Design, synthesis, biological evaluation, and molecular docking of novel quinazolinone EGFR inhibitors as targeted anticancer agents

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ABSTRACT

A novel series of 2-methyl-3-phenylquinazolin-4-one derivatives were synthesized and biologically evaluated for their cytotoxic potential against MCF-7, HepG2, and PC-3 cancer cells. Most of the tested compounds showed reasonable safety in the normal human skin melanocyte HFB4 cell line. Compound 4 showed potent cytotoxicity on Hep-G2 cell lines, while compound 9 showed potent cytotoxicity on the MCF-7 cell line, whereas compounds 10 and 12 showed potent cytotoxicity on Hep-G2 cell lines using 5-fluorouracil as a reference standard. Cell division analysis on the tested cell lines revealed that compounds 4, 9, 10, and 12 have potent antiproliferative properties. An \textit{in vitro} enzymatic inhibition assay against EGFR-TK confirmed that those compounds have potent EGFR inhibitory activity. The target compounds arrested the cell cycle at the pre-G1 and G2/M phases. Molecular docking simulations showed that all the target compounds possess a common binding pattern like that of Erlotinib.

GRAPHICAL ABSTRACT

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**Introduction**

Chemotherapy is one of the most regularly used cancer therapies. Despite the plethora of anticancer medicines, traditional chemotherapy has significant limitations.\(^1\) The fundamental disadvantage of classical cytotoxic drugs is their inability to distinguish between malignant and normal cells, which results in significant side effects.\(^2\) As a result, researchers are working hard to develop novel anticancer treatments that target cancer-specific molecules that have been changed by overexpression or mutation.

The epidermal growth factor receptor (EGFR) is a well-known tyrosine kinase that initiates several signaling pathways that control a variety of events in the cell cycle, proliferation, differentiation, migration, apoptosis, and gene transcription.\(^3,4\) EGFR signaling pathways have been thoroughly studied for their remarkable role in the progression of several types of malignant tumors. Thus, the development of small-molecule drugs targeting EGFR is a well-established strategy for the design of novel targeted anticancer agents.\(^5\)

Quinazoline is a privileged scaffold exhibiting several pharmacological activities, in particular, anticancer activity.\(^6\) Furthermore, quinazoline derivatives represent the main class of promising targeted anticancer agents targeting tyrosine kinases, especially EGFR. Thus, after the FDA approved the\(^7\) quinazoline derivative, Gefitinib, for the treatment of non-small cell lung cancer in 2003, several quinazoline derivatives were approved as EGFR inhibitors for cancer treatment, such as Erlotinib, Vandetanib, lapatinib, and Dacomitinib (Fig. 1), either alone or in combination with other anticancer drugs.\(^7\)

![Figure 1](image)

**Figure 1.** The FDA approved drugs as EGFR inhibitors based on quinazoline nucleus and the quinazoline derivatives that were approved to inhibit EGFR.
Thenceforth, quinazolines and quinazolinones have drawn the focus of researchers, which led to the discovery of many novel quinazoline derivatives with potent cytotoxic efficacy through inhibition of the EGFR tyrosine kinases. Quinazoline and quinazolnone compounds are synthesized using a variety of synthetic methodologies. Kostakis et al. developed an effective three-step reaction from anthranilic acid for the synthesis of a variety of 2,3-disubstituted-quinazolin-4-(3H)-ones (Scheme 1).[8] In another attempt, Kshirsagar et al. described a novel, simple, and general method to synthesize natural and unnatural quinazolinones in high yields from the reactions of isatoic anhydride/sulfinamide anhydride with several different amines.[9]

The quinazolinone derivative I showed a comparable affinity toward the inhibition of the EGFR to Erlotinib, exhibiting potent anticancer activity against breast cancer cell lines.[10] Furthermore, 7-chloroquinazolinone derivative II displayed selective inhibition against wild-type EGFR and possessed more potent cytotoxic efficacy than the reference drug, Gefitinib, against the NCI-H1975 cell line.[11] Recently, compound III displayed a good inhibitory effect against EGFR and proved to be more active and safer than the reference drugs Erlotinib and Gefitinib. It also showed a remarked apoptotic activity toward many cancer cell lines (Fig. 1).[12]

These findings and a comprehensive review of the rationale for the EGFR-TKS inhibitors prompt us to synthesize some new quinazolinones hybridized with different heterocyclic or aromatic moieties using different chemical synthetic approaches. We evaluate the anticancer potential of these novel derivatives, hoping to obtain more potent and selective anticancer agents.

**Results and discussion**

**Chemistry**

The starting compound 1 was successfully obtained by refluxing the initially prepared 3-(4-acetylphenyl)-2-methylquinazolin-4-one[13] in an excess dimethylformamide-dimethyl acetal (DMF-DMA). The $^1$HNMR spectrum of compound 1 revealed singlet signals owing to the protons of dimethyl-imino group at $\delta = 2.95$, 3.19 ppm, and two doublet signals at $\delta = 5.71$, 7.85 ppm confirming the formation of the CH = CH bond. Also, its mass spectrum exhibited a molecular ion peak at $m/z=333$ (M$^+$, 46%). Treatment of compound 1 with 3-fluoroaniline in ethanol/acetic acid mixture at room temperature yielded compound 2 while refluxing of compound 1 with 4-aminopyridine and 2-aminothiazole yielded compounds 3 and 4, respectively. The IR spectra of compounds 2, 3, and 4 displayed the absorption bands expected for (NH) group at the range of 3214–3424 cm$^{-1}$, while their $^1$HNMR spectra confirmed the disappearance of

![Scheme 1](image-url)

**Scheme 1.** Reagents and conditions: (a) (R1 = Me): acetic anhydride, MW (200 W), 130°C, 10 min; (R1 = Et): propionic anhydride, MW (200 W), 160°C, 10 min; (b) aliphatic amine (R2−NH$_2$) (2 equiv.), CH$_2$Cl$_2$, rt, 10–40 min; (c) formamide, MW (200 W), 170°C, 10 min.
the two methyl groups of the dimethyl-imino groups. On the other hand, the nucleophilic substitution reactions involved the attack of the amino group at the enamine carbon was accomplished by reactions of compound 1 with an excess amount of the appropriate secondary amine, namely, morpholine, piperidine, and N-methyl piperazine under reflux to obtain derivatives 5–7 (Scheme 2). The spectroscopic data and elemental analysis confirmed the formation of the new compounds (see Supporting Information).

On the other hand, Pyrazolyl derivatives 8 and 9 were synthesized by reacting compound 1 with hydrazine hydrate and phenyl hydrazine, respectively. However, the condensation reaction of compound 1 with hydroxyl amine hydrochloride in ethanolic sodium acetate solution afforded the oxazolyl derivative 10, while pyrimidinyl derivative 11 was successfully obtained on reaction with urea. Finally, the target compound 12 was obtained by refluxing a solution of compound 1 with barbituric acid in acetic acid (Scheme 3).

The $^1$H NMR spectrum of compound 8 revealed the existence of the two doublet peaks owing to the CH=CH at $\delta = 6.57$ and 7.97 ppm. NH group also has a peak at $\delta = 12.08$ ppm (see Supporting Information). The pyrazole ring of compound 9 was elucidated by the two doublet peaks in its $^1$H NMR at $\delta = 6.84$ and 8.01 ppm, while its three carbons appeared at 105.42, 129.20, and 151.77 ppm in the $^{13}$C NMR spectrum.

Scheme 2. (i) DMF/DMA, reflux, 0.5 h; (ii) 3-fluoroaniline, rt, 2 h, AcOH/EtOH; (iii) 4-aminopyridine, reflux, 5 h, gla.ACOH; (iv) 2-aminothiazole, reflux, 16 h, dioxane; (v) appropriate cyclic amine, reflux, 4–6 h.
In addition, compound 10 revealed the existence of the two doublet peaks in its $^1$H NMR at $= 6.74$ and $8.53$ ppm, representing the oxazole ring, in addition to the molecular ion peak at $m/z = 303$ (M$^+$, 100%) in its mass spectrum. Furthermore, the IR spectrum of compound 11 showed a broad band at $3437$ cm$^{-1}$ deducting the cyclization of the parent compound with urea to give the 2-hydroxypyrimidine ring rather than the keto-form. Finally, the IR spectrum of compound 12 showed three bands representing the three carbonyl groups at $1869$, $1839$, and $1679$ cm$^{-1}$ in addition to the presence of molecular ion peak in the mass spectrum of the appointed compound at $m/z = 401$ (M$^+$, 60%).

The proposed mechanism of cyclization for the synthesized compounds (8–12) is postulated as shown in Scheme 4.$^{[14]}$

**Biological evaluation**

**In vitro cytotoxic activity**

The anti-proliferative activities of the newly synthesized compounds were *in vitro* evaluated against MCF-7, HepG2, and PC-3 cancer cell lines as well as the normal cell line

\[
\text{Scheme 3. (i) } \text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O, reflux, 6 h, EtOH; (ii) phenyl hydrazine, reflux, 6 h, EtOH; (iii) } \text{NH}_2\text{OH}\cdot\text{HCl, reflux, 5 h, AcONa, EtOH; (iv) } \text{NH}_2\text{CONH}_2, \text{reflux, 4 h, gla.AcOH; (v) barbituric acid, reflux, 5 h, gla.AcOH.}
\]
human normal melanocyte (HFB4) by MTT assay. 5-Fluorouracil was used as a standard chemotherapeutic drug. The results indicated that most of the tested compounds exhibited no activity against the growth of HFB4. However, most of the compounds showed a remarkable significant reduction in the cell viability of at least one of the three cell lines, MCF-7, HepG2, and PC-3. The effect of the synthesized derivatives on the tested cell lines is illustrated in Figures 2a–c.

The fast screening was performed to determine the derivatives which showed a significant reduction in the cell viabilities against the three cell lines used (Fig. 3). MTT assay was performed to determine the IC50 of all compounds. MTT can produce false positive or negative results due to the interference of the investigated compound with the mitochondrial enzymes without affecting the cell viability.

**IC50 determination**

The obtained viabilities were then used to plot the dose-dependent response (Figs. 3a–c) from which, the IC50 was carried out to determine the cytotoxic potential in the three cell lines for all compounds using 5-Fluorouracil (5-FU) as a reference drug. The results showed that compound 4 is the most active compound compared to the reference drug in the three cell lines with IC50 of 4.65 to 6.2 (μM). In addition, other compounds possessed good cytotoxic effect against one at least of the tested cell lines. However, compound 9 displayed anticancer selectivity toward MCF-7 cell line at (IC50 = 5.43 μM) while it has no effect on the other two cell lines. Compounds 10 and 12 showed good cytotoxicity against the HepG2 cell line at (IC50 = 6.1 and 6.04 μM), respectively (Table 1) (C.f. supplementary content section of this article’s webpage).

**Cell division analysis**

Aiming to study the effect of the most active compounds on the cell cycle progression as well as their apoptotic tendency, compounds 4, 9, 10, and 12 underwent cell division analysis on the cell lines which demonstrated the most potency on utilizing CFSE assay. The effect of compounds 4 and 9 on MCF-7 cell division was depicted in Figure 4 where, the IC50 of compound 4, showed a slight reduction in the cell division from 6 to 12%. On doubling the concentration; the cell division was further reduced to 24%.
Compound 9, showed an IC50 reduction in the cell division from 6 to 14% and by doubling the concentration, the cell division was further reduced to 26%.

This fluctuation in curves in Figure 4 can be due to the different effects of the newly synthesized compounds in different concentrations. We planned to add error bars, but the figure seems not good presenting. For your consideration, an example was shown below.

Furthermore, we record the effect of compounds 4, 10, and 12 on HepG2 cell division (Fig. 5) where, HepG2 cell division was slightly affected by treating with compounds 4, 10, and 12. Compound 4, showed a slight reduction in the cell division at

Figure 2. Cell viability of the three cell lines after 48 h treatment with the newly synthesized derivatives, (a) MCF-7; (b) HepG2; (c) PC-3.
IC$_{50}$ from 6 to 10% and by doubling the concentration, the cell division was further reduced to 22%. For compound 10, showed a reduction in the cell division at IC$_{50}$ from 6 to 12%, while doubling the concentration causes a further reduction to 24%. On other hand, compound 12 at IC$_{50}$ showed a reduction in the cell division from 6 to 14% and by doubling the concentration the cell division was further reduced to 26%.

PC-3 cell division was slightly affected by treating with compounds 4 and 10, where, compound 4, showed a slight reduction in the cell division from 6 to 11% at IC$_{50}$ and by doubling the concentration, the cell division was further reduced to 23%. For compound 10, IC$_{50}$ showed a reduction in the cell division from 6 to 13% and by doubling the concentration, the cell division was further reduced to 25%. The effect of compounds 4 and 10 on PC-3 cell division is shown in Figure 6.

**Figure 3.** The fast screening was performed to determine the derivatives which showed a significant reduction in the cell viabilities against the three cell lines used (Fig. 3a-c).
Flow cytometry: Cell cycle analysis

Cell cycle analysis was performed in MCF-7 human breast cancer cell line treated with compounds 4 and 9. The percentage of cells of MCF-7 cell line in G0/G1 phase of the cell cycle in the control was 55.51%, which recorded a noteworthy decrease to 24.23%, while, upon treatment with compound 4, the percentage of cells in the S phase was slightly reduced up to 33.29% compared to the control 39.22%. The percentage of
MCF-7 human breast cancer cell line at the G2/M phase was apparently increased to 43.78% upon treatment with compound 4 compared to the control (9.67%) (Fig. 7).

Furthermore, it is observable that the apoptotic cell percentage in the Pre-G1 phase was increased from 2.73% for control untreated MCF-7 human breast cancer cells to 34.19 and
Table 1. IC50 values of the prepared compounds against MCF-7, HepG2, PC-3, and normal HFB4 cell lines.

| Compound | MCF-7       | HEPG-2      | PC-3        | HFB4       |
|----------|-------------|-------------|-------------|------------|
| Control  | 34.65 ± 5.43| 32.86 ± 0.11| 36.6 ± 5.24 | 27.59 ± 1.80|
| 1        | 37.5 ± 0.35 | 33.02 ± 0.40| 32.86 ± 0.11| 35.03 ± 2.14|
| 2        | 14.87 ± 1.15| 12.16 ± 2.23| 16.44 ± 1.74| 37.35 ± 1.76|
| 3        | 15.61 ± 1.86| 17.60 ± 0.02| 22.24 ± 5.87| 30.91 ± 2.06|
| 4        | 4.65 ± 0.83 | 5.77 ± 0.99 | 6.20 ± 1.08 | 31.97 ± 2.30|
| 5        | 23.05 ± 1.17| 32.86 ± 0.11| 40.43 ± 1.10| 61.94 ± 3.4 |
| 6        | 19.02 ± 0.20| 15.61 ± 1.86| 33.12 ± 2.01| 44.63 ± 2.20|
| 7        | 24.21 ± 2.10| 31.44 ± 2.28| 29.80 ± 1.73| 41.08 ± 2.35|
| 8        | 35.59 ± 3.10| 45.19 ± 1.31| 36.30 ± 10.68| 38.55 ± 2.80|
| 9        | 5.44 ± 1.05 | 43.42 ± 2.32| 36.37 ± 6.87| 119.55 ± 6.02|
| 10       | 23.05 ± 1.17| 6.10 ± 11.28| 23.61 ± 2.79| 113.76 ± 7.54|
| 11       | 29.92 ± 1.22| 30.17 ± 1.19| 31.74 ± 1.34| 122.89 ± 8.77|
| 12       | 25.15 ± 6.26| 6.04 ± 0.43 | 28.38 ± 1.64| 135.21 ± 8.30|
| 5-FU     | 3.97 ± 0.10 | 4.27 ± 0.58 | 5.05 ± 0.66 | 153.01 ± 10.21|

MTT assay results of HFB4 normal cell line will be added to figures and in Table 1 added to the Table 1.

Figure 4. Proliferation inhibition potential of compounds 4 and 9 on the MCF-7 cell line (CFSE, 48 h).

Figure 5. Proliferation inhibition potential of compounds 4, 10, and 12 on the HepG2 cell line (CFSE, 48 h).
22.17% in cells striated with the compounds 4 and 5-FU, respectively. According to the above results, it is clear that compound 4 exhibited mainly cell cycle arrest at the Pre-G1 and G2/M phases. Moreover, it is obvious that compound 4 is not only cytotoxic but anti-proliferative causing programmed cell death and cell cycle arrest (Figs. 8a–c).

**EGFR-TK inhibitory assay**

In an attempt to study the mechanism of action of the most active new quinazoline derivatives, compounds 4, 9, 10, and 12, were subjected to EGFR inhibitory assay. The tested compounds displayed moderate EGFR inhibitory activity with IC$_{50}$ ranging from 18.29 to 55.52 nM (Table 2) (C.f. supplementary content section of this article’s webpage).

However, compound 4, the most active derivative in the cytotoxicity assay also exhibited potent activity against all the tested cell lines (IC$_{50}$ = 18.29 nM). Despite of their higher IC$_{50}$ against EGFR enzyme than the reference drug Erlotinib, the tested compounds inhibitory concentration was relative to their cytotoxic efficacy.

**Structure–activity relationship (SARs)**

The amine derivatives 2–4 showed the most prominent activity in the synthesized compounds. Both the fluorophenyl-and pyridinyl derivatives 2,3 exhibited moderate cytotoxic activity against the MCF-7, HepG2, and PC-3 cancer cell lines, while the amino-thiazole
derivative 4 was the most potent compound in the synthesized series. Contrary to that, the cyclic amine derivatives 5–7 showed low or no antitumor activity against the tested cancer cell lines. Although the cyclized pyrazolyl compound 8 was inactive, the insertion of the phenyl ring through the compound 9 synthesis resulted in selective cytotoxic activity against the MCF-7 cancer cell line, while the oxazolyl compound 10 showed selectivity against the HepG2 cancer cell line and low activity toward the two other cell lines. Similarly, the pyranopyrazole derivative 12 was also selectively cytotoxic against the HepG2 cancer cell line. The hydroxypyrimidinyl compound 11 showed almost no cytotoxic activity. In conclusion, the derivatives with the flexible acrolyl side chain displayed

Figure 8. Percentage of apoptosis and necrosis for the most active compounds and control in the three tested cell lines (a) compounds 4 and 9 on MCF-7 (b) compounds 4, 10, and 12 on HEPG-2 (c) compounds 4 and 10 on PC-3.
better anticancer activity, as a result of the amino-thiazole compound 4. When the amino group is incorporated into the ring, the biological activity drops drastically. The more rigid cyclized side chains exhibited lower cytotoxicity as in the compound 9 results.

**Molecular docking**

Molecular docking simulations were carried out for the target compounds to investigate their binding mode in the EGFR active site. The molecular docking protocol was first validated by self-docking of the co-crystallized ligand (Erlotinib) in the vicinity of the active site of the target protein giving an energy score (S) of $-10.89 \text{kcal/mol}$ and an RMSD of 1.47 Å. Moreover, it reproduced all the interactions achieved by Erlotinib with the key amino acids in the active site; H-bonding with Met769, water-mediated H-bonding with Thr766, and cation-π interaction with Lys721 (Figs. 9 and 10a,b).

Generally, the target compounds showed a common binding pattern similar to that of the co-crystalized ligand (Erlotinib) with their quinazolinone ring aligned to the quinazoline ring of Erlotinib achieving an H-bond interaction with the key amino acid Met769. Through hydrophobic interaction, the phenyl ring in position 3 of the quinazolinone ring interacts with the hydrophobic side chains of amino acids Val702 and Leu820. Moreover, by their side chain extending from position 3 of the quinazolinone ring, they interact through H-bonding with the key amino acids Lys721 and/or Asp831.

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**Table 2.** IC₅₀ values for the inhibitory activity of compounds 4, 9, 10, and 12 against EGFR.

| Compound | EGFR IC₅₀ (nM) |
|----------|---------------|
| 4        | 18.29         |
| 9        | 20.53         |
| 10       | 25.59         |
| 12       | 55.52         |
| Erlotinib| 3.183         |

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**Figure 9.** 2D interaction diagram showing Erlotinib docking pose interactions with the key amino acids in the EGFR binding site.
The capability of the target compounds to interact with the key amino acids in the active site rationalizes their promising activity as indicated by their binding pattern and docking score (−9.81 to −12.80 kcal/mol) compared to that of Erlotinib (−10.89 kcal/mol) (Figs. 11a,b) (Table 3) (C.f. supplementary content section of this article’s webpage).

Table 3. Docking energy scores (S) in kcal/mol of target compounds 1–12, and Erlotinib in EGFR active site, amino acids involved in binding.

| Compound | Energy score (S) kcal/mol | Amino acids involved in binding                  |
|----------|---------------------------|-------------------------------------------------|
| 1        | −11.72                    | Met769 and Lys721                               |
| 2        | −9.81                     | Met769 and Lys721                               |
| 3        | −10.57                    | Met769 and Asp831                               |
| 4        | −12.22                    | Met769 and Asp831                               |
| 5        | −11.69                    | Met769                                          |
| 6        | −11.06                    | Met769                                          |
| 7        | −12.80                    | Met769 and Asp831                               |
| 8        | −10.88                    | Met769, Lys721 and Asp831                       |
| 9        | −11.66                    | Met769                                          |
| 10       | −10.91                    | Met769 and Lys721                               |
| 11       | −12.14                    | Met769, Lys721 and Asp831                       |
| 12       | −10.54                    | Met769                                          |
| Erlotinib| −10.89                    | Met769 and Thr766                               |
Compound 4 (Fig. 11) showed the best experimental cytotoxic activity and EGFR inhibitory activity with IC₅₀ of 18.29 µM, and showed the second highest predicted binding affinity with a docking score of −12.22 kcal/mol. It exhibited a promising binding pattern interacting through hydrogen bonding with the key amino acid Met769 by its quinazolinone nitrogen at position 1 and with the amino acids Lys721 and Asp831 by its aminothiazole substituent extends at position 3 of the quinazolinone ring. Moreover, through hydrophobic interaction, the phenyl ring in position 3 of the quinazolinone ring interacts with the hydrophobic side chains of amino acids Val702, and Leu820.

**Conclusion**

In an attempt to find a potent and safer new anticancer agent among quinazoline derivatives based upon many approved drugs related to this moiety, we designed and synthesized novel quinazolinones linked to heterocyclic/aromatic structures aiming to target Tyrosine Kinase, EGFR, in a trial to inhibit cancer cell proliferation of MC-7, HepG2, and PC-3. Our new compounds proved their potential effect as cell proliferation inhibitors, especially compound 4, which exhibited mainly cell cycle arrest at the pre-G1 and G2/M phases. These results were supported by the tyrosine kinase assay and molecular docking simulations. All the target compounds showed a common binding pattern aligned to the quinazoline ring of Erlotinib.

**Experimental**

**Chemistry**

**Materials and methods**

All chemicals were provided by Aldrich. Elemental microanalyses were carried out at Micro Analytical Unit, Central Services Laboratory, National Research Center, Dokki, Giza, Egypt, using Vario Elementary and were found within ±0.4% of the theoretical values. All melting points were uncorrected and were taken in open capillary tubes using electrothermal apparatus 9100. FT-IR spectra were recorded with a Perkin-Elmer Frontier. Routine NMR spectra were recorded at room temperature on a Bruker Avance TM 500 MHz spectrometer as solutions in dimethyl sulfoxide (DMSO-d₆). All chemical shifts are quoted in δ relative to the trace resonance of protonated dimethyl sulfoxide (δ 2.50 ppm), DMSO (δ 39.51 ppm). The mass spectra were measured with a GC Finnegan MAT SSQ-7000 mass spectrometer. The reactions were followed using TLC on silica gel-percolated aluminum sheets (Type 60, F 254, Merck) and the spots were detected by exposure to a UV lamp at λ₂54 nm. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated. solvents were dried/purified according to conventional procedures.

3-(4-(3-(Dimethylamino)acryloyl)phenyl)-2-methylquinazolin-4(3H)-one (1). A solution of 3-(4-acetylphenyl)-2-methylquinazolin-4(3H)-one (2.78 g, 10 mmol) in N,N-dimethylformamide dimethyl acetal (3 ml) was refluxed for 1/2 h, and then cooled to room temperature. The formed solid was filtered, washed several times with ethyl ether, and dried. The resulting solid was crystallized from acetic acid.
Yield: 75% (2.5 g); m.p. 218–219°C. IR (KBr; cm⁻¹) 1682, 1639 (C=O). ¹H NMR (500 MHz, CDCl₃, δ ppm) 2.25 (s, 3H, CH₃), 2.95 (s, 3H, -NCH₃), 3.19 (s, 3H, -NCH₃), 5.72 (d, 1H, J = 12.32 Hz, CH-CO), 7.32 (d, 2H, J = 8.36 Hz, C₈H₂), 7.47 (t, 1H, J = 7.16 Hz, C₆H), 7.69 (d, 1H, J = 7.96 Hz, C₈H), 7.77 (t, 1H, J = 7.0 Hz, C₇H), 7.86 (d, 1H, J = 12.28 Hz, C₈H), 8.06 (d, 2H, J = 8.36 Hz, C₈H₂). ¹³C NMR (126 MHz, CDCl₃, δ ppm): 24.36 (CH₃), 37.40 (CH₃N), 45.19 (CH₃N), 92.09 (CH-CO), 120.73, 126.65, 126.87, 126.97, 127.97 (2CPh), 129.12 (2CPh), 134.61, 139.77, 141.54, 147.52, 153.91, 154.76 (CH-N), 162.11 (CO), 187.21 (CO). MS (EI, 70 eV) m/z (%): M⁺ 333.17 (45.95). Anal. Calcd for C₂₀H₁₉N₃O₂ (333.39) C, 72.05; H, 5.74; N, 12.60; Found: 72.07; H, 5.72; N, 12.61.

3-(4-(3-(3-Fluorophenyl)amino) acryloyl) phenyl-2-methylquinazolin-4(3H)-one (2). A mixture of compound 1 (0.33 g, 1 mmol) and 3-fluoroaniline (0.11 g, 1 mmol) in absolute ethanol/glacial acetic acid (4 ml) was stirred at room temperature for 2 h, then the solution was poured on ice water, filtered, washed several times with water and dried. The resulting solid was crystallized from ethanol.

Yield: 85% (0.34 g); m.p. 168–169°C. IR (KBr; cm⁻¹) 3214 (NH), 1656 (C=O), 1584 (C=N). ¹H NMR (500 MHz, CDCl₃, δ ppm) 2.30 (s, 3H, CH₃), 6.09 (d, 1H, J = 7.84 Hz, CH-CO), 6.81 (t, 1H, J = 8.32 Hz, C₆H), 6.86 (d, 1H, J = 10.24 Hz, C₇H₂), 6.90 (d, 1H, J = 8.08 Hz, C₈H), 7.32 (q, 1H, J = 8.08 Hz, C₅H), 7.39 (d, 2H, J = 7.82 Hz, C₆H₂), 7.52 (m, 2H, C₅H₂), 7.74 (d, 1H, J = 7.96 Hz, CH-NH), 7.80 (t, 1H, J = 7.12 Hz, CH-CO), 8.82 (d, 2H, J = 8.32 Hz, C₈H₂), 8.28 (d, 1H, J = 7.80 Hz, C₅H), 12.14 (s, 1H, NH). ¹³C NMR (126 MHz, CHCl₃, δ ppm) 24.26 (CH₃), 94.30 (CH-CO), 103.48 (d, JCF = 25 Hz, C₆F), 110.60 (d, JCF = 21 Hz, C₆F), 112.31 (d, JCF = 12 Hz, C₆F), 120.59, 126.73, 126.96 (d, JCF = 18 Hz, C₆F), 128.36 (2CPh), 129.07 (2CPh), 131.08, 131.17, 134.80, 139.82, 140.47, 141.72 (d, JCF = 10 Hz, C₆F), 145.07, 147.18, 151.80 (CH-N), 162.05 (CO). MS (EI, 70 eV) m/z (%): M⁺ 398.34 (100). Anal. Calcd for C₂₄H₁₈N₃O₂ (399.43) C, 72.17; H, 4.54; N, 10.52; Found: C, 72.21; H, 4.55; N, 10.50.

General procedure for the preparation of compounds (5–7)

A solution of compound 1 (0.33 g, 1 mmol) in 1 ml appropriate cyclic amine, namely, N-methyl piperazine, morpholine, and/or piperidine was heated under reflux for 4–6 h, then the solution was cooled and the excess solvent was evaporated then the formed solid mass treated ice water, washed several times with water then filtered and dried. The resulting solid was crystallized from ethanol.

Yield: 45% (0.18 g); m.p. 193°C decom. IR (KBr; cm⁻¹) 1682 (CO), 1592 (C=N). ¹H NMR (500 MHz, DMSO, δ ppm) 2.12 (s, 3H, N-CH₃), 2.22 (s, 3H, CH₃), 2.64 (t, 4H, 2CH₂), 3.04 (t, 4H, 2CH₂), 6.01 (d, 1H, J = 12.35 Hz, CH-CO), 7.17 (m, 3H, 2C₈H₂), 7.65 (d, 1H, J = 8.04 Hz, C₆H), 7.71 (d, 1H, J = 12.32 Hz, CH-N), 7.82 (t, 1H, J = 7.44 Hz, C₇H), 8.04 (d, 2H, J = 8.08 Hz, C₈H₂), 8.08 (d, 1H, J = 8.24 Hz, C₅H), ¹³C NMR (126 MHz, DMSO, δ ppm): 24.52 (CH₃), 43.44 (NCH₃), 45.93 (CH₂), 51.64 (CH₂), 91.53 (CH-CO), 120.95, 126.83 (2CPh), 127.02, 127.21, 128.90, 128.96 (2CPh), 134.61, 147.52, 151.80 (CH-N), 162.05 (CO), 162.37 (d, JCF = 245 Hz, C₆F). MS (EI, 70 eV) m/z (%): M⁺ 398.34 (100). Anal. Calcd for C₂₄H₁₈N₃O₂ (399.43) C, 72.17; H, 4.54; N, 10.52; Found: C, 72.21; H, 4.55; N, 10.50.
135.19, 140.45, 141.09, 147.86, 153.56 (CH-N), 154.67, 161.83 (C=O), 185.87 (C=O). Anal. Calcd for C\textsubscript{23}H\textsubscript{24}N\textsubscript{4}O\textsubscript{2} (388.47) C, 71.11; H, 6.23; N, 14.42; found; C, 71.10; H, 6.25; N, 14.43.

**Biology**

**Cell lines**

Three cancer cell lines, human breast cancer cell line (MCF-7), liver cancer cell line (HepG2), and prostate cancer cell line (PC-3) were obtained from National Cancer Institute, Cairo University. The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), penicillin (100 U/ml), and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere containing 5% CO\textsubscript{2}. Cells at a concentration of 0.50 × 10\textsuperscript{6} were grown in a 25 cm\textsuperscript{2} flask in 5 ml of culture medium.

**Cell viability**

*Fast screening.* Cells were seeded in 96 well plates. The synthesized compounds were applied to the two cell lines to test their anticancer activity. The compounds were tested in two different concentrations (0.05 μg/mL and 5 μg/mL). The two working solutions were prepared using the complete medium. Three technical replicates were carried out for each concentration. The treated cells were incubated for 48 h at 37°C and 5% CO\textsubscript{2}. Afterward, the cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The comparison was performed between the treated cells to the positive control (reference drugs) and the negative control (DMEM). The assays were performed in biological replicates.

*IC\textsubscript{50} determination.* Cells were seeded in 96 wells plates. The compounds that showed a significant reduction in cell viability were chosen for further analysis. Each compound was tested on the two cell lines in seven different concentrations (5, 10, 15, 25, 50, and 100 μg/mL). The working solutions were prepared using the complete medium. Three technical replicates were performed for each concentration. The treated cells were incubated for 48 h at 37°C and 5% CO\textsubscript{2}. The viability of the cells was determined using an MTT assay. IC\textsubscript{50} (50% inhibitory concentration) values were calculated with a four-parameter logistic function and presented in a mean. The assay was performed in biological replicates.

*MTT assay.* The cells were washed with 50 μL of PBS and then the PBS was discarded. Afterward, 50 μL of MTT working solution was applied to each well and the cells were incubated for 15–30 min at 37°C and 5% CO\textsubscript{2}. The cells were examined microscopically for formazan (black precipitate) development. The supernatant was discarded from each well and the formazan was dissolved using DMSO. The absorbance of the developed color was measured using an automated plate reader at 570 nm with a background wavelength of 670 nm. The results were presented in percentage to the values obtained from untreated cells (negative control).[8]
Cell cycle analysis. The impact of the synthesized compounds on the regulation of the cell cycle progression was explored using the Propidium Iodide Flow Cytometry Kit assay. Changes in the cell cycle were detected after the incubation of the MCF-7, HepG2, and PC-3 cells with compounds 4, 9, 10, and 12, selected for their potent cytotoxic activities in 24 h.

EGFR-TK inhibitory assay. EGFR enzyme inhibition was measured using a BPS Biosciences Colorimetric 96-well EGFR assay kit (catalog no. 40321), according to the manufacturer’s instructions. Percent inhibition was calculated by the comparison of compounds treated to control incubations. The concentration of the test compound causing 50% inhibition (IC50) was calculated from the concentration–inhibition response curve (triplicate determinations) and the data were compared with Erlotinib as a standard EGFR inhibitor.

Molecular docking. Using MOE 19.0901 Software, the co-crystallized ligand has been used to create the binding sites inside the crystal protein (PDB code: IMI7). Chem-Bio Draw Ultra 14.0 was used to create 2D structures of the chemicals studied, which were then stored in MDL-SD format. The energy of 3D structures was reduced by using RMSD at 0.05 kcal/mol MMFF94 force field. Following the prepared ligand protocol, the structures were minimized for docking. The CDOCKER protocol was used to carry out the molecular docking procedure. The receptor was kept inflexible during the refinement, while the ligands were permitted to be flexible. Each molecule was given ten possible interaction postures with the protein. Using Discovery Studio 2019 Client software, docking scores of the best-fitted postures with the active site at (EGFR bounded by Erlotinib) were recorded, and a 3D view was produced. Redocking of the co-crystallized ligand into the active site of the corresponding receptor with the calculation of root mean square deviation (RMSD).

Disclosure statement
No potential conflict of interest was reported by the author(s).

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