

## Synthesis, characterization, and biological evaluation of new quinazolin-4-one derivatives hybridized with pyridine or pyran moiety

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**Abstract** A new series of 2-(furan-2-yl)-4-oxoquinazolin-3-phenyl derivatives hybridized with pyridine **3a–d**, **4a–d** or pyran moiety **5a–d** were synthesized; their structures were confirmed by spectral and elemental analysis. Cytotoxicity was evaluated on three cancer cell lines (HEPG2, HCT116 and MCF7) using the sulphorodamine-B assay method and doxorubicin as a reference drug. Compound **5d** showed a closely similar activity to doxorubicin on MCF7; their IC<sub>50</sub> values are 4.6 and 4.4 nmol/mL, respectively. In addition, compounds **4b** and **4c** exhibited a closely similar activity to doxorubicin on HEPG2 cancer cells; their IC<sub>50</sub> values expressed in nmol/mL are 6.6, 6.7, and 5.7, respectively. Moreover, compound **4b** (IC<sub>50</sub> = 1.2 nmol/mL) was four times more potent than doxorubicin (IC<sub>50</sub> = 4.8 nmol/mL) on HCT116, and compound **5d** (IC<sub>50</sub> = 0.2 nmol/mL) revealed potency equal to 24 times the potency of doxorubicin on the same cancer cell line. The most active compounds were screened against EGFR TK, and results showed that compound **5d** was the most potent inhibitor; its percentage of inhibition was 95.6. Furthermore, compound **5d** was docked into the EGFR binding site to explore its possible interactions with EGFR TK.

**Keywords** Quinazoline derivatives · Pyran · Pyridine · Antitumor · Tyrosine kinase inhibition · Molecular docking

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## Introduction

Cancer is the second cause of death in the US, with an expected 1,665,540 cancer cases in 2014 and 585,720 Americans expected to die of cancer in the same year; nearly 1600 people per day. External factors (chemicals, radiation, infectious microorganisms, and tobacco) and internal factors (mutations, hormones, and immune conditions) are the two reported causes of cancer. These two factors may act together or sequentially to initiate cancer. Cancers can be treated with hormone therapy, surgery, immune therapy, radiation, and chemotherapy [1]; however, the undesirable side effects and availability of anticancer drugs are major limitations [2]. This proves the urgent need for developing novel chemotherapeutic agents with less side effects and more potent antitumor activities.

Quinazolinone is a privileged heterocyclic system, which plays a major role in medicinal chemistry due to its diverse biological activities [3–8]. Methaqualone and its analogue mebroqualone are quinazolinone derivatives reported to have sedative and hypnotic properties [9]. In addition, several quinazolinones were reported to have anti-HIV [10], anti-inflammatory [11, 12], antibacterial [13, 14], selective modulators for estrogen receptor beta subtype [15], antidepressant [16], and anticonvulsant [17] properties. Moreover, quinazolinones proved to possess a promising antitumor activity [18, 19] in addition to pyran and pyridine moieties which represent two important pharmacophores in several anticancer active agents [20, 21]. All these facts encouraged us to hybridize the quinazolinone-4-one scaffold bearing two lipophilic moieties at positions 2 (furan-2-yl) and 3 (phenyl ring) with the pyran/pyridine nucleus in a trial to obtain promising new anticancer active agents.

The epidermal growth factor receptor (EGFR) is a tyrosine kinase that exists on the cell surface and plays a role in regulating essential functions, e.g. differentiation, proliferation, and apoptosis [22]. EGFR are noted usually to be over-expressed in many types of cancers, hence their inhibition represents a useful strategy in cancer treatment [23, 24]. Several quinazolinone derivatives as erlotinib, gefitinib, and lapatinib were reported to act as inhibitors for these kinases [25], which has guided us to evaluate the inhibitory activity of the most active synthesized quinazolines **4a–d** and **5d** against EGFR TK. Moreover, molecular docking studies for the most potent EGFR inhibitor **5d** were performed in the ATP binding site of EGFR to explore its mode of binding to amino acids and other possible interaction with EGFR active site.

## Results and discussion

### Chemistry

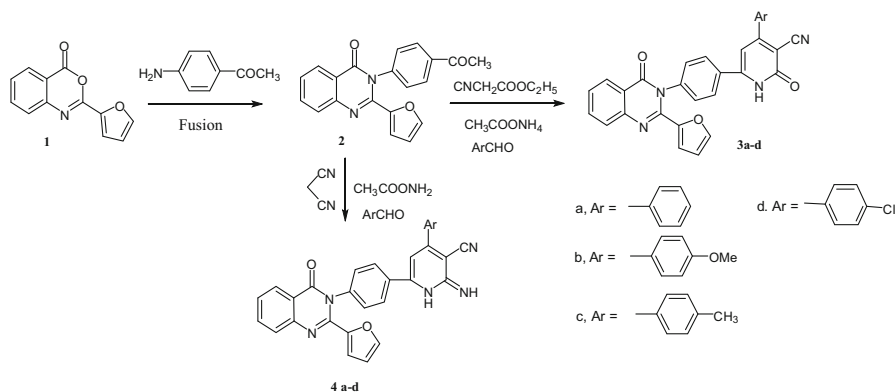
Compound **1** was prepared according to reported literature [26], synthesis of the starting compound, namely 3-(4-acetylphenyl)-2-(furan-2-yl)quinazolin-4(3H)-one **2** was achieved by fusion of the known, 2-(furan-2-yl)-4H-benzo[d] [1, 3] oxazin-4-

one **1** with p-aminoacetophenone. The facile one-pot reaction of ketone **2** with the appropriate aromatic aldehyde, namely benzaldehyde, p-anisaldehyde, p-tolualdehyde, and/or p-chlorobenzaldehyde with ethyl cyanoacetate in the presence of anhyd. ammonium acetate in n-butanol, afforded the corresponding pyridine-2(1H)-ones **3a–d**, respectively (Scheme 1). In the same manner, the one pot reaction of **2** with the same aldehydes and malononitrile in the presence of anhyd. ammonium acetate in n-butanol afforded the corresponding 2(1H)-iminopyridine derivatives **4a–d**, respectively (Scheme 1), while the same one-pot reaction of **2** with aromatic aldehydes and malononitrile in piperidine, gave the corresponding 2-aminopyrans **5a–d** (Scheme 2). Structures of the newly synthesized compounds were confirmed by IR, Mass,  $^1\text{H}$  NMR, and  $\text{C}^{13}$  NMR in addition to elemental analysis (Exp. Section).

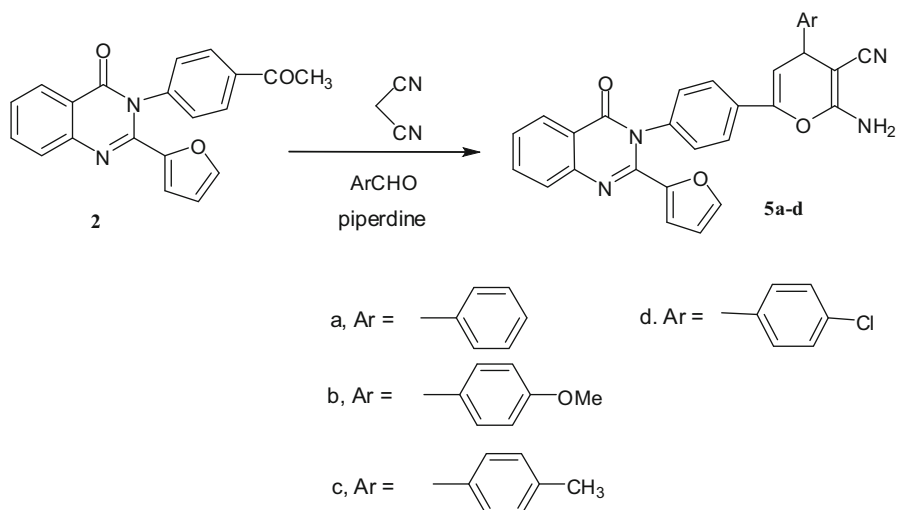
## Pharmacological activity

### Cytotoxicity

In this work, cytotoxicity of the newly synthesized derivatives was evaluated on three cancer cell lines [liver (HEPG2), colon (HCT116) and breast (MCF7)] using the sulphorhodamine-B assay method [27] and doxorubicin as a reference drug. The tested compounds and doxorubicin were used in dosages of 0, 6.25, 12.5, 25, 50, and 100  $\mu\text{g}/\text{mL}$  to detect percent of viability.  $\text{IC}_{50}$  values, which represent the concentration of the compound that inhibits the growth of 50 % of cancer cells, were calculated (Table 1). As for activity of the tested compounds on HEPG2, all of them showed remarkable anticancer activity except compounds **5a–c** (their  $\text{IC}_{50}$  values exceeded 100 nmol/mL); however, compounds **4b** and **4c** exhibited a closely similar activity to doxorubicin, with  $\text{IC}_{50}$  values of 6.6, 6.7, and 5.7 nmol/mL, respectively. Compounds **5d** and **4b** showed to be 24 times and four times more potent than doxorubicin on HCT116 cancer cells, with  $\text{IC}_{50}$  values of 0.2, 1.2, and 4.8 nmol/mL, respectively; however, compounds **3a**, **b** and **5a**, **c** were the least



**Scheme 1** Synthesis of quinazolin-4-one derivatives hybridized with pyridine **3a–d** and **4a–d**



**Scheme 2** Synthesis of quinazolin-4-one derivatives hybridized with pyran **5a-d**

**Table 1**  $IC_{50}$  (nmol/mL) of the newly synthesized quinazolines on liver (HEPG-2), colon (HCT116), and breast (MCF-7) cancer cell lines

Comp. No.	$IC_{50}$ (nmol/mL)		
	HEPG-2	HCT116	MCF-7
<b>3a</b>	31.1	1299	25.8
<b>3b</b>	41	373	150
<b>3c</b>	64	14.5	35.5
<b>3d</b>	13	81.7	53.8
<b>4a</b>	47	21.7	9.7
<b>4b</b>	6.6	1.2	11.6
<b>4c</b>	6.7	79.5	17.7
<b>4d</b>	19	66	13.4
<b>5a</b>	122	175	27.6
<b>5b</b>	136	43	162
<b>5c</b>	105	112	29.4
<b>5d</b>	40.3	0.2	4.6
Dox.	5.7	4.8	4.4

active compounds on this cell line. All the tested compounds revealed good activity on MCF7 cancer cells except compounds **3b** and **5b**; compounds **4b-d** showed a very good inhibitory activity, with  $IC_{50}$  in the range of 11.6–13.4 nmol/mL. Moreover, compound **5d** showed equal potency as doxorubicin on MCF7, and compound **4a** showed half potency of doxorubicin on the same cancer cell line, with  $IC_{50}$  values of 4.6, 9.7, and 4.4 nmol/mL, respectively.

**Table 2** Percent of inhibition of the best active quinazolines against EGFR tyrosine kinase at single dose (10  $\mu$ M)

Comp. No.	% of inhibition
<b>4a</b>	49.24
<b>4b</b>	71.44
<b>4c</b>	58
<b>4d</b>	44
<b>5d</b>	95.6
Erlotinib	100

### *EGFR inhibition assay*

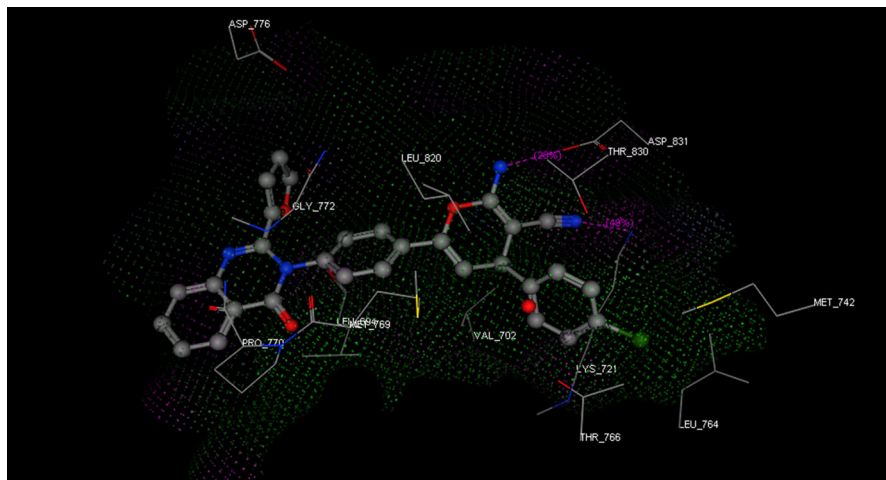
The previous literature proved the important role of quinazolines as EGFR TK inhibitors [25], which encouraged us to investigate the inhibitory activity of the best active quinazoline derivatives, **4a–d**, **5d**, against EGFR using erlotinib as a reference drug. These compounds revealed a remarkable inhibitory activity against these kinases; they showed an inhibition percent range from 44 to 95.6 at a concentration of 10  $\mu$ M (Table 2). The best activity was assigned for compound **5d** which is 24 times more potent than doxorubicin on HCT116 and has the same potency as doxorubicin on MCF7; its inhibition percent on EGFR was 95.6, followed by compound **4b** with inhibition percent equal 77.4. This compound showed to be four times more potent than doxorubicin on HCT116 and closely similar potency with doxorubicin on HEPG2 cancer cells.

### *Molecular docking studies*

The best active inhibitor of EGFR was compound **5d**; it showed inhibition activity equal 95.6 % at 10  $\mu$ M. The Protein Data Bank file 1M17 containing EGFR cocrystallized with 4-AQ [28] was downloaded, refined, verified, and saved as an moe file to be used in performing the docking process. Compound **5d** was docked into the active site of EGFR (Fig. 1), and the observed score energy was  $-21.43$ . Compound **5d** showed two types of interactions with Lys721 amino acid: one hydrogen bond with the CN group, and the other is an arene-cation interaction with the p-Cl-phenyl moiety. In addition, there was another hydrogen bonding with Asp 831 amino acid through the NH<sub>2</sub> group. These results proved the high affinity of compound **5d** to the binding site of EGFR and also that it can form interactions with two amino acid residues. This encouraged us to consider this compound as a promising EGFR TK inhibitor that deserves to be the subject of further investigation aiming to prove it as a new potent anticancer agent.

## **Conclusion**

From all the obtained results we can conclude that: (1) Hybridizing 4-quinazolinone with 2-imino-pyridine **4a–d** gave better activity than hybridizing it with 2-oxo-pyridine **3a–d**. (2) Hybridization of 4-quinazolinone with pyran decreased the



**Fig. 1** Compound **5d** (colored by atoms) in the ATP binding site of EGFR TK

activity more than pyridine except the *p*-chloro derivative **5d** that showed to be a broad spectrum anticancer agent against the three tested cell lines. (3) 2-amino-4-(4-chlorophenyl)-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4H-pyran-3-carbonitrile **5d** revealed 24 times the potency of doxorubicin on HCT116 and similar activity to doxorubicin on MCF7. (4) Hybridization of 2-furan-2-yl-quinazolin-4-one-3-phenyl with *p*-chloro pyran derivative afforded a very potent inhibitor **5d** to EGFR TK.

## Experimental

### Chemistry

All melting points are uncorrected; elemental analyses were carried out in the microanalytical unit of the National Research Centre and Cairo University, Egypt. IR spectra were recorded on a Nexus 670-Nicolet FT-IR spectrophotometer (USA) and Perkin Elmer-9712 spectrophotometer.  $^1\text{H}$  NMR spectra were determined on a Varian-Gemini 300 MHz and Joel-Ex 270 MHz NMR spectrometer using TMS as an internal standard.  $^{13}\text{C}$  NMR ( $\text{DMSO-d}_6$ ) spectra were recorded at 100.62 MHz at the aforementioned research center in Cairo University. Mass spectra were determined on Finnigan Mat SSQ 7000, mode EI 70 eV (Thermo Inst. Sys. Inc., USA). Thin layer chromatography was carried out on silica gel 60 F254 (Merck) plates.

#### *3-(4-Acetylphenyl)-2-(furan-2-yl)quinazolin-4(3H)-one (2)*

A mixture of the benzoxazine **1** (2.13 g, 0.01 mol) and *p*-aminoacetophenone (1.35 g, 0.01 mol) was heated together upon fusion at 160 °C on a sand bath for 2 h.

After cooling, the crude mass was crystallized from ethanol twice to give dark brown crystals of **II**, m.p. 250 °C in 80 % yield. Analysis for  $C_{20}H_{14}N_2O_3$ , M.wt (330.34) Calcd.: %C, 72.72; H, 4.27; N, 8.48; Found: % C, 72.52; H, 4.17; N, 8.40, IR spectrum (KBr,  $cm^{-1}$ ) showed characteristic absorption bands at 3390 (C–H aromatic), 1700 (C=O of acetyl), at 1680 (C=O ofquinazolinone), 1633 (C=N), and at 1590 (C=C).  $^1H$ NMR spectrum (DMSO- $d_6$ , ppm) showed signals at 2.5 (3H, s, COCH<sub>3</sub>) and 6.5–8.1 (m, 11H, Ar–H and furan-H).  $^{13}C$  NMR (DMSO- $d_6$ ) d ppm: 197, 164, 160.6, 144.5, 143, 141.7, 137.1, 136.6, 133.4, 129, 127.3, 124.3, 120.7, 109.9, 109.4, 26.6. MS (m/z, R.I.): calcd for  $C_{20}H_{14}N_2O_3$ : 330.10; found: 330.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4-(4-substituted aryl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3a-d)*

**General method** A mixture of the ketone **2** (0.66 g; 0.002 mol), ethyl cyanoacetate (0.23 mL, 0.002 mol), anhyd. ammonium acetate (1.24 g; 0.016 mol), and the appropriate aldehydes, namely benzaldehyde, p-anisaldehyde, p-tolualdehyde, and/or p-chlorobenzaldehyde in 10 mL n-butanol, was refluxed for 5 h. The reaction mixture was concentrated to half its volume under reduced pressure. After cooling, the formed ppt was filtered off, air dried, and recrystallized from the proper solvent to give compounds **3a-d**, respectively.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (3a)*

Crystallized from glacial acetic acid to give yellow crystals, m.p. 172 °C in 80 % yield. Analysis for  $C_{30}H_{18}N_4O_3$ , M.wt. (482.49), Calcd: %C, 74.68; H, 3.76; N, 11.61; Found: %C, 74.58; H, 3.64; N, 11.59, IR spectrum (KBr,  $cm^{-1}$ ) showed absorption bands at 3370 (NH), 2220 (–CN), 1740 (C=O, pyridone), 1685 (C=O, quinazolinone), and 1600 (C=C).  $^1H$ NMR spectrum (DMSO- $d_6$ , ppm) showed 6.13 (1H, s, 1H of pyridine), 6.53–8.1 (m, 16H, Ar–H and furan-H), and at 8.5 (1H, s, NH proton).  $^{13}C$  NMR (DMSO- $d_6$ ) d ppm: 164, 160.6, 160.4, 159.4, 157.4, 144.5, 143, 141.7, 135.6, 133.4, 131.9, 128.9, 127.9, 127.3, 126.6, 126.4 124.3, 120.8, 115.8, 115.3, 109.9, 109.4, 104.8. MS (m/z, R.I.): calcd for  $C_{30}H_{18}N_4O_3$ : 482.14; found: 482.14.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3b)*

Crystallized from ethanol to give brown crystals, m.p. 200 °C in 75 % yield. Analysis for  $C_{31}H_{20}N_4O_4$ , M.wt. (512.51), Calcd: %C, 72.65; H, 3.93; N, 10.93; Found: %C, 72.55; H, 3.90; N, 10.90, IR spectrum (KBr,  $cm^{-1}$ ) showed absorption bands at 3375 (NH), 2220 (–CN), 1745 (C=O, pyridone), 1680 (C=O, quinazolinone), and 1600 (C=C).  $^1H$ NMR spectrum (DMSO- $d_6$ , ppm) showed 3.83 (3H, s, OCH<sub>3</sub>), 6.13 (1H, s, 1H of pyridine), 6.53–8.1 (m, 15H, Ar–H and furan-H), and at 8.5 (1H, s, NH proton).  $^{13}C$  NMR (DMSO- $d_6$ ) d ppm: 163.4, 160.5, 160.2, 159.8, 159.4, 156.4, 144, 143.2, 141.7, 135.4, 131.9, 130.1, 127.7, 127.3, 126.7, 126.6,

126.4, 123.2, 120.8, 114.8, 114.3, 107.9, 107.4, 55.8. MS (m/z, R.I.): calcd for  $C_{31}H_{20}N_4O_4$ : 512.15; found: 512.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-oxo-4-p-tolyl-1,2-dihydropyridine-3-carbonitrile (3c)*

Crystallized from ethanol to give yellowish brown crystals, m.p. 190 °C in 75 % yield. Analysis for  $C_{31}H_{20}N_4O_3$ , M.wt. (496.52), Calcd: %C, 74.99; H, 4.06; N, 11.28; Found: %C, 74.94; H, 4.12; N, 11.31, IR spectrum (KBr,  $cm^{-1}$ ) showed absorption bands at 3370 (NH), 2220 (–CN), 1740 (C=O, pyridone), 1675 (C=O, quinazolinone), and 1590 (C=C).  $^1H$ NMR spectrum (DMSO- $d_6$ , ppm) showed 2.3 (3H, s,  $CH_3$ ), 6.13 (1H, s, 1H of pyridine), 6.52–7.9 (m, 15H, Ar–H and furan–H), and at 8.2 (1H, s, NH proton).  $^{13}C$  NMR (DMSO- $d_6$ ) d ppm: 162.9, 159.5, 159.2, 158.8, 158.4, 156.9, 144.2, 143.5, 141.4, 137.6, 134.5, 132.6, 130.9, 128.9, 127.3, 125.7, 125.6, 125.2, 123.2, 120.7, 115.8, 115.3, 108.7, 108.4, 21.3. MS (m/z, R.I.): calcd for  $C_{31}H_{20}N_4O_3$ : 496.15; found: 496.15.

*4-(4-Chlorophenyl)-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3d)*

Crystallized from ethanol to give yellowish brown crystals, m.p. 135 °C in 70 % yield. Analysis for  $C_{30}H_{17}ClN_4O_3$ , M.wt. (516.93), Calcd: %C, 69.70; H, 3.31; N, 10.84; Found: %C, 69.50; H, 3.11; N, 10.62; IR spectrum (KBr,  $cm^{-1}$ ) showed absorption bands at 3375 (NH), 2225 (–CN), 1745 (C=O, pyridone), 1675 (C=O, quinazolinone), and 1600 (C=C).  $^1H$ NMR spectrum (DMSO- $d_6$ , ppm) showed 6.2 (1H, s, 1H of pyridine), 6.52–8.2 (m, 15H, Ar–H and furan–H), and at 8.5 (1H, s, NH proton).  $^{13}C$  NMR (DMSO- $d_6$ ) d ppm: 164, 160.5, 159.8, 158.8, 158.2, 155.4, 144.5, 142.9, 141.7, 135.7, 133.7, 133.4, 131.9, 130, 128.7, 127.3, 126.7, 126.6, 126.2, 123.9, 120.4, 114.8, 114.3, 108.4, 108.2. MS (m/z, R.I.): calcd for  $C_{30}H_{17}ClN_4O_3$ : 516.93; found: 516.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-imino-4-(4-substituted aryl)-1,2-dihydropyridine-3-carbonitrile (4a-d)*

**General method** A mixture of compound **2** (0.66 g, 0.002 mol), malononitrile (0.12 mL, 0.002 mol), anhyd. ammonium acetate ((1.24 g, 0.016 mol), and the appropriate aldehydes, namely benzaldehyde, p-anisaldehyde, p-tolualdehyde, and/or p-chlorobenzaldehyde in 10 mL n-butanol, was refluxed for 5 h. After cooling, the reaction mixture was filtered of and crystallized from the proper solvent to give the iminopyridines **4a–d**, respectively.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-imino-4-phenyl-1,2-dihydropyridine-3-carbonitrile (4a)*

Crystallized from glacial acetic acid to give brown crystals, m.p. 160 °C in 75 % yield. Analysis for  $C_{30}H_{19}N_5O_2$ , M.wt. (516.93), Calcd: %C, 74.83; H, 3.98; N,



14.54; Found: %C, 74.70; H, 3.84; N, 14.43; IR spectrum (KBr,  $\text{cm}^{-1}$ ) absorption bands at 3330–3220 (NH, =NH), 2200 (C=N), 1700 (C=O, quinazolinone), and at 1600 (C=N).  $^1\text{H}$ NMR spectrum (DMSO- $d_6$ , ppm) showed 6.52–7.9 (m, 17H, Ar-H and furan-H) and 9.60, 11.70 (2H, 2 s, 2NH).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 169.3, 164, 160.7, 160.6, 144.5, 143, 141.4, 135.6, 133.4, 131.7, 128.9, 128.6, 127.7, 127.3, 126.7, 126.6, 126.4, 123.4, 120.5, 115.8, 115.3, 109.9, 109.4, 102.6. MS (m/z, R.I.): calcd for  $\text{C}_{30}\text{H}_{19}\text{N}_5\text{O}_2$ : 481.15; found: 481.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-imino-4-(4-methoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (4b)*

Crystallized from glacial acetic acid to give brown crystals, m.p. 150 °C in 75 % yield. Analysis for  $\text{C}_{31}\text{H}_{21}\text{N}_5\text{O}_3$ , M.wt. (511.53), Calcd: %C, 72.79; H, 4.14; N, 13.69; Found: %C, 72.75; H, 4.12; N, 13.52; IR spectrum (KBr,  $\text{cm}^{-1}$ ) absorption bands at 3335–3220 (NH, =NH), 2205 (C=N), 1710 (C=O, quinazolinone), and at 1600 (C=N).  $^1\text{H}$ NMR spectrum (DMSO- $d_6$ , ppm) showed 3.7 (3H, s, OCH<sub>3</sub>), 6.52–8.3 (m, 16H, Ar-H and furan-H), and 9.60, 11.70 (2H, 2 s, 2NH).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 170.2, 165.6, 163.7, 163.6, 159.8, 143.9, 143.2, 141.7, 134.4, 131.9, 127.9, 127.3, 126.3, 125.6, 125.5, 124.2, 114.8, 114.2, 107.7, 107.4, 56.1 MS (m/z, R.I.): calcd for  $\text{C}_{31}\text{H}_{21}\text{N}_5\text{O}_3$ : 511.16; found: 511.16.

*6-(4-(2-(Furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-imino-4-p-tolyl-1,2-dihydropyridine-3-carbonitrile (4c)*

Crystallized from ethanol to give brown crystals, m.p. 165 °C in 75 % yield. Analysis for  $\text{C}_{31}\text{H}_{21}\text{N}_5\text{O}_2$ , M.wt. (495.53), Calcd: %C, 75.14; H, 4.27; N, 14.13; Found: %C, 75.10; H, 4.11; N, 14.00; IR spectrum (KBr,  $\text{cm}^{-1}$ ) absorption bands at 3330–3215 (NH, =NH), 2200 (C=N), 1710 (C=O, quinazolinone), and at 1600 (C=N).  $^1\text{H}$ NMR spectrum (DMSO- $d_6$ , ppm) showed 2.3 (3H, s, CH<sub>3</sub>), 6.51–8.2 (m, 16H, Ar-H and furan-H), and 9.60, 11.70 (2H, 2 s, 2NH).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 167.4, 162.9, 159.9, 159.7, 142.5, 142, 140.7, 137.6, 134.5, 133.4, 132.6, 131.9, 129.9, 126.6, 126.2, 125.6, 125.6, 124.3, 120.7, 119.3, 113.8, 107.9, 107.4, 102.4, 25.3. MS (m/z, R.I.): calcd for  $\text{C}_{31}\text{H}_{21}\text{N}_5\text{O}_2$ : 495.17; found: 495.17.

*4-(4-Chlorophenyl)-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (4d)*

Crystallized from glacial acetic acid to give brown crystals, m.p. 240 °C in 70 % yield. Analysis for  $\text{C}_{30}\text{H}_{18}\text{ClN}_5\text{O}_2$ , M.wt. (515.95), Calcd: %C, 69.84; H, 3.52; N, 13.57; Found: %C, 69.72; H, 3.42; N, 13.50; IR spectrum (KBr,  $\text{cm}^{-1}$ ) absorption bands at 3340–3225 (NH, =NH), 2210 (C=N), 1720 (C=O, quinazolinone), and at 1600 (C=N).  $^1\text{H}$ NMR spectrum (DMSO- $d_6$ , ppm) showed 6.55–8.4 (m, 16H, Ar-H and furan-H) and 9.70, 11.90 (2H, 2 s, 2NH).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 170.4, 164.9, 160.9, 160.7, 144.5, 142.9, 133.7, 133.5, 133.4, 132.6, 130.9, 129.7, 129.4, 127.2, 126.6, 126.4, 125.3, 120.9, 119.1, 114.7, 110.9, 110.4, 100.2. MS (m/z, R.I.): calcd for:  $\text{C}_{30}\text{H}_{18}\text{ClN}_5\text{O}_2$ : 515.11; found: 515.

*2-Amino-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4-(4-substituted phenyl)-4H-pyran-3-carbonitrile (5a-d)*

**General method** A mixture of compound **2** (0.66 g, 0.002 mol), malononitrile (0.12 mL, 0.002 mol), and the appropriate aromatic aldehydes, namely benzaldehyde, p-anisaldehyde, p-tolualdehyde, and/or p-chlorobenzaldehyde in few drops of piperidine, was refluxed for 4 h. The reaction mixture was reduced to half its volume under reduced pressure and cooled. The precipitated crude was filtered off, washed with cold water, and crystallized from the proper solvent to give the aminopyrans **5a-d**, respectively.

*2-Amino-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4-phenyl-4H-pyran-3-carbonitrile (5a)*

Crystallized from glacial acetic acid to give brown crystals, m.p. 135 °C in 70 % yield. Analysis for C<sub>30</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub>, M.wt. (484.50), Calcd: %C, 74.37; H, 4.16; N, 11.56; Found: %C, 74.30; H, 4.12; N, 11.50; IR spectrum (KBr, cm<sup>-1</sup>) absorption bands at 3430–3350 (NH<sub>2</sub>), 2220 (–CN), 1680 (C=O quinazolinone), and at 1600 (C=N). <sup>1</sup>HNMR spectrum (DMSO-d<sub>6</sub>, ppm) showed 4.2 (1H, d, 1H of pyran), 5.1 (1H, d, 1H of pyran), 6.55–8.2 (m, 16H, Ar–H and furan-H), and 9.40 (2H, s, NH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 164.2, 159.9, 157.7, 145.5, 143.9, 142.2, 133.4, 131.9, 130.7, 127.9, 127.5, 126.9, 126.5, 125.9, 125.7, 123.9, 121.1, 110.7, 110.4, 91.5, 59.1, 29.6. MS (m/z, R.I.): calcd for: C<sub>30</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub>: 484.15; found: 484.15.

*2-Amino-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4-(4-methoxy phenyl)-4H-pyran-3-carbonitrile (5b)*

Crystallized from glacial acetic acid to give yellow crystals, m.p. 120 °C in 70 % yield. Analysis for C<sub>31</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>, M.wt. (514.53), Calcd: %C, 72.36; H, 4.31; N, 10.89; Found: %C, 72.29; H, 4.27; N, 10.79; IR spectrum (KBr, cm<sup>-1</sup>) absorption bands at 3435–3355 (NH<sub>2</sub>), 2225 (–CN), 1680 (C=O quinazolinone), and at 1600 (C=N). <sup>1</sup>HNMR spectrum (DMSO-d<sub>6</sub>, ppm) showed 3.8 (3H, s, OCH<sub>3</sub>), 4.3 (1H, d, 1H of pyran), 5.2 (1H, d, 1H of pyran), 6.5–8.4 (m, 15H, Ar–H and furan-H), and 9.40 (2H, s, NH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 165, 160.9, 159.7, 157.6, 144.9, 143.5, 141.7, 140.5, 134.5, 133.9, 131.7, 129, 127.3, 125.7, 125.6, 124.9, 123.3, 119.1, 114.2, 112.4, 111.2, 95.5, 60.1, 55.9, 30.6. MS (m/z, R.I.): calcd for: C<sub>31</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>: 514.16; found: 514.

*2-Amino-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4-p-tolyl-4H-pyran-3-carbonitrile (5c)*

Crystallized from ethanol to give dark brown crystals, m.p. 110 °C in 70 % yield. Analysis for C<sub>31</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>, M.wt. (498.53), Calcd: %C, 74.69; H, 4.45; N, 11.24; Found: %C, 74.67; H, 4.42; N, 11.20; IR spectrum (KBr, cm<sup>-1</sup>) absorption bands at 3430–3355 (NH<sub>2</sub>), 2220 (–CN), 1680 (C=O quinazolinone), and at 1600 (C=N). <sup>1</sup>HNMR spectrum (DMSO-d<sub>6</sub>, ppm) showed 2.5 (3H, s, CH<sub>3</sub>), 4.0 (1H, d,

1H of pyran), 5.0 (1H, d, 1H of pyran), 6.5–7.9 (m, 15H, Ar–H and furan-H), and 9.0 (2H, s, NH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 165.4, 162.9, 159.2, 153.6, 145.9, 140.5, 139.5, 135.5, 133.4, 131.9, 129.3, 127.3, 126.7, 126.5, 125.9, 124.3, 121.1, 119.1, 92.7, 59.4, 59.1, 29.6, 21.3 MS (m/z, R.I.): calcd for: C<sub>31</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>: 498.17; found: 498.

*2-Amino-4-(4-chlorophenyl)-6-(4-(2-(furan-2-yl)-4-oxoquinazolin-3(4H)-yl)phenyl)-4H-pyran-3-carbonitrile (5d)*

Crystallized from ethanol to give dark brown crystals, m.p. 210 °C in 70 % yield. Analysis for C<sub>30</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>3</sub>, M.wt. (518.95), Calcd: %C, 69.43; H, 3.69; N, 10.80; Found: %C, 69.33; H, 3.59; N, 10.75; IR spectrum (KBr, cm<sup>-1</sup>) absorption bands at 3435–3355 (NH<sub>2</sub>), 2225 (–CN), 1685 (C=O quinazolinone), and at 1610 (C=N). <sup>1</sup>H NMR spectrum (DMSO-d<sub>6</sub>, ppm) showed 4.0 (1H, d, 1H of pyran), 5.0 (1H, d, 1H of pyran), 6.5–7.9 (m, 15H, Ar–H and furan-H), and 9.0 (2H, s, NH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ ppm: 163.4, 160.2, 159.2, 144.9, 143, 141.7, 140.5, 140.3, 133.7, 132.4, 132.2, 130.4, 128.7, 127.5, 126.9, 126.7, 126.5, 125.9, 124.3, 120.1, 119.6, 109.7, 109.4, 91.5, 58.4, 29.6, 28.8 MS (m/z, R.I.): calcd for: C<sub>30</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>3</sub>: 518.11; found: 518.11.

## Pharmacological screening

### *In vitro* cytotoxicity

Cytotoxicity of the new derivatives **3a–5d** was evaluated using the sulphorhodamine-B (SRB) assay method that was previously reported [27]. HEPG2, HCT116, and MCF7 cancer cell lines were obtained from the American Type Culture Collection (ATCC, MN, USA) through the Tissue Culture Unit, the Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt). Cytotoxicity evaluation was performed at the Center for Genetic Engineering, Al-Azhar University, Cairo, Egypt. Reagents, and chemicals were purchased from Sigma Aldrich Chemical Company (St. Louis, MO, USA). Seeding cells for 24 h in a 96 well microtiter plate at a concentration of 1000–2000 cells/well was performed, then cells were incubated for 48 h with various concentrations (0, 6.25, 12.5, 25, 50, 100 µg/mL) of the tested compounds and doxorubicin in DMSO. Three wells were used for each concentration, and after incubation for 48 h the cells were fixed with 10 % trichloro acetic acid for 1 h at 4 °C, and washed with distilled water three times. Wells were stained for 10–30 min at room temperature with 0.4 % SRB, dissolved in 1 % acetic acid. Washed with acetic acid 1 % to remove unbound dye until colorless drainage obtained. The plates were subjected to air drying, 24 h not exposed to UV. The dye was solubilized with 10 mM Tris-EDTA (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 545 nm with an ELISA microplate reader. The percent of surviving cells was calculated and plotted against different concentrations of the tested compounds to obtain the survival curve. The IC<sub>50</sub> values was calculated using sigmoidal concentration—response curve fitting models (Sigmaplot software).

### *EGFR TK inhibition assay*

Compounds **4a–d** and **5d** were tested *in vitro* for inhibition of EGFR tyrosine kinase using Kinase-Glo Plus luminescence kinase assay kit. The tested compounds were dissolved in DMSO. They were then added to reaction plates containing the EGFR tyrosine kinase in assay buffer [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM ethylene glycol tetraacetic acid (EGTA), 0.02 % Brij35, 0.02 mg/mL bovine serum albumin (BSA), 0.1 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM dithiothreitol (DTT), 1 % DMSO]. Reactions were initiated by addition of a mixture of ATP (Sigma, St. Louis, MO, USA) and 33P ATP (Perkin Elmer, Waltham, MA, USA) to a final concentration of 10 μM of the tested compounds. Reactions were carried out at room temperature for 2 h, followed by spotting of the reactions onto P81 ion exchange filter paper (Whatman Inc., Piscataway, NJ, USA). Unbound phosphate was removed by extensive washing of filters in 0.75 % phosphoric acid [29]. Results are presented as percentage enzyme inhibition in comparison to erlotinib as a reference EGFR TK inhibitor.

### *Molecular docking*

Molecular modeling studies were carried out on an Intel Core i5, 2.53 GHz processor, 4 GB ram memory with Windows XP 32-bit operating system using Molecular Operating Environment software. Energy minimization was performed with MOE, RMSD gradient of 0.05 kcal/mol, MMFF94X force field, and the partial charges were automatically calculated. The Protein Data Bank (PDB ID: 1M17) was used in this study. The ATP binding site of EGFR was prepared for docking studies where erlotinib was removed from the active site, hydrogen atoms were added to the structure with their standard geometry, MOE Alpha Site Finder was used for the active sites detection, and the obtained model was saved as an moe file then used in predicting interactions at the active site between the selected compounds and EGFR. The 2D structures of the docked compounds were generated by chem. draw, transformed to their 3D by the MOE program, protonated, and subjected to energy minimization, then saved as an mdb file to be docked into the active site of EGFR.

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