Synthesis, Crystal Structure and Anticancer Activity of Substituted Quinazoline Derivatives

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Summary: News series of substituted quinazoline derivatives has been synthesized from 3,4-dihydro-7-methoxy-4-oxoquinazoline-6-yl acetate (**1**) by five-step procedures including chlorination, amination, hydrolysis and etherification. The structures of target compounds were confirmed by IR, ^{1}H-NMR, element analysis and single-crystal X-ray diffraction. The results showed that the compound **8c** exhibited remarkable inhibitory activity against MCF-7 cell lines with inhibition rate value of 38.45 %, which was comparable to that of the positive control Gefitinib (inhibition rate = 13.25 % for MCF-7). The initial relationship between structure and activity was worth further exploration.

Keywords: Quinazoline, Synthesis, X-ray diffraction, Crystal structure, anticancer activity.

Introduction

Quinazoline is an important and widely used pharmacophore in drug discovery research [1]. The quinazoline alkaloids [2-11] and quinazoline derivatives [12] were isolated from natural products which has been reported to have a variety of physiological and pharmacological activities, such as anticancer [13], antimalaria, antiparasite, antibacterial, cardiovascular protection and antiplatelet aggregation activity, anti-virus, anti-inflammatory and regulatory immune function activity, anti-Alzheimer's disease and other neurological diseases, herbicidal activity, etc. [9, 11, 13-17]. Moreover, many quinazolines contributed to the quest for an ultimate antitumor chemotherapeutic agent [18], such as it was reported that 2-thioxo-3-substituted quinazolinones and anilino-quinazoline, as well as the 6-substituted quinazolinone derivatives, which showed potential antitumor potency [19]. During the last decades, an intensive research was dedicated to the discovery of more effective, selective, and nontoxic new anilino-quinazoline derivatives. Anilino-quinazoline compounds are representative structures of EGFR and its EGFR-TK inhibitors [20]. Many researchers demonstrated 4-aminoquinazoline derivatives as specific kinase inhibitors, including tyrosine kinase and serine/theronine kinases, the selective inhibition of EGFR-TK phosphorylation can achieve anticancer effect and a number of anilinoquinazoline compounds have been successfully used in clinical practice. 4-aminoquinazoline derivatives are applied for target specific treatment of lung, breast, colon, prostate cancers [21]. Above all, the quinazoline has a lot effect in anticancer activities which can be seen from that. In view of this several types of quinazoline derivatives were synthesized in our laboratory [22]. In order to obtain new quinazoline derivatives, as well as to establish their spatial structure and to study their anticancer activity, we obtained a series of quinazoline derivatives **8a~8h** bases as potential antiviral and antitumor activities. Of course, biological experiments showed that the compounds had a significant inhibitory effect on SKBr-3 and MCF-7 cancer cell lines.

Experimental

Chemistry

General

All solvents used and reagents were obtained from commercial sources and were used without purification. The melting points of all compounds were measured with a Beijing Taike X-4 microscopy melting point apparatus and were uncorrected. ^{1}H-NMR spectra were obtained on a Bruker Biospin 400 MHz instrument using TMS as internal standard. IR spectra were taken on a Bruker Platinum ART Tensor II FT-IR spectrometer. Crystal data was obtained on a Bruker D8 VENTURE X-diffractometer. Elemental analysis of the newly synthesized compounds was carried out on Carlo Erba 1108 analyzer.

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General procedure for preparation of 7-methoxy-4-(substituted phenylamino) quinazolin-6-yl acetate **4a-4d**:

A solution of compound **1** (8.54 mmol), triethylamine (17.32 mmol), phosphorus oxychloride (26.22 mmol) and toluene (25 mL) was heated to 78 ℃ for 6 h. Then toluene (5 mL) was mixed with 3a-3d (8.55 mmol) was added to the above reaction solution and stirred for 5 h. Upon completion of the reaction (TLC), the mixture was cooled to 0 ℃. The solid was obtained by filtration under reduced pressure and washed with toluene (30 mL). Added isopropanol (40 mL) to the solid and stirred for 2 h. The solid was filtered and washed with cold isopropanol (20 mL). The solid obtained was dried in an oven at 50 ℃.

*7-methoxy-4-(phenylamino)quinazolin-6-yl acetate***(4a)**: Yield 0.80g(89.39%); grayish white solid; M.p.: 176.5-178.2 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2748.02, 2117.27, 1776.91, 1628.40, 1427.58, 1289.24, 1217.73, 991.34, 744.82, 493.47; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 11.64 (s, 1H，-NH), 8.89 (d, *J* = 15.2 Hz, 2H, -ArH), 7.71 (d, *J* = 8.0 Hz, 2H, -ArH), 7.59 (s, 1H, -ArH), 7.49 (t, *J* = 7.6 Hz, 2H, -ArH), 7.33 (t, *J* = 7.2 Hz, 1H, -ArH), 4.01 (s, 3H, -OCH_3), 2.39 (s, 3H, -CH_3).

*7-methoxy-4-(p-tolylamino)quinazolin-6-yl acetate***(4b)**: Yield 0.92g(84.47%); white solid; M.p.: 168.5-170.2 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2752.03, 1771.34, 1640.13, 1509.73, 1395.25, 146.59, 1007.35, 884.14, 653.03, 538.45, 507.40; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 11.62 (s, 1H, -NH), 8.89 (d, *J* = 4.0 Hz, 2H, -ArH), 7.60 (d, *J* = 1.2 Hz, 2H, -ArH), 7.58 (s, 1H, -ArH), 7.27 (d, *J* = 8.0 Hz, 2H, -ArH), 4.00 (s, 3H, -OCH_3), 2.38 (s, 3H, -CH_3), 2.35 (s, 3H, -CH_3).

*4-((2-fluorophenyl)amino)-7-methoxyquinazolin-6-yl acetate***(4c)**: Yield 0.95g (92.39%); white solid; M.p.: 196.5-198.2 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2945.98, 2689.27, 2496.25, 1760.39, 1627.89, 1457.88, 1370.45, 1203.77, 992.71, 757.49, 523.70, 460.74; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 10.31 (s, 1H, -NH), 8.90 (s, 1H, -ArH), 8.79 (d, *J* = 6.6 Hz, 1H, -ArH), 7.58 (s, 1H, -ArH), 7.57 – 7.52 (m, 1H, -ArH), 7.50 – 7.45 (m, 1H, -ArH), 7.45 – 7.41 (m, 1H, -ArH), 7.36 - 7.31 (m, 1H, -ArH), 4.02 (s, 3H, -OCH_3), 2.40 (s, 3H, -CH_3)

*4-((2,4-dimethylphenyl)amino)-7-methoxyquinazolin-6-yl acetate***(4d)**:Yield 1.04g(95.73%); white solid; M.p.: 154.5-156.2 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3014.64, 2837.02, 2495.87, 1770.36, 1637.46, 1434.26, 1276.00, 1181.06, 994.07, 864.60, 759.92, 630.79, 530.34, 450.28, 1434.26; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 11.73 (s, 1H, -NH), 8.90 (s, 1H, -ArH), 8.77 (s, 1H, -ArH), 7.64 (s, 1H, -ArH), 7.20 – 7.16 (m, 2H, -ArH), 7.10 (d, *J* = 7.6 Hz, 1H, -ArH), 4.00 (s, 3H, -OCH_3), 2.38 (s, 3H, -CH_3), 2.33 (s, 3H, -CH_3), 2.17 (s, 3H, -CH_3).

General procedure for preparation of 4-((substituted phenyl) amino)-7-methoxy-6-ol (**5a-5d)**:

The ammonia solution (15 mL) was added to a mixture of **4a-4d** (9.3 mmol) and methanol (15 mL) in a round-bottomed flask at 0 ˚C, then stirred at 25 ˚C for 2 h. The resulting solid was filtered and washed with a mixture of cold methanol (10 mL) and water (10 mL), and was dried in an oven at 50˚C.

*7-methoxy-4-(phenylamino)quinazolin-6-ol***(5a)**: Yield 0.65g(75.22%); yellow solid; M.p.: 226.5-227.9 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3389.77, 2116.32, 1831.63, 1604.56, 1469.82, 1405.69, 1338.78, 1242.69, 1188.55, 955.71, 860.05, 775.21, 527.42, 484.92; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 9.37 (s, 1H, -NH), 8.43 (s, 1H, -ArH), 7.89 – 7.70 (m, 3H, -ArH), 7.36 (t, *J* = 7.6 Hz, 2H, -ArH), 7.20 (s, 1H, -ArH), 7.07 (t, *J* = 7.2 Hz, 1H, -ArH), 3.98 (s, 3H, -OCH_3).

*7-methoxy-4-(p-tolylamino)quinazolin-6-ol***(5b)**: Yield 1.5g(36.37%); yellow solid; M.p.: 216.8-217.3 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3304.96, 1795.30, 1630.07, 1469.36, 1430.15, 1287.67, 1063.50, 921.85, 813.00, 687.47, 571.89, 536.19, 498.40; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 9.61 (s, 1H, -OH), 9.29 (s, 1H, -NH), 8.40 (s, 1H, -ArH), 7.80 (s, 1H, -ArH), 7.73 (s, 1H, -ArH), 7.71 (s, 1H, -ArH), 7.18 (d, *J* = 5.6 Hz, 2H, -ArH), 7.15 (s, 1H, -ArH), 3.97 (s, 3H, -OCH_3), 2.30 (s, 3H, -CH_3).

*4-((2-fluorophenyl)amino)-7-methoxyquinazolin-6-ol***(5c)**: Yield 1.46g(33.33%); white solid; M.p.: 247.2-249.3 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3334.86, 3013.96, 2343.21, 1701.48, 1623.78, 1502.69, 1427.23, 1256.97, 1185.39, 927.20, 751.88, 526.87, 445.89; ^{1}H-NMR (DMSO-d_6, 400MHz): δ 9.70 (s, 1H, -OH), 9.34 (s, 1H, -NH), 8.31 (s, 1H, -ArH), 7.61 (d, *J* = 60.0 Hz, 2H, -ArH), 7.24 (d, *J* = 31.6 Hz, 4H, -ArH), 3.97 (s, 3H, -OCH_3).

*4-((2,4-dimethylphenyl)amino)-7-methoxyquinazolin-6-ol***(5d)**: Yield 0.49(58.32%); yellow solid; M.p.: 236.4-238.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3348.79, 2724.36, 1714.29, 1623.11, 1519.39, 1455.70, 1249.11, 1060.12, 894.68, 854.35, 784.32, 685.69, 508.75; ^{1}H-NMR (DMSO-d_6, 400MHz): 9.57 (s, 1H, -OH), 9.10 (s, 1H, -NH), 8.21 (s, 1H, -ArH), 7.70 (s, 1H, -ArH), 7.17 (d, *J* = 7.6 Hz, 2H, -ArH), 7.10 (s, 1H, -ArH), 7.03 (d, *J* = 7.6 Hz, 1H, -ArH),

3.96 (s, 3H, -OCH_3), 2.31 (s, 3H, -CH_3), 2.12 (s, 3H, -CH_3).

General procedure for preparation of 4-(3-chloropropyl) morpholine **(8'a)**:

1-chloro-3-bromopropane (31.76 mmol), morpholine (63.52 mmol), potassium carbonate (0.32 mmol) and toluene (50 mL) put into a 100 mL round bottom flask, stirred and heated to reflux. The filtrate was washed twice with water, dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to obtain brown yellow oil 4-(3-chloropropyl) morpholine (2.945 g, Yield (56.94%), which was used directly in further reactions.

General procedure for preparation of N-(substituted phenyl)-7-methoxy-6-(3-morpholinopropoxy) quinazoline-4-amin*e* **(8a-8d)**:

A mixture of **5a-5d** (1.85 mmol), K_2CO_3(3.7 mmol) and **8'a** (3.7 mmol) was added to 30 mL dried *N, N*-dimethylformamide (DMF) and stirred at 70 ℃ for 10 h. The reaction mixture was diluted with water (50 mL), extracted by ethyl acetate three times (30 mL×3), and dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give crude product, which was purified by column chromatography.

7-methoxy-6-(3-morpholinopropoxy)-N-phenylquinazolin-4-amine **(8a)**: Yield 0.78g(52.85%); yellow solid; M.p.: 89.4-91.5 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3329.25, 2923.14, 2116.65, 1620.01, 1572.88, 1499.79, 1419.81, 1354.20, 1258.33, 1140.41, 919.50, 787.87, 692.73, 578.98, 507.61, 476.09; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.50 (s, 1H, -NH), 8.45 (s, 1H, -ArH), 7.87 (s, 1H, -ArH), 7.80 (d, *J* = 8.0 Hz, 2H, -ArH), 7.40 (t, *J* = 7.6 Hz, 2H, -ArH), 7.19 (s, 1H, -ArH), 7.12 (t, *J* = 7.2 Hz, 1H, -ArH), 4.20 (t, *J* = 6.0 Hz, 2H, -CH_2), 3.94 (s, 3H, -OCH_3), 3.59 (s, 4H, -CH_2), 2.42 (s, 4H, -CH_2), 2.05 – 1.97 (m, 2H, -CH_2), 1.20 (d, *J* = 21.2 Hz, 2H, -CH_2). Calculated for $C_{22}H_{26}N_4O_3$: C 66.99, H 6.64, N 14.20; Found C 66.88, H 6.77, N 14.13.

7-methoxy-6-(3-morpholinopropoxy)-N-(p-tolyl)quinazolin-4-amine **(8b)**: Yield 0.12g(41.32%); yellow solid; M.p.: 83.8-86.2 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2920.67, 2356.63, 1663.40, 1576.79, 1470.01, 1236.69, 1113.99, 1034.12, 919.62, 853.55, 787.02, 655.11, 553.13, 503.65; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.43 (s, 1H, -NH), 8.42 (s, 1H, -ArH), 7.84 (s, 1H, -ArH), 7.65 (d, *J* = 8.4 Hz, 2H, -ArH), 7.23 – 7.16 (m, 3H, -ArH), 4.18 (t, *J* = 6.4 Hz, 2H, -CH_2), 3.94 (s, 3H, -OCH_3), 3.62 – 3.55 (m, 4H, -CH_2), 2.48 (d, *J* = 6.8 Hz, 2H, -CH_2), 2.40 (s, 4H, -CH_2), 2.32 (s, 3H, -CH_3), 2.05 – 1.95 (m, 2H, -CH_2). Calculated for $C_{23}H_{28}N_4O_3$: C 67.63, H 6.91, N 13.72; Found C 67.56, H 6.82, N 13.83.

N-(2-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine **(8c)**: Yield 0.13g(42.82%); white solid; M.p.: 110.5-112.9 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3596.11, 2925.20, 2364.30, 1619.87, 1578.11, 1499.12, 1425.81, 1252.14, 1115.14, 853.03. 755.25, 554.88, 461.66; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.51 (s, 1H, -NH), 8.35 (s, 1H, -ArH), 7.82 (s, 1H, -ArH), 7.54 (t, *J* = 7.8 Hz, 1H, -ArH), 7.32 (dd, *J* = 12.6, 6.0 Hz, 2H, -ArH), 7.27 – 7.22 (m, 1H, -ArH), 7.19 (s, 1H, -ArH), 4.17 (t, *J* = 6.6 Hz, 2H ,-CH_2), 3.94 (s, 3H, -OCH_3), 3.58 (d, *J* = 3.6 Hz, 4H, -CH_2), 2.50 – 2.46 (m, 2H, -CH_2), 2.39 (s, 4H, -CH_2), 2.03 – 1.93 (m, 2H, -CH_2). Calculated for $C_{22}H_{25}FN_4O_3$: C 64.06, H 6.11, N 13.58. Found C 63.98, H 6.19, N 13.44.

*N-(2,4-dimethylphenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine***(8d)**: Yield 0.11g(38.44%); yellow solid; M.p.: 96.3-98.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2917.93, 2359.28, 1619.37, 1498.85, 1382.85, 1280.64, 1143.55, 1042.77, 946.92, 850.21, 616.21, 432.68; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.33 (s, 1H, -NH), 8.25 (s, 1H, -ArH), 7.83 (s, 1H, -ArH), 7.15 (s, 2H, -ArH), 6.86 (s, 1H, -ArH), 6.59 (d, *J* = 8.8 Hz, 1H, -ArH), 4.16 (s, 2H, -CH_2), 3.93 (s, 3H, -OCH_3), 3.66 (d, *J* = 11.2 Hz, 2H, -CH_2), 3.59 (s, 4H, -CH_2), 2.40 (s, 4H, -CH_2), 2.32 (s, 3H, -CH_3), 2.13 (s, 3H, -CH_3), 2.01 – 1.96 (m, 2H, CH_2). Calculated for $C_{24}H_{30}N_4O_3$:C 68.22, H 7.16, N 13.26; Found C 68.11; H 7.22; N 13.14.

General procedure for preparation of 6-(3-chloropropoxy)-N-(substituted phenyl)-7-methoxyquinazolin-4-amine **(6e-6h)**:

A solution of **5a-5d** (2.43 mmol), K_2CO_3 (4.86 mmol), 1-chloro-3-bromopropane (4.86 mmol) and DMF (10 mL) was heated to 65 ℃ for 6 h. The reaction mixture was diluted with water (20 mL), extracted by ethyl acetate three times (15 mL×3), and concentrated in vacuo to give crude product, which was purified by column chromatography.

*6-(3-chloropropoxy)-7-methoxy-N-phenylquinazolin-4-amine***(6e)**: Yield 0.15g(17.94%); white solid; M.p.: 136.4-137.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3063.04, 2843.45, 2362.54, 1727.02, 1619.47, 1579.29, 1475.19, 1356.35, 1239.37, 1064.11, 945.43, 781.67, 758.38, 591.18, 465.38; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.50 (s, 1H, -NH), 8.46 (s, 1H, -ArH), 7.90 (s, 1H, -ArH), 7.79 (d, *J* = 7.6 Hz, 2H, -ArH),

7.40 (t, *J* = 7.6 Hz, 2H, -ArH), 7.20 (s, 1H, -ArH), 7.12 (t, *J* = 7.2 Hz, 1H, -ArH), 4.28 (t, *J* = 5.6 Hz, 2H, -CH_2), 3.95 (s, 3H, -OCH_3), 3.87 (t, *J* = 6.0 Hz, 2H, -CH_2), 2.35 – 2.25 (m, 2H, -CH_2).

*6-(3-chloropropoxy)-7-methoxy-N-(p-tolyl)quinazolin-4-amine***(6f)**: Yield 0.16g(41.93%); white solid; M.p.: 120.4-122.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3204.23, 2962.07, 1737.70, 1619.58, 1513.96, 1450.10, 1304.46, 1241.24, 1142.38, 1035.56, 918.31, 778.39, 665.09, 501.08, 411.21; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.43 (s, 1H, -NH), 8.43 (s, 1H, -ArH), 7.89 (s, 1H, -ArH), 7.67 (s, 1H, -ArH), 7.64 (s, 1H, -ArH), 7.21 (s, 1H, -ArH), 7.19 (s, 2H, -ArH), 4.28 (t, *J* = 6.0 Hz, 2H, -CH_2), 3.95 (s, 3H, -OCH_3), 3.86 (t, *J* = 6.4 Hz, 2H, -CH_2), 2.32 (s, 3H, -CH_3), 2.31 – 2.24 (m, 2H, -CH_2).

*6-(3-chloropropoxy)-N-(2-fluorophenyl)-7-methoxyquinazolin-4-amine***(6g)**: Yield 0.38g(47.56%); white solid; M.p.: 110.5-112.2 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3212.32, 2915.11, 2362.54, 1727.02, 1579.29, 1499.64, 1386.61, 1141.43, 945.43, 851.00, 716.30, 591.18, 552.32, 465.38; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.53 (s, 1H, -NH), 8.35 (d, *J* = 13.6 Hz, 1H, -ArH), 7.87 (d, *J* = 13.2 Hz, 1H, -ArH), 7.54 (s, 1H, -ArH), 7.39 – 7.19 (m, 4H, -ArH), 4.26 (s, 2H, -CH_2), 3.94 (d, *J* = 13.6 Hz, 3H, -OCH_3), 3.85 (s, 2H, -CH_2), 2.29 (s, 2H, -CH_2).

*6-(3-chloropropoxy)-N-(2,4-dimethylphenyl)-7-methoxyquinazolin-4-amine***(6h)**: Yield 0.22g(35.66%); white solid; M.p.: 120.4-122.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2914.18, 2361.60, 1738.35, 1574.91, 1499.29, 1425.97, 1284.26, 1144.38, 893.66, 734.75, 615.70, 529.18, 442.73; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.32 (s, 1H, -NH), 8.25 (s, 1H, -ArH), 7.86 (s, 1H, -ArH), 7.19 – 7.11 (m, 3H, -ArH), 7.06 (d, *J* = 8.0 Hz, 1H, -ArH), 4.26 (d, *J* = 5.6 Hz, 2H, -CH_2), 3.94 (s, 3H, -OCH_3), 3.86 (t, *J* = 6.4 Hz, 2H, -CH_2), 2.32 (s, 3H, -CH_3), 2.31 – 2.19 (m, 2H, -CH_2), 2.13 (s, 3H, -CH_3).

General procedure for preparation of N-(substituted phenyl)-7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy) quinazoline-4-amine **8e-8h**

A mixture of **6e-6h**(0.44 mmol), 1-Methylpiperazine **7'e** (0.87 mmol), K_2CO_3 (0.87 mmol), potassium iodide (0.04 mmol) was added to DMF (5 mL) and heated to 70 ℃ and stirred for 9 h. The reaction mixture was diluted with water (20 mL), extracted by ethyl acetate three times (10 mL×3), and concentrated to give crude product, which was purified by column chromatography.

*7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy)-N-phenylquinazolin-4-amine***(8e)**: Yield 0.1g(54.10%); yellow solid; M.p.: 189.4-190.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2940.36, 2860.92, 2117.83, 1622.35, 1578.46, 1474.39, 1423.93, 1283.72, 1209.53, 1145.77, 972.08, 839.06, 695.01, 505.09; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.52 (s, 1H, -NH), 8.45 (s, 1H, -ArH), 7.83 (d, *J* = 25.2 Hz, 3H, -ArH), 7.40 (s, 2H, -ArH), 7.16 (d, *J* = 24.4 Hz, 2H, -ArH), 4.19 (s, 2H, -CH_2), 3.95 (s, 3H, -OCH_3), 2.51 (s, 2H, -CH_2), 2.41 (d, *J* = 24.0 Hz, 8H, -CH_2), 2.15 (s, 3H, -CH_3), 1.99 (s, 2H, -CH_2). Calculated for $C_{23}H_{29}N_5O_2$.: C 67.79, H 7.17, N 17.19; Found C 67.68, H 7.24, N 17.04.

*7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy)-N-(p-tolyl)quinazolin-4-amine***(8f)**: Yield 0.11g(46.69%); yellow solid; M.p.: 178.8-180.3 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3097.18, 2947.61, 2817.20, 2342.11, 1625.64, 1509.51, 1422.62, 1328.70, 1306.25, 1162.42, 1046.01, 847.25, 705.52, 613.06, 510.00, 467.80, 412.07; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.45 (s, 1H, -NH), 8.42 (s, 1H, -ArH), 7.85 (s, 1H, -ArH), 7.65 (d, *J* = 7.6 Hz, 2H, -ArH), 7.24 – 7.14 (m, 3H, -ArH), 4.17 (s, 2H, -CH_2), 3.94 (s, 3H, -OCH_3), 2.51 (s, 2H, -CH_2), 2.46 (d, *J* = 6.4 Hz, 3H, -CH_3), 2.32 (s, 8H, -CH_2), 2.14 (s, 3H, -CH_3), 1.98 (s, 2H, -CH_2). Found, %: C 68.33; H 7.56; N 16.54. $C_{24}H_{31}N_5O_2$. Calculated, %: C 68.38; H 7.41; N 16.61.

*N-(2-fluorophenyl)-7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy)quinazolin-4-amine***(8g)**: Yield 0.14g(70.02%); yellow solid; M.p.: 194.2-195.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2934.08, 2789.88, 2343.47, 1621.11, 1501.10, 1425.74, 1286.66, 1163.36, 944.39, 810.61, 743.28, 618.41, 486.53; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.52 (s, 1H, -NH), 8.35 (s, 1H, -ArH), 7.82 (s, 1H, -ArH), 7.55 (t, *J* = 7.2 Hz, 1H, -ArH), 7.36 – 7.24 (m, 3H, -ArH), 7.19 (s, 1H, -ArH), 4.16 (s, 2H, -CH_2), 3.94 (s, 3H, -OCH_3), 2.47 (s, 2H, -CH_2), 2.47 – 2.20 (m, 8H, -CH_2), 2.14 (s, 3H, -CH_3), 1.99 (d, *J* = 5.6 Hz, 2H, -CH_2). Calculated for $C_{23}H_{28}FN_5O_2$: C 64.92, H 6.63, N 16.46; Found C 64.88; H 6.72; N 16.34.

*N-(2,4-dimethylphenyl)-7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy)quinazolin-4-amine***(8h)**: Yield 0.11g (78.26%); yellow solid; M.p.: 176.3-178.4 ˚C; IR (υ_{max}, cm^{-1}) KBr: 2941.77, 2114.57, 1618.84, 1499.35, 1421.49, 1228.18, 1142.95, 1062.43, 851.91, 786.42, 651.20, 444.34; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 9.36 (s, 1H, -NH), 8.24 (s, 1H, -ArH), 7.85 (s, 1H, -ArH), 7.15 (d, *J* = 6.8 Hz, 2H, -ArH), 7.12 (s, 1H, -ArH), 7.05 (d, *J* = 8.0 Hz, 1H, -ArH), 4.15 (t, *J* = 6.4 Hz, 2H, -CH_2), 3.92 (s, 3H, -OCH_3), 2.51 (d, *J* = 1.6 Hz, 10H, -CH_2), 2.32 (s, 3H, -CH_3), 2.27 (s, 3H, -CH_3), 2.13 (s, 3H, -CH_3), 2.03 – 1.95 (m, 2H, -CH_2). Calculated for $C_{25}H_{33}N_5O_2$.: C 68.94, H 7.64, N 16.08; Found C 68.87, H 7.55, N 16.02.

*Crystal data structure determination of the compound **8c**.*

The white solid of the obtained target compound was dissolved in ethyl acetate solvent. Some single crystals suitable for X-ray analysis were obtained after the solvent was evaporated slowly. A white crystal ($C_{24}H_{30}FN_4O_4$) with dimensions of 0.120 x 0.110 x 0.10 mm^3 was selected for data collection which was performed on a Bruker D8 VENTURE diffractometer equipped with a graphite-monochromatic Mo *Kα* radiation (λ = 0.71073 Å) by using an ω scan mode at 100(2) K. A total of 21205 reflections were collected in the range of 4.672<2θ< 61.974 (index ranges: -17<h<16, -14<k<15, -41<l<35) and 6036 were independent (R_{int} = 0.0408), of which 4588 observed reflections with $I > 2\sigma(I)$ were used in the structure determination and refinements. The structure was solved by intrinsic phasing methods with SHELXT 2014 program [23] and expanded by Fourier technique. The hydrogen atoms bound to carbon were determined with theoretical calculations. The structure was refined by full-matrix Least-squares techniques on F^2 with SHELXL-2017 [23]. The final refinement gave the final R = 0.0600 and wR = 0.1241 (w=1/ [σ^2 (Fo^2) + (0.0606P)^2 + 1.5230P) where P = (Fo^2+2Fc^2)/3). S = 1.06, (Δ/σ) max = 0.053, ($\Delta\rho$) max = 0.411 and ($\Delta\rho$) min = –0.807e/Å^{-3}. All calculations were performed using the Crystal Structure crystallographic software package except for the refinement. Crystallographic data and experimental details of structural analyses for compound **8c** was summarized in Table 1. CCDC 2053649 contains the supplementary data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via https://summary.ccdc.cam.ac.uk /structure-summary-form.

Table-1: Crystal data for the compound **8c.**

Crystal size	0.12x 0.11 x 0.1 mm^3
Formula	$C_{24}H_{30}FN_4O_4$
fw	457.52
T/K	100 K
Crystal system	*Orthorhombic*
Space group	Pbca
a/Å	12.9586(4)
b/Å	14.4144(4)
c/Å	28.5651(8) Å
α/°	90
β/°	90
γ/°	90
V/Å^3	4595.4(2)
Z	8
Dx/g . cm^{-3}	1.323
F(000)	1944.0
GOF on F^2	1.061
Reflection/unique	30890/6036
R_1,wR_2 [I >=2 (I)]	0.0451, 0.1151
R_1,wR_2 (all data)	0.0644, 0.1241

R_1=$\sum(||F_o|-|F_c||)/\sum|F_o|$ wR_2=[$\sum w(F_o^2-F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$

Crystal data for the compound **8c**

Biological activity determination.

The target compounds (**8a-8h)** were evaluated against two cancer cell lines (SKBr-3 and MCF-7) by the MTT-based assay *in vitro* using Gefitinib as a positive control (Table-2). The cells in logarithmic growth phase were digested by 0.25% trypsin (suspension cells need not be digested) and suspended in the culture medium containing 10% calf serum. The single cell suspension was blown by glass dropper, and the living cells were counted by blood cell counting board under microscope. The 96 well plate was inoculated with 90 μL cell suspension (cell concentration was 8 ~ 15 × 104 cells / mL) in each well. After pre culture for 24 h in the incubator containing 5% CO_2 and 95% air at 37 ℃, 100% relative humidity, 10 μL drug solution was added to each well (the final concentration was set as 10 μ / mL). In addition, negative control (equal concentration of DMSO) and blank background (without cells) were set for each concentration, and 6 multiple pores were set in each group. After continuous culture for 24 h, 10 μL and 5 mg / mL MTT solution was added to each well. After 4 h of culture, the supernatant was carefully aspirated. 100 μL DMSO was added into each well, and the microoscillator was placed for 5 min to dissolve the crystal completely. The OD value was determined by colorimetry at 492 nm. The evaluation index of pharmacological experiment was cell growth inhibition rate, and the calculation method was as follows: inhibition rate (%) = [1 - (OD mean value of experimental group OD mean value of blank group) / (OD mean value of control group OD mean value of blank group)] × 100%.

Results and Discussion

Chemistry

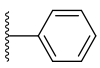
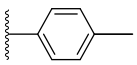
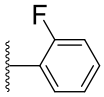
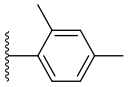
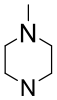
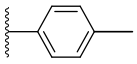
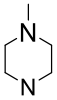
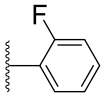
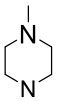
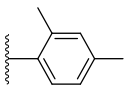
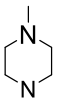
The synthetic methods for compounds **8a~8h** are outlined in Scheme 1 [24-27]. The intermediate **2** was synthesized from compound **1** by chlorinating in toluene solvent. The key intermediate 7-methoxy-4-(substituted phenylamino) quinazolin-6-yl acetate (**4a-4d**) were prepared by nucleophilic substitution reaction of intermediate **2** with aniline substituted by different groups. Then, the important intermediate 4-((substituted phenyl) amino)-7-methoxy-6-ol (**5a-5d**) were produced by hydrolysis with ammonia in the methanol solvent and 6-(3-chloropropoxy)-N-(substituted phenyl)-7-methoxyquinazolin-4-amine (**6e-6h**) were synthesized by substitution reaction. The target compounds *N*-(substituted phenyl)-7-methoxy-6-(3-morpholinopropoxy) quinazoline-4-amine (**8a-**

8d) were synthesized by direct substitution reaction of 4-(3-chloropropyl) morpholine (**8'a**) with K_2CO_3, and *N*-(substituted phenyl)-7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy) quinazoline-4-amine (**8e-8h**) were synthesized by substitution reaction of piperazine compounds (**7'e**) with KI as catalyst in DMF solvent. The structures of target compounds were confirmed by IR, ^{1}H-NMR and element analysis. The crystal structure of **8c** was determined by X-ray single-crystal diffraction analysis. All data of the target compounds confirmed its structural integrity.

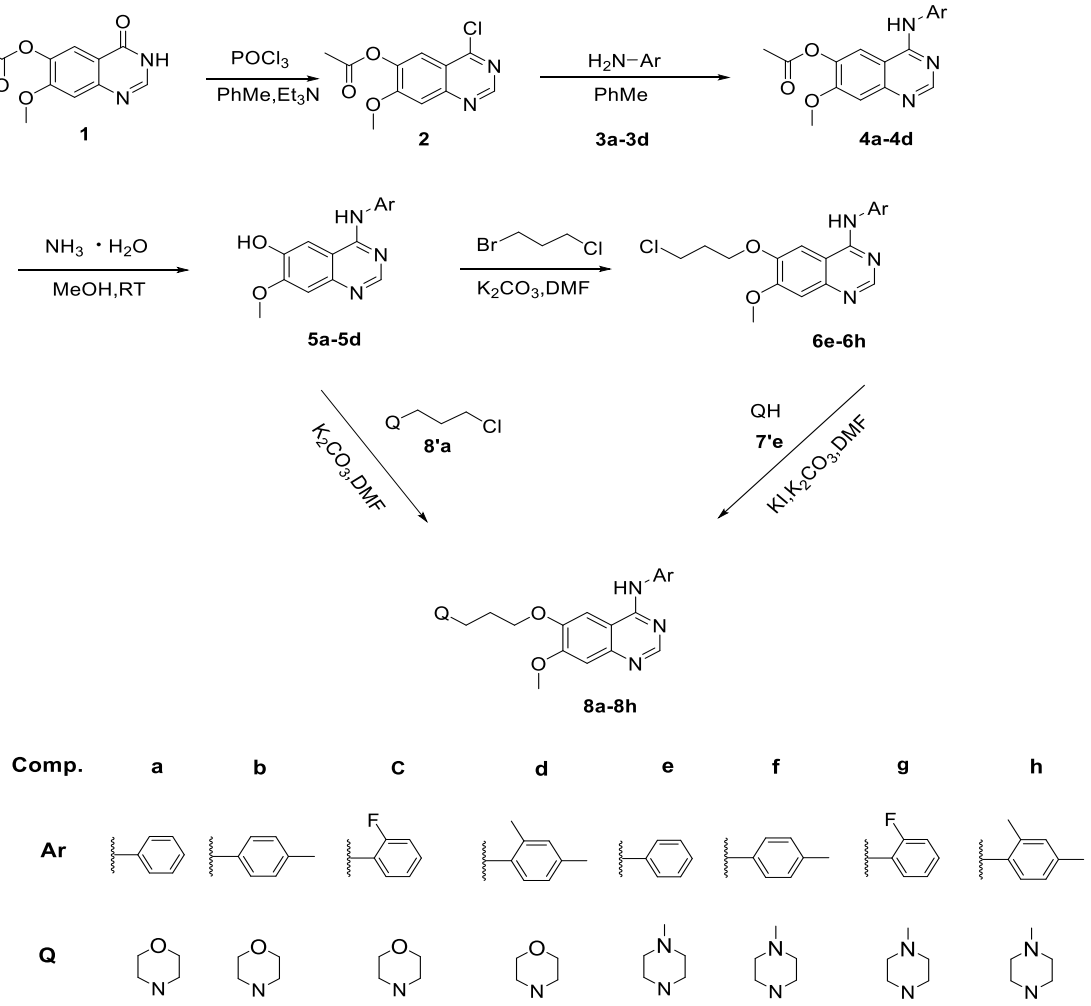
*Crystal of the compound **8c***

The molecular structure of the target compound **8c** is illustrated in Fig. 1 and Fig. 2, and the crystal data are shown in Table-1, which shows the molecular accumulation and hydrogen bond in the unit cell. As can be seen from Fig. 1, the molecular structure of the *N*-(2-fluorocyclohexyl)-7-methoxy-6-(3-morpholinopropoxy) quinazolin-4-amine is very exciting, because its molecule form a complex structure with the ethyl acetate molecules by intermolecular force. In the crystal, the bond lengths of F(1)-C(2), O(1)-C(15), N(1)-C(7) and C(1)-C(2) are 1.3509(17) Å, 1.4356(16) Å, 1.3570(16) Å and 1.3863(19) Å. The bond angles of C(11)-O(1)-C(15), C(7)-N(1)-C(1) and C(12)-O(2)-C(16) are 116.07(11)º, 123.39(11)º and 118.87(10)º, respectively. The bond C(2)-F(1) in 1.3509 Å belongs to the typical C-F single bond. From these results, we can know that all bond lengths and bond angles are in the normal range. The torsion angles C(10)-C(9)-C(14)-C(7) and N(3)-C(9)-C(14)-C(13) are 174.55(12)º and 173.45(12)º. The results show that the quinazoline nucleus is almost coplanar. In addition, intermolecular hydrogen bond N (1)-H (1)···N (3) also exists, as shown in Table 3, which helps to form stable molecules in the unit cell.

Table-2: Inhibitory effect of target compounds (**8a-8h**) against cancer cell lines[a]

Compounds	Structure		Inhibition rate (%)	
	Ar	Q	SKBr-3	MCF-7
8a			**15.78**	**9.67**
8b			**11.16**	**14.16**
8c			**18.32**	**38.45**
8d			**Not determined**	**20.35**
8e			**16.56**	**20.40**
8f			**12.55**	**26.32**
8g			**19.61**	**36.07**
8h			**Not determined**	**21.22**
Gefitinib	-	-	**42.13**	**13.25**

[a] Each experiment was carried out in triplicate.



Scheme 1: Reagents and conditions. (A)$POCl_3$, toluene, Et_3N at 80℃; (B) substituted anilines, toluene; (C) ammonia solution, methanol at 80℃; (D) Bromoacetyl chloride, K_2CO_3, DMF at 70℃. (E) K_2CO_3, DMF, at 75℃ (F) K_2CO_3, IK, DMF, at 75℃

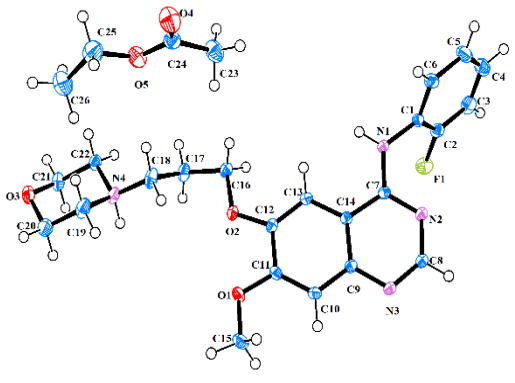


Fig. 1: The structure of $C_{24}H_{30}FN_4O_4$ and ellipsoids drawn at the 50% probability level.

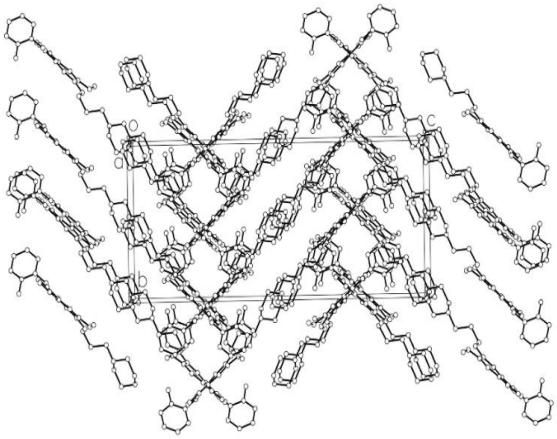


Fig. 2: A packing diagram of $C_{24}H_{30}FN_4O_4$

Table-3: Hydrogen Bond Lengths (Å) and Bond Angles (°) in compound **8c**[a]

D–H···A	d(D–H)	d(H···A)	D (D···A)	∠DHA
N(1)-H(1)···N(3)#1	0.88	2.07	2.8812(15)	153.1

[a]Symmetry code: (#1) 1/2+X, +Y, 3/2-Z

Anticancer activity and structure-activity relationship

The anticancer activities of all the newly synthesized compounds (**8a~8h**) were evaluated against human breast cancer cell line SKBr-3 and MCF-7 using the standard MTT-based assay in vitro, with Gefitinib used as the positive control. The inhibition rate of the compounds against these cancer cells were presented in Table-2. It can be seen from table 2 that the activity of the compounds to cancer cells can be affected by the connection of different groups in the benzene ring of aromatic amines and there is no significant difference in the activity between the two series of compounds for SKBr-3 cancer cell lines. When the benzene ring of 4-aminoquinazoline is replaced by a lipophilic group, the antitumor activity of the compound is significantly enhanced, and especially the 2-position of benzene ring is substituted by fluorine atom (for **8c** and **8g**). The results revealed that the activity of compound **8c** was the best for SKBr-3 cancer cell lines and MCF-7 cancer cell lines. It can be assumed the the affinity of EGFR to aromatic amines can be improved by the F element at 2 position in benzene ring. When the 2-fluorolaniline group is linked to the nucleus of quinazoline (**8c**, inhibition rate = 38.45% for MCF-7), the inhibitory activity against breast cancer MCF-7 cell line was relatively high, which was better to the positive drug gefitinib. It was obtained as more promising inhibitor among them.

Conclusion

Some novel quinazoline derivatives were synthesised as EGFR inhibitors. The novel compounds were measured for their inhibition activity on MCF-7 and SKBr-3. The activity of compound **8c** was the best for SKBr-3 cancer cell lines and MCF-7 cancer cell lines, which was better to the positive drug Gefitinib. The anticancer activities of these compounds need to be further studied

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