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Design, Synthesis, QSAR, Molecular Docking Study and Antitumor Activity of some Novel Quinazolin-4(3H)-One Derivatives

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Abstract: In view of the effective range of biological activities exhibited by quinazolines, a novel series of 2,3-disubstitutedquinazolin-4-(3H)-ones were designed, synthesized and evaluated for *in vitro* anticancer activity against human breast carcinoma cell line (MCF-7). The results of this study showed that 3-(4-aminophenyl)-2-(chloromethyl)quinazolin-4(3H)-one **2,** 2-{[4-(2-chloromethyl-4-oxo-4H-quinazolin-3-yl)-phenyl]diazoenyl} malononitrile **11a,** 2-(2,4-dichlorophenyl)-4-oxoquinazoline-3(4*H*)-carboxamide **16a** and *N*-(2-(2,4-dichlorophenyl)-4-oxoquinazolin-3(4*H*)-yl) benzamide **18** possessed an inhibitory activity against human breast carcinoma with IC_{50} (2.84, 6.21, 4.19, and 2.48 ug/well) respectively. Compounds **2, 16a,** and **18** were more potent compared with the positive control Imatinib with IC_{50}, 6.06 ug/well. Molecular docking methodology was performed for compounds **2**, **11a, 16a,** and **18** into ATP binding site of the epidermal growth factor receptor-tyrosine kinase (EGFR-TK), using gefitinib as a lead compound which proved that the docking results were in coincidence with the biological activity.

Keywords: Imatinib, gefitinib, in vitro antitumor evaluation, MCF-7, molecular docking, EGFRTK, quinazolinones

1 Introduction

Cancer represents one of the most severe health problems [1]. It remains one of the most difficult diseases to treat, and is responsible for about 13% of all deaths worldwide [2]. As chemotherapeutic drugs have a wide range of nonspecific effects, there is an urgent need to develop safe and cost effective anticancer agents [3]. Growth factors and their transmembrane receptor kinase e.g. EGFR (epidermal growth factor receptor) play important roles in cell proliferation, survival, adhesion, migration and differentiation [4]. Breast cancer is the most common tumor among women worldwide. Its incidence is increasing around the world, and it is believed to be the leading cause of cancer mortality among women, according to American Cancer Society [5]. Quinazolines are classes of fused heterocycles that are of considerable interes and play a major role in the field of medicinal chemistry. They are of particular interest due to their diverse pharmacological activities and are considered as promising scaffolds in the search for new bioactive agents [6-19]. Some quinazoline derivatives are potent inhibitors of epidermal growth factor receptor (EGFR). The EGFR is a cellular trans-membrane tyrosine kinases that are over-expressed in a significant number of human tumors (e.g., breast, ovarian, colon, renal, and prostate) [20].

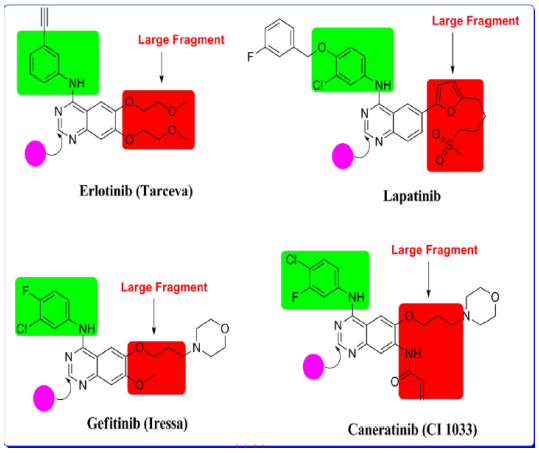


Figure1. EGFR Tyrosine Kinase inhibitors [20]

A number of anilinoquinazoline containing compounds were evaluated as EGFR kinase inhibitors in cancer clinical trials such as gefitinib (Iressa), erlotinib (Tarceva),

lapatinib and caneratinib (C11033) (Figure 1). They were recently approved for the treatment of breast cancer and non-small-cell lung cancer [20-23]

Herein, we have designed a number of new quinazoline derivatives, the substitution pattern at the 2,3-disubstituted quinazolinones pharmacophore was selected so as to confer different electronic environment that would affect the lipophilicity, and hence the activity of the target molecules. Moreover, QSAR study was performed to identify the structural features required for the antitumor properties of these new series.

2 Materials and methods

2.1 Chemistry

Melting points were measured in open capillary tubes using Griffin apparatus and were uncorrected. Elemental microanalyses were carried out at the Regional Centre for Mycology and Biotechnology, Al-Azhar University. The infrared (IR) spectra were recorded using potassium bromide disc technique on a Schimadzu 435 IR spectrophotometer at Micro analytical Center, Ain Shams University and Al-Azhar University.The proton nuclear magnetic resonance (^{1}HNMR) spectra were performed on a Varian Mercury VX-300 NMR spectrophotometer 300 MHz at The Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defence. DMSO-*d6* was used as a solvent, and the chemical shifts were measured in ppm, relative to TMS as an internal standard. As for the proton magnetic resonance, D_2O was carried out for NH, NH_2 and OH exchangeable protons. Mass spectra were recorded on a DI-50 unit of Shimadzu GC/ MS-QP 2010 plus Spectrometer(Japan) or on single quadrpole mass Spectrometer ISQ LT (Thermo scientific) at the Regional Centre for Mycology and Biotechnology, Al-Azhar University.

Compounds 2-(chloromethyl)-4*H*-benzo[*d*][1,3] oxazin-4-one **1** and 2-(2,4-dichlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **13** were prepared according to reported procedures [24,25].

3-(4-aminophenyl)-2-(chloromethyl)quinazolin-4(3H)-one. (2)

A mixture of 2-(chloromethyl)-4*H*-benzo[*d*][1,3] oxazin-4-one **1** (2 mmol, 0.5gm) and 1,4-phenylene diamine (2 mmol, 0.22 gm) in pyridine (10 ml) was refluxed for 4h. The precipitated solid obtained on hot was filtered off, washed with ethanol, dried and crystallized from ethanol.

Yield: (43.8 %); m.p. 200-202ºC. Analysis % for $C_{15}H_{12}ClN_3O$, Calcd. (Found) C: 63.05 (63.27), H: 4.23 (4.31), N: 14.71 (14.93). IR (KBr) (cm^{-1}): 3410-3325 (NH_2), 3136-3020 (aromatic CH str.), 1697 (C=O). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 5.82 (s, 2H, CH_2Cl), 7.01-7.16 (m, 2H, p-amino phenyl-$H_{3,5}$), 7.53-7.58 (t, 1H, quinazolinone-H_6), 7.92 (d, 1H, quinazolinone-H_8), 8.21-8.24 (m, 2H, p-amino phenyl-$H_{2,6}$), 8.68- 8.73 (t,1H, quinazolinone-H_7), 9.09 (d, 1H, quinazolinone-H_5), 10.53 (s, 2H, NH_2; exchangeable with D_2O). MS(*m/z*): 285 (M^+, 14.73 %),287 (M^++2, 4.31%), 52.06 (100%).

General procedure for the synthesis of 2(chloromethyl)-3-(substituted-phenyl) quinazolin-4(3*H*)-one. (3-9)

Equimolar amounts of compound **1** (2 mmol, 0.5 gm), and appropriate primary aromatic amine, (2 mmol), were dissolved in pyridine (10 ml) then the mixture was refluxed. The precipitated solid obtained after cooling and pouring on ice water, containing a few drops of HCl, was filtered off, washed with ethanol, dried and crystallized from ethanol.

2(chloromethyl)-3-(2-hydroxyphenyl)quinazolin-4(3*H*)-one.(3)

Yield: (30%); m.p. 240-241ºC. Reflux time= 20h. Analysis % for $C_{15}H_{11}ClN_2O_2$, Calcd.(Found), C: 62.84 (63.12), H: 3.87 (3.91), N: 9.77 (9.86). IR (KBr) (cm^{-1}): 3421- 3367 (broad band of OH), 3136- 3024 (aromatic CH str.), 1639 (C=O). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 5.79 (s, 2H, CH_2Cl), 7.08-7.13 (t, 1H, 2-OH-ph-$H_{5'}$), 7.40-7.46 (t, 1H, H_6), 8.01 (d, 1H, H_8), 8.21-8.29 (m, 3H, 2-OH-ph-$H_{6',4',3'}$), 8.67- 8.72 (t, 1H, H_7), 9.01 (d, 1H, H_5), 13.69 (s, 1H, OH; D_2O exchangeable). MS (m/z): 286 (M^+,0.97 %), 288 (M^++2, 0.29%), 52.06 (100 %).

2-(chloromethyl)-3-(2-ethylphenyl)quinazolin-4(3*H*)-one. (4)

Yield: (78.9%); m.p. 230-232ºC. Reflux time=25h. Analysis % for $C_{17}H_{15}ClN_2O$, Calcd. (Found), C: 68.34 (68.53), H: 5.06 (5.14), N: 9.38 (9.47). IR (KBr) (cm^{-1}): 3136 (aromatic CH str.),1697 (CO), ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 1.20-1.24 (t, 3H, CH_3 of ethyl gp), 2.62-2.65 (q, 2H, CH_2 of ethyl gp.), 3.99 (s, 2H, CH_2Cl), 6.36 (d, 1H, 2-ethyl-ph-$H_{6'}$), 6.57-6.62 (t, 1H, 2-ethyl-ph-$H_{4'}$), 6.95-7.02 (t, 1H, 2-ethyl-ph-$H_{5'}$), 7.09-7.27 (m, 2H, H_6 & 2-ethyl-ph-$H_{3'}$), 7.46-7.56 (t, 1H, H_7), 8.01 (d, 1H, H_8), 8.73 (d, 1H, H_5). MS (*m/z*): 298 (M^+, 2.73 %), 300 (M^++2, 1.01 %), 151.09 (100%).

2-(chloromethyl)-3-(naphthalen-1-yl)quinazolin-4(3*H*)-one. (5)

Yield: (43.8 %); m.p. 200-202ºC. Reflux time=15h. Analysis % for $C_{15}H_{12}ClN_3O$, Calcd. (Found), C: 71.14(71.42), H: 4.08 (4.16), N: 8.73 (8.97). IR(KBr) (cm^{-1}): 3136-3020 (aromatic CH str.), 1685 (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 5.77 (s, 2H, CH_2Cl), 7.09-7.14 (m, 3H, naphthyl-$H_{5',6',7'}$), 7.41-7.46 (t, 1H, H_6), 8.01 (d, 1H, H_8), 8.20-8.29 (m, 4H, naphthyl-$H_{2',3',4',8'}$), 8.67-8.72 (t, 1H, H_7), 9.09 (d,1H, H_5). MS(*m/z*): 320 (M^+, 4.41 %), 322 (M^++2, 2.83%), 43.17(100%).

3-Benzyl-2-(chloromethyl)quinazolin-4(3*H*)-one. (6)

Yield: (41.6%); m.p. 240-242ºC. Reflux time= 15h. Analysis % for $C_{16}H_{13}ClN_2O$ (284.74), Calcd. (Found) C: 67.49 (67.62), H: 4.60 (4.67), N: 9.84 (9.98). IR(KBr) (cm^{-}

[1]): 3136 (aromatic CH str.), 1697 (CO),1635 (C=C).^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 3.38 (s, 2H, CH_2 of benzyl-H), 3.82 (s, 2H, CH_2Cl), 7.08-7.13 (t,1H, H_6), 7.24-7.54 (m, 6H, 5-ph-H & H_7), 7.99 (d, 1H, H_8), 8.62 (d, 1H, H_5).MS(*m/z*): 284 (M^+, 4.64 %), 286 (M^++2, 2.94 %),91.08 (100 %).

2-(chloromethyl)-3-(2,3-dimethylphenyl) quinazolin-4(3*H*)-one. (7)

Yield: (31.5%); m.p. 142-144ºC. Reflux time=30h. Analysis % for $C_{17}H_{15}ClN_2O$, Calcd.(Found) C:68.34 (68.51), H:5.06 (5.12), N:9.38 (9.53). IR (KBr) (cm^{-1}): 3062- 3028 (aromatic CH str.), 2924- 2854 (alphatic CH str.), 1685(CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 2.48 (s, 6H, $2CH_3$), 5.65 (s, 2H, CH_2Cl), 7.08-7.13 (t, 1H, 2,3-di methyl-ph-$H_{5'}$), 7.40-7.46 (t, 1H, H_6), 8.01 (d, 1H, H_8), 8.20-8.29 (m, 2H, 2,3-di methyl-ph-$H_{6',4'}$), 8.67-8.72 (t, 1H, H_7), 9.08 (d, 1H, H_5). MS (m/z): 298 (M^+, 5.36 %), 300(M^++2, 0.89%) 237.07 (100 %).

3-(2-amino-4-methylphenyl)-2-(chloromethyl) quinazolin-4(3H)-one. (8)

Yield: (27.6%); m.p. 239-240ºC. Reflux time =10h. Analysis % for $C_{16}H_{14}ClN_3O$, Calcd.(Found) C:64.11 (64.37), H:4.71 (4.79), N:14.02 (14.23). IR (KBr) (cm^{-1}): 3383- 3136 (NH_2), 3093-3020 (aromatic CH str.), 2974-2850 (alphatic CH str.), 1681 (C=O). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 2.49 (s, 3H, CH_3), 5.87 (s, 2H, CH_2Cl), 7.04-7.13 (m, 2-amino4-methyl-phenyl), 7.44-7.46 (t, 1H, H_6), 8.01(d, 1H, H_8), 8.21-8.29 (m, 2-amino4-methyl-phenyl), 8.67-8.73 (t, 1H, H_7), 9.10 (d, 1H, H_5), 13.59 (s, 2H, NH_2; exchangeable with D_2O). MS (m/z): 299 (M^+, 4.91 %), 301 (M^++2, 0.60 %), 123.15 (100%).

3-(2-amino-4-chlorophenyl)-2-(chloromethyl) quinazolin-4(3*H*)-one. (9)

Yield: (33.3%); m.p.241-243ºC. Reflux time=7h. Analysis % for $C_{15}H_{11}$ Cl_2N_3O, Calcd.(Found) C:56.27 (56.48), H:3.46 (3.41), N:13.12 (13.29). IR (KBr) (cm^{-1}): 3402-3216 (NH_2), 1689 (C=O). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 5.81 (s, 2H, CH_2Cl), 7.16-7.19 (m, 2-amino4-Chloro-phenyl), 7.48-7.53 (t, 1H, H_6), 8.01 (d, 1H, H_8), 8.21-8.28 (m, 2-amino4- Chloro-phenyl), 8.68-8.73 (t, 1H, H_7), 9.10 (d, 1H, H_5), 13.69 (s, 2H, NH_2; exchangeable with D_2O). MS (m/z): 319 (M^+, 3.61 %), 323 (M^++4, 1.25 %), 151.08 (100%).

3-(4-(2-hydroxybenzylideneamino)phenyl)-2-(chloromethyl)quinazolin-4(3H) one. (10)

A mixture of 3-(4-aminophenyl)-2-(chloromethyl) quinazolin-4(3H)-one **(2),** and 2-hydroxy- benzaldehyde, (2 mmol), and glacial acetic acid, was refluxed for 8h. The precipitated solid formed after cooling was filtered off, washed and crystallized from ethanol.

Yield: (30%); m.p. 211-213ºC. Analysis % for $C_{22}H_{16}ClN_3O_2$, Calcd. (Found) C: 67.78 (68.03), H: 4.14 (4.19), N: 10.78 (10.85). IR(KBr) (cm^{-1}): 3448-3421 (OH), 1650 (C=O), 1608 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 4.01 (s, 2H, CH_2Cl), 7.12-7.16 (t, 1H, 2-hydroxy-ph-$H_{5'}$), 7.27 (d, 1H, 2- hydroxy-ph-$H_{6'}$), 7.44 (s, 1H, N=CH), 7.49-7.53 (t, 1H, 2- hydroxyl- ph-$H_{4'}$), 8.01 (d, 1H, H_8), 8.14-8.17(t, 1H, H_6), 8.55-8.59 (t, 1H, H_7), 9.02 (d, 1H, H_5), 9.83 (s, 1H, OH; exchangeable with D_2O). MS (*m/z*): 389 (M^+, 10.24%), 391 (M^++2, 1.50), 45.05 (100 %).

General procedure for the synthesis of 2-{[4-(2-chlormethyl-4-oxo-4*H*-quinazolin-3-yl)-phenyl] diazoen -yl} derivatives. (11a-c & 12)

Diazotisation of compound **2** (2 mmol, 0.57 gm) was performed using a mixture of sodium nitrite (1.1 mmol, 0.15 gm), and HCl at 0-5 ºC over a period of 30 min . The diazonium salt thus obtained, was treated in ethanol (15ml) in the presence of sodium hydroxide (6 mmol, 0.25gm) with calculated amounts (3 mmol), of some active methylene compounds, namely, malononitrile, ethyl acetoacetate, acetylacetone and α-naphthol. After complete addition, the reaction mixture was stirred for further 2h. The resulting solid was collected by filtration, and crystallized from ethanol to afford the corresponding hydrazono derivatives.

2-{[4-(2-chloromethyl-4-oxo-4H-quinazolin-3-yl)-phenyl]diazoenyl} malononitrile. (11a)

Yield: (30.8%); m.p. 390-392 ºC. Analysis % for $C_{18}H_{11}ClN_6O$, Calcd. (Found) C: 59.59 (59.67), H: 3.06 (3.12), N: 23.17 (23.42). IR(KBr) (cm^{-1}): 3344 (NH), 2222 (CN), 1685 (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 5.76 (s, 2H, CH_2Cl), 5.78 (s, 1H, NH; exchangeable with D_2O), 7.11-7.43 (m, 4H, ph-H), 7.57-7.60 (d, 1H, H_6), 7.99(d, 1H,H_8), 8.21-8.25 (t,1H, H_7), 9.03 (d, 1H, H_5). MS (*m/z*): 362 (M^+, 5.71 %), 364 (M^++2, 0.77%), 153.23 (100%).

3-{[4-(2-chloromethyl-4-oxo-4*H*-quinazolin-3-yl)phenyl]hydrazono}-pentane-2,4-dione. (11b)

Yield: (35%); m.p. 300-302 ºC. Analysis % for $C_{22}H_{21}ClN_4O_3$, Calcd. (Found) C: 60.53 (60.78), H: 4.32 (4.39), N: 14.12 (14.37). IR(KBr) (cm^{-1}): 3371 (NH), 1670 (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 2.79 (s, 3H, OCH_3), 2.95 (s, 3H, OCH_3), 5.83 (s, 2H, CH_2Cl), 7.73-7.51 (m, 4H, ph-H), 8.01-8.07 (m, 3H, $H_{6,7,8}$), 9.15(d, 1H, H_5). 14.17 (s, 1H, NH; exchangeable with D_2O). MS (*m/z*): 424 (M^+, 5.71 %), 426 (M^++2, 0.77%), (100%).

2-{[4-(2-chloromethyl-4-oxo-4*H*-quinazolin-3-yl)-phenyl]hydrazono}-3-oxobutyric acid ethyl ester. (11c)

Yield: (40%); m.p. 280-281 ºC. Analysis % for $C_{25}H_{31}ClN_4O_4$, Calcd. (Found) C: 61.66 (61.65), H: 6.42 (6.40), N: 11.50 (11.52). IR(KBr) (cm^{-1}): 3367 (NH), 1678 (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 1.26 (t, 3H, CH_3 of ethyl ester), 2.70 (s, 3H, OCH_3), 4.25 (q, 2H, CH2 of ethyl ester), 5.74 (s, 2H, CH_2Cl), 7.36-7.41 (m, 8H, 4ph-H & quinazolinone-$H_{5,6,7,8}$). MS (*m/z*): 486 (M^+, 5.71 %), 488 (M^++2, 0.77%), (100%).

2-{[4-(2-chloromethyl-4-oxo-4*H*-quinazolin-3-yl)-

phenyl]hydrazono}-3-Naphthol. (12)

Yield: (40%); m.p. 250-252 °C. Analysis % for $C_{25}H_{17}ClN_4O_2$, Calcd. (Found) C: 68.11(68.26), H: 3.89(3.94), N: 12.71(12.89). IR(KBr) (cm^{-1}): 3367 (OH), 1681 (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 5.69 (s, 2H, CH_2Cl), 6.65 (d, 1H, naphthyl-H_2), 6.94-6.97 (t, 1H, naphthyl-H_7), 7.05 (d, 1H, naphthyl-H_3), 7.14-7.24 (t, 1H, naphthyl-H_6), 7.22-7.79 (m, 5H, naphthyl-H_5 & ph-H), 8.02(d, 1H, H_8), 8.18-8.21 (t, 1H, H_6), 8.29 (d, 1H, naphthyl-H_8),8.64-8.68 (t, 1H, H_7), 8.92 (s, 1H, OH; exchangeable with D_2O), 9.06 (d, 1H, H_5). MS(*m/z*): 440 (M^+, 1.20 %), 442 (M^++2, 1.07), 77.10 (100%).

2-(2,4dichlorophenyl)-3-amino-4(3H)-quinazolinone. (14)

Compound 2-(2,4-dichlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **13** (3mmol, 0.9gm) was dissolved in absolute ethanol (25ml). Excess Hydrazine hydrate was added to it. The reaction mixture was refluxed for 4h. The solid precipitated after cooling was filtered off and recrystallized from ethanol.

Yield: (34.18%); m.p.180-181 °C. Analysis % for $C_{14}H_9Cl_2N_3O$, Calcd. (Found) C: 54.92 (55.09), H: 2.96 (2.98), N: 13.73 (13.87). IR(KBr) (cm^{-1}): 3324-3258 (NH_2), 3054.19 (aromatic CH str.), 1659 (CO).^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 5.53 (s, 2H, NH_2; exchangeable with D_2O), 7.54-7.62 (m, 3H, H_6 & 2,4-dichloro-ph-$H_{3',5'}$), 7.71 (d, 1H, H_8), 7.75 (d, 1H, 2,4-dichloro-ph-$H_{6'}$), 7.84-7.88 (t, 1H, H_7), 8.21 (d, 1H, H_5), MS(*m/z*): 305(M^+, 6.45 %), 309 (M^++4, 2.14%), 43.06 (100%).

3-N-phenylamine-2(2,4-dichlorophenyl) quinazolin-4(3H)-one. (15)

A mixture of compound **13** (3mmol, 0.9 gm), phenyl hydrazine (3mmol, 0.32 gm) and pyridine (10ml) was refluxed for 10h. The precipitated solid formed after cooling was filtered off, washed and crystallized from ethanol

Yield: (17.90%); m.p. 135-137 °C. Analysis % for $C_{20}H_{13}Cl_2N_3O$, Calcd. (Found) C: 62.84 (63.01), H: 3.43(3.46), N: 10.99 (11.24). IR(KBr) (cm^{-1}): 3282 (NH), 3186-3062 (aromatic CH str.), 1697 (CO). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 6.87-6.89 (t, 2H, ph-$H_{3,4}$), 7.27-7.30 (t, 2H, H_6), 7.39 (d, 1H, 2,4- dichloro--ph-$H_{5'}$), 7.53-7.74 (m, 4H, ph-$H_{2,5,6}$ & 2,4- dichloro--ph-$H_{3'}$), 7.84 (d, 1H, H_8), 7.88-7.92 (t, 1H, H_7), 8.21 (d, 2H, 2,4-dichloro-ph $H_{6'}$), 8.27 (d, 1H, H_5), 10.30 (s, 1H, NH; exchangeable with D_2O). MS(*m/z*): 381 (M^+, 7.83 %), 385 (M^++4, 1.30%), 136.11 (100%).

General procedure for the synthesis of 2-(2,4-dichlorophenyl)-4-oxoquinazoline-3(4H)-carboxamide, (16a) &2-(2,4-dichlorophenyl)-4-oxoquinazoline-3(4H)-carbothioamide, (16b):

Equimolar amount of compound **13** (2 mmol, 0.58 gm), and urea or thiourea (2 mmol, 0.12 gm ,0.15 gm), respectively, were dissolved in pyridine (10ml). The mixture was refluxed for 8-10 h. The reaction mixture was poured on ice-water, the precipitated solid obtained was filtered off, washed with ethanol, dried and crystallized out from ethanol.

2-(2,4-dichlorophenyl)-4-oxoquinazoline-3(4*H*)-carboxamide. (16a)

Yield: (42%); m.p. 177-180°C. Analysis % for $C_{15}H_9Cl_2N_3O_2$, Calcd.(Found) C:53.91 (54.16), H:2.71 (2.69), N:12.57 (12.65). IR (KBr) (cm^{-1}): 3383, 3302 (NH_2), 1662, 1612 (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 7.14-7.23 (t, 1H, H_6), 7.55-7.78 (m, 4H, H_7 &2,4-dichloro-ph-$H_{3',5',6'}$), 7.87 (d, 1H, H_8), 8.56 (d, 1H, H_5), 12.30 (s, 2H, NH_2; D_2O exchangeable).MS (m/z): 333 (M^+, 10.44 %), 337 (M^++4, 3.56 %), 108.59 (100%).

2-(2,4-dichlorophenyl)-4-oxoquinazoline-3(4H)-carbothioamide. (16b)

Yield: (20%); m.p.183-185°C. Analysis % for $C_{15}H_9Cl_2N_3OS$, Calcd.(Found) C:51.44 (51.60), H:2.59 (2.62), N:12.00 (12.17). IR (KBr) (cm^{-1}): 3387, 3336 (NH_2), 1662, (CO). ^{1}H-NMR (DMSO-d_6-D_2O) δ (ppm): 7.18-7.27 (t, 1H, H_6), 7.54-7.77 (m, 4H, H_7 &2,4- dichloro-ph-$H_{3',5',6'}$),), 7.87 (d, 1H, H_8), 8.56 (d, 1H, H_5), 12.23 (s, 2H, NH_2; D_2O exchangeable). MS (m/z): 348 (M^+, 4.13 %), 352 (M^++4, 3.20 %), 80.07 (100%).

4-(2-(2,4-dichlorophenyl)-4-oxoquinazolin-3(*4H)-yl*)benzoic acid. (17)

A mixture of compound **13** (3mmol), 4-amino benzoic (3mmol), and pyridine (10ml) was refluxed for 3h. The precipitated solid formed after cooling was filtered off, washed and crystallized from ethanol.

Yield: (26.66%); m.p. 270-273 °C. Analysis % for $C_{21}H_{12}Cl_2N_2O_3$, Calcd. (Found) C: 61.33 (61.62), H: 2.94 (2.92), N: 6.81 (6.92). IR(KBr) (cm^{-1}): 3290-3236 (OH), 1662, 1693 (CO). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 7.31-7.36 (t, 1H, H_6), 7.54-7.60 (t, 1H, H_7), 7.63 (s, 1H, 2,4-dichloro-ph-$H_{3'}$), 7.66-7.77 (m, 3H, H_8 & 2,4-dichloro-ph-$H_{5',6'}$), 7.82 (d, 2H, ph-$H_{3,5}$ J = 8.7 Hz), 7.91 (d, 2H, $ph_{2,6}$ J =8.7 Hz), 8.06 (d, 1H, H_5), 12.71 (s, 1H, OH; exchangeable with D_2O). MS(*m/z*): 410 (M^+, 23.11 %), 412 (M^++2, 16%), 414 (M^++4, 4.86%), 139.07 (100%).

***N*-(2-(2,4-dichlorophenyl)-4-oxoquinazolin-3(4*H*)-yl) benzamide. (18)**

An equimolar amount of 2-(2,4dichlorophenyl)-3-amino-4(3H)-quinazo-linone **14** (5mmol, 1.5gm), and benzoyl chloride ,(5 mmol, 0.7gm), in pyridine was stirred for 2h then refluxed for 15 h. The reaction mixture was poured on ice-water then acidified with HCl. The solid precipitate filtered off, washed with water, dried and crystallized from ethanol.

Yield: (30%); m.p. 218-220 °C. Analysis % for $C_{21}H_{13}Cl_2N_3O_2$, Calcd. (Found) C: 61.48 (61.72), H: 3.19 (3.17), N:

10.24 (10.39). IR(KBr) (cm^{-1}): 3313 (NH), 1685, 1672 (CO). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 5.53 (s, 1H, NH; exchangeable with D_2O), 7.54 (m, 7H, 2,4- dichloro-ph-$H_{5',6'}$ & 5ph-H), 7.71 (d, 1H, H_8), 7.74 (s, 1H, 2,4-dichloro-ph-$H_{3'}$), 7.83-7.87 (2t, 2H, $H_{6,7}$), 8.21 (d, 1H, H_5). MS(*m/z*): 409 (M^+, 1.39 %), 413 (M^++4, 0.75%), 40.15 (100%).

General procedure for the synthesis of 3-(4-(4-substituted-benzylideneamino) phenyl)-2-(2,4-dichlorophenyl)quinazolin-4(3*H*)-one (19a-d) & 3-(1-(4-phenyl) substituted thylidene-amino)-2-(2,4dichloro-phenyl)quinazolin-4(3*H*)-one.(19e-g)

A mixture of compound **14** and the appropriate substituted aromatic aldehyde or ketone (1 mmol) and glacial acetic acid, was refluxed. The precipitated solid formed after cooling was filtered off, washed and crystallized from ethanol.

3-(4-methylbenzylideneamino)-2-(2,4-dichlorophenyl)quinazolin-4(3H)-one. (19a)

Yield: (25.60%); m.p. 172- 173 °C. Reflux time=8h. Analysis % for $C_{22}H_{15}Cl_2N_3O$, Calcd. (Found) C: 64.72 (64.95), H: 3.70 (3.76), N: 10.29 (10.48). IR(KBr) (cm^{-1}): 3059 (aromatic CH str.), 1678 (CO), 1600 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 2.40 (s, 3H, CH_3), 7.28 (d, 2H, ph-$H_{3',5'}$ *J*= 8.1Hz), 7.52 (d, 2H, ph-$H_{2',6'}$, *J*= 8.1Hz), 7.56-7.66 (m, 2H, H_6 & 2,4- dichloro-ph-$H_{5'}$), 7.68 (s, 1H, 2,4- dichloro-ph-$H_{3'}$), 7.71 (d, 1H, H_8), 7.77 (d, 1H, 2,4-dichloro-ph-$H_{6'}$), 7.89-7.94 (t, 1H, H_7), 8.25 (d, 1H, H_5), 9.00 (s, 1H, N=CH). MS(*m/z*): 407(M^+, 7.37 %), 409 (M^++2, 2.28 %), 411(M^++4, 1.36), 273.06 (100 %).

3-(4-chlorobenzylideneamino)-2-(2,4-dichlorophenyl)quinazolin-4(3*H*)-one. (19b)

Yield: (24.39%); m.p. 205-206 °C. Reflux time=8h. Analysis % for $C_{21}H_{12}Cl_3N_3O$, Calcd. (Found) C: 58.83 (59.04), H: 2.82 (2.80), N: 9.80 (9.95). IR(KBr) (cm^{-1}):3070 (aromatic CH str.), 1678 (CO), 1593 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 7.55 (d, 2H, ph-$H_{3',5'}$ *J*=9 Hz), 7.64 (d, 2H, ph-$H_{2',6'}$ *J*=9 Hz), 7.70 (s,1H, 2,4-dichloro-ph-$H_{3'}$), 7.72-7.78 (m, 3H, $H_{6,8}$ & 2,4- dichloro-ph-$H_{5'}$), 7.80 (d, 1H, $H_{6'}$), 7.90-7.95 (t, 1H, H_7), 8.28 (d, 1H,H_5), 9.14 (s, 1H, N=CH). MS (*m/z*): 427 (M^+, 1.13 %), 433 (M^++6, 1.86), 76.02 (100 %).

3-(4-methoxybenzylideneamino)-2-(2,4-dichlorophenyl)quinazolin-4(3H)-one. (19c)

Yield: (24.39%); m.p.160-162°C. Reflux time=18h. Analysis % for $C_{22}H_{15}Cl_2N_3O_2$, Calcd. (Found) C: 62.28 (62.41), H: 3.56 (3.58), N: 9.90 (9.97). IR(KBr) (cm^{-1}): 3074 (aromatic CH str.), 1678 (CO), 1604 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 3.81 (s, 3H, OCH_3), 7.02 (d, 2H, ph-$H_{3',5'}$ *J*=8.4 Hz), 7.55-7.63 (m, 3H, H_6 & 2,4-dichloro-ph-$H_{3',5'}$), 7.66 (d,2H, ph-$H_{2',6'}$, *J*=8.4 Hz), 7.73 (d, 1H, H_8), 7.79 (d, 1H, 2,4- dichloro-ph-$H_{6'}$), 7.89-7.94 (t, 1H, H_7), 8.27 (d, 1H, H_5), 8.92 (s, 1H, N=CH). MS(*m/z*): 423 (M^+, 29.47%), 427 (M^++4, 13.50), 299.64 (100 %).

3-((furan-2-yl)methyleneamino)-2-(2,4-dichlorophenyl)quinazolin-4(3*H*)-one. (19d)

Yield: (27%); m.p. 149-150°C. Reflux time=18h. Analysis % for $C_{19}H_{11}Cl_2N_3O_2$, Calcd. (Found) C: 59.39 (59.62), H: 2.89 (2.93), N: 10.94 (11.07). IR(KBr) (cm^{-1}): 3082-3032 (aromatic CH str.), 2962-2927 (alphatic CH str.),1678 (CO), 1597 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 6.62-6.63 (t, 1H, furan-H_3), 7.21 (d, 1H, furan-H_4), 7.55-7.95 (m, 7H, $H_{6,7,8}$ & 2,4- dichloro-ph-$H_{3',5',6'}$ & furan-H_2), 8.27 (d, 1H, H_5), 8.87 (s, 1H, N=CH). MS(*m/z*): 383 (M^+, 14.75%), 385(M^++2, 3.87), 387 (M^++4, 0.98), 185.16 (100 %).

3-(1-p-tolylethylideneamino)-2-(2,4-dichlorophenyl)quinazolin-4(3H)-one. (19e)

Yield: (35%); m.p. 200-201°C. Reflux time=8h. Analysis % for $C_{23}H_{17}Cl_2N_3O$, Calcd. (Found) C: 65.41 (65.62), H: 4.06 (4.11), N: 9.95 (10.09). IR(KBr) (cm^{-1}): 3163-3059 (aromatic CH str.), 2962 (alphatic CH str.), 1670 (CO), 1600 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 1.80 (s, 6H, ph-CH3 & N=C-CH3), 7.43-7.45 (m, 4H, p-methyl-ph-H), 7.53-7.59 (m, 2H, H_8 & 2,4- dichloro-ph-$H_{5'}$), 7.63-7.70 (t, 1H, H_6), 7.76-7.80 (m, 2H, 2,4- dichloro-ph-$H_{3',6'}$), 7.90-7.96 (t, 1H, H_7), 8.23 (d, 1H, H_5). (MS(*m/z*): 421 (M^+, 8.82%), 425(M^++4, 6.51), 79.27 (100 %).

3-(1-(4-chlorophenyl)ethylideneamino)-2-(2,4-dichlorophenyl)quinazolin-4(*3H*)-one. (19f)

Yield: (29.9%); m.p. 230-232°C. Reflux time=12h. Analysis % for $C_{22}H_{14}Cl_3N_3O$, Calcd. (Found) C: 59.68 (59.91), H: 3.19 (3.26), N: 9.49 (9.67). IR(KBr) (cm^{-1}): 3155-3062 (aromatic CH str.), 2951 (alphatic CH str.), 1701 (CO), 1600 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 1.81 (s, 3H, N=C-CH3), 7.33-7.43 (m, 4H, p-chloro-ph-H), 7.53-7.58 (m, 2H, H_8 & 2,4- dichloro-ph-$H_{5'}$), 7.63-7.69 (t, 1H, H_6), 7.76-7.81 (m, 2H, 2,4- dichloro-ph-$H_{3',6'}$), 7.91-7.97 (t, 1H, H_7), 8.23 (d, 1H, H_5). (MS(*m/z*): 441 (M^+, 3.14%), 445(M^++4, 1.24), 447 (M^++6, 0.87), 238.25 (100 %).

3-(1-(4-methoxyphenyl)ethylideneamino)-2-(2,4-dichlorophenyl)quinazolin-4(3H)-one (19g).

Yield: (35%); m.p. 226-229°C. Reflux time=10h. Analysis % for $C_{23}H_{17}Cl_2N_3O_2$ (438.31), Calcd. (Found) C: 63.03 (63.25), H: 3.91 (3.97), N: 9.95 (9.72). IR(KBr) (cm^{-1}): 3155-3062 (aromatic CH str.), 2954 (alphatic CH str.), 1697 (CO), 1600 (C=N). ^{1}H-NMR(DMSO-d_6-D_2O) δ (ppm): 1.80 (s, 3H, N=C-CH3), 3.79 (s, 3H, p-methoxy-phenyl), 7.35-7.43 (m, 4H, p-methoxy-ph-H), 7.53-59 (m, 2H, H_8 & 2,4- dichloro-ph-$H_{5'}$), 7.63-7.69 (t, 1H, H_6), 7.75-7.81 (m, 2H, 2,4- dichloro-ph-$H_{3',6'}$), 7.91-7.97 (t, 1H, H_7), 8.23 (d, 1H, H_5). (MS (*m/z*): 437 (M^+, 7.94%), 441(M^++4, 6.57), 69.05 (100 %).

2.2. Antitumor screening

The *in vitro* anti-tumor activity against human breast cancer

cells (MCF7) of the 26 tested compounds was achieved in the cell culture lab, the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Imatinib was used as a reference standard and showed IC_{50} 6.06 µg/well.

The anticancer MCF7 profile suggested that, the tested compounds showed variable activity compared to the reference drug as shown in Table1**,** on the basis of the following method:

Mammalian cell lines: MCF-7 cells (human breast cancer cell line was obtained from VACSERA Tissue Culture Unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH_2O and filtered through a Whatmann No.1 filter paper.

Cell line Propagation**:** The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell mono layers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software [26].

2.3 Docking methodology

Docking studies have been performed using MOE 2008.10. Docking procedure was followed using the standard protocol implemented in MOE 2008.10 and the geometry of resulting complexes was studied using the MOE's Pose Viewer utility

3 Results and Discussion

3.1 Chemistry

Quinazoline is one of the most important heterocyclic compounds, possessing varied biological activities, which make it of great scientific interest. Our target in the present work is to design and synthesize new series of quinazoline-4-(3H)-ones as possible anticancer agents.

The known starting materials 2-(chloromethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **1,** and 2-(2,4-dichlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **13,** were prepared by reaction of anthranilic acid with chloroacetyl chloride / 2,4-dichlorobenzoyl chloride respectively, in pyridine[24-25].

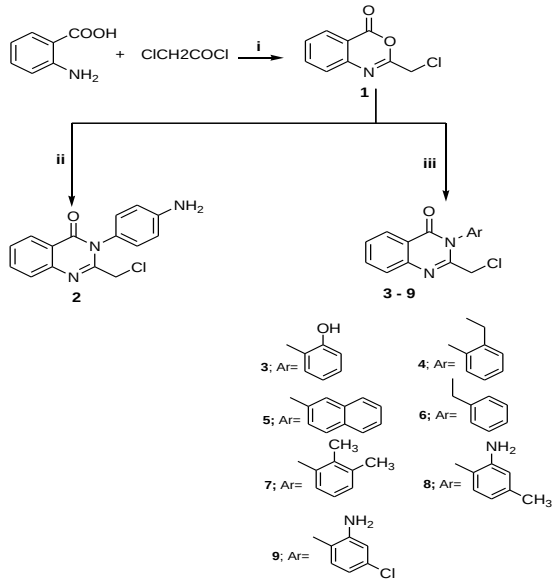
A series of 2,3-disubstituted 4(3*H*)-quinazolinones were prepared through cyclo-condensation reaction, this was performed by intrarmolecular cyclization reaction, *via* ring opening followed by ring closure of compound **1,** with different aromatic amines[27] affording compounds **2-9**.

The spectral and analytical data of compounds **2-9** were in accordance with the proposed structures. The main characteristic feature for the formation of 4(3*H*)-quinazolinone ring, was the disappearance of the etherial C-O band at 1178 cm^{-1}, and the shift of the carbonyl C=O band from 1710 to 1697 cm^{-1}.

A representative of this group is compound **2,** its **IR** spectrum displayed a sharp band at 1697 cm^{-1} corresponding to the carbonyl C=O group, in addition to

another band ranging from 3410 -3325 cm^{-1}, corresponding to the NH_2 stretching, thus confirming the structure of this compound.

Further confirmation was obtained by its **^{1}HNMR** spectrum, which exhibited the D_2O exchangeable singlet at δ 10.53, attributed to the NH_2 group, in addition to the usual singlet signal of CH_2Cl protons, which appeared at δ 5.65 ppm.



Scheme 1

Reagents and conditions : **(i)** Dry acetone stirring at 0-5 ^{0}C for 3h. **(ii)** 1,4 Phenelene diamine / pyridine, reflux 4h. **(iii)** Aromatic amine / pyridine, reflux and cooling.

The **IR** spectra of compounds **8** and **9** were in agreement with the predicted structures as they shared the presence of an amino group, which displayed a characteristic absorption band in the range of 3425-3163 cm^{-1}.

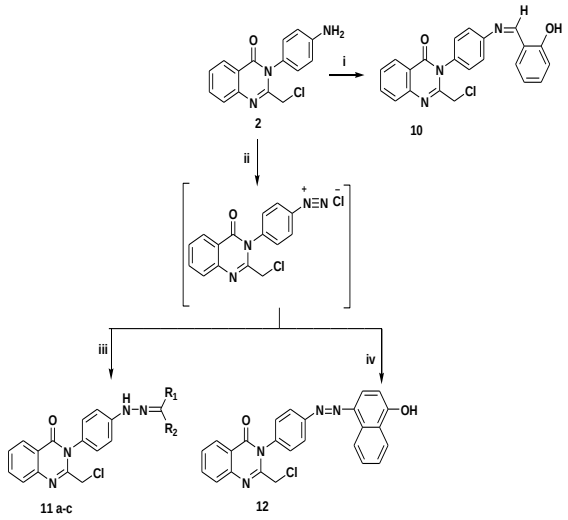
The structure of compound **3** was proved through its **IR** spectrum which showed a broad absorption band corresponding to the OH group at 3525- 3367 cm^{-1}.

Examining the **^{1}H-NMR** spectrum for compound **4,** revealed the existence of the triplet quartet pattern of CH_2CH_3 group at δ 1.18 and 3.99 ppm respectively, while the **^{1}H-NMR** spectrum of compound **5** exhibited an increase in the number of aromatic protons by 7H belonging to the naphthyl group.

The configuration of compound **6** was based on its **^{1}H-NMR** spectrum, which displayed the presence of a singlet at δ 3.50 ppm representing the aliphatic CH_2 protons of the hetero aromatic ring, while for compound **7** an appearance of a characteristic singlet at δ 2.61 ppm, attributed to the 6 protons of two methyl groups, established its structure. established its structure.

Reagents and conditions : (**i**) 2-hydroxybenzaldhyde / glacial acetic acid, reflux 8h. (**ii**) NaNO2 /HCl / 0 ^{0}C, stirring for 30 min. (**iii**) Active methylene compounds (malononitrile, acetylacetone and ethyl acetoacetate) / NaOH /EtOH, stirring for 2h. / cooling. (**iv**) Alpha-naphthol / NaOH /EtOH, stirring for 2h. /cooling.

Schiff's bases were obtained by the reaction of aldehydes / ketones with amines in acidic medium [28]. In the present work the newly synthesized compound **10** was successfully prepared by refluxing compound **1** with 2-hydroxy benzaldehyde, in glacial acetic acid.



Comp.	R1	R2
a	CN	CN
b	CH_3CO	CH_3CO
c	CH_3CO	$COOC_2H_5$

Scheme 2

The common feature for this compound, was the disappearance of the NH_2 band in its **IR** spectra, in addition to the presence of a singlet attributed to the imine proton (CH=N) at δ7.49 ppm in its **^{1}H-NMR** spectra, denoting the formation of the Schiff's bases.

The **IR** of compound **10** was characterized by the existence of the OH absorption bands at 3448-3421 cm^{-1}, in addition to the common features of this group.

The presence of an aromatic amino group in compound **2** encouraged the diazotization reaction. This was achieved by using a mixture of sodium nitrite and HCl at 0-5 ^{o}C [29,30]. The diazonium salt thus obtained, was coupled with active methylene compounds, namely malononitrile, acetylacetone, ethyl acetoacetate and α-naphthol, in ethanol in the presence of sodium hydroxide affording compounds **11a-c** and **12**.

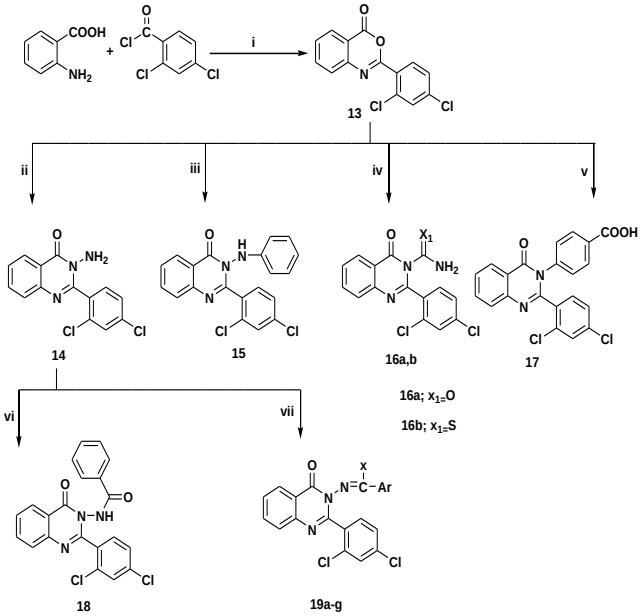
The above mentioned structures were confirmed based on their elemental and spectral data. Their structures were established by **IR** spectra, which displayed the disappearance of the characteristic NH_2 band**..**

Compound **11a,** as one of the formed hydrazono compounds**,** exhibited a characteristic absorption bands at 2222 cm^{-1} corresponding to the cyano group, along with the

disappearance of NH_2 bands in its **IR** spectrum. Furthermore, its **^{1}H-NMR** spectrum showed the D_2O exchangeable singlet at δ 5.78, attributed to the NH group.

On the other hand, the structure of compound **11b,c** was confirmed through the spectral data mainly the **^{1}H-NMR.**

The **^{1}H-NMR** spectrum of compound **11b** displayed a singlet at δ 2.70 and 2.86 ppm representing the two methyl groups, while the classical signals of triplet-quartet pattern at δ 1.26 and 4.25 ppm were exhibited for compound **11c,** establishing its proposed structure.



Comp.	X	Ar
a	H	P-CH_3
b	H	P- Cl
c	H	P- OCH_3
d	H	Furan
e	CH_3	P-CH_3
f	CH_3	P- Cl
g	CH_3	P- OCH_3

Scheme 3

Reagents and conditions : (**i**) dry acetone/pyridine, stirring at 0-5 ^{0}C for 3h., then stirring for 1h at room temp. (**ii**) NH_2-NH_2.H_2O/EtOH, reflux 4h. (**iii**) Phenyl hydrazine/ pyridine, reflux 10h./ cooling. .(**iv**) NH_2CONH_2 or NH_2CSNH_2/ pyridine, reflux 8-10h./ cooling.(**v**) p-aminobenzoic acid/ pyridine, reflux 3h./ cooling. (**vi**) benzoyl chloride/ pyridine, stirring for 2h., reflux 15h./cooling. (**vii**) aromatic aldehydes or ketones/ glacial acetic acid, reflux 10-18h./cooling.

Scheme **3** describes the preparation of compounds **14, 15, 16a,b, 17, 18** and **19a-g** respectively.

Condensation of compound **13** with hydrazine hydrate in boiling ethanol resulted in the 3-amino-4(3H)-quinazolinone derivative, **14,** which was used as a precursor for construction of biologically active heterocycles.[31]

In a similar manner, refluxing compound **13** with phenyl hydrazine in pyridine afforded compound **15.**

These compounds were confirmed on the basis of their spectral data, the most characteristic feature was the absorption bands at 3324-3258 and 3282 cm^{-1} belonging to NH_2 and NH groups respectively in their **IR** spectra.

Compound **13** was converted to 4-oxoquinazoline-3(4*H*)-carbamide derivatives, **16a, b** by its nucleophilic substitution reaction with urea [32] and thiourea.

Studying the **IR** spectra of compounds **16a, b** revealed that the two compounds shared the presence of NH_2 group absorption band at 3383-3302 cm^{-1}. Further conformation for compound **16a** was the appearance of a characteristic absorption bands at 1662, 1612 cm^{-1}, corresponding to the two C=O groups.

Compound **17** was prepared successfully according to the literature procedure *via* the cyclo-condensation reaction of compound **13** with an equimolar amount of p-amino benzoic acid in pyridine [27]. Its IR spectrum displayed an absorption band at 1693 cm^{-1} assigned to the C=O group, in addition to another broad band of the OH group at 3290-3236 cm^{-1}.

Its **^{1}H-NMR** spectrum exhibited two doublets representing the para-substituted system of the incorporated amine with *J* constant = 9 Hz, and the D_2O exchangeable singlet at δ 12.71 ppm.

Benzoylation reaction was achieved by refluxing compound **14** with benzoyl chloride in pyridine to afford compound **18** [33].

The proposed structure of compound **18** was in agreement with the spectral data, as it showed in its **IR** spectrum the appearance of a sharp band at 3313 cm^{-1} attributed to NH group with disappearance of NH_2 absorption band, in addition to two absorption bands at 1685, 1672 cm^{-1} corresponding to the two C=O groups.

Its **^{1}H-NMR** spectrum revealed an increase in the number of aromatic protons by 5 belonging to the phenyl ring.

Condensation of the amino group of compound **14** with aldhehydes / ketones gave a series of Schiff's bases **19a-g** [28].

The **IR** spectra for all derivatives showed the disappearance of NH_2 band and the appearance of the characteristic band belonging to C=N at 1600 cm^{-1}, and this was taken as an evidence for the formation of the imines compounds.

In addition, the **^{1}H-NMR** spectra for the derivatives **19a-d** exhibited a singlet corresponding to the imine proton (CH=N) at the range δ 8.81 to 9.14, while for the derivatives **19e-g,** the presence of a singlet at δ 1.80 ppm. attributed to (N=C-CH_3) denoted the formation of the Schiff's bases.

Examining the **^{1}H-NMR** spectrum of compound **19a** as a representative of this series, showed a common feature, which is the appearance of a singlet at δ 2.40 ppm representing the CH_3 protons, in addition to two doublets representing para-substituted system at δ7.28 and 7.52 ppm, with *J* constant = 8.1Hz. Furthermore, the existence of a signal corresponding to the imine proton (CH=N) at δ

Table 1: % Viability of MCF-7 cells.

Comp.	Viability %					
	1.56	**3.125**	**6.25**	**12.5**	**25**	**50ug/well**
2	64.85	46.78	31.29	20.31	12.84	6.39
3	87.39	81.72	67.34	50.92	39.47	14.75
4	98.65	93.13	82.59	69.87	45.19	27.48
5	98.73	94.73	87.12	70.95	59.81	37.24
6	100	97.68	86.14	72.39	41.82	34.37
7	96.84	92.71	81.36	70.52	40.95	27.82
8	94.22	85.96	74.53	57.32	37.86	26.71
9	100	100	100	100	98.89	91.46
10	98.61	92.83	84.19	75.41	68.27	45.62
11a	90.71	78.56	49.62	42.31	36.72	21.08
11b	93.22	85.14	79.36	58.29	40.17	21.98
11c	95.16	89.23	78.41	62.34	35.06	18.21
12	100	99.12	94.06	86.57	75.14	68.32
14	81.54	76.43	68.12	51.74	43.82	28.97
15	93.02	84.75	69.47	43.78	35.96	21.34
16a	85.31	57.52	35.49	27.18	19.43	10.59
16b	100	100	98.79	91.43	79.52	43.64
17	98.61	92.56	86.43	70.85	47.82	34.21
18	60.48	42.75	36.94	32.81	21.52	8.37
19a	100	94.86	83.41	70.87	64.98	32.24
19b	98.49	92.64	83.42	64.21	52.87	38.93
19c	96.73	94.68	89.35	81.43	74.91	34.56
19d	100	99.12	96.73	90.64	78.52	65.67
19e	97.13	90.82	73.65	59.71	45.64	37.98
19f	92.39	86.45	72.82	50.89	31.32	18.96
19g	100	95.05	84.59	65.84	45.61	27.04
Imatinib	79.46	67.52	48.89	37.18	26.72	17.65

Table 2: IC_{50}'s of the tested compounds against human breast cancer cells MCF-7.

Compound	IC_{50} (µg/well)	IC_{50} (µM)
2	**2.84**	**9.96**
3	13.5	47.20
4	22.6	75.84
5	35.9	112.19
6	21.7	76.41
7	21.2	71.14
8	17.2	57.53
9	>50	156.74
10	45.2	116.20
11a	**6.21**	**17.15**
11b	18.2	38.7
11c	18.2	43.4
12	>50	113.64
14	15.2	49.84
15	11	28.87
16a	**4.19**	**12.58**
16b	45.6	131.03
17	23.8	58.05
18	**2.48**	**5.89**
19a	36.4	89.43
19b	30.1	70.49
19c	40.4	95.51
19d	>50	130.55

19e	21.1	50.12
19f	13.1	29.71
19g	19.1	43.72
Imatinib	**6.06**	**12.28**

9.00 ppm, established the structure of the successfully prepared Schiff's base.

3.2. Antitumor activity

The newly synthesized compounds were screened against breast cell cancer (MCF-7) using the mentioned technique (viability assay) [26], where the anticancer profile of MCF-7 suggested that, the tested compounds showed variable activity compared to the reference drug.

The close examination of the results representing the percentage of growth inhibition and IC_{50} in Table 1, 2 are resumed in **Figure** 2, 3 respectively.

From the above-mentioned results we can deduce that the most active compounds were **2, 16a, 11a** and **18** exhibiting IC_{50} (2.84, 4.19, 6.21 and 2.48 ug/well respectively), compared with the reference Imatinib possessing IC_{50} 6.06 ug/well.

The presence of the p-amino phenyl group in compound **2,** the amide group in **16a** and the **NH** group in **18** respectively attached to the Nitrogen in the position **3** of the quinazolinone ring, contributed to the high anti-breast cancer activity in comparison with the reference Imatinib. This emphasizes the importance of the amino group, either primary or secondary amine, for the activity.

Compound **11a** was nearly equipotent to the reference drug, Imatinib, against MCF-7 cell line. This illustrated the significant role of the azide group, additionally, the presence of the azide group increased the binding with the amino acids residues of the receptor as shown in the docking study.

Moderate anti-breast cancer activity was demonstrated by compounds **3, 4, 6, 7, 8, 14, 15, 17** and **19e-g.** Compounds **9, 12** and **19d** possessed no significant anti-breast cancer activity.

Examining the SARs of compounds **2** and **9** revealed that, replacement of the p- amino group in **2** by electron withdrawing group (p-Cl), abolished the activity.

In the two analogues **16a,b** introducing the S instead of O resulted in abolishing the activity of the later.

Comparing the tested compounds, **14** and **15,** revealed that**,** replacement of the amino group attached to the Nitrogen at position **3** of quinazolinone ring by the NH-ph, increased the activity by 1.3 folds.

On the contrary the abolished anti-breast carcinoma activity was observed in compound **11a** (IC_{50}=45.2 µg/well).

Furthermore, compound **12** exhibited no anticancer activity against the breast cell line MCF-7, this could be attributed the bulkiness of the naphthyl group in contrast to its analogue (**11a**) which possessed a [$(CN)_2CH$-CH=N-] moiety, and exhibited a potent anti-breast cancer activity (IC_{50}= 6.21 ug/well).

Referring to the anti-breast cancer activity results of the Schiff's bases, we can conclude that, the Schiff's bases **19a-c** resulting from reaction of amine with aldehydes are less potent (IC_{50} = 36.4, 30.1 and 40.4 µg/well, respectively), than their counterparts **19e-g,** resulting from reaction of amine with ketones and possessing moderate anticancer activity (IC_{50} = 21.1, 13.1 and19.1 µg/well respectively).

This activity was completely abolished in compound **19d,** and this supposes that the six memberd ring of the phenyl substituent, might be considered as an important element for the activity.

It was obvious that, Schiff's base with p-Cl substituent, compounds **19b** and **19f,** (IC_{50} = 30.1 and 13.1 µg/well respectively), remarkably enhanced the anticancer activity over their analogues possessing p- CH_3 or p-OCH_3 group, This could be attributed to the electron withdrawing effect of the Cl group.

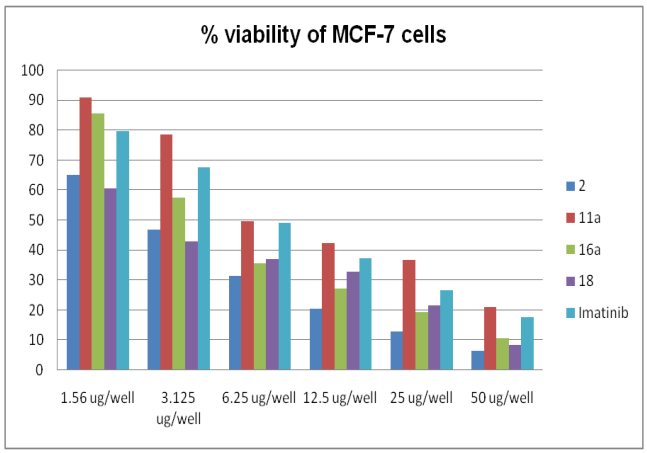


Figure 2: % viability of MCF-7 cells

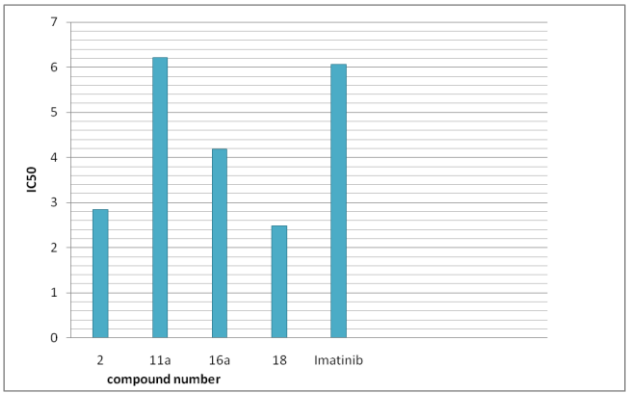


Figure 3: Cytotoxicity of compounds **2, 11a, 16a, 18 and Imatinib** against human breast cancer cells MCF-7.

3.3 Molecular docking studies

In order to understand the obtained biological data on a structural basis, the most active compounds; **2, 11a, 16a** and **18** were evaluated through molecular modelling and docking techniques.

Table 3: The docking energy scores of compounds **2, 16a, 11a** and **18** with the amino acid residues in the EGFR active site forming hydrogen and arene-cation bonds in comparison with ligand **IRE**.

Cpd. No.	Docking score (Kcal/mol)	Amino acid residues (bond length A°)	Atoms of cpd.	Type of bond
IRE	-2.73	Asp855(2.4) Lys745	Aliphatic NH group Phenyl group	H-bond Arene-cation
2	-4.56	Met793(2.4) Lys745	H of NH_2 of phenyl Benzyl group	H-bond Arene-cation
16a	-6.14	Asp855(2.7) Thr854(2.9) Lys745	H of NH_2 of amide O of C=O Phenyl group	H-bond H-bond Arene-cation
11a	-4.47	Lys745(2.7) Asp 855(2.8) Thr 854(2.9)	N of CN N of CN N of CN	H-bond H-bond H-bond
18	-5.21	Lys 745(3.12) Ser 719(2.96) Lys 745	N of quinazolinone O of C=O Di-chloro phenyl	H-bond H-bond Arene-cation

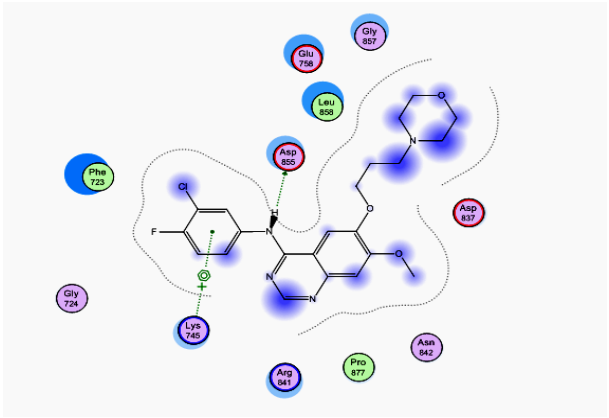
The level of antitumor activities of the compounds **2, 11a, 16a** and **18** over breast cancer cell, in which EGFR kinase is highly expressed, prompted us to perform the molecular docking into the ATP binding site of EGFR kinases to predict if these compounds had analogous binding mode to the EGFR kinase inhibitor. We assumed that the active target compounds **2, 11a, 16a** and **18** might demonstrate antiproliferative activity against breast cancer cell lines through inhibition of EGFR.

In the present study, the target compounds were docked into receptor active site of EGFR. [5,34] All calculations were performed using MOE 2008.10 software.The crystal structure of EGFR with gefitinib (Iressa) (IRE), (PDB code: 2ito) was obtained from protein data bank (PDB) and used as the receptor model in the docking simulation to predict binding modes, affinities and orientation at the active site of the enzyme.

The binding energies of compounds **2, 11a, 16a, 18** and gefitinib docked into the active site of EGFR were -4.56, -4.47, -6.14, -5.21 and -2.37 kcal/mol, respectively (Table 3).

These docking studies of the ligand IRE, as shown in Figure 4, revealed the following binding modes:

- Arene-cation interaction between the phenyl group and Lys745.
- Hydrogen bond interaction between the Aliphatic NH group and O in Asp855.
- Hydrophobic interaction between the ethyl morpholine and Leu 858, Glu 758 and Phe 723, both Lys 745 and Asp 855 were present in the catalytic core of the enzyme active site.



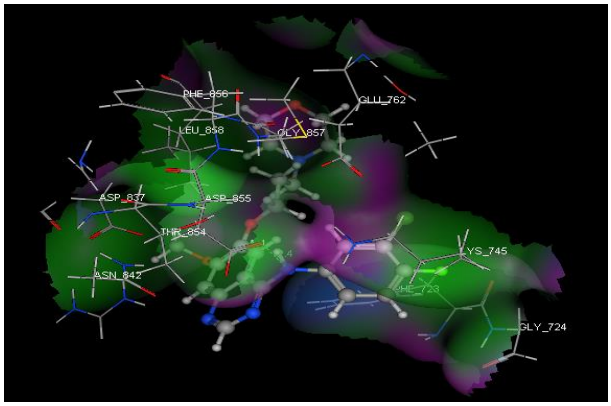


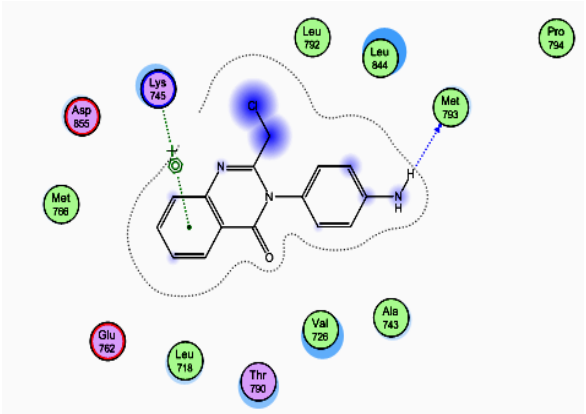
Figure 4: The proposed binding mode of compound **IRE** docked in the active site of EGFR; (2D and 3D ligand-

receptor interactions) (hydrogen bonds are illustrated as arrows; C atoms are colored gray, N blue and O red).

The conserved lysine in the N-lobe was found to be important for the stabilization. [35]

The docked model of compound **2** (Figure 5) showed an arene-cation interaction of the benzyl group of quinazolinone ring with Lys745, present in the catalytic core, in addition to a hydrogen bond interaction between the amino phenyl group, and the oxygen of Met793, present in the backbone residues of the connecting hinge. [34] Moreover, the methylene chloride formed a hydrophobic interaction with Leu844, Val 726 and Thr 790.

Concerning compound **16a (Figure 6),** the NH_2 of the amide group formed a hydrogen bond with Asp 855. A second hydrogen bond was also formed between oxygen of carbonyl group of quinazolinone, and Thr845. Furthermore, an arene-cation interaction of the benzyl group of the quinazolinone with Lys745. Hydrophobic interactions were also demonstrated in its docking model between the amide group and Val726, Leu 844, Leu 718 and Thr790.



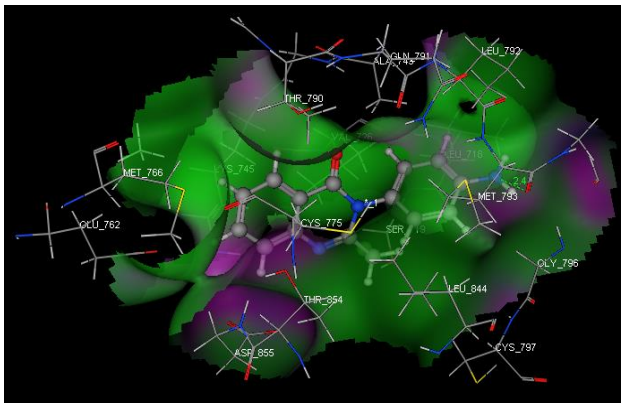
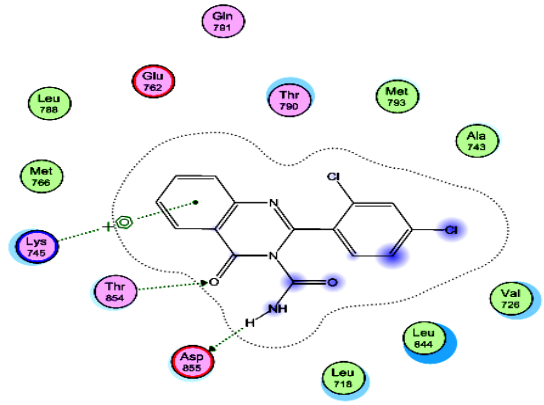


Figure 5: The proposed binding mode of compound **2** docked in the active site of EGFR.

On the other hand, the docked model of compound **11a** showed three hydrogen bonds between the two cyano groups and three amino acids residues in EGFR (Lys745, Asp855 and Thr845) (**Figure 7**). In addition, the hydrophobic interactions were observed between compound **11a** and Asp 800, Arg 841 and Ser 719.

The docking study of compound **18** (**Figure 8**)**,** revealed a hydrogen bond between N1 of the quinazolinone and Lys 745. Another hydrogen bond also formed between oxygen of carbonyl group of the quinazolinone ring, and Ser719.



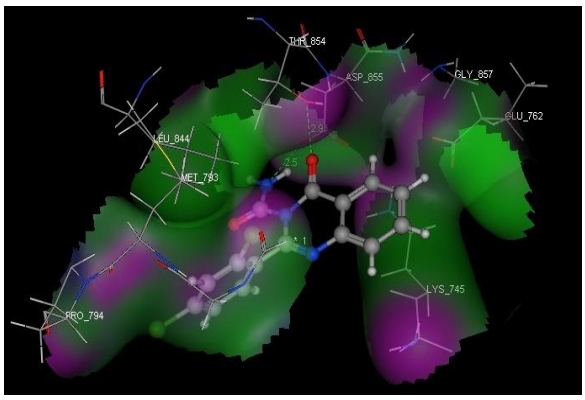
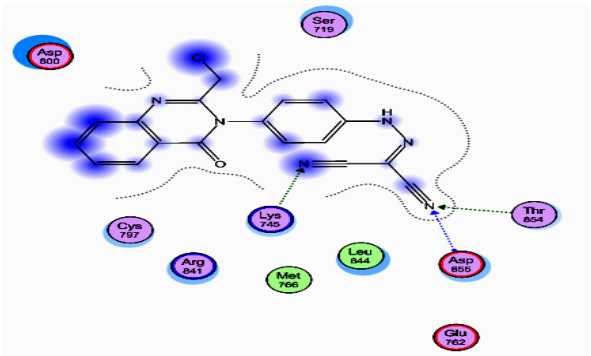


Figure 6: The proposed binding mode of compound **16a** docked in the active site of EGFR



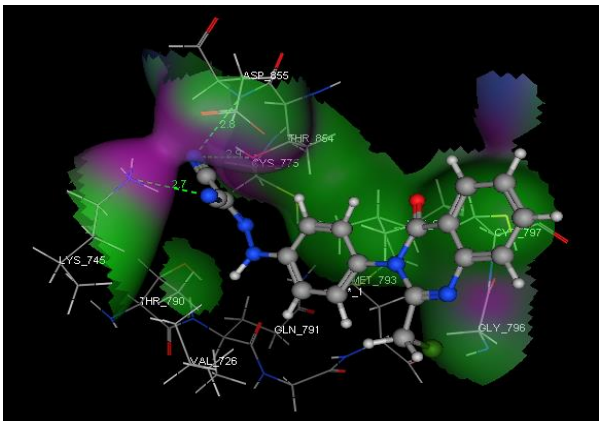
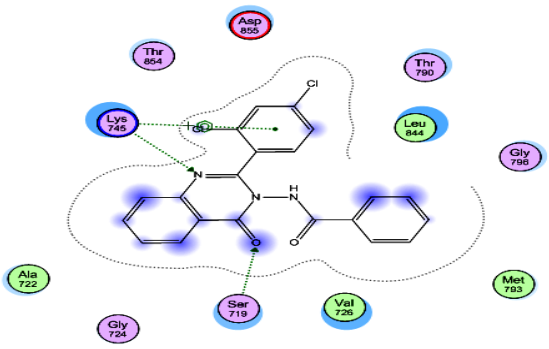


Figure 7: The proposed binding mode of compound **11a** docked in the active site of EGFR.

The di-chloro phenyl moiety formed an arene-cation interaction with Lys 745. Hydrophobic interaction was demonstrated between compound **18** and Val 726, Leu 844 and Asp 855.

These results support the postulation of the ability of these compounds to act as EGFR-TKIs



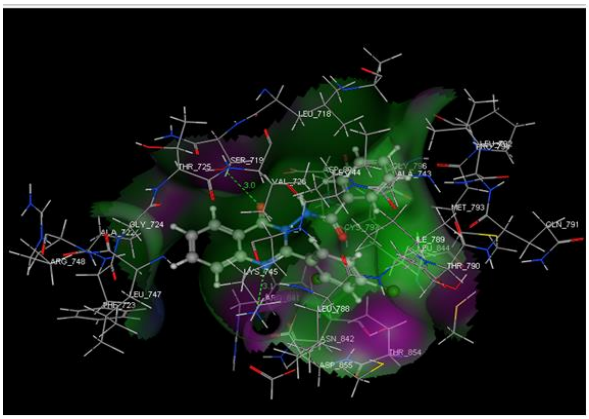


Figure 8: The proposed binding mode of compound **18** docked in the active site of EGFR;

3.4. 2D QSAR Study

3.4.1 Development of QSAR model

QSAR studies are undoubtedly important in drug design. Once a correlation between structure and activity is established, newly designed compounds, including those not yet synthesized, can be readily screened on the computer to select the structures with desired properties. Then, it is possible to select the most promising compounds to synthesize and test in the laboratory. [36]

2D-QSAR analysis for anti-proliferative activity by the novel synthesized quinazolinones derivatives was performed in order to correlate the biochemical data with synthesized structures, and to identify positive and negative structural features within the designed structures. The QSAR study was performed using Discovery Studio 2.5 software. The training set was composed of 23 synthesized compounds from the present study with their measured pIC_{50} (-Log IC_{50}) against MCF-7 cancer cell line for QSAR modeling. The remaining three compounds **(2, 3, 16b)** were adopted as an external test subset for validating the QSAR model.

"Calculate Molecular Properties" module was used for calculating different molecular properties of the training set compounds. 2D Descriptors involved: AlogP, molecular properties, molecular property counts, surface area and volume and topological descriptors, while the 3D descriptors involved: Dipole, jurs descriptors, principle moments of inertia, shadow indices and surface area and volume.

Genetic function approximation (GFA) was utilized to search for the best possible QSAR regression equation capable of correlating the variations in the biological activities of the training set compounds with variations in the generated descriptors, *i.e.*, multiple linear regression modeling (MLR).

QSAR model was validated employing leave one-out cross-validation by setting the folds to a number much larger than the number of samples, *r*2 (squared correlation coefficient value) and *r*2 prediction (predictive squared correlation coefficient value), residuals between the predicated and experimental activity of the test set and training set.

3.4.2 QSAR study results

Table 4: Experimental activities of the synthesized derivatives against the predicted activities according to the equation.

Compound	Experimental activity (-logIC_{50})	Predicted Activity (-logIC_{50})	Residual
4	-1.8799	-1.8799	-2.27778e-10
5	-2.04995	-2.04995	1.31721e-10
6	--1.88315	--1.88315	2.68252e-12
7	-1.85211	-1.85211	1.4164e-10
8	-1.75989	-1.75989	-1.31151e-11
9	-2.19518	-2.19518	--1.25959e-10
10	-2.06521	-2.06521	-1.47189e-10

11a	-1.23426	-1.23426	-6.65998e-11
11b	-1.58771	-1.58771	9.65794e-11
11c	-1.63749	-1.63749	6.67e-11
12	-2.05553	-2.05553	-5.51355e-11
14	-1.69758	-1.69758	3.43812e-10
15	-1.46045	-1.46045	-7.54952e-15
16a	-1.09968	-1.09968	-2.03961e-11
17	-1.7638	-1.7638	2.45763e-11
18	-0.770115	-0.770115	-6.28578e-11
19a	-1.95148	-1.91668	-0.0348025
19b	-1.84813	-1.84813	-3.25534e-09
19c	-1.98005	-1.960	0.0348025
19d	-2.11578	-2.11578	--6.76161e-11
19e	-1.70001	-1.70001	2.83622e-09
19f	-1.4729	-1.5077	0.0348025
19g	-1.64068	-1.60588	-0.0348025

Equation 1 represents the best performing QSAR model

-logIC$_{50}$ = -2.1315 - 1.771 [ALogP_ AtomClassName] + 0.6367 [ALogP_AtomScore] + 0.7918 [ES _Count_ ssNH]

In this equation, -logIC$_{50}$ is the negative logarithmic value of the concentration required to produce 50% inhibition of MCF-7 cancer cells.

According to the equation the QSAR model was represented graphically by scattering plots of the experimental (pIC$_{50}$) *versus* the predicted bioactivity (MLRT1) values −logIC$_{50}$ for the training set compounds as shown in **Figure 9**. The method used to build the model was Least-Squares, $r2$ = 0.999, $r2$ (adj) = 0.773, r^2(prd) =0.714, Least- Squared error = 0.172. Where r^2 (adj) is r^2 adjusted for the number of terms in the model; r^2 (pred) is the prediction *c*, equivalent to q^2 from a leave-1-outcross validation.

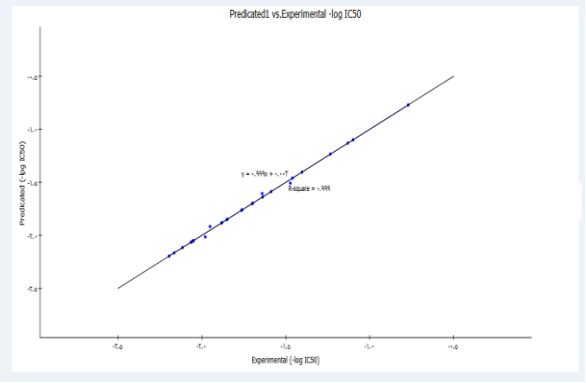


Figure 9: Predicted versus experimental pIC$_{50}$ of the tested compounds against MCF-7 cell line. r2 = 0.999

In conclusion, the equation (1) suggested that the anti-proliferative activity of the synthesized compounds is mainly affected by hydrophobicity of the molecule (ALogP) and the E-state count of nitrogen atom (ES _Count_ ssNH).

ALogP is a measure of the hydrophobicity of the molecule; it is calculated in Discovery Studio as the Log of the octanol-water partition coefficient using Ghose and Crippen's method. [37].

The estate keys calculate the sums of electrotopological state (E-state) values and/or the counts of each atom type. ES _Count_ ssNH calculates the E-state count for nitrogen. [38].

It was found that the anti-proliferative activity is negatively correlated with the hydrophobicity (ALogP) and positively correlated with the estate keys of the synthesized compounds

3.4.3 QSAR Validation

Robustness of the established QSAR model was verified by using; Leave-one-out (LOO) internal validation ($r2$ = 0.999). Cross-validation was also employed where $q2$, which is equivalent to $r2$ (pred), 0.714 in addition, validation was employed by measuring the residuals between the experimental and the predicted activities of the training set. Table 4

Moreover, the experimental and expected activities as well as the residuals of the compounds, used as statistical outliers in building the three models, are presented in (Table 5). Interestingly, the predicted activities by the generated QSAR models were very close to those observed experimentally, indicating that these models could be applied for further prediction of more effective hits having the same skeletal framework.

Table 5: Experimental activities of compounds **2**, **3** and **16b**, used as statistical outliers against the predicted activities according to the equation.

Compound	Experimental activity (-logIC$_{50}$)	Predicted Activity (-logIC$_{50}$)	Residual
2	00.998259	00.998259	4.17129e-11
3	-1.67394	-1.67394	1.81322e-12
16b	-2.11737	-2.11727	-5.41469e-11

4 Conclusion

In the present work twenty six novel derivatives of 2, 3-disubstitutedquinazolin-4-(3H)-ones were synthesized and evaluated as anti-breast cancer agents, using Imatinib as reference drug. Amongst these novel compounds, four derivatives (2, 11a, 16b and 18) were displayed high anti breast cancer activity against the reference compound.

The later compounds were evaluated through molecular modelling and docking techniques and compared with IRE (gefitinib) in the binding site of EGFR.

The 2D QSAR models generated by Discovery studio 2.5 software, showed some important geometric and molecular

descriptors that might be controlling the activities of these novel compounds. These results suggest that the novel 2,3-disubstitutedquinazolin-4-(3H)-ones derivatives could be further investigated for their inhibitory activity against human breast carcinoma cell line (MCF-7).

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