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Benzo[g]quinazolin-based scaffold derivatives as dual EGFR/HER2 inhibitors

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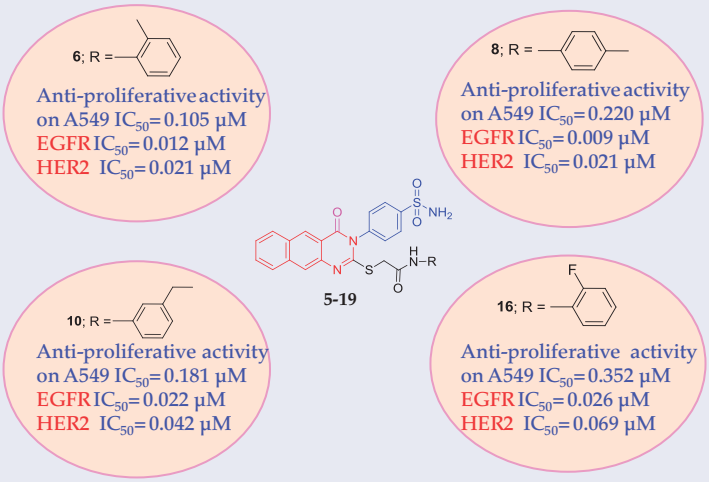
ABSTRACT

Targeting EGFR has proven to be beneficial in the treatment of several types of solid tumours. So, a series of novel 2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-*N*-substituted acetamide **5**–**19** were synthesised from the starting material 4-(2-mercapto-4-oxobenzo[g]quinazolin-3(4*H*)-yl) benzenesulfonamide **4,** to be evaluated as dual EGFR/HER2 inhibitors. The target compounds **5**–**19,** were screened for their cytotoxic activity against A549 lung cancer cell line. The percentage inhibition of EGFR enzyme was measured and compared with erlotinib as the reference drug. Compounds **6, 8, 10**, and **16** showed excellent EGFR inhibitory activity and were further selected for screening as dual EGFR/HER2 inhibitors. The four selected compounds showed IC_{50} ranging from 0.009 to 0.026 μM for EGFR and 0.021 to 0.069 μM for the HER2 enzyme. Compound **8** was found to be the most potent in this study with IC_{50} 0.009 and 0.021 μM for EGFR and HER2, respectively.



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Benzo[g]quinazolin; benzenesulfonamide; EGFR; HER2

Introduction

Tyrosine kinases (TK) are involved in signalling transduction pathways which make them the candidates of choice for the treatment of cancer[1,2]. Epidermal growth factor receptor (EGFR) and human epidermal growth factor 2 (HER2) are members of the TK family. The EGFR family has four members: human epidermal growth factor receptor-2 (HER2; also known as erbB2) and its relatives HER1 (epidermal growth factor receptor; EGFR), HER3, and HER4[3]. Each member of the epidermal growth factor receptor (EGFR) family plays a key role in normal development, homeostasis, and a variety of pathophysiological conditions[4]. Upon overexpression, they form a mono or heterodimers with other ERB receptors leading to activation of signalling pathways and hence tumour growth[5,6]. ERB inhibition can block TK phosphorylation leading to the loss of tumour regulatory functions[5]. The overexpression of EGFR and HER2 has been reported in a variety of solid tumours as non-small cell lung cancers (NSCLC)[7,8]. They are also accompanied with postoperative adverse, radiotherapy, and chemotherapy resistance[9]. So, it is more effective to dual target EGFR/HER2 rather than EGFR inhibition alone[10].

Quinazolines are heterocyclic compounds having diverse biological activities including anticancer activity[11,12]. The wide reported biological activities of quinazoline derivative might have derived from the fact that its corresponding monocyclic counterpart, pyrimidines are being prebiotic in nature to living cells in biodiversity which made them be highly privileged motifs for the

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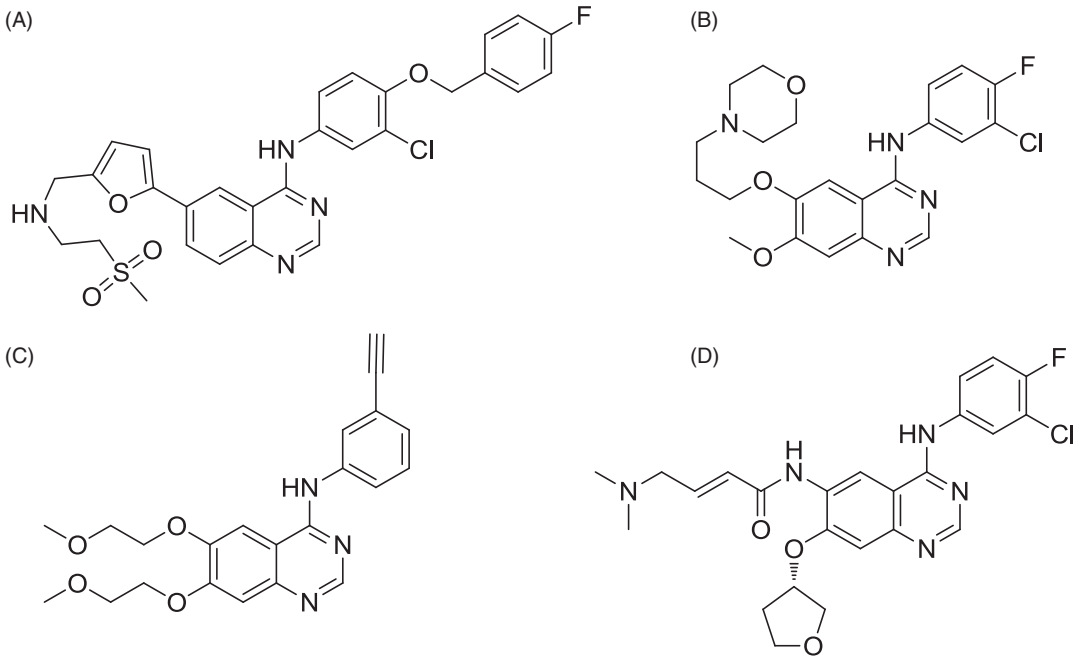


Figure 1. Structures of EGFR inhibitors.

development of molecules of biological and pharmaceutical interest[3,13].

The 4-anilinoquinazoline is an important nucleus in targeting EGFR/HER2 dual inhibitors, the most common examples are lapatinib (Tykerb™) (**A**), used for patients with HER2 overexpression metastatic breast cancer[10,14]. Gefitinib (Iressa™) (ZD1839) (**B**), was approved for the treatment of patients with EGFR mutation-positive NSCLC[15]. Erlotinib (Tarceva™) (**C**), the reference drug used in this study, is an EGFR inhibitor that is used in the therapy of advanced or metastatic NSCLC after the failure of at least one prior chemotherapy[15]. And afatinib (Gilotrif™) (**D**), used for the treatment of cancers resistant to gefitinib and erlotinib[16,17] (Figure 1). They act through competitive binding to the ATP binding pocket of EGFR site, blocking EGFR downstream signalling required for tumour survival and proliferation[18,19].

In order to design our targeted compounds, the essential requirements for EGFR receptor bearing quinazoline inhibitor (PDB ID: **1M17**) were studied[20] (Figure 2). It was found that a hydrogen bond acceptor at N-1 of quinazoline ring interacts with Met 769[20]. After docking of the targeted compounds, it is obvious that the oxygen of the sulfonamide group favours this interaction than the N-1 atom of the benzo[g]quinazolin and forms a hydrogen bond with Met 769.

In this respect, we designed novel compounds based on the benzo[*g*]quinazoline core and sulfonamide moiety. These derivatives were subjected to *in vitro* cytotoxic evaluation against A549, followed by EGFR inhibitory profile and measuring the IC_{50} towards both EGFR and HER2 in comparison with the reference drug erlotinib.

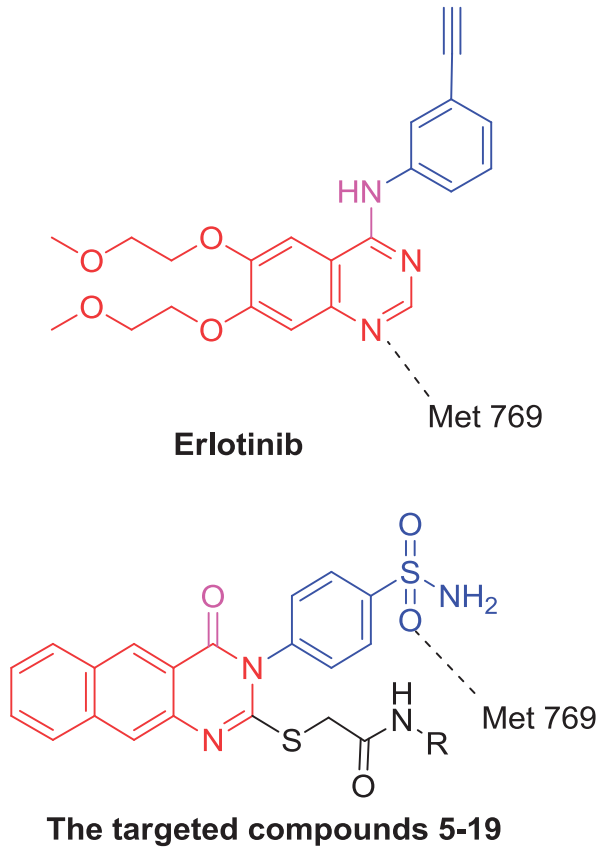


Figure 2. The design concept of the targeted compounds.

Materials and methods

Melting points (uncorrected) were determined in an open capillary on a Gallen Kamp melting point apparatus (Sanyo Gallen Kamp, UK). Pre-coated silica gel plates *(Kieselgel* 0.25 mm, 60 F254, Merck, Germany) were used for thin layer chromatography. A developing solvent system of chloroform/methanol (8:2) was used and the spots were detected by ultraviolet light. IR spectra (KBr discs) were recorded using a FT-IR spectrophotometer (Perkin Elmer, Waltham, MA). ^{1}H-NMR spectra were scanned on an NMR spectrophotometer (Bruker AXS Inc., Switzerland), operating at 500 MHz for ^{1}H- and 125.76 MHz for ^{13}C. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using DMSO-d_6 as a solvent. Elemental analyses were done on a model 2400 CHNSO analyser (Perkin Elmer, Waltham, MA). All the values were within

±0.4% of the theoretical values. All reagents used were of AR grade.

Chemistry

4-(2-Mercapto-4-oxobenzo[g]quinazolin-3(4H)-yl) benzenesulfonamide (4)

A mixture of 3-amino-2-naphthoic acid **3** (1.87 g, 0.01 mol) and 4-isothiocyanatobenzenesulfonamide **2** (2.14 g, 0.01 mol) in absolute ethanol (30 ml) containing 3 drops of triethylamine, was refluxed for 2 h, then left to cool. The solid product formed was collected by filtration and crystallised from ethanol to give **4**.

Yield, 92%; m.p. 210.5 °C. IR: 3390, 3278 (NH_2), 3068 (arom.), 1703 (CO), 1633 (CN), 1357, 1159 (SO_2). ^{1}H-NMR: 2.0 (s, 1H, SH), 7.5–8.1 (m, 10H, Ar-H), 8.7 (s, 2H, SO_2NH_2).^{13}C-NMR: 111.8, 116.5 (2), 126.3, 126.9, (2), 127.8, 129.8 (2), 130.0, 130.1, 130.2, 130.5, 135.7, 136.7, 144.1, 160.3, 176.0. MS *m/z* (%): 383 (M^+) (9.22), 226 (100). Anal. Calcd. for $C_{18}H_{13}N_3O_3S_2$ (383.44): C, 56.38; H, 3.42; N, 10.96. Found: C, 56.55; H, 3.65; N, 11.27.

2-(4-Oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-N-substituted acetamide (5–19)

General procedure

A mixture of **4** (3.83 g, 0.01 mol) and 2-chloro-*N*-substituted acetamide derivatives (0.01 mol) in dry acetone (50 ml) and anhydrous K_2CO_3 (0.5 g) was stirred at room temperature for 10 h, filtered and the product formed was crystallised from ethanol to give **5**–**19**, respectively.

2-(4-Oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-N-phenylacetamide (5)

5: Yield, 80%; m.p. 278.9 °C. IR: 3291, 3265, 3140 (NH_2, NH), 3053 (arom.), 2950, 2837 (aliph.), 1678, 1664 (2CO), 1598 (CN), 1398, 1157 (SO_2). ^{1}H-NMR: 4.2 (s, 2H, CH_2), 7.5–8.7 (m, 15H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 9.0 (s, 1H, NH). ^{13}C-NMR: 27.2, 119.3, 119.9 (2), 120.6 (2), 125.7, 126.8 (2), 127.0, 127.2, 127.9 (2), 128.7, 128.9, 129.9, 130.0, 131.1, 133.6, 135.0, 136.2, 137.8, 143.2, 160.6, 161.7, 167.4. MS *m/z* (%): 516 (M^+) (20.47), 362 (100). Anal. Calcd. for $C_{26}H_{20}N_4O_4S_2$ (519.59): C, 60.45; H, 3.90; N, 10.85. Found: C, 60.12; H, 3.64; N, 10.58.

2-(4-Oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-N-o-tolylacetamide (6)

6: Yield, 86%; m.p. 255.5 °C. IR: 3261, 3267, 3192 (NH_2, NH), 3053 (arom.), 2936, 2877 (aliph.), 1691, 1660 (2CO), 1568 (CN), 1325, 1157 (SO_2). ^{1}H-NMR: 2.2 (s, 3H, CH_3), 4.3 (s, 2H, CH_2), 6.8–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.3 (s, 1H, NH). ^{13}C-NMR: 21.6, 30.1, 116.8, 117.0 (2), 119.4, 120.2, 123.4, 124.6 (2), 125.0, 126.6, 127.4 (2), 128.1, 129.1, 129.9, 131.0, 131.1, 136.8, 138.4, 138.5, 138.8, 142.8, 161.3, 165.0, 165.9. MS *m/z* (%): 530 (M^+) (9.21), 366 (100). Anal. Calcd. for $C_{27}H_{22}N_4O_4S_2$ (530.62): C, 61.12; H, 4.18; N, 10.56. Found: C, 60.87; H, 3.80; N, 10.22.

2-(4-Oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-N-m-tolylacetamide (7)

7: Yield, 79%; m.p. 246.5 °C. IR: 3290, 3220, 3170 (NH_2, NH), 3093 (arom.), 2976, 2912 (aliph.), 1691, 1664 (2CO), 1610 (CN), 1330, 1159 (SO_2). ^{1}H-NMR: 2.2 (s, 3H, CH_3), 4.2 (s, 2H, CH_2), 7.1–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.3 (s, 1H, NH). ^{13}C-NMR: 21.3, 30.0, 119.4, 119.7, 119.8, 123.4 (2), 126.6, 127.4, 128.1 (2), 128.8, 129.4, 129.6 (2), 129.7, 129.8, 131.0, 132.9, 133.3, 136.4, 136.8, 139.1, 145.8, 155.4, 164.8, 165.7. MS *m/z* (%): 530 (M^+) (2.93), 91 (100). Anal. Calcd. for $C_{27}H_{22}N_4O_4S_2$ (530.62): C, 61.12; H, 4.18; N, 10.56. Found: C, 61.44; H, 4.52; N, 10.88.

2-(4-Oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-N-p-tolylacetamide (8)

8: Yield, 75%; m.p. 318.0 °C. IR: 3302, 3255, 3132 (NH_2, NH), 3089 (arom.), 2920, 2861 (aliph.), 1691, 1668 (2CO), 1604 (CN), 1332, 1161 (SO_2). ^{1}H-NMR: 2.1 (s, 3H, CH_3), 4.3 (s, 2H, CH_2), 7.0–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 9.7 (s, 1H, NH). ^{13}C-NMR: 18.3, 30.1, 119.4, 123.5 (2), 125.4 (2), 125.5, 125.9 (2), 126.1, 126.4 (2), 127.4 (2), 128.8, 129.4, 130.7, 131.0, 132.3, 136.0, 136.5, 139.1, 142.9, 161.4, 165.3, 166.1. MS *m/z* (%): 530 (M^+) (32.72), 106 (100). Anal. Calcd. for $C_{27}H_{22}N_4O_4S_2$ (530.62): C, 61.12; H, 4.18; N, 10.56. Found: C, 61.32; H, 4.37; N, 10.70.

N-(2-ethylphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (9)

9: Yield, 88%; m.p. 299.1 °C. IR: 3392, 3273, 3191 (NH_2, NH), 3051 (arom.), 2966, 2833 (aliph.), 1693, 1653 (2CO), 1568 (CN), 1354, 1157 (SO_2). ^{1}H-NMR: 1.1 (t, 3H, CH_3 ethyl), 2.6 (q, 2H, CH_2 ethyl), 4.3 (s, 2H, CH_2), 6.9–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.7 (s, 1H, NH). ^{13}C-NMR: 15.9, 28.6 (2), 117.1, 119.0 (2), 123.4, 123.5, 123.8, 126.6, 127.4 (2), 128.1, 128.8, 129.1 (2), 129.2, 129.4, 129.9, 131.0 (2), 136.8, 138.9, 139.1, 144.8, 155.4, 161.3, 165.9. MS *m/z* (%): 544 (M^+) (22.41), 360 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_4S_2$ (544.64): C, 61.75; H, 4.44; N, 10.29. Found: C, 61.48; H, 4.11; N, 10.04.

N-(3-ethylphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (10)

10: Yield, 81%; m.p. 201.3 °C. IR: 3273, 3155, 3103 (NH_2, NH), 3087 (arom.), 2929, 2870 (aliph.), 1680, 1666 (2CO), 1616 (CN), 1334, 1159 (SO_2). ^{1}H-NMR: 1.0 (t, 3H, CH_3 ethyl), 2.6 (q, 2H, CH_2 ethyl), 4.2 (s, 2H, CH_2), 7.3–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 9.6 (s, 1H, NH). ^{13}C-NMR: 14.6, 24.1, 30.0, 119.4, 123.5, 126.4, 126.5 (2), 126.6, 126.7, 127.4 (2), 128.1, 128.9, 129.1 (2), 129.4, 129.9, 131.1, 135.3, 135.8, 136.9, 138.4, 142.9, 145.9, 155.4, 161.4, 166.4. MS *m/z* (%): 544 (M^+) (10.35), 121 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_4S_2$ (544.64): C, 61.75; H, 4.44; N, 10.29. Found: C, 61.99; H, 4.69; N, 9.95.

N-(4-ethylphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (11)

11: Yield, 89%; m.p. 238.2 °C. IR: 3336, 3210, 3153 (NH_2, NH), 3099 (arom.), 2986, 2844 (aliph.), 1691, 1662 (2CO), 1612 (CN), 1377, 1161 (SO_2). ^{1}H-NMR: 1.1 (t, 3H, CH_3 ethyl), 2.6 (q, 2H, CH_2 ethyl), 4.2 (s, 2H, CH_2), 7.1–8.2 (m, 14H, Ar-H), 8.8 (s, 2H, SO_2NH_2), 10.3 (s, 1H, NH). ^{13}C-NMR: 16.1, 28.0, 31.1, 119.4, 119.8 (2), 119.9 (2), 123.4, 126.6 (2), 127.4 (2), 128.1, 128.4 (2), 128.8, 129.3, 131.0, 131.1, 136.6, 136.8, 137.0, 142.8, 145.9, 155.4, 161.3, 164.8. MS m/z (%): 544 (M^+) (4.83), 154 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_4S_2$ (544.64): C, 61.75; H, 4.44; N, 10.29. Found: C, 61.50; H, 4.21; N, 10.02.

N-(4-methoxyphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (12)

12: Yield, 78%; m.p. 318.0 °C. IR: 3412, 3290, 3138 (NH_2, NH), 3055 (arom.), 2971, 2833 (aliph.), 1692, 1681 (2CO), 1627 (CN), 1338,

1161 (SO_2). ^{1}H-NMR: 1.1 (s, 3H, CH_3), 3.8 (s, 3H, CH_2), 7.1–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.5 (s, 1H, NH). ^{13}C-NMR: 18.2, 30.0, 56.5, 108.2 (2), 115.6, 119.4 (2), 123.4 (2), 126.6, 127.4 (2), 128.1, 128.8 (2), 129.4, 129.8, 131.0, 131.1, 136.8, 139.1, 145.8, 150.3, 155.3, 161.3, 165.9. MS *m/z* (%): 546 (M^+) (51.12), 122 (100). Anal. Calcd. for $C_{27}H_{22}N_4O_5S_2$ (546.62): C, 59.33; H, 4.06; N, 10.25. Found: C, 59.04; H, 3.85; N, 9.91.

N-(4-ethoxyphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (13)

13: Yield, 88%; m.p. 266.7 °C. IR: 3367, 3271, 3130 (NH_2, NH), 3049 (arom.), 2978, 2916 (aliph.), 1695, 1651 (2CO), 1602 (CN), 1398, 1153 (SO_2). ^{1}H-NMR: 1.1 (s, 3H, CH_3), 4.0 (s, 2H, CH_2), 4.3 (q, 2H, OCH_2), 7.0–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.6 (s, 1H, NH). ^{13}C-NMR: 16.1, 30.9, 68.0, 119.4 (2), 119.7, 119.8 (2), 123.4 (2), 123.9, 124.3 (2), 126.6, 127.4 (2), 128.1, 128.8, 129.2, 129.3, 131.0, 136.8, 138.9, 142.8, 155.3, 161.3, 165.1, 166.0. MS *m/z* (%): 560 (M^+) (6.44), 361 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_5S_2$ (560.64): C, 59.98; H, 4.31; N, 9.99. Found: C, 59.61; H, 4.04; N, 9.69.

N-(3,4-dimethoxyphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (14)

14: Yield, 72%; m.p. above 398 °C. IR: 3412, 3314, 3200 (NH_2, NH), 3100 (arom.), 2961, 2841 (aliph.), 1678, 1645 (2CO), 1560 (CN), 1394, 1157 (SO_2). ^{1}H-NMR: 3.6 (s, 6H, $2OCH_3$), 4.1 (s, 2H, CH_2), 7.3–8.0 (m, 13H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 11.8 (s, 1H, NH). ^{13}C-NMR: 28.9, 59.2 (2), 87.4, 110.3, 112.6, 119.3, 121.8 (2), 123.4, 126.7 (2), 127.4, 128.1 (2), 129.4, 129.8, 130.6, 131.0, 136.8, 137.9, 139.1, 142.8, 145.9, 155.3 (2), 161.3, 166.3. MS *m/z* (%): 576 (M^+) (0.98), 151 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_6S_2$ (576.64): C, 58.32; H, 4.20; N, 9.72. Found: C, 58.68; H, 4.49; N, 9.98.

2-(4-Oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl)acetamide (15)

15: Yield, 83%; m.p. 285.8 °C. IR: 3343, 3315, 3181 (NH_2, NH), 3081 (arom.), 2954, 2857 (aliph.), 1690, 1684 (2CO), 1613 (CN), 1388, 1167 (SO_2). ^{1}H-NMR: 3.6, 3.7 (2 s, 9H, $3OCH_3$), 4.1 (s, 2H, CH_2), 7.0–8.2 (m, 12H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.3 (s, 1H, NH). ^{13}C-NMR: 27.8, 56.1 (2), 60.5, 97.3 (2), 119.4, 123.4 (2), 126.7, 127.4 (2), 128.0, 128.8 (2), 129.4, 129.9, 131.1 (2), 134.0, 135.5, 136.8, 139.1, 142.8, 153.2 (2), 155.4, 161.3, 165.8. MS *m/z* (%): 606 (M^+) (33.04), 433 (100). Anal. Calcd. for $C_{29}H_{26}N_4O_7S_2$ (606.67): C, 57.41; H, 4.32; N, 9.24. Found: C57.71; H, 4.63; N, 9.48.

N-(2-fluorophenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (16)

16: Yield, 72%; m.p. 264.4 °C. IR: 3431, 3302, 3196 (NH_2, NH), 3086 (arom.), 2955, 2862 (aliph.), 1691, 1676 (2CO), 1624 (CN), 1396, 1163 (SO_2). ^{1}H-NMR: 4.3 (s, 2H, CH_2), 6.9–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.8 (s, 1H, NH). ^{13}C-NMR: 30.0, 106.7, 115.5, 119.4, 123.4 (2), 126.6 (2), 127.4 (2), 128.1, 128.8, 129.3 (2), 129.8, 130.9 (2), 131.0, 131.1, 136.8, 139.1, 145.9, 155.2, 161.3, 165.4, 166.5. MS *m/z* (%): 534 (M^+) (14.23), 94 (100). Anal. Calcd. for $C_{26}H_{19}FN_4O_4S_2$ (534.58): C, 58.42; H, 3.58; N, 10.48. Found: C, 58.11; H, 3.23; N, 10.21.

N-(3-fluorophenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (17)

17: Yield, 66%; m.p. 251.5 °C. IR: 3381, 3320, 3211 (NH_2, NH), 3075 (arom.), 2963, 2844 (aliph.), 1692, 1681 (2CO), 1613 (CN), 1390, 1166 (SO_2). ^{1}H-NMR: 4.2 (s, 2H, CH_2), 7.0–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.6 (s, 1H, NH). ^{13}C-NMR: 31.1, 115.4, 115.5, 119.4 (2), 123.4 (2), 126.6, 127.4 (2), 128.1, 128.8 (2), 129.3, 129.8, 130.9, 131.0, 136.8, 139.1 (2), 140.6, 145.9, 155.2, 161.3, 163.5, 165.4. MS *m/z* (%): 534 (M^+) (28.19), 94 (100). Anal. Calcd. for $C_{26}H_{19}FN_4O_4S_2$ (534.58): C, 58.42; H, 3.58; N, 10.48. Found: C, 58.69; H, 3.93; N, 10.13.

N-(4-fluorophenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (18)

18: Yield, 75%; m.p. 258.0 °C. IR: 3267, 3212, 3154 (NH_2, NH), 3099 (arom.), 2976, 2833 (aliph.), 1678, 1654 (2CO), 1614 (CN), 1327, 1153 (SO_2). ^{1}H-NMR: 4.1 (s, 2H, CH_2), 6.8–8.2 (m, 14H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.2 (s, 1H, NH). ^{13}C-NMR: 27.9, 114.9 (2), 119.4 (2), 121.2, 123.4 (2), 126.7, 127.4 (2), 128.1, 128.8 (2), 128.4, 129.9, 131.0, 131.1 (2), 132.4, 136.8, 145.8, 155.1, 155.4, 161.3, 165.4. MS *m/z* (%): 534 (M^+) (21.09), 94 (100). Anal. Calcd. for $C_{26}H_{19}FN_4O_4S_2$ (534.58): C, 58.42; H, 3.58; N, 10.48. Found: C, 58.60; H, 3.75; N, 10.91.

N-(3-fluoro-4-methoxyphenyl)-2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[g]quinazolin-2-ylthio) acetamide (19)

19: Yield, 79%; m.p. 253.0 °C. IR: 3268, 3209, 3143 (NH_2, NH), 3078 (arom.), 2965, 2845 (aliph.), 1691, 1679 (2CO), 1562 (CN), 1348, 1161 (SO_2). ^{1}H-NMR: 4.0 (s, 3H, OCH_3), 4.3 (s, 2H, CH_2), 7.1–8.2 (m, 13H, Ar–H), 8.8 (s, 2H, SO_2NH_2), 10.3 (s, 1H, NH). ^{13}C-NMR: 30.0, 61.0, 115.8, 115.9, 116.0, 119.4, 123.4 (2), 124.3, 125.8 (2), 126.3, 127.4 (2), 128.8, 129.4, 131.0, 136.8 (2), 139.1 (2), 142.8, 145.9, 153.0, 161.3, 165.6, 166.7. MS *m/z* (%): 564 (M^+) (17.92), 407 (100). Anal. Calcd. for $C_{27}H_{21}FN_4O_5S_2$ (564.61): C, 57.44; H, 3.75; N, 9.92. Found: C, 57.11; H, 3.46; N, 9.67.

Biological evaluation

MTT cytotoxicity assay

A549 lung cancer cells (obtained from VACSERA, Cairo, Egypt) were obtained from American Type Culture Collection, cells were cultured using DMEM (Dulbecco's Modified Eagle's Medium) (Invitrogen/Life Technologies) supplemented with 10% foetal bovine serum (Hyclone), 10 μg/ml of insulin (Sigma), and 1% penicillin–streptomycin. The 96-well plate was incubated for 24 h before the MTT assay. Then, briefly rinse the cell layer with 0.25% (w/v) Trypsin, 0.53 mM EDTA solution. Add reconstituted MTT in an amount equal to 10% of the culture medium volume. Incubate for 2–4 h. Measure absorbance at a wavelength of 570 nm. The IC_{50} values were calculated according to the equation for Boltzmann sigmoidal concentration–response curve using the non-linear regression fitting models (GraphPad, Prism, GraphPad Software Inc., La Jolla, CA).

In vitro *enzymatic activity assay*

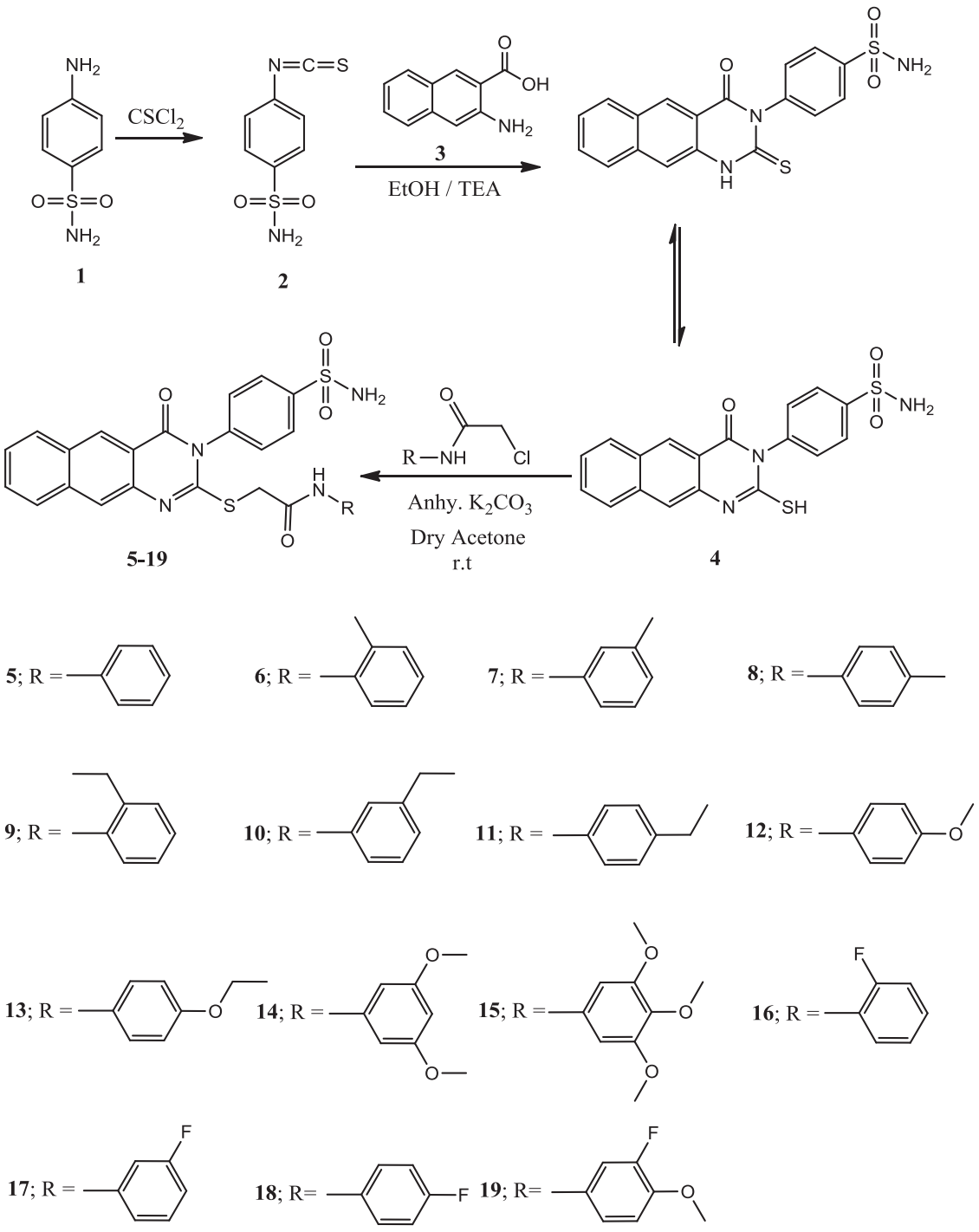
EGFR and HER2 kinase kit were purchased from Invitrogen. The experiments were performed according to the manufacturer's instructions. Briefly, EGFR (PV3872), 0.200 mg/ml and HER2 (PV3366), 0.192 mg/ml were used. Six concentration gradients were set for all the tested compounds in DMSO. An ATP solution and a kinase/peptide mixture were prepared right before use. The solutions on the plate were mixed thoroughly, and the plate was incubated for 1 h at room temperature. After that, 5 ml of the developing solution was added to each well. The plate was

incubated for 1 h at room temperature and then read by ELISA Reader (PerkinElmer, Waltham, MA). Curve fitting and data presentations were performed using Graph Pad Prism 5.0. Every experiment was repeated three times. Data represented as means ± SD from three independent experiments.

Results and discussion

Chemistry

Scheme 1 reports the synthetic pathway utilised to obtain the targeted compounds **5**–**19**, from the reaction of 4-isothiocyanatobenzenesulfonamide **2** with 3-amino-2-naphthoic acid **3**[21] in ethanol containing triethylamine to yield the 4-(2-mercapto-4-oxobenzo[*g*]-quinazolin-3(4*H*)-yl) benzenesulfonamide **4**. ^{1}H-NMR of **4** revealed a singlet at 2.0 ppm attributed to the SH. ^{13}C-NMR exhibited two signals at 160.3 and 176.0 ppm attributed to C–SH and CO, respectively. The reaction of **4** with 2-chloro-*N*-substituted acetamide in dry acetone and anhydrous K_2CO_3 gave the corresponding 2-(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydrobenzo[*g*]quinazolin-2-ylthio)-*N*-substituted acetamide **5**–**19**. IR of **5** revealed bands at 1678, 1664 cm^{-1} for the CO groups. ^{1}H-NMR displayed singlet at 4.2 ppm attributed to CH_2 and another singlet at 9.0 ppm for the NH proton. ^{13}C-NMR exhibited signal at 27.2 ppm for the CH_2, and another signal at 167.4 ppm due to the CO acetamide. ^{1}H-NMR of



Scheme 1. Formation of the benzoquinazolinone derivatives **4**–**19**.

6–8 displayed three singlets at the range of 2.1–2.2 ppm attributed to CH_3 group, 4.2–4.3 ppm for the CH_2, and 9.7–10.3 ppm for the NH proton. ^{13}C-NMR of **6–8** exhibited new signals in the range of 18.3–21.6 ppm for the CH_3, 30.0–30.1 ppm for the CH_2, and another signal at 165.7–166.1 ppm due to the CO acetamide. ^{1}H-NMR of **9–11** displayed triplet at the range of 1.0–1.1 ppm attributed to CH_3 ethyl, the quartet at 2.6 ppm for the CH_2 ethyl, singlet at the range of 4.2–4.3 ppm for the CH_2, and a singlet at 9.6–10.7 ppm for the NH proton. ^{13}C-NMR of **9–11** exhibited new signals in the range of 14.6–16.1 ppm for the CH_3 ethyl, 24.1–28.6 ppm for the CH_2 ethyl, 28.6–31.1 ppm for the CH_2, and 164.8–166.4 ppm due to the CO acetamide. ^{1}H-NMR of **12** and **13** displayed singlet at 1.1 ppm attributed to CH_3, singlet at the range of 3.8–4.0 ppm for the CH_2, the quartet at 4.1–4.3 ppm due to OCH_2, and a singlet at 10.5–10.6 ppm for the NH proton. ^{13}C-NMR of **12** and **13** exhibited new signals in the range of 16.1–18.2 ppm for the CH_3, 30.0–30.9 ppm for the CH_2, 56.5–68.0 ppm for the OCH_2, and another signal at 165.9–166.0 ppm due to the CO acetamide. ^{1}H-NMR of **14** displayed three singlets at 3.6 ppm due to the $2OCH_3$, 4.1 ppm attributed to CH_2 and at 11.8 ppm for the NH proton. ^{13}C-NMR of **14** exhibited signal at 28.9 ppm for the CH_2, 59.2 ppm for the $2OCH_3$, and at 166.3 ppm due to the CO acetamide. ^{1}H-NMR of **15** displayed two singlets at 3.6 and 3.7 ppm due to the $3OCH_3$, singlet at 4.1 ppm attributed to CH_2 and singlet at 10.3 ppm for the NH proton. ^{13}C-NMR of **15** exhibited signal at 27.8 ppm for the CH_2, 56.1, 60.5 ppm for the $3OCH_3$, and at 165.8 ppm due to the CO acetamide. ^{1}H-NMR of **16–18** displayed two singlets at the range of 4.1–4.3 ppm for the CH_2, and 10.2–10.6 ppm for the NH proton. ^{13}C-NMR of **16–18** exhibited new signals in the range of 27.9–31.1 ppm for the CH_2, and another signal at 165.4–166.5 ppm due to the CO acetamide. ^{13}C-NMR of **16** displayed a signal at 155.2 ppm attributed to the C–F carbon at the *ortho* position, while for **17** and **18** the C–F carbon appeared at 163.5 and 161.3 ppm due to its presence at the *meta* and *para* position, respectively. ^{1}H-NMR of **19** displayed three singlets at 4.0 ppm attributed to OCH_3, 4.3 ppm for CH_2 and 10.3 ppm for the NH proton. ^{13}C-NMR of **19** exhibited signal at 30.0 ppm for the CH_2, 61.0 ppm for the OCH_3 and another signal at 166.7 ppm due to the CO acetamide.

Biological evaluation

In vitro *cytotoxic activity against A549*

The newly synthesised compounds were evaluated for their *in vitro* cytotoxic activity through MTT cytotoxicity assay against human Lung (A549) cancer cell line, and erlotinib was used as the reference drug. In a closer look to Table 1, we can see that compounds **5–19** cytotoxicity ranges from 0.105 to 1.992 μM, in comparison with erlotinib (IC_{50}= 0.727 μM). Compounds **6, 8, 10**, and **14–16** were more active than the reference drug, with IC_{50} ranging from 0.105 to 0.711 μM. the *O*-methyl derivative **6** was the most active followed by the *m*-ethyl **10,** the *p*-methyl **8,** the *O*-fluoro **16,** the 3,4,5-trimethoxy **15** and the 3,5-dimethoxy derivative **14** (IC_{50} values 0.105, 0.181, 0.220, 0.352, 0.545, and 0.711 μM, respectively). The EGFR inhibitory activity of the tested compounds **5–19** was measured. Results showed that most of the tested compounds have high inhibitory activity ranging from 90.21% to 34.37%. Compounds **6, 8, 10**, and **16** showed the highest inhibition percentages ranging from 90.21% to 84.19%.

EGFR and HER2 inhibition

The IC_{50} values for the most potent compounds **6**, **8**, **10**, and **16** were determined on both EGFR and HER2 enzymes. Compound **8** was found to be the most potent on both EGFR and HER2 with IC_{50} values of 0.009 and 0.021 μM, respectively. Followed by compound **6** with IC_{50} values of 0.012 and 0.021 μM. Compound **10** showed IC_{50} values of 0.022 and 0.044 μM and compound **16** of 0.026 and 0.069 μM. Erlotinib IC_{50} values were 0.047 and 0.071 μM towards EGFR and HER2 enzymes, respectively (Table 2).

Table 1. EGFR inhibitory activity and anti-proliferative activity against A549 cell line.

Compoun no.	IC_{50} on A549 (μM)	% inhibition of EGFR
5	1.017 ± 0.010	55.58
6	0.105 ± 0.012	84.31
7	1.271 ± 0.002	66.85
8	0.220 ± 0.120	90.21
9	1.992 ± 0.006	35.97
10	0.181 ± 0.101	84.91
11	0.812 ± 0.009	63.02
12	0.996 ± 0.008	42.17
13	0.912 ± 0.112	41.83
14	0.711 ± 0.151	35.52
15	0.545 ± 0.003	58.04
16	0.352 ± 0.007	84.19
17	1.341 ± 0.103	52.89
18	1.122 ± 0.121	34.37
19	0.766 ± 0.021	55.40
Erlotinib	0.727 ± 0.008	70.97

The values represent the mean ± SD of three independent experiments.

Table 2. Inhibition activities of the most potent compounds against EGFR and HER2.

Compound no.	EGFR IC_{50} (μM)	HER2 IC_{50} (μM)
6	0.012 ± 0.005	0.021 ± 0.008
8	0.009 ± 0.002	0.021 ± 0.012
10	0.022 ± 0.011	0.042 ± 0.010
16	0.026 ± 0.004	0.069 ± 0.015
Erlotinib	0.047 ± 0.003	0.071 ± 0.006

The values represent the mean ± SD of three independent experiments.

Conclusion

In summary, a novel series of benzo[*g*]quinazolin bearing sulfonamide was designed and synthesised. All the compounds showed very potent anticancer activity against A549 lung cancer cell line and high inhibition percentage towards EGFR in A549 cancer cells. Compounds **6, 8, 10**, and **16** that showed the highest cytotoxic activity and inhibition percentages were further screened against EGFR and HER2 enzymes. The IC_{50} values for those four compounds were found to be better than that of erlotinib against both EGFR and HER2 enzymes. Compound **8** was found to be the most potent on both EGFR and HER2 with IC_{50} values of 0.009 and 0.021 μM, respectively. Followed by compound **6** with IC_{50} values of 0.012 and 0.021 μM.

Disclosure statement

No potential conflict of interest was reported by the authors.

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