokinetic/ADME attributes of the derivatives investigated are given in Table 5. All the test compounds except for molecule 11b have shown that they are easily absorbed gastrointestinal (GI) and are non-inhibitors P-gp (P-glycoprotein). Except for 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).

The assorted laws regulating drug-likeness, i.e. Lipinski et al. [49], Ghose et al. [50], Veber et al. [51], Egan and Lauri [52], and Muegge et al. [53] had to analyse the molecule as an important candidate for drugs. The number of violations of the rules previously called along with their bioavailability scores is 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 one 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 Fig. 5.

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 (Å²), 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.

### 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-likeness, pharmacodynamic and pharmacokinetic properties, respectively. The analysed compounds are prospective drug-like candidates according to the outcomes presented. In future work, we can study the in vivo physicochemical drug-likeness, pharmacodynamic, and pharmacokinetic properties.

### Experiments

#### 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. $^1$H NMR and

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

| Cpd. no. | Pim-1 (Pim-1S (33 kDa)) kinase % inh. (Conc. 10 µg) | 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    |

### 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$_c$ | iLog $P_d$ | Log.S$_e$ | TPSAf | 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       |

$a$The ratio of sp$^3$ hybridized carbons over the total carbon count of the molecule  
$^b$Number of hydrogen bond acceptors  
$^c$Number of hydrogen bond donors  
$^d$Lipophilicity  
$^e$Water solubility (SILICOS-IT; $PS$ poorly soluble, $MS$ moderately soluble, $S$ soluble)  
$^f$Topological polar surface area ($Å^2$)

### 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 Yes | −5.90 |
| 11b      | Low No No Yes Yes No Yes No Yes | −6.41 |
| 13d      | High No Yes Yes Yes Yes Yes No | −5.51 |
| DOX      | Low No Yes Yes Yes Yes Yes No | −8.71 |

$^a$Gastro Intestinal absorption  
$^b$Blood–brain barrier permeant  
$^c$P-glycoprotein substrate  
$^d$CYP1A2: Cytochrome P450 family 1 subfamily A member 2 (PDB:2HI4)  
$^e$CYP2C19: Cytochrome P450 family 2 subfamily C member 19 (PDB:4GQS)  
$^f$CYP2C9: Cytochrome P450 family 2 subfamily C member 9 (PDB:1OG2)  
$^g$CYP2D6: Cytochrome P450 family 2 subfamily D member 6 (PDB:5TFT)  
$^h$CYP3A4: Cytochrome P450 family 3 subfamily A member 4 (PDB:4K9T)  
$^i$Skin permeation in cm/s
13C NMR spectra were measured on a Bruker AC 500 (500 MHz) in DMSO-d6 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 Micro-analytical 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 ±0.4% of calculated value.

2-Chloroquinoline-3-carbaldehyde (1a) and 2-oxo-1,2-dihydroquinoline-3-carbaldehyde (1b) were prepared according to a reported method [39, 42].

Mp. for 1a 135 °C, and for 1b 290 °C.

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

A mixture of 2-substituted-quinoline-3-carbaldehyde 1a or b (1.91 g, or 1.73 g, 0.01 mol) and aryl cyanoacetanilides 2a–c (0.01 mol) in ethanol (20 mL), pipridine 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-toly)acrylamide (3b) Yellow crystals; Yield (2.37 g, 68%); mp. 270–272 °C; IR (KBr, cm⁻¹): 3316 (OH-br.), 3116 (NH), 3000 (CH-arom.), 2944 (CH-aliph.), 2217 (C≡N), 1658 (C=O), 1555 (CH=N); 1H NMR (DMSO-d6) δ 6.72, 6.92 (2d, 4H, J = 6 Hz, Ar-H), 7.50, 7.56 (2t, 2H, J = 7 Hz, 6H,7H-quinoline), 7.59, 7.97 (2d, 2H, J = 6 Hz, 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-d6): 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. Caled. for C18H12ClN3O2 (347.57): C, 65.24; H, 3.46; N, 12.01; Found: C, 65.23; H, 3.38; N, 12.10.

3-(2-Chloroquinolin-3-yl)-2-cyano-N-(4-hydroxyphenyl)acrylamide (3c) Orange crystals; Yield (2.52 g, 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-d6) δ 4.42 (s, J = 7 Hz, 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-d6): 36.91, 107.85, 108.21, 111.03, 115.86, 118.79, 123.30, 124.63, 130.01, 133.50, 140.22, 141.26, 145.43, 152.01, 160.80; Anal. Caled. for C19H16ClN3O2 (337.76): C, 64.01; H, 3.58; N, 12.44; Found: C, 64.23; H, 3.81; N, 12.15.

3-(2-Chloroquinolin-3-yl)-2-cyano-N-(furan-2-yl)methyl)acrylamide (3d) Yellow crystals; Yield (2.23 g, 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-d6) 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 = 5 Hz, 6H,7H-quinoline), 7.82, 7.84 (2d, 2H, J = 5.5 Hz, 5H,8H-quinoline), 8.40 (s, 1H, methine-H), 8.79 (s, 1H, 4H-quinoline), 10.43, 12.35 (2s, 2H, 2NH;
Cancelled with D$_2$O; $^{13}$C 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 C$_{20}$H$_{15}$N$_3$O$_2$ (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 (2.64 g, 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 (C=O), 1618 (CH=C); $^1$H NMR (DMSO-$d_6$) $\delta$ 6.77, 7.36 (2d, 4H, $J = 6$ Hz, Ar-H), 7.46,
5-Acetyl-4-(2-chloroquinolin-3-yl)-6-methyl-2-oxo-1-(p-tolyl)-1,2-dihydropyridine-3-carbonitrile (6a) Yellow crystals, Yield (2.56 g, 60%), mp. >320 °C; IR (KBr, cm⁻¹): 3043 (CH-aro.), 2948 (CH-aliph.), 2228 (C=O), 1730 (CH₃-C=O), 1664 (N=C=O); ¹H NMR (DMSO-d₆): δ 2.45 (s, 3H, CH₃), 7.26, 7.39 (2d, J = 5.5 Hz, 4-tolyl), 7.61, 7.83 (2t, 2H, J = 5.5 Hz, 6H,7H-quinoline), 7.66, 8.17 (2d, 2H, J = 6 Hz, 5H,8H-quinoline), 9.00 (s, 1H, 4H-quinoline), 9.10 (s, 1H, 4H-pyridine); ¹³C NMR (DMSO-d₆): 21.36, 107.98, 115.22, 115.91, 125.00, 126.44, 128.27, 129.77, 131.00, 132.00, 135.15, 140.28, 147.00, 148.22, 149.08, 160.02; Anal. Calcd. for C₂₃H₁₉ClN₃O₃ (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)-6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6b) Yellow crystals, Yield (2.87 g, 69%), mp. 280–282 °C; IR (KBr, cm⁻¹): 3039 (CH-aro.), 2229 (C=O), 1732 (CH₃-C=O), 1665 (N=C=O); ¹H NMR (DMSO-d₆): δ 2.74, 2.90 (2s, 6H, 2CH₃), 5.71 (s, 2H, CH₂), 6.37–6.44 (m, 3H, furyl), 7.54–8.99 (m, 4H, Ar-H), 9.04 (s, 1H, 4H-quinoline); ¹³C NMR (DMSO-d₆): 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 C₂₃H₁₉ClN₃O₃ (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-tetrahydropyran-[2,3-c]pyrazole-5-carboxamide (7) 5-Methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (1.74 g, 0.01 mol) was added to a solution of 3a (3.47 g, 0.01 mol) and piperidine (0.01 mol) in ethanol (20 mL) and the reaction mixture was heated under reflux for 8 h. The solid formed was filtered, washed with ethanol, dried and recrystallized from a mixture of EtOH/DMF.
N-(4-(2-Substitutedquinolin-3-yl)methylene)amino)cyanoacetanilide (9a,b)

The aldehyde derivative 1a or b (1.91 g, or 1.73 g, 0.01 mol) was added to a solution of p-aminocyanaceta-
nilide 8 (1.75 g, 0.01 mol) in ethanol (20 mL) containing acetic acid (5 mL). The mixture was heated under reflux for 1 h 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)cyanoacetanilide (9a)
Brown crystals, Yield: (2.01 g, 60%), mp. 270–272 °C; IR (KBr, cm⁻¹): 3280 (NH), 2261 (C=C=O), 1658 (N–C=O), 1596 (CH=N); ¹H NMR (DMSO-d₆) δ 3.85 (s, 2H, CH₂), 7.48–8.20 (m, 8H, Ar-H), 8.50 (s, 1H, CH=NH), 9.03 (s, 1H, 4H-quinoline), 10.29 (s, 1H, NH; Cancellled with D₂O); MS m/z (%): 350 (M⁺, 5.95), 348 (M⁺–CH₃, 13.01), 340 (5.80), 313 (50.68), 70 (17.37), 65 (16.80), 57 (100.00); Anal. Calcd. for C₁₉H₁₃ClN₅O₂ (348.79): C, 65.43; H, 3.76; N, 16.06; Found: C, 65.23; H, 3.55; N, 16.35.

N-(4-(2-Oxobenzaldehyde)amino)cyanoacetanilide (9b)
Red crystal; Yield: (2.10 g, 71%); mp. 290–292 °C; IR (KBr, cm⁻¹): 3270, 3157 (NH), 3007 (CH-arom.), 2949 (CH-aliph.), 2212 (C=C=O), 1656 (N–C=O), 1552 (CH=N); ¹H NMR (DMSO-d₆) δ 3.90 (s, 2H, CH₂), 7.00–8.20 (m, 8H, Ar-H), 8.50 (s, 1H, CH=NH), 9.00 (s, 1H, 4H-quinoline), 10.20, 10.40 (2s, 2H, 2NH; Cancellled with D₂O); ¹³C NMR (DMSO-d₆): 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-cyano-3-(1H-2-dihydroquinolin-3-yl)acrylamide (11a)
Pale green crystal; Yield: (3.26 g, 65%); mp. 260–262 °C; IR (KBr, cm⁻¹): 3317, 3165 (NH), 3024 (CH, arom.), 2950 (CH, aliph.), 2213 (C=C=O), 1659 (C=O), 1615 (CH=N); ¹H NMR (DMSO-d₆) δ 7.23 (t, 1H, Ar-H), 7.28 (d, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 7.58 (t, 1H, Ar-H), 7.65 (d, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 8.42–8.67 (m, 5H, 4Ar-H & CH=N), 8.83 (s, 1H, methine-H), 9.10 (s, 1H, 4H-quinoline), (10.20, 10.80 (2s, 2H, 2NH; Cancellled with D₂O); ¹³C NMR (DMSO-d₆): 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, 147.92, 160.80, 161.91, 162.04; Anal. Calcd. for C₂₀H₁₇ClN₅O₂ (503.95): C, 69.12; H, 3.60; N, 13.90; Found: C, 69.23; H, 3.44; N, 13.85.

N-(4-((2-Chloroquinolin-3-yl)methylene)amino)phenyl)-2-cyano-3-(1H-2-dihydroquinolin-3-yl)acrylamide (11b)
Pale green crystal; Yield: (3.80 g, 80%); mp. 320 °C; IR (KBr, cm⁻¹): 3346, 3157, 3102 (3NH), 3038 (CH-arom.), 2999 (CH-aliph.), 2218 (C=C=O), 1649 (C=O), 1534 (CH=N); ¹H NMR (DMSO-d₆) δ 7.24 (t, 1H, Ar-H), 7.30 (d, 2H, Ar-H), 7.38 (d, 2H, Ar-H), 7.60 (t, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.92 (d, 1H, Ar-H), 8.34–8.84 (m, 5H, 4Ar-H & CH=N), 8.81 (s, 1H, methine-H), 8.82 (s, 1H, 4H-quinoline), 8.84 (s, 1H, 4H-quinoline),
3-(2-Chloroquinolin-3-yl)-2-cyano-4-(4-methoxybenzylideneamino)phenyl)-2-cyanoacrylamide (13a)

Yield (3.90 g, 85%); mp. 247 °C; IR (KBr, cm−1): 3344, 3320 (2NH), 3060 (CH-arom.), 2982 (CH-aliph.), 1682, 1668 1665 1645 (C=O), 1591 (CH=O, 1H NMR (DMSO-d6) δ 3.88 (s, 3H, OCH3), 6.55 (t, 1H, Ar-H), 7.48 (d, 2H, Ar-H), 7.80 (d, 1H, Ar-H), 8.10 (d, 1H, Ar-H), 8.14–8.79 (m, 4H, Ar-H), 8.79, 8.83 (2s, 2H, 2-methine-H), 8.84 (s, 1H, 4H-quinoline), 10.23, 10.56 (2s, 2H, NH; C cancelled with D2O); 13C NMR (DMSO-d6): 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)-3-(2-chloro-1,2-dihydroquinolin-3-yl)-2-cyanoacrylamide (13d)

Yield (3.16 g, 70%); mp. >320 °C; IR (KBr, cm−1): 3344, 3320 (2NH), 3060 (CH-arom.), 2982 (CH-aliph.), 1682, 1668 1665 1645 (C=O), 1591 (CH=O, 1H NMR (DMSO-d6) δ 7.25 (t, 1H, Ar-H), 7.40 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 7.80 (d, 1H, Ar-H), 8.10 (d, 1H, Ar-H), 8.14–8.79 (m, 4H, Ar-H), 8.79, 8.83 (2s, 2H, 2-methine-H), 8.84 (s, 1H, 4H-quinoline), 10.23, 10.56 (2s, 2H, NH; C cancelled with D2O); 13C NMR (DMSO-d6): 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.

Antitumor activity of compounds against cancer cells

Maintenance of cancer cells in culture

Mammary gland breast cancer cell line (MCF-7), human intestinal cancer cells (Caco-2), human hepatocellular carcinoma cell line (Hep G-2), and breast cancer cell line (HCT116) 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. FBS 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) [36]. 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).

Cytotoxicity screening  The cells were cultured in RPMI-1640 medium with 10% FBS. 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 1.0 × 104 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% TCA 150 μL/well for 1 h at 4 °C. Wash by water 3 times (TCA reduce SRB protein binding). The wells were 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: colourless drainage). The plates will be air dried 24 h. The dye will be solubilized with 50 μL/well of 10 mM trias 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) × 100 and The IC50 values will be calculated using sigmoidal concentration response curve fitting models (Sigmaplot software) [36].

Apoptosis detection studies

Determination of Caspase-3 The HepG-2 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 h before the enzymes test) [35].

Determination of BAX and Bcl-2 Cells HepG-2 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 μL of the compounds tested in a 96-well platform 24 h before human BAX/Bcl-2 measurement.) [36].

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 1 mM 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 min, and add 5 μL of ADP-Glo™. Incubate at room temperature for 40 min, add 10 μL of Kinase Detection Reagent and incubate at room temperature for 30 min. Record luminescence (integration time 0.5–1 s) [54].

Method of computation

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 [28,55–57].

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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