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Design, synthesis, and antiproliferative activity of new 2-amino-pyrano[3,2-c]quinoline-3-carbonitriles as potential EGFR, BRAF^{V600E}, and HER-2 inhibitors

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A novel series of pyrano-quinoline compounds 5a-l was designed, synthesized, and investigated for antiproliferative efficacy as multi-EGFR/HER-2/BRAF^{V600E} inhibitors. This work addresses the reaction between 4-hydroxy-2-oxo-1,2-dihydroquinolines and 2-benzylidenemalononitriles, which produces a new series of 2-amino-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile derivatives 5a-I, giving good yields. The suggested mechanism was considered. The structures of 5a-I were elucidated using NMR spectroscopy, mass spectrometry, and elemental analysis. The cell viability assay of 5a-l against a normal cell line showed that none of the studied compounds exhibited cytotoxicity, and all hybrids retained above 90% cell viability at a dose of 50 µM. The antiproliferative activity of 5a-I was assessed against a panel of four cancer cell lines using the MTT assay. Compounds 5e and 5h had the most antiproliferative activity, with GI_{50} values of 26 and 28 nM, respectively, making them more efficient than erlotinib ($GI_{50} = 33$ nM). Inhibitory assays on EGFR, HER-2, and BRAF^{V600E} indicated that compounds 5e and 5h were the most efficacious derivatives, with IC50 values of 71 nM (EGFR), 62 nM (BRAF V600E), and 21 nM (HER-2) for compound **5e**, whereas compound **5h** displayed IC₅₀ values of 75 nM (EGFR), 67 nM (BRAF^{V600E}), and 23 nM (HER-2). Molecular docking studies were conducted on a series of quinoline-based compounds to evaluate their binding affinity with EGFR and HER-2 kinases. Compound 5e showed promising interactions, forming stable complexes with key residues like Met769 (EGFR) and Asp863 (HER-2). The docking simulations revealed critical hydrogen bonding, $\pi - \pi$ stacking, and hydrophobic interactions, supporting its potential as a kinase inhibitor for cancer treatment.

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1. Introduction

Cancer is a complex disease whose increasing prevalence is significantly impacting human health.¹ Chemotherapy, a pivotal therapeutic modality for cancer, has consistently garnered significant attention from researchers and clinicians.² The advancement of anticancer drugs exhibiting enhanced therapeutic efficacy and reduced clinical adverse effects has

garnered increasing interest from medicinal chemists.^{3,4} Oncology has experienced significant breakthroughs since establishing targeted therapy utilizing small-molecule inhibitors.^{5,6} The latter refers to the treatment that directly targets the primary causes of cancer formation, which may include dysregulated enzymes and proteins.

The protein kinase family, recognized as a prominent class of carcinogenic drug targets, is responsible for the phosphorylation reaction essential in various physiological functions. The overexpression, disruption, mutations, and translocation of these proteins led to various disorders, particularly cancer. Recently, 80 small compounds that inhibit various kinases have received FDA approval, establishing kinase family proteins among the most targeted proteins in cancer research. 11,12

Quinoline has emerged as a significant scaffold in drug development in recent decades, especially within cancer research. Quinoline, a nitrogen-containing heterocyclic molecule, has a variety of biological activities. ^{13–17} Compounds containing quinoline exhibit markedly increased basicity owing to

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NOH SO₂CH₃

Compound I [Ref. 21]
Dual EGFR/HER-2 inhibitor

Compound II [Ref. 25]
Dual EGFR/BRAF inhibitor

Compound III [Ref. 26]
Dual EGFR/BRAF inhibitor

Fig. 1 Structures of compounds I-III, quinoline-based moieties, as inhibitors of protein kinase.

the presence of nitrogen atoms. Clinical trials are presently investigating numerous anticancer agents that feature the quinoline moiety. Page 18-20 Quinoline derivatives effectively combat cancer by multiple mechanisms, including inhibiting protein kinase, epidermal growth factor receptor (EGFR), and mitogenactivated protein kinases. Paticancer agents developed from quinoline encompass bosutinib, lenvatinib, and cabozantinib, all of which function as protein kinase inhibitors. Quinoline derivatives have demonstrated potential in several cancer cell lines, including those originating from the breast, colon, lung, colorectal, and renal tissues.

We recently²¹ reported the design and synthesis of a new class of EGFR/HER-2 dual-target inhibitors derived from quinoline compounds. The new compounds were evaluated for antiproliferative efficacy against four cancer cell lines, demonstrating considerable antiproliferative activity, particularly in breast (MCF-7) and lung (A-549) cancer cell lines, which exhibited the highest sensitivity. Compound I (Fig. 1) had the most pronounced antiproliferative effect. Compound I demonstrated the highest efficacy as a dual-target inhibitor of EGFR and HER-2, with inhibitory (IC50) values of 71 and 31 nM, respectively. It surpassed the reference erlotinib (IC50 = 80 nM) as an EGFR inhibitor but was comparable to the clinically utilized drug lapatinib (IC50 = 26 nM) as a HER-2 inhibitor. Furthermore, results show that compound I promotes

apoptosis by activating caspase-3, 8, and Bax while down-regulating the expression of the anti-apoptotic protein Bcl2.

In a different study completed in our laboratory, 25 we continued developing and synthesizing innovative quinoline-derived compounds as prospective antiproliferative medicines. We assessed the antiproliferative effect of the newly synthesized compounds against four human cancer cell lines. Compound II (Fig. 1) demonstrated superior efficacy to the standard medication doxorubicin against the four cancer cell lines (GI $_{50}=1.40~\mu\text{M}~\nu\text{s}.~1.20~\mu\text{M}$ for II). Compound II exhibited the highest efficacy in inhibiting EGFR and BRAF V600E , with IC $_{50}$ values of 105 \pm 10 and 140 \pm 12 nM, respectively. The values were comparable to those of the conventional medication erlotinib, which exhibited IC $_{50}$ values of 80 \pm 10 and 60 \pm 10 nM, respectively.

In 2021, 26 we detailed synthesizing a new series of quinoline-based compounds employed as antiproliferative agents targeting EGFR and BRAF V600E . Compound III (Fig. 1) exhibited enhanced antiproliferative efficacy relative to doxorubicin (GI $_{50}$ = 1.15 μ M). It demonstrated a GI $_{50}$ value of 3.30 μ M against four human cancer cell lines. Compound III demonstrated inhibitory effectiveness against EGFR and BRAF V600E , with IC $_{50}$ values of 1.30 \pm 0.12 μ M and 3.80 \pm 0.15 μ M, respectively. In contrast, the reference erlotinib exhibited IC $_{50}$ values of 0.08 \pm 0.005 μ M for EGFR and 0.06 \pm 0.01 μ M for BRAF V600E .

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$$\begin{array}{c} \text{NH}_2 \text{ O} \\ \text{NH}_2 \text{ O} \\ \text{NH}_2 \text{ O} \\ \text{NH}_2 \text{ CN} \\ \text{Normal of the properties of$$

Fig. 2 Structures of previously described compound IV and newly developed compounds 5a-l.

1.1. Rational design

Aly *et al.*,²⁷ reported the synthesis and anticancer efficacy of a novel series of 2-amino-4-(furan-2-yl)-4*H*-pyrano[3,2-*c*] quinoline-3-carboxylates. Compound **IV** (Fig. 2) has been identified as the most efficient derivative, with an IC₅₀ value of 35 μ M against A-549 lung epithelial cancer cells. The *in vitro* inhibitory profile of topoisomerase II for the most efficient derivative, compound **IV**, was examined. Compound **IV** had moderate to weak inhibitory action with an IC₅₀ value of 45.19 μ M.

The present study continues our endeavor to develop dual or multi-targeted protein kinase inhibitors, 21,22,28,29 presenting the design, synthesis, and antiproliferative effects of a novel series of pyrano-quinolines 5a-l, Fig. 2. The newly synthesized compounds were developed through structural changes of compound IV, as illustrated in Fig. 2. The ester group at position 3 of 2-amino-pyrano[3,2-c]quinoline was substituted with a nitrile group. The carbonitrile group has notable and varied biological actions, with anticancer properties being the most prominent. It is defined by its rigidity, stability under in vivo conditions, capacity for hydrogen bonding with diverse protein targets, and moderate dipole features.30 The second modification involves substituting the furan moiety with a benzene ring. These modifications were incorporated, hopefully, to enhance the pharmacokinetics and/or pharmacodynamics of novel 5a-l compounds.

2. Experimental

2.1. Chemistry

General details: see Appendix A (SI).

4-Hydroxy-2-oxo-1,2-dihydroquinoline derivatives **1a-e**,³¹ and 2-benzylidenemalononitriles **4a-c** (ref. 32) were prepared according to reported procedures.

2.1.1. Synthesis of 2-amino-5-oxo-5,6-dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitriles 5a–l

2.1.1.1. Methods I and II. A mixture of 1a-e (1 mmol) and 4a-c (1 mmol) in 50 mL of absolute ethanol and a few drops of Et₃N (method I) or anhydrous K_2CO_3 (method II) (1.5 mmol, 0.192 g) was refluxed for 12 h. The reaction was observed via TLC analysis. Upon completion of the reaction, allow it to cool to room

temperature. The resultant compounds 5a-1 were further filtered and washed several times with ethanol (method I) or a mixture of ethanol and water (method II to eliminate K_2CO_3) and then dried. The obtained products were recrystallized from the specified solvents to yield pure 5a-1.

2.1.1.2. Method III. A mixture of 1a-e (1 mmol) and 4a-c (1 mmol) in 40 mL of DMF, together with anhydrous K_2CO_3 (1.5 mmol, 0.192 g), was stirred at ambient temperature for 24 h. The reaction was observed via TLC analysis. Upon completion of the reaction, 100 g of crushed ice was introduced with stirring, and the products were filtered, washed repeatedly with water, and dried. The resultant products were recrystallized from the specified solvents to yield pure 5a-l.

2.1.1.2.1. 2-Amino-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5a). Colorless crystals (EtOH) (80%); m.p.: 304–306 °C; ¹H NMR (DMSO- d_6): $\delta_H = 11.77$ (s, 1H, NH), 7.93 (d,J = 8.0 Hz; 1H), 7.58 (t,J = 7.7 Hz, 1H), 7.42–7.06 (m, 9H, Ph-H, Q-H and NH₂), 4.51 ppm (s, 1H, H-4); ¹³C NMR (DMSO- d_6): $\delta_C = 160.94$ (CO), 159.45 (C-2), 151.69 (C-10b), 144.84 (Ph-C), 138.26 (C-6a), 131.69, 128.86, 127.85, 127.20, 122.46, 122.25 (Ar-CH), 120.31 (CN), 115.83 (C-10a), 112.48 (C-4a), 110.07 (C-7), 58.28 (C-3), 37.19 ppm (C-4): anal. calcd for C₁₉H₁₃N₃O₂: C, 72.37; H, 4.16; N, 13.33; found: C, 72.47; H, 4.21; N, 13.49.

2.1.1.2.2. 2-Amino-9-methyl-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5b). Colorless crystals (EtOH) (90%); m.p.: 309–311 °C; 1 H NMR (DMSO- 4 6): 6 H = 11.68 (s, 1H, NH), 7.73 (s, 1H, H-10), 7.42 (d, 4 J = 6 Hz, 1H, H-8), 7.25 (m, 8H, H-7, NH2, Ph-CH), 4.49 (s, 1H, H-4), 2.40 ppm (s, 3H, Me); 13 C NMR (DMSO- 4 6): 6 C = 160.80 (CO), 159.50 (C-2), 151.56 (C-10b), 144.91 (Ph-C), 136.30 (C-6a), 132.89 (C-9), 131.60 (C-8), 128.85, 127.81, 127.18, 121.70 (Ph-CH), 120.30 (CN), 115.78 (C-10a), 112.38 (C-7), 110.02 (C-4a), 58.30 (C-3), 37.20 (C-4), 21.13 ppm (Me): anal. calcd for 13 C C₂₀H₁₅N₃O₂: C, 72.94; H, 4.59; N, 12.76; found: C, 72.88; H, 4.77; N, 12.71.

2.1.1.2.3. 2-Amino-9-methoxy-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5c). Colorless crystals (EtOH) (92%); m.p.: 312–314 °C; ^1H NMR (DMSO- d_6): $\delta_{\text{H}}=11.66$ (s, 1H, NH), 7.44 (d, J=2.6 Hz, 1H, H-8), 7.32–7.18 (m, 9H, q-H-7,10, Ph-H, NH2), 4.51 (s, 1H, H-4), 3.85 ppm (s, 3H, OCH3); ^{13}C NMR (DMSO- d_6): $\delta_{\text{C}}=159.99$ (CO), 159.06 (C-2), 154.42 (C-9),

150.88 (C-10b), 144.42 (Ph-C), 132.33 (C-6a), 128.42, 127.39, 126.74, 120.46 (Ph-CH, q-C-7,10), 120.00 (CN), 116.86 (C-10a), 112.50 (C-8), 109.92 (C-4a), 57.68 (C-3), 55.57 (OMe), 36.76 ppm (C-4): anal. calcd for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.38; N, 12.17; found: C, 69.74; H, 4.29; N, 12.09.

2.1.1.2.4. 2-Amino-8-methyl-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5d). Colorless crystals (EtOH) (90%); m.p.: 300–302 °C; ¹H NMR (DMSO- d_6): $\delta_H = 11.74$ (s, 1H, NH), 7.74–7.19 (m, 10H, H-7,9,10, Ph-CH, NH₂), 4.53 (s, 1H, H-4), 2.78 ppm (s, 3H, Me); ¹³C NMR (DMSO- d_6): $\delta_C = 160.94$ (CO), 159.45 (C-2), 151.69 (C-10b), 144.84 (Ph-C), 138.26 (C-8), 131.69 (C-6a), 128.86, 127.85, 127.20, 122.66, 122.46 (Ar-CH), 120.31 (CN), 115.93 (C-7), 112.88 (C-10a), 110.07 (C-4a), 58.28 (C-3), 37.19 (C-4), 21.23 ppm (Me): anal. calcd for C₂₀H₁₅N₃O₂: C, 72.94; H, 4.59; N, 12.76; found: C, 73.01; H, 4.66; N, 12.61.

2.1.1.2.5. 2-Amino-6-methyl-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5e). Colorless crystals (EtOH) (78%); m.p.: 290–292 °C; 1 H NMR (DMSO- d_6): $\delta_H = 8.03$ (d, J = 8.0 Hz, 1H), 7.70 (t, J = 7.9 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.27 (d, J = 5.5 Hz, 4H), 7.20 (dd, J = 11.7, 7.1 Hz, 1H, NH₂), 4.52 (s, 1H, H-4), 3.53 ppm (s, 3H, N-Me); 13 C NMR (DMSO- d_6): $\delta_C = 160.84$ (CO), 159.45 (C-2), 151.64 (C-10b), 144.84 (Ph-C), 138.26 (C-6a), 131.69, 128.80, 127.85, 127.24, 122.46, 122.25 (Ar-CH), 120.30 (CN), 115.83 (C-10a), 112.48 (C-4a), 110.07 (C-7), 58.20 (C-3), 37.30 (C-4), 29.69 ppm (N-Me): anal. calcd for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.59; N, 12.76; found: C, 72.87; H, 4.76; N, 12.88.

2.1.1.2.6. 2-Amino-4-(4-methoxylphenyl)-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5f). Yellow crystals (EtOH) (86%); m.p.: 301–303 °C; 1 H NMR (DMSO- 1 d): $\delta_{\rm H} = 11.76$ (s, 1H, q-NH), 7.92 (d, J = 8.0 Hz, 1H, q-H-7), 7.57 (t, J = 7.7 Hz, 1H, q-H-8), 7.37–7.18 (m, 4H, q-H-9,10, NH₂), 7.14 (d, J = 8.6 Hz, 2H, Ph-H-o), 6.85 (d, J = 8.6 Hz, 2H, Ph-H-m), 4.47 (s, 1H, H-4), 3.71 ppm (s, 3H, OCH₃); 13 C NMR (DMSO- 1 d): $\delta_{\rm C} = 160.56$ (CO), 158.95 (C-2), 158.11 (Ph-C-OMe), 150.96 (C-10b), 137.75 (Ph-C), 136.52 (C-6a), 131.17, 128.55 (Ph-CH), 121.99, 121.79 (q-CH), 120.02 (CN), 115.38 (C-10a), 113.77 (C-7), 109.93 (C-4a), 58.05 (C-3), 55.05 (OMe), 35.96 ppm (C-4): anal. calcd for C₂₀H₁₅N₃O₃: C, 69.56; H, 4.38; N, 12.17; found: C, 69.43; H, 4.44; N, 12.29.

2.1.1.2.7. 2-Amino-4-(4-methoxylphenyl)-9-methyl-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5g). Yellow crystals (EtOH) (89%); m.p.: 317–319 °C; 1 H NMR (DMSO- 1 G): 1 H NP-H-o), 6.85 (d, 1 H = 8.5 Hz, 2H, Ph-H-m), 4.44 (s, 1H, H-4), 3.70 (s, 3H, OCH₃), 2.39 ppm (s, 3H, CH₃); 1 C NMR (DMSO- 1 G): 1 C = 160.40 (CO), 158.99 (C-2), 158.08 (Ph-C-OMe), 150.80 (C-10b), 136.59 (Ph-C), 135.79 (C-6a), 132.35 (C-9), 131.09 (Ph-CH), 128.50, 121.22 (q-CH), 120.01 (CN), 115.30 (C-10a), 113.75 (C-7), 111.97 (Ph-CH), 109.86 (C-4a), 58.02 (C-3), 55.03 (OMe), 35.96 (C-4), 2070 ppm (Me): anal. calcd for C₂₁H₁₇N₃O₃: C, 70.18; H, 4.77; N, 11.69; found: C, 69.99; H, 4.70; N, 11.86.

2.1.1.2.8. 2-Amino-9-methoxyl-4-(4-methoxylphenyl)-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5h).

Colorless crystals (EtOH) (90%); m.p.: 320–322 °C; ¹H NMR (DMSO- d_6): $\delta_{\rm H}=11.64$ (s, 1H, q-NH), 7.42 (d, J=2.5 Hz, 1H, q-H-7), 7.29 (s, 1H, q-H-10), 7.27 (bs, 2H, NH₂), 7.25 (d, J=8.7, 1H, q-H-8), 7.12 (d, J=8.6 Hz, 2H, Ph-H-o), 6.85 (d, J=8.6 Hz, 2H, Ph-H-m), 4.44 (s, 1H, H-4), 3.84 (s, 3H, OCH₃), 3.71 ppm (s, 3H, OCH₃); ¹³C NMR (DMSO- d_6): $\delta_{\rm C}=160.01$ (CO), 159.00 (C-2), 158.07 (Ph-C-OMe), 154.40 (C-9), 150.57 (C-10b), 136.50 (Ph-C), 132.25 (C-6a), 128.48 (Ph-CH), 120.36 (q-C-7), 120.00 (CN), 116.82 (q-C-8), 113.74 (Ph-CH), 112.54 (q-C-10), 110.23 (C-4a), 58.00 (C-3), 55.55 (OMe), 55.04 (OMe), 35.96 ppm (C-4): anal. calcd for C₂₁H₁₇N₃O₄: C, 67.19; H, 4.56; N, 11.19; found: C, 67.28; H, 4.72; N, 11.13.

2.1.1.2.9. 2-Amino-6-methyl-4-(4-methoxylphenyl)-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5i). Colorless crystals (EtOH) (78%); m.p.: 296–298 °C; 1 H NMR (DMSO- 4 6): $\delta_{\rm H}$ = 8.02 (d, J = 7.2 Hz, 1H, q-H-10), 7.70 (t, J = 8.2 Hz, 1H, q-H-8), 7.55 (d, J = 8.5 Hz, 1H, q-H-7), 7.39 (t, J = 7.5, 1H, q-H-8), 7.24 (s, 2H, NH₂), 7.13 (d, J = 8.6 Hz, 2H, Ph-H- $^{\circ}$ 0), 6.84 (d, J = 8.6 Hz, 2H, Ph-H- $^{\circ}$ 0), 6.84 (d, J = 8.6 Hz, 2H, Ph-H- $^{\circ}$ 0), 4.47 (s, 1H, H-4), 3.71 (s, 3H, OCH₃), 3.54 ppm (s, 3H, N-CH₃); 13 C NMR (DMSO- $^{\circ}$ 6): $\delta_{\rm C}$ = 159.77 (CO), 158.8 (C-2), 158.08 (Ph-C-OMe), 149.80 (C-10b), 138.50 (q-C-6a), 136.45 (Ph-C), 131.46 (Ph-CH), 128.65, 121.10 (q-CH), 119.98 (CN), 114.82 (Ph-CH), 113.67 (C-7), 109.25 (C-4a), 58.16 (C-3), 55.01 (OMe), 36.54 (C-4), 29.22 ppm (N-Me): anal. calcd for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69; found: C, 70.31; H, 4.63; N, 11.55.

2.1.1.2.10. 2-Amino-4-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5j). Colorless crystals (EtOH) (70%); m.p.: 298–300 °C; 1 H NMR (DMSO- d_6): δ_H = 11.79 (s, 1H, NH), 7.92 (d, J = 7.9 Hz, 1H, q-H-10), 7.58 (t, J = 7.6 Hz, 1H, q-H-7), 7.32 (dd, J = 18.1, 5.9 Hz, 6H, Ph-H, NH₂), 7.26 (t, J = 8.1, 2H, q-H-8,9), 4.53 ppm (s, 1H, H-4). Anal. calcd for C₁₉H₁₂ClN₃O₂: C, 65.24; H, 3.46; N, 12.01; found: C, 65.37; H, 3.39; N, 12.18.

2.1.1.2.11. 2-Amino-9-methyl-4-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5k). Colorless crystals (EtOH) (68%); m.p.: 300–302 °C; $^1\mathrm{H}$ NMR (DMSO- d_6): $\delta_{\mathrm{H}}=11.71$ (s, 1H, NH), 7.73 (s, 1H, q-H-10), 7.42 (dd, J=8.5, 1.8 Hz, 1H, q-H-8), 7.35 (d, J=8.5 Hz, 2H, ph-H-o), 7.25 (dd, J=16.1, 9.6 Hz, 5H, q-H-7, Ph-H-m, NH₂), 4.51 (s, 1H, H-4), 2.40 ppm (s, 3H, Me); $^{13}\mathrm{C}$ NMR (DMSO- d_6): $\delta_{\mathrm{C}}=160.30$ (CO), 158.97 (C-2), 151.12 (C-10b), 143.48 (Ph-C), 135.92 (C-6a), 132.55 (C-9), 131.27 (Ph-C-Cl), 131.16, 129.36 (Ph-CH), 128.35 (q-C-8), 121.28 (q-C-7), 119.75 (CN), 115.36 (C-10a), 109.00 (C-4a), 57.27 (C-3), 36.28 (C-4), 20.70 ppm (Me): anal. calcd for $\mathrm{C}_{20}\mathrm{H}_{14}\mathrm{ClN}_3\mathrm{O}_2$: C, 66.03; H, 3.88; N, 11.55; found: C, 65.91; H, 4.02; N, 11.49.

2.1.1.2.12. 2-Amino-9-methoxyl-4-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (5l). Colorless crystals (EtOH) (65%); m.p.: 310–312 °C; ¹H NMR (DMSO- d_6): δ_H = 11.68 (s, 1H, NH), 7.33 (d, J = 2.5 Hz, 1H, q-H-7), 7.37–7.21 (m, 8H, q-H-8,9,10, ph-H, NH $_2$), 4.52 (s, 1H, H-4), 3.85 ppm (s, 3H, OMe); ¹³C NMR (DMSO- d_6): δ_C = 159.97 (CO), 159.02 (C-2), 154.46 (C-9), 150.93 (C-10b), 143.43 (Ph-C), 132.40 (Ph-C-Cl), 131.30 (q-C-6a), 129.38, 128.34 (Ph-CH), 120.56 (q-C-7), 119.77

(CN), 116.90 (C-10a), 112.45 (q-C-8), 109.37 (C-4a), 57.22 (C-3), 55.58 (OMe), 36.31 ppm (C-4): anal. calcd for C₂₀H₁₄ClN₃O₃: C, 63.25; H, 3.72; N, 11.06; found: C, 63.33; H, 3.89; N, 10.92.

2.2. Biology

- **2.2.1. Cell viability assay.** The human mammary gland epithelial (MCF-10A) normal cell line was used to examine the viability effects of new derivatives **5a–l** using the MTT test.^{33,34} Refer to Appendix A for more details.
- **2.2.2. Antiproliferative assay.** The MTT assay was used to assess the antiproliferative activity of **5a-l** against four human cancer cell lines, with erlotinib serving as a control. ^{35,36} Appendix A has more information.
- **2.2.3. EGFR inhibitory assay.** The EGFR-TK assay³⁷ assessed the inhibitory activity of the most potent antiproliferative derivatives, **5a**, **5d**, **5e**, **5h**, and **5i**, against the EGFR. For more details, see Appendix A.
- **2.2.4.** BRAF^{V600E} inhibitory assay. Compounds 5a, 5d, 5e, 5h, and 5i were assessed for their ability to inhibit BRAF^{V600E}, with erlotinib as the reference agent.³⁸ The outcomes are presented as IC_{50} values. Appendix A outlines additional experimental details.
- **2.2.5. HER-2 inhibitory assay.** The kinase assay³⁹ was used to assess the inhibitory activity of compounds **5a**, **5d**, **5e**, **5h**, and **5i** against HER-2. The results are presented as IC_{50} values. Lapatinib served as the reference medication. Appendix A describes more experimental details.

Results and discussion

3.1. Chemistry

Aly *et al.*, 27 previously reported the synthesis of pyrano[3,2-c] quinoline-3-carboxylate derivatives. The synthesis of compounds **3a-h** was achieved through the reaction of 4-hydroxy-2-oxo-1,2-dihydroquinoline derivatives **1a-h** with ethyl (E)-2-cyano-3-(furan-2-yl)acrylate (2), and their biological properties were investigated as dual-function anticancer and

antibacterial drugs, potentially serving as topoisomerase II and DNA-gyrase inhibitors (Scheme 1).

Also, Kadu et al., obtained the similar 4H-pyrano[3,2-c] quinoline-3-carbonitriles via three-component multicomponent reactions. 40 In this manuscript, to prevent the possibility of side reactions, between quinolones-C-3 and aldehyde, we developed a new series of 2-amino-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile derivatives 5a-l via direct interaction between 4-hydroxy-2-oxo-1,2dihydroquinoline derivatives 2-benzylidene-1a-e and malononitriles 4a-c (Scheme 2).41 The reaction was conducted under several conditions: ethanol/Et₃N under reflux (method I), ethanol/K2CO3 (method II), and DMF with stirring at room temperature (method III) (Scheme 2). Nevertheless, when the reaction was conducted using method III, the optimal approach afforded substantial yields of products 5a-l with exceptional purity (65-92%). Additionally, all the reactions were carried out under the same conditions and through three different methods, but a higher yield was obtained when using method III. In general, across the three methods used, there was a clear effect on the yield percentage in the case of substitutes with electron-withdrawing group's 5j-l comparing with others obtained compounds 5a-i as shown in Table 1.

NMR spectroscopy, elemental analysis, and mass spectrometry were performed on all samples to validate the structures of our new compounds. For example, compound $5\mathbf{b}$ was assigned as 2-amino-9-methyl-5-oxo-4-phenyl-5,6-dihydro-4*H*-pyrano[3,2-c]quinoline-3-carbonitrile and $5\mathbf{h}$, designated as 2-amino-9-methoxy-4-(4-methoxyphenyl)-5-oxo-5,6-dihydro-4*H*-pyrano[3,2-c]quinoline-3-carbonitrile (Fig. 3). The elemental analysis and mass spectrometry of $5\mathbf{b}$ gave its molecular weight as m/z 329, with the molecular formula $C_{20}H_{15}N_3O_2$. The 1H NMR spectrum for $5\mathbf{b}$ showed four singlet signals at $\delta_H = 2.40$, 4.49, 7.73, and 11.68 ppm, which were assigned as CH_3 group, H-4, H-10, and quinolinone-NH, respectively, in addition to aromatic protons. Another doublet signal at $\delta_H = 7.42$ ppm (J = 8.4 Hz; 1H) as H-8. The NH_2 protons resonated as a broad singlet at $\delta_H = 7.27$ ppm,

R¹ = H, Cl, Br, F, and Me R² = H, OMe R³ = H, Me

Scheme 1 Synthesis of pyrano[3,2-c]quinoline-3-carboxylates 3a-h.

$$R^{2} \longrightarrow R^{1} \longrightarrow R^{1} \longrightarrow R^{2} \longrightarrow R^{2$$

Scheme 2 Synthesis of 2-amino-pyrano[3,2-c]quinoline-3-carbonitriles 5a-l.

which appeared clearly in compound 5h (Fig. 3). As $^{13}\mathrm{C}$ NMR spectrum revealed the methyl carbon signals at $\delta_\mathrm{C}=21.13$ (CH $_3$) and 58.30 ppm (C-3), while the CH-pyran appeared as a singlet at $\delta_\mathrm{C}=37.20$ ppm (C-4). The two signals resonated at $\delta_\mathrm{C}=160.80$ and 159.50 ppm, were assigned as carbonyl group and pyran-C-2, respectively.

Compound **5h** gives similar chemical shifts for all protons, unlike the aromatic ring, which contains a doublet of doublet

Table 1 Yields of 2-amino-6-methyl-4-(subst. phenyl)-5-oxo-5,6-dihydro-4H-pyrano-[3,2-c]quinoline-3-carbonitriles using methods I, II and III

Compound	Method I	Method II	Method III	
5a	60%	70%	80%	
5 b	63%	77%	90%	
5c	65%	78%	92%	
5 d	67%	80%	90%	
5e	57%	66%	78%	
5 f	70%	70%	86%	
5g	61%	77%	89%	
5 h	69%	79%	90%	
5i	57%	67%	78%	
5j	50%	65%	70%	
5k	48%	63%	68%	
5 l	45%	61%	65%	

system due to a 1,4-disubstituted benzene ring at $\delta_{\rm H}=7.12$ (d, J=8.6 Hz; 2H), 6.85 ppm (d, J=8.6 Hz; 2H). Also, the $^1{\rm H}$ NMR spectrum for **5h** clarified five singlet signals at $\delta_{\rm H}=11.64$, 7.29, 7.27, 4.44 and 3.84 ppm, which were assigned as NH, quinolinone-H-10, amino-group, pyran-CH and methoxy group, respectively. However, the signal resonated at $\delta_{\rm H}=7.25$ ppm, which appeared as dt-coupling with two different J-coupling (J=8.7, 6.7 Hz, 1H), was assigned quinolinone-H-8, resulting two different coupling system, the first one as AB-coupling system with the quinolinone-H-7 and the second coupling as AX-coupling system with two methoxy-proton in the same direction due to the sp³-configuration of methoxy group. The $^{13}{\rm C}$ NMR spectrum of compound **5b** confirmed its $^{1}{\rm H}$ NMR spectral data.

5k, $R^1 = R^3 = H$, $R^2 = Me$, X = Cl5l, $R^1 = R^3 = H$, $R^2 = OMe$, X = Cl

For example, the 13 C NMR spectrum revealed the methyl carbon signals at $\delta_{\rm C}=21.13$ (CH₃) and 37.20 (C-4), in addition to the carbonyl group and cyano-group, which appeared as a singlet at $\delta_{\rm C}=160.80$ and 115.78 ppm, respectively. Also, the pyran-C-2 and C-3 are resonated at $\delta_{\rm C}=159.50$ and 58.30 ppm, respectively, by observed trends in d values for C-atoms in pushpull alkenes. However, elemental analyses and mass spectrometry for compound 5h revealed that it was formed through an interaction between one molecule of 4-hydroxy-6-methoxy-quinolin-2(1H)-one 1c and another molecule of 2-(4-

Fig. 3 Distinctive carbons and protons of compounds 5b and 5h.

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1} \\
1a-e
\end{array}$$

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1} \\
R^{2} \\
R^{3}
\end{array}$$

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1} \\
R^{2} \\
R^{3}
\end{array}$$

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1} \\
R^{2} \\
R^{3}
\end{array}$$

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1} \\
R^{2} \\
R^{3}
\end{array}$$

$$\begin{array}{c}
R^{1} \\
R^{2} \\
R^{3} \\
R^{1}
\end{array}$$

$$\begin{array}{c}
R^{1} \\
R^{2} \\
R^{3} \\
R^{1}
\end{array}$$

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1}$$

$$\begin{array}{c}
R^{2} \\
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$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1}$$

$$\begin{array}{c}
R^{2} \\
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R^{1}
\end{array}$$

$$\begin{array}{c}
R^{2} \\
R^{3} \\
R^{1}$$

$$\begin{array}{c}
R^{3} \\
R^{3} \\
R^{3} \\
R^{3}
\end{array}$$

Scheme 3 Suggested mechanism for pyrano[3,2-c]quinoline-3-carbonitriles 5a-l.

Table 2 IC_{50} values of compounds 5a-l and erlotinib against four cancer cell lines

$$R^{2} \longrightarrow NH_{2} CN$$

$$R^{3} \longrightarrow NH_{2} CN$$

$$R^{3} \longrightarrow NH_{2} CN$$

$$R^{3} \longrightarrow NH_{2} CN$$

Compound 5a-l

	Cell viability (%)	R^1	R^2	R^3	X	Antiproliferative activity $IC_{50} \pm SEM$ (nM)				
Comp.						A-549	MCF-7	Panc-1	HT-29	Average (GI ₅₀)
5a	92	Н	Н	Н	Н	32 ± 2	27 ± 2	32 ± 2	32 ± 2	31
5 b	90	H	Me	Н	Н	48 ± 4	44 ± 4	48 ± 4	50 ± 5	48
5 c	91	H	ОМе	Н	Н	66 ± 6	59 ± 5	68 ± 6	70 ± 6	66
5d	93	Н	Н	Me	Н	35 ± 3	31 ± 2	36 ± 3	37 ± 3	35
5e	90	Me	Н	H	Н	26 ± 2	24 ± 2	28 ± 2	27 ± 2	26
5 f	92	H	H	H	ОМе	54 ± 4	50 ± 4	56 ± 5	56 ± 5	54
5g	93	Н	Me	H	OMe	71 ± 6	67 ± 6	74 ± 6	74 ± 6	72
5 h	91	Н	OMe	H	OMe	28 ± 2	26 ± 2	29 ± 2	29 ± 2	28
5i	90	Me	Н	H	ОМе	30 ± 2	27 ± 2	31 ± 2	33 ± 2	30
5j	91	H	H	H	Cl	43 ± 4	38 ± 3	44 ± 4	46 ± 4	43
5k	93	Н	Me	H	Cl	39 ± 3	34 ± 3	42 ± 3	42 ± 3	39
51	92	Н	ОМе	Н	Cl	60 ± 5	56 ± 5	62 ± 5	63 ± 5	61
Erlotinib	N.D.	_	_	_	_	30 ± 3	40 ± 3	30 ± 3	30 ± 3	33

methoxybenzylidene)malononitrile **4b** without any elimination, with the chemical formula $C_{21}H_{17}N_3O_4$ and molecular weight m/z=375.

The proposed mechanism for the obtained products **5a-l** begins with a nucleophilic attack of the active C-3, which is formed by triethylamine **1a-e**, to bond in compound **4a-c** *via* Michael addition to produce intermediates **6**. Further internucleophilic attack of the hydroxyl lone pair, followed by cyclization occurs and forms the intermediate **8**. Finally, the rearrangement of the intermediate **8** gives the final products **5a-l** (Scheme 3).

3.2. Biology

3.2.1. Cell viability assay. The human mammary gland epithelium (MCF-10A) normal cell line was employed to evaluate the viability effects of novel derivatives 5a–1. The cell viability of 5a–1 was assessed via the MTT assay following four days of incubation with $50 \mu M$ of each compound on MCF-10A cells. 33,34 The results in Table 2 indicate that none of the investigated compounds exhibited cytotoxicity, and all hybrids maintained over 90% cell viability at a concentration of $50 \mu M$.

3.2.2. Antiproliferative assay. The MTT assay was employed to evaluate the antiproliferative effects of targeting **5a-l**, using erlotinib as a reference, against four human cancer cell lines: colon cancer (HT-29), pancreatic cancer (Panc-1), lung cancer (A-549), and breast cancer (MCF-7). Table 2 presents each compound's median inhibitory concentration (IC₅₀) and GI_{50} (mean IC_{50}) against the four cancer cell lines.

Target 5a–I displayed significant antiproliferative efficacy, with GI_{50} values ranging from 26 nM to 72 nM, compared to the reference erlotinib ($GI_{50} = 33$ nM). Furthermore, all evaluated compounds showed pronounced sensitivity to the breast cancer (MCF-7) cell line relative to other cell lines examined. Compounds 5a, 5d, 5e, 5h, and 5i exhibited the highest antiproliferative potency, with GI_{50} values of 31, 35, 26, 28, and 30 nM, rendering these compounds (except 5d) more effective than erlotinib. Furthermore, these five derivatives exhibited superior efficacy to erlotinib against the MCF-7 cancer cell line, with IC_{50} values between 24 nM and 31 nM, while erlotinib's IC_{50} value is 40 nM.

Compound 5e ($R^1 = Me$, $R^2 = R^3 = X = H$), N-Me derivative, was the most effective of all the synthesized derivatives, with a GI_{50} value of 26 nM. It was 1.3 times more effective than erlotinib ($GI_{50} = 33$ nM) against the four cancer cell lines that were tested. Furthermore, 5e exhibited an IC_{50} value of 24 nM against the MCF-7 breast cancer cell line, demonstrating a potency 1.7-fold greater than erlotinib ($IC_{50} = 40$ nM) against the MCF-7 cancer cell type. Also, compound 5e has marginally greater effectiveness than erlotinib against the other three cell lines, as shown in Table 2. These findings countered recent findings from related compounds, showing that N-alkyl derivatives had the lowest activity relative to derivatives containing a free nitrogen atom at quinoline position-1.21

The antiproliferative efficacy of compounds 5a-l is markedly influenced by the substitution pattern at positions one (N-1) and six of the quinoline scaffold. For instance, compound

Table 3 IC₅₀ values of compounds **5a**, **5d**, **5e**, **5h**, and **5i** against EGFR, BRAF^{V600E}, and HER-2

Compound	EGFR inhibition $IC_{50} \pm SEM (nM)$	$BRAF^{V600E}$ inhibition $IC_{50} \pm SEM$ (nM)	HER-2 inhibition $IC_{50} \pm SEM (nM)$
5a	84 ± 5	79 ± 5	36 ± 2
5d	87 ± 5	83 ± 5	39 ± 2
5e	71 ± 4	62 ± 3	21 ± 1
5h	75 ± 4	67 ± 3	23 ± 1
5i	78 ± 4	74 ± 4	31 ± 2
Erlotinib	80 ± 5	60 ± 3	_
Lapatinib	_	_	26 ± 1
•			

5a ($R^1 = R^2 = R^3 = X = H$), a derivative containing a free nitrogen atom, had inferior efficacy as an antiproliferative agent compared to the *N*-methyl derivative, 5e ($R^1 = Me$, $R^2 = R^3 = X = H$). Compound 5a had a GI₅₀ value of 31 nM against four cancer cell lines, placing it fourth in activity compared to 5e ($GI_{50} = 26$ nM). An additional example is the 6-methyl derivative, compound 5b ($R^2 = Me$, $R^1 = R^3 = X = H$), and the 6-methoxy derivative, 5c ($R^2 = OMe$, $R^1 = R^3 = X = H$), both of which were demonstrated to be less efficacious than the unsubstituted derivative, 5a ($R^1 = R^2 = R^3 = X = H$). Compounds 5b and 5c demonstrate IC₅₀ values of 48 and 66 nM, respectively, which are 1.5 and 2.1 times less efficient than 5a ($GI_{50} = 31$ nM). The findings indicate that derivatives with an unsubstituted phenyl group of the quinoline moiety exhibit greater efficiency than those substituted with electron-donating methyl and methoxy groups.

Also, the substitution pattern at position 4 of the phenyl group in the pyran moiety may significantly influence the antiproliferative activity of these compounds. Compound 5h ($R^1 = R^3 = H, R^2 = X = OMe$) exhibited the second highest activity, with a GI_{50} value of 26 nM, demonstrating a potency 1.25-fold greater than the reference erlotinib, which has a GI_{50} value of 33 nM. Compound 5h exhibits antiproliferative activity comparable to that of 5e, with both demonstrating more potency against the MCF-7 cell line than the reference erlotinib, Table 2.

Compounds $\mathbf{5c}$ ($\mathbf{R}^1 = \mathbf{R}^3 = \mathbf{H}$, $\mathbf{R}^2 = \mathbf{OMe}$, $\mathbf{X} = \mathbf{H}$) and $\mathbf{5l}$ ($\mathbf{R}^1 = \mathbf{R}^3 = \mathbf{H}$, $\mathbf{R}^2 = \mathbf{OMe}$, $\mathbf{X} = \mathbf{Cl}$) possess identical structural characteristics to compound $\mathbf{5h}$, differing just in the substitutions at the four position of the phenyl group of the pyran moiety, with hydrogen for $\mathbf{5c}$ and chlorine for $\mathbf{5l}$. Compounds $\mathbf{5c}$ and $\mathbf{5l}$ exhibited \mathbf{GI}_{50} values of $\mathbf{66}$ and $\mathbf{61}$ nM, respectively, demonstrating a potency reduction of 2.5- and 2.3-fold compared to $\mathbf{5h}$. This underscores the significance of the substitution pattern at this position on antiproliferative efficacy, with increasing activity in the sequence $\mathbf{OMe} > \mathbf{Cl} > \mathbf{H}$.

Finally, the substitution of the 6-OMe group in compound 5h with a 6-Me group, as observed in compound 5g ($R^1 = R^3 = H$, $R^2 = Me$, X = OMe), led to a notable reduction in antiproliferative activity. Compound 5g demonstrated a GI_{50} value of 72 nM, making it the least effective derivative and 2.6-fold less potent than 5h. These findings indicate that the methoxy group is more tolerated at the six-position for antiproliferative action than the methyl group.

3.2.3. Assay for EGFR inhibitory activity. The EGFR-TK assay³⁷ has been performed to evaluate the inhibitory efficacy of the most potent antiproliferative derivatives 5a, 5d, 5e, 5h, and 5i against EGFR, with results (IC50 values) presented in Table 3. The results from this in vitro assay were consistent with those from the antiproliferative assay. Compound 5e ($R^1 = Me$, $R^2 = R^3 = X = H$), the most effective antiproliferative derivative, exhibited the highest efficiency as an EGFR inhibitor with an IC₅₀ value of 71 nM, demonstrating 1.2-fold more potency than the reference medication erlotinib, which has an IC50 value of 80 nM. Compounds **5h** ($R^1 = R^3 = H$, $R^2 = X = OMe$) and **5i** (R^1 = Me, $R^2 = R^3 = H$, X = OMe) exhibited second and thirdhighest EGFR inhibitory activity, with IC50 values of 75 and 78 nM, respectively. Both compounds exhibit lower potency than 5e, although they remain more potent than the reference erlotinib, as indicated in Table 3.

Compound 5a ($R^1 = R^2 = R^3 = X = H$), featuring a free nitrogen atom at position-1 of the quinoline moiety, exhibited lower efficacy as an EGFR inhibitor than the *N*-methyl derivative, compound 5e. Compound 5a demonstrated an IC₅₀ value of 84 nM, rendering it 1.2-fold less potent than 5e and even less potent than the reference erlotinib (IC₅₀ = 80 nM), suggesting that the presence of a free nitrogen atom in the quinoline moiety does not confer any benefits for the antiproliferative or anti-EGFR activities of this class of compounds. Compound 5d ($R^1 = R^2 = X = H$, $R^3 = Me$) exhibited significant inhibitory activity as an EGFR inhibitor, with an IC₅₀ value of 87 nM. These data indicated that compounds 5e, 5h, and 5i displayed significant antiproliferative activity and may function as EGFR inhibitors.

3.2.4. Assay for BRAF^{V600E} **inhibitory action.** The *in vitro* anti-BRAF^{V600E} efficacy of compounds **5a**, **5d**, **5e**, **5h**, and **5i** was evaluated.³⁸ The enzyme assays indicated that the evaluated hybrids exhibited substantial BRAF^{V600E} inhibitory activity, with IC₅₀ values between 62 and 83 nM, as presented in Table 3. In every instance, the IC₅₀ of the examined compounds exceeds that of the reference erlotinib (IC₅₀ = 60 nM).

Once again, compound $5\mathbf{e}$, the most effective antiproliferative and EGFR inhibitor, demonstrated superior efficacy as a mutant-BRAF (BRAF^{V600E}) inhibitor, with an IC₅₀ value of 62 nM, equivalent to the reference erlotinib (IC₅₀ = 60 nM). Compounds $5\mathbf{h}$ and $5\mathbf{i}$ exhibited remarkable anti-BRAF^{V600E} inhibitory efficacy, with IC₅₀ values of 67 and 74 nM, respectively. Compounds $5\mathbf{h}$ and $5\mathbf{i}$ displayed 1.1- and 1.3-fold reduced

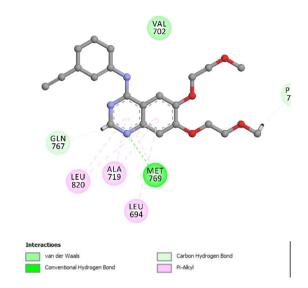


Fig. 4 Two-dimensional schematic illustration depicting the binding orientation of erlotinib within the EGFR active site, highlighting key interactions that stabilize the ligand in the binding pocket.

potency compared to the reference erlotinib as BRAF^{V600E} inhibitors. Although the $\rm IC_{50}$ values of the studied compounds as BRAF^{V600E} inhibitors are lower than their $\rm IC_{50}$ values as EGFR inhibitors, all investigated compounds had lesser potency as BRAF^{V600E} inhibitors than erlotinib, necessitating additional structural modifications to improve their efficacy against BRAF^{V600E}.

3.2.5. HER-2 inhibitory assay. Compounds **5a, 5d, 5e, 5h,** and **5i** were evaluated for their capacity to inhibit HER-2 by the kinase assay. The findings are displayed in Table 3. Lapatinib functioned as the reference drug.

The findings indicated that the investigated compounds markedly suppressed HER-2, exhibiting IC $_{50}$ values between 21 and 39 nM, compared to lapatinib's IC $_{50}$ of 26 nM. Compounds 5e and 5h exhibited the highest potency as HER-2 inhibitors, with IC $_{50}$ values of 21 and 23 nM, respectively, surpassing the reference lapatinib, which has an IC $_{50}$ value of 26 nM. Compounds 5a, 5d, and 5i exhibited HER-2 activity with IC $_{50}$ values of 36, 39, and 31 nM, respectively, which are less efficient than the reference compound lapatinib. These findings indicated that compounds 5e and 5h may serve as lead compounds exhibiting significant antiproliferative activity, potentially operating as multi-targeted protein kinase inhibitors.

3.3. Molecular docking studies

To explore the molecular interactions of compound **5e** with EGFR and HER-2 kinases, docking simulations were carried out utilizing the crystal structures of EGFR (PDB ID: 1M17) and HER-2 (PDB ID: 3PP0).⁴³ These structural templates served as the basis for *in silico* analysis using Discovery Studio.⁴⁴ Erlotinib and lapatinib were selected as benchmark inhibitors for EGFR and HER-2, respectively, to provide a comparative assessment of binding efficiency. Prior to docking, comprehensive preparation of the protein structures was conducted, including protonation

state adjustments and energy minimization steps to optimize the geometry. The OPLS-AA force field was employed during the minimization phase to ensure stable ligand–protein conformations and improve docking precision. To confirm the robustness of the docking protocol, erlotinib, co-crystallized in the EGFR structure, was subjected to a re-docking procedure. The reproduced binding pose showed strong concordance with the crystallographic orientation, yielding a binding affinity score of -8.56 kcal mol $^{-1}$ and a root-mean-square deviation (RMSD) of 0.95 Å. This level of agreement validated the reliability of the docking workflow. Importantly, the re-docked pose highlighted a key hydrogen bond between the pyrimidine moiety of erlotinib and the Met769 residue in the EGFR active site, a critical interaction for ligand stabilization (Fig. 4).

The docking evaluation of compound **5e** within the ATP-binding cleft of EGFR yielded a favorable binding pose, reflected by a docking score of -7.76 kcal mol⁻¹ and an RMSD value of 1.40 Å. These results support the reliability of the computational approach and suggest a meaningful correlation between the predicted binding conformation and potential

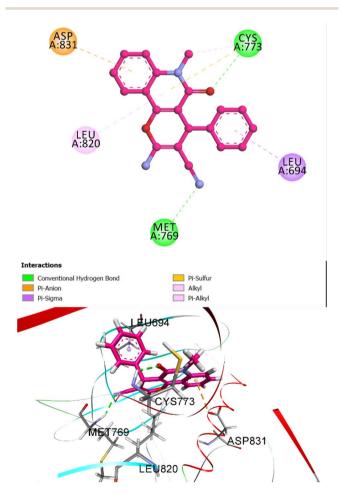


Fig. 5 Two- and three-dimensional representations of the binding mode of compound 5e within the EGFR active site. The ligand forms a key hydrogen bond with Met769, along with additional interactions including π -alkyl contacts with Leu694 and Leu820, π -sulfur and π -anion interactions with Cys773 and Asp831, respectively, and a π -sigma interaction contributing to overall binding stability.

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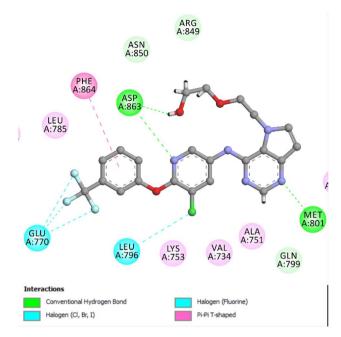


Fig. 6 Two-dimensional schematic representation of the binding interactions formed by the validated co-crystallized ligand within the HER-2 active site. Key interactions include hydrogen bonds with Met801 and Asp863, π - π T-shaped stacking with Phe864, and halogen bonding with Glu770 and Leu796, all contributing to the stabilization of the ligand in the binding pocket.

biological activity. Compound 5e adopts a stable orientation in the active site. The cyano group of pyran ring forms a key conventional hydrogen bond with the Met769 residue,

a hallmark interaction for effective EGFR inhibition. Additionally, the ligand is anchored through multiple non-covalent interactions, including π -alkyl interactions with Leu694 and Leu820. Also, the oxo group of quinolone ring forms essential hydrogen bond with Cys773. In addition, the aromatic system of quinolone moiety forms π -sulfur and π -anion contacts with Cys773 and Asp831, respectively. These contacts collectively enhance the overall binding stability. The spatial accommodation of compound 5e within the EGFR pocket is further reinforced by favorable hydrophobic interactions, supporting its firm positioning within the enzymatic cleft. The binding profile observed in the 2D, and 3D interaction diagrams (Fig. 5) illustrates the multifaceted interaction network contributing to ligand affinity and specificity.

To ensure the reliability of the docking approach for HER-2, the co-crystallized ligand was subjected to a re-docking procedure within the active site. The validation yielded a binding affinity score of -9.21 kcal mol⁻¹ and an RMSD of 1.38 Å, indicating strong agreement with the experimentally observed binding pose and confirming the robustness of the docking methodology. The re-docking analysis revealed a key hydrogen bond between the pyrimidine nitrogen of the ligand and the Met801 residue, a critical interaction that contributes to anchoring the ligand within the active site. Additional stabilization was provided through a hydrogen bond between the pyridine nitrogen and Asp863, reinforcing the binding affinity. Furthermore, the simulation identified supplementary noncovalent interactions, including an additional hydrogen bond with Met801, a π - π T-shaped interaction involving Phe864, and halogen bonds with Glu770 and Leu796. These interactions

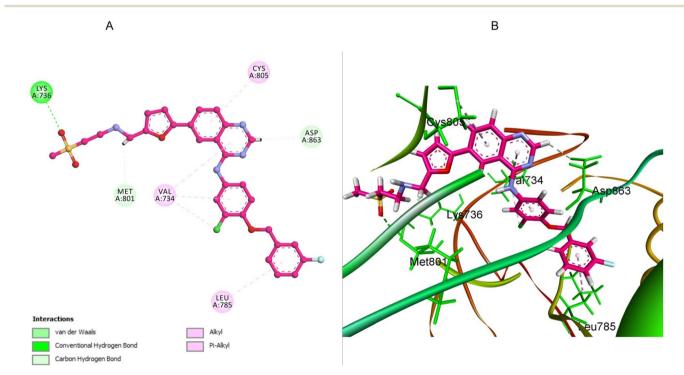


Fig. 7 (A) 2D-docking representation of lapatinib within the HER-2 active site, (B) 3D representation, highlighting key interactions including hydrogen bonding with Lys753, carbon-hydrogen bonds with Met801 and Asp863, hydrophobic contacts with Leu785, and π -alkyl interactions with Val734, all contributing to stable conformation and inhibitory activity.

collectively contribute to a stable and well-oriented ligand conformation within the HER-2 binding pocket (Fig. 6).

The binding behavior of lapatinib, employed as a reference inhibitor in the in vitro evaluation of HER-2, was examined through molecular docking studies. The results revealed a stable binding conformation, with a calculated binding energy of -8.64 kcal mol⁻¹ and an RMSD of 1.01 Å, suggesting effective accommodation within the HER-2 active site. A key stabilizing interaction was observed between the sulfone oxygen atoms of lapatinib and the Lys736 residue, forming a crucial hydrogen bond. This was complemented by carbon-hydrogen bonding interactions with both Met801 and Asp863, which further reinforced the positioning. Additionally, lapatinib established extensive hydrophobic interactions, most notably with Leu785, forming a compact hydrophobic environment that enhances overall stability. The presence of π -alkyl interactions with Val734 contributed to the formation of a robust interaction network, securing lapatinib firmly within the HER-2 binding pocket. These collective interactions provide structural insights into the strong binding affinity and inhibitory potential of lapatinib against HER-2 (Fig. 7).

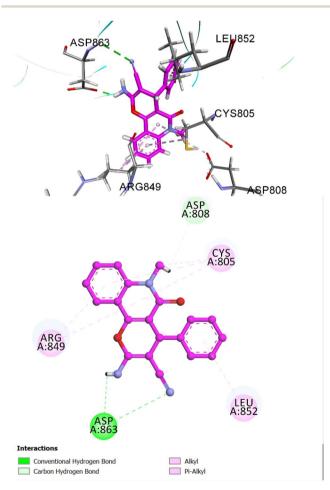


Fig. 8 Two- and three-dimensional docking models showing the binding interactions of compound 5e within the HER-2 active site. Key interactions include a conventional hydrogen bond with Asp863, carbon-hydrogen bonding with Asp808, and π -alkyl contacts with Arg849, Leu852, and Cys805. These interactions collectively support stable orientation and potential inhibitory activity against HER-2.

The molecular docking of compound 5e within the HER-2 active site revealed a favorable binding profile, with a calculated binding energy of -7.84 kcal mol⁻¹ and an RMSD value of 1.37 Å. These values indicate a stable ligand conformation and reliable fit within the binding pocket. Compound 5e demonstrated a wellanchored pose. The cyano and amino groups of pyran moiety stabilize compound 5e through a conventional hydrogen bond with Asp863. Additional stabilization arose from carbon-hydrogen bonding with Asp808, as well as, the quinolone moiety forms multiple π -alkyl interactions involving residues such as Arg849, and Cys805. These interactions contribute to the formation of a robust interaction network within the binding site. The 3D docking visualization highlights the deep insertion of compound 5e into the HER-2 binding cavity, where it forms several essential contacts that enhance its binding affinity and structural integrity. These interactions collectively suggest that compound 5e may serve as a promising scaffold for HER-2 inhibition (Fig. 8).

These molecular interactions observed in both EGFR and HER-2 highlight the structural compatibility and binding strength of compound **5e**, supporting its potential as an effective kinase inhibitor. In conclusion, compound **5e** demonstrated stable and favorable binding within both targets, making it a promising lead candidate for the development of EGFR/HER-2-targeted anticancer therapies.

3.4. Structural activity relationship (SAR) analysis

The following are some key-points outline the SAR of the newly synthesized compounds 5a-l

$$R^2$$
 N^{H_2}
 N^{CN}
 N^{R_2}
 N^{R_2}
 N^{R_2}
 N^{R_2}
 N^{R_2}
 N^{R_2}
 N^{R_2}
 N^{R_2}

Compound 5a-l

- (1) The cyano group of pyran ring forms a key conventional hydrogen bond with the Met769 residue, a crucial interaction for effective EGFR inhibition.
- (2) Additionally, the cyano and amino groups of pyran moiety stabilize compound **5e** within HER-2 active site through a conventional hydrogen bond with Asp863 as well as carbonhydrogen bonding with Asp808.
- (3) The oxo group of quinolone ring forms essential hydrogen bond with Cys773 which stabilize **5e** with EGFR binding site.
- (4) The quinolone moiety forms π -sulfur and π -anion contacts with Cys773 and Asp831, respectively in EGFR binding sites, while forms multiple π -alkyl interactions involving residues such as Arg849, and Cys805 in HER-2 active site.
- (5) Quinoline derivatives with an unsubstituted phenyl group demonstrate superior efficacy compared to those substituted with electron-donating methyl and methoxy groups.

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(6) The substitution pattern of the X group (OMe, H, and Cl) significantly influences activity, with the following order of increasing activity: OMe > Cl > H.

Conclusion

This study involved the design, synthesis, and biological evaluation of a novel series of 2-amino-pyrano[3,2-c]quinoline-3carbonitriles (5a-l) as multi-target inhibitors with significant antiproliferative activity. Compounds 5e and 5h had the most potent antiproliferative activities (GI₅₀ = 26 and 28 nM, respectively), exceeding the efficacy of erlotinib and lapatinib by combined inhibition of EGFR and HER-2. The investigation of structure-activity relationships emphasized the essential function of the quinoline moiety and electron-donating substituents in strengthening activity. The multi-target profile (EGFR/HER-2/ BRAF V600E inhibitors) of these compounds highlights their potential as adaptable therapeutic agents for cancer treatment. Docking simulations identified compound 5e as a strong dualtarget inhibitor for EGFR and HER-2. Its favorable binding interactions and affinity suggest its potential as an effective therapeutic agent. Additional research is needed to evaluate their in vivo effectiveness, pharmacokinetics, and toxicity. Future investigations will be focusing on enhancing BRAF V600E inhibition while preserving the anticancer efficacy of the compounds. Furthermore, these lead compounds will be presented to kinase profiling experiments.

In summary, these quinoline-based compounds constitute a promising category of multi-targeted inhibitors with considerable potential for the development of novel therapeutic medicines for cancer, a serious global health concern. The multi-EGFR/HER-2/BRAF^{V600E} inhibition mechanism puts these compounds as adaptable candidates for advanced development in medicinal chemistry and drug discovery pipelines.

Author contributions

Bahaa G. M. Youssif, Essmat M. El-Sheref, and Aliaa M. Mohassab: conceptualization, methodology, writing, editing and revision. Safwat M. Rabea: writing, editing and revision. S. Bräse: writing and editing. Lamya H. Al-Wahaibi: funding requirements, editing and revision.

Conflicts of interest

The authors reported no potential conflicts of interest(s).

Data availability

Samples of compounds 5a-l are available from the authors. Supplementary information is available. See DOI: https:// doi.org/10.1039/d5ra04276c.

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