Synthesis and Evaluation of Some Novel 6-Substituted Quinazoline Derivatives as Antitumor Agents

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Summry: A series of novel 6-substituted quinazoline derivatives were synthesised as epidermal growth factor receptor(EGFR) and Human epidermal growth factor receptor 2 (HER2)inhibitors in our lab. The novel compounds were measured for their dual enzyme inhibition as well as their cytotoxic activity on MCF7 cell line. The results revealed that all the compounds showed inhibition of both enzymes. Compound **5c** showed the best inhibitory activity against both enzymes and IC_{50} of its was 2.6 nM against EGFR kinases and 4.3 nM against HER2 kinases, respectively. Most of the measured compounds showed to antitumor activity on MCF7.

Keywords: Antitumor, Poziotinib, Quinazoline, Synthesis, HER2.

Introduction

Quinazoline-incorporating aromatic rings, which show a range of latent biological activities and are found in medicinal chemistry and organic chemistry, have appealed to interest of synthetic chemists[1-5]. In particular, 6-substituted quinazoline derivatives have been demonstrated to have potential antitumor activity. For example, Lapatinib, which contains the quinazoline core with furan moiety linking to the C6 position of quinazoline, is a inhibitor with potent activity in EGFR-dependent cancer cells and HER2-dependent breast cancer cells[6-11]. The human epidermal growth factor receptor (HER) family consists of four members: epidermal growth factor receptor (EGFR), HER2, HER3, and HER4, and these proteins regulate the growth and differentiation of malignant cells.The tyrosine kinase inhibitors(TKIs) can inhibit the catalytic kinase function of the HER family. These drugs include EGFR-specific inhibitors such as gefitinib and erlotinib. TKIs also include the dual reversible inhibitor of EGFR and HER2 such as Lapatinib and Poziotinib.

We have previously synthesized several quinazolin-4-amines containing p-toluenesulfonate moiety as potent inhibitors for epidermal growth factor receptor (EGFR)[12]. In this paper, six novel quinazoline derivatives were designed and synthesised on the basis of essential requirements for SAR. These compounds were measured for their enzyme inhibition and their cytotoxic activity on MCF7 cell line.

Experimental

Chemistry

General

Unless specified otherwise, all starting materials and reagents were obtained from commercial supplies without further purification, and all chemicals and solvents were purchased from Alfa Aesar. The human breast adenocarcinoma cell line (MCF7) was obtained as a gift from Tianjin Institute of Pharmaceutical Research. All chemicals and solvents were purchased from Sigma-Aldrich. All melting points were taken on a Beijing Taike X-4 microscopy melting point apparatus and were uncorrected. ^{1}H-NMR spectra were recorded on a Bruker Biospin 400 MHz instrument using TMS as the internal standard. All chemical shifts were reported in ppm. IR spectra were recorded on a Bruker Platinum ART Tensor II FT-IR spectrometer. Elemental analysis of the newly synthesized compounds was carried out on Carlo Erba 1108 analyzer and is found within the range of theoretical value. Compounds **4a~f** were synthesized according to reported procedures [12].

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General procedure for Preparation of methyl 3-[3-[[[4-(4-*substituted anilines*)-7-methoxyquinazolin-6-yl] oxy]sulfonyl]phenyl] acrylate (**5a~f**):

A mixture of 7-methoxy-4-(substituted anilines)quinazolin-6-ol (4.5 mmol) **4a~f**, Et_3N (9.0 mmol) and methyl 3-[3-(chlorosulfonyl) phenyl]acrylate (4.5 mmol) in 80 mL dried *N,N*-Dimethylethanamide (DMA) was stirred at 75 ˚C for 8 h. The reaction mixture was diluted with water (200 mL), extracted by dichloromethane three times (200 mL×3), and dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to give crude product, which was purified by column chromatography to afford **5a~5f** as powder.

methyl 3-[3-[[[4-[(4-chlorophenyl)amino] -7-methoxyquinazolin-6-yl]oxy]sulfonyl]phenyl]acrylate (**5a**): Yield 1.93 g(81.7%); M.p.: 211.5-212.8 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3425, 1708, 1626, 1600, 1494, 1466, 1374, 1199, 762, 499; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 3.532(s, 3H, -OCH_3), 3.734(s, 3H, -OCH_3), 6.783-6.823(d, *J*=16.0, 1H, -CH), 7.226(s, 1H, -CH), 7.442-7.464(d, *J*=8.8, 2H, -ArH), 7.694-7.732(t, *J*=15.2, 1H, -ArH), 7.783-7.832(d, *J*=19.6, 1H, -ArH), 7.868-7.905(m, 3H, -ArH), 8.212-8.234(d, *J*=8.8, 2H, -ArH), 8.580-8.584(d, *J*=1.6, 2H, -ArH), 9.879(s, 1H, -NH); Anal. calcd for $C_{25}H_{20}ClN_3O_6S$: C 57.09, H 3.83, N 7.99; found C 57.12, H 3.87, N7.90.

methyl 3-[3-[[[4-[(4-chloro-3- fluorophenyl) amino]-7-methoxyquinazolin-6-yl]oxy] sulfonyl] phenyl] acrylate(**5b**): Yield 1.68 g(68.8%); M.p.: 216.3-217.5 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3523, 1703.78, 1635.29, 1505.44, 1501.15, 1422.67, 1372.63, 1184.37, 1004.58, 936.08, 857.60, 763.42, 672.10, 557.94; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 3.534(s, 3H, -OCH_3), 3.730-3.750(t, *J*=8.0, J=3.2, 3H, -OCH_3), 6.771-6.811(d, *J*=16.0, 1H, -CH), 7.225-7.237(d, *J*=4.8, 1H, -ArH), 7.423-7.469(m, 1H, -ArH), 7.693-7.732(t, *J*=15.6, 1H, -ArH), 7.775-7.833(m, 2H, -ArH), 7.869-7.895(m, 1H, -CH), 8.145-8.168(dd, *J*=2.4, *J*=6.8, 1H, -ArH), 8.199-8.228(m, 2H, -ArH), 8.547(s, 1H, -ArH), 8.600(s, 1H, -ArH), 8.891(s, 1H, -NH); Anal. calcd for $C_{25}H_{19}ClFN_3O_6S$: C 55.20, H 3.52, N 7.72; found C 55.22, H 3.55, N7.81.

methyl 3-[3-[[[7-methoxy-4-[(3-methoxyphenyl)amino]quinazolin-6-yl]oxy]sulfonyl]phenyl] acrylate(**5c**): Yield 1.34 g(57.1%); M.p.: 222.3-222.5 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3416, 1707, 1572, 1456, 1434, 1226, 912, 864, 619, 528; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 3.526(s, 3H, -OCH_3), 3.734(s, 3H, -OCH_3), 3.792(s, 3H, -OCH_3), 6.712-6.736(m, 1H, -ArH), 6.782-6.822(d, *J*=16, 1H, -CH), 7.213(s, 1H, -ArH), 7.278-7.319(t, *J*=16.4, 1H, -ArH), 7.448-7.506(m, 2H, -ArH), 7.691-7.730(t, *J*=15.6, 1H, -ArH), 7.787-7.827(d, *J*=16, 1H, -CH), 7.868-7.888(d, *J*=8.0, 1H, -ArH), 8.211-8.231(t, *J*=8.0, 2H, -ArH), 8.575-8.603(d, *J*=11.2, 2H, -ArH), 9.749(s, 1H, -NH); Anal. calcd for $C_{26}H_{23}N_3O_7S$: C 59.88, H 4.45, N 8.06; found C 59.97, H 4.50, N8.13.

methyl 3-[3-[[[7-methoxy-4-[(4-methoxyphenyl)amino]quinazolin-6-yl]oxy]sulfonyl]phenyl] acrylate(**5d**): Yield 1.87 g(79.7%); M.p.: 194.2-195.4 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3407, 1710, 1640, 1623, 1532, 1512, 1470, 1433, 1282, 1245, 926, 736, 642, 495; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 3.519(s, 3H, -OCH_3), 3.739(s, 3H, -OCH_3), 3.780(s, 3H, -OCH_3), 6.787-6.827(d, *J*=16.0, 1H, -CH), 6.955-6.995(m, 2H, -ArH), 7.178(s, 1H, -ArH), 7.632-7.672(m, 2H, -ArH), 7.693-7.732(t, *J*=15.6, 1H, -ArH), 7.789-7.830(d, *J*=16.4, 1H, -CH), 7.864-7.891(m, 1H, -ArH), 8.208-8.231(m, 2H, -ArH), 8.479(s, 1H, -ArH), 8.550(s, 1H, -ArH), 9.735(s, 1H, -NH); Anal. calcd for $C_{26}H_{23}N_3O_7S$: C 59.88, H 4.45, N 8.06; found C 59.93, H 4.56, N8.08.

methyl 3-[3-[[[4-[(3,4-dichloro-2-fluorophenyl)amino]-7-methoxyquinazolin-6-yl]oxy]sulfonyl] phenyl]acrylate(**5e**): Yield 1.31 g(50.5%); M.p.: 210.3-211.4 ˚C; IR (υ_{max}, cm^{-1}) KBr: 3572, 1707, 1627, 1469, 1187, 877, 745, 572; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 3.563(s, 3H, -OCH_3), 3.739(s, 3H, -OCH_3), 6.791-6.831(d, *J*=16.0, 1H, -CH), 7.214-7.259(d, *J*=18.0, 1H, -ArH), 7.587(s, 1H, -ArH), 7.701-7.757(m, 2H, -ArH), 7.787-7.827(d, *J*=16.0, 1H, -CH), 7.866-7.905(t, *J*=15.6, 1H, -ArH), 8.217-8.232(t, *J*=6.0, 2H, -ArH), 8.486-8.503(d, *J*=6.8, 2H, -ArH), 10.073(s, 1H, -NH); Anal. calcd for $C_{25}H_{18}Cl_2FN_3O_6S$: C 51.91, H 3.14, N 7.26; found C 51.99, H3.20, N7.33.

methyl 3-[3-[[[4-[(2,4-difluorophenyl) amino]-7-methoxyquinazolin-6-yl]oxy]sulfonyl]phenyl] acrylate(**5f**): Yield 1.08 g(45.5%); M.p.: 206.3-207.7 ˚C; IR (υ_{max}, cm^{-1}) KBr: 1709.49, 1625.30, 1535.40, 1504.01, 1415.54, 1384.14, 1178.66, 1001.72, 963.19, 866.16, 787.68, 507.99; ^{1}H-NMR (DMSO-d_6, 400MH_Z): δ 3.547(s, 3H, -OCH_3), 3.743(s, 3H, -OCH_3), 6.798-6.839(d, *J*=16.4, 1H, -CH), 7.141-7.188(m, 1H, -ArH), 7.230(s, 1H, -ArH), 7.370-7.419(m, 1H, -ArH), 7.521-7.581(m, 1H, -ArH), 7.700-7.741(m, 1H, -ArH), 7.795-7.835(d, *J*=16.0, 1H, -CH), 7.877-7.901(m, 1H, -ArH), 8.217-8.235(m, 2H, -ArH), 8.443-8.477(d, *J*=13.6, 2H, -ArH), 9.859(s, 1H, -NH); Anal. calcd for $C_{25}H_{19}F_2N_3O_6S$: C 56.92, H 3.63, N 7.97; found C 56.96, H3.67, N8.05.

Measurement of in vitro EGFR and HER2 inhibition

The enzymes inhibition was determined using CUSABIO human EGFR ELISA kit(catalog number CSB-E12124h) and human HER2 ELISA kit(catalog number KE00053). Briefly, the reactions were performed in 96 well polystyrene round-bottomed plates (Nunc, Denmark) containing kinase buffer composed of 100 mM HEPES (pH 7.4), 25 mM $MgCl_2$, 10 mM $MnCl_2$ and 250μM Na_3VO_4. The reactions were initiated by the addition of 100 ng/assay enzyme, 100μM ATP, and 10 ng/ml poly(Glu, Tyr). After 1 h of incubation at room temperature, the reactions were terminated by adding 6 mM EDTA solution and then anti-phosphotyrosine antibody, PTK Green Tracer, and FP dilution buffer mixtures. The fluorescence polarization values were then measured after 30 min at room temperature using a Victor3 microplate reader (Perkin Elmer).

Measurement of potential cytotoxicity

The cytotoxic activity of the newly synthesized compounds was measured in vitro on human breast adenocarcinoma cell line (MCF7) using sulforhodamine-B stain assay applying the method of Skehan et al[13]. Cells were plated in 96-multiwell plate (104 cells per well) for 24 h before treatment with the test compounds to allow attachment of the cells on the surface of the plate. The cell line (MCF7) was cultured in RPMI-1640 medium (WelGENE Inc.Daegu, Korea) supplemented with 10% fetal bovineserum and gentamicin (10mg/ml). The test compounds were dissolved in DMSO and diluted in culture media to the appropriate volume. Different concentrations of the test compound (0, 5, 12.5, 25 and 50μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37 °C in atmosphere of 5% CO_2. After 48 h, the cells were fixed with trichloroacetic acid, washed with water, and stained for 30min with 0.4% (wt/vol) sulforhodamine-B stain dissolved with 1% acetic acid. Excess stain was removed by four washes with 1% acetic acid and attached stain was recovered with Tris-ethylene diamine tetraacetic acid buffer. The color intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration was plotted, and IC_{50} (the concentration required for 50% inhibition of cell viability) was calculated for each compound and the results are given in Table-1.

Results and Discussion

Chemistry

The synthetic route of novel compounds was summarizingd in the Schemes 1. In order to prepare these novel compounds, the starting materials were were prepared previously in our lab, such as **4a-f** (Scheme-1). The commercially available compounds methyl 3-[3-(chlorosulfonyl) phenyl]acrylate reacted with **4a-f** in the presence of DMF at 90 ˚C for 3 h to afford the compounds (**5a-f**) with high yields, respectively. Compounds **5a-f** was appropriately established by spectroscopic and analytical methods. IR shows the peak at about 3425~3634 cm^{-1} results from the stretching vibration of aromatic amino in all products, and C=O band at 1703-1710 cm^{-1}. ^{1}H-NMR data were consistent with structures **5a~f,** for example, the ^{1}H-NMR spectrum for compound **5c** exhibits three sharp singlets at 3.526, 3.734, 3.792 ppm, corresponding to the methoxy proton at ester group and to the methoxy proton at quinazoline ring and benzene ring, respectively. The carbon-carbon double bond protons appeared as double peaks at 6.782-6.822 and 7.787-7.827 ppm, respectively. Aromatic protons resonate as multiples at δ = 6.712-8.603 ppm.

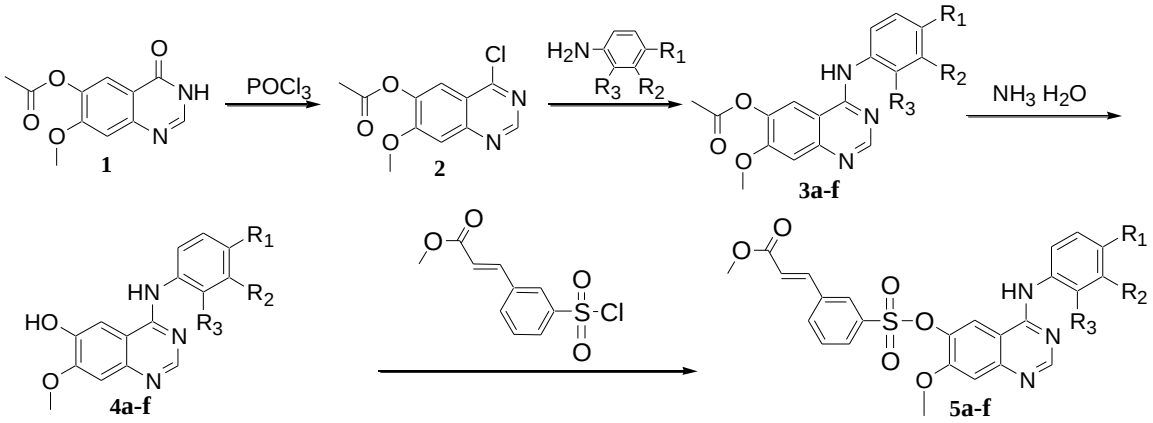
Table-1: Results of enzyme inhibition and cytotoxic activity in vitro of the synthesized compounds on MCF7 cell line.

Compound no.	IC_{50}		
	EGFR (nM*)	HER2 (nM*)	MCF7(μM*)
5a	**10.2**	**8.7**	**25**
5b	**8.4**	**7.2**	**6**
5c	**2.6**	**4.3**	**13**
5d	**8.5**	**9.1**	**22**
5e	**6.2**	**3.9**	**9**
5f	**12.5**	**15.7**	**8**
Poziotinib	**4.1**	**4.8**	**19**
	3.2**	**5.3****	**>10*****

*The average values given are means of three experiments.

** The values given are in the literature [13].

*** The values given are in the literature [14].



Comp.	a	b	c	d	e	f
R_1	Cl	Cl	H	OCH_3	Cl	F
R_2	H	F	OCH_3	H	Cl	H
R_3	H	H	H	H	F	F

Scheme 1**:** Reagents and conditions. (A)$POCl_3$, toluene, Et_3N at 80˚C; (B) substituted anilines, toluene; (C) ammonia solution, methanol at 80˚C; (D) methyl 3-(3-(chlorosulfonyl)phenyl)acrylate, Et_3N, DMA, at 75˚C.

Measurement of EGFR and HER2 inhibition in vitro

The compounds were tested for their inhibitory activity against EGFR and HER2 kinases, and the results of IC_{50} were showed in Table-1. Poziotinib was employed as positive control in this assay.

The results displayed that all the compounds showed potent inhibition of both enzymes with IC_{50} 2.6-12.5 nM against EGFR enzyme and IC_{50} 3.9-15.7 nM against HER2 enzyme. Compound **5c** displayed the best inhibitory activity against both enzymes with IC_{50} at 2.6 nM against EGFR kinases and 4.3 nM against HER2 kinases, respectively. The introduction of alkoxy in 4-substituted quinazolines enhanced both enzyme inhibitions significantly, especially the introduction of methoxy side chains in the interposition(Compare **5c**). While the introduction of halogens decreased the inhibitory activity against both enzymes(Compare **5c** and **5f**).

Anticancer screening in vitro

The novel synthesized compounds were evaluated for their cytotoxic activity *in vitro* against MCF7 using sulforhodamine-B stain assay in accordance with the method of Skehan *et al*[13]. The parameter used here was IC_{50}, which corresponds to the concentration required for 50% inhibition of the cell viability. The IC_{50} of the compounds are showed in Table-1. From the results in Table-1, it displayed that most of the test compounds showed to potent anticancer activity on MCF7.

Compound **5a** and **5d** showed weak cytotoxic activity consistent with its weak enzymes inhibitory activities. Compound **5c** showed weak cytotoxic activity on MCF7 cell line(IC_{50}=13 μM), but it is good enzymes inhibitory activities. Significant improvement in the cytotoxic activity was observed with compound **5b**, **5e** and **5f** (IC_{50}=6, 9 and 8 μM, respectively). These results suggested that compound **5c** is more sensitive than poziotinib to control the antitutor activity.

Conclusion

Some novel 6-substituted quinazoline derivatives were synthesised as EGFR and HER2 inhibitors. The novel compounds were measured for their enzyme inhibition as well as their cytotoxic

activity on MCF7 cell line. Almost all the compounds exerted inhibition activity of both enzymes and the best activity was explored by compound **5c**, which IC_{50} was 2.6 nM against EGFR kinases and 4.3 nM against HER2 kinases, respectively. But compound **5c** showed weak cytotoxic activity on MCF7 cell line(IC_{50}=13 μM). Three compounds (**5b, 5e** and **5f**) showed potent cytotoxic activity with IC_{50} values between 6 and 9 μM. Due to the small number of compounds, we will synthesize more compounds to verify this conclusion. Meanwhile, further studies will be needed to improve the enzymes inhibitory activity and the cytotoxic activity of the compounds.

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References

1. J. A. Palop, D. Plano, E. Moreno, and C. Sanmartín, Novel Quinazoline and Pyrido[2,3-d]Pyrimidine Derivatives and their Hydroselenite Salts as Antitumoral Agents. *Arkivoc*, **2**, 187 (2014).
2. Z. Q. Cai, W. Y. Ma, J. Y. Wang, Z. Y. Li, H. B. Guo, A. F. Cai, B. Xue, Synthesis and Antitumor Activities of Novel Quninazoline Derivatives Based on HER2 Receptor. *Chin. J. Syn. Chem.*, **23**, 194(2015).
3. Z. Q. Cai, J. Liu, M. X. Shao, Y. Wang, M. R. Zhang, Y. Chen, L. F. Xu M. Gong, A Cup-like Structure: Synthesis, Crystal Structure and Anti-Cancer Activity of 2-(2-(4,5-diphenyl-1H-imidazol-1-yl)acetamido)ethyl adamantane-1-carboxylate. *J. Chem. Soc. Pak.*, **36**, 717(2014).
4. Z. Q. Cai, X. Hou, L. Hou, Z. Q. Hu, B. Zhang, Z. S. Jin, One-Pot Palladium(II)-Catalyzed Synthesis of Fluoren -ones via Decarboxylative Cyclization. *Synlett*, **27**, 395(2016).
5. J. Liu, X. C. Hao, Y. Chen, S. Ding, H. S. Liu, D. Wang, Synthesis of New Substituted 5-amino-8H-phthalazino[1,2-b] quinazolin-8-one Derivatives. *J. Chem. Res.*, **41**, 18(2017).
6. H. K. Rhee, J. H. Yoo, E. Lee, Y. J. Kwon, H. R. Seo, Y. S. Lee, H. Y. Choo, Synthesis and Cytotoxicity of 2-phenylquinazolin-4(3H)-one Derivatives. *Eur. J. Med. Chem.*, **46**, 3900(2011).
7. N. Jiang, X. Zhai, Y. Zhao, Y. Liu, B. Qi, H. Tao, P. Gong, Synthesis and Biological Evaluation of Novel 2-(2-arylmethylene)hydrazinyl-4-aminoquinazoline Derivatives as Potent Antitumor Agents. *Eur. J. Med. Chem.*, **54**, 534(2012).
8. S. Das, N. Chatterjee, D. Bose, S. K. Dey, R. N. Munda, A. Nandy, S. Bera, S. K. Biswas, K. S. Das, Anticancer Potential of 3-(Arylideneamino)-2-Phenylquinazoline-4(3H)-One Derivatives. *Cell. Physiol. Biochem.*, **29**, 251(2012).
9. D. D. Li, F. Fang, J. R. Li, Q. R. Du, J. Sun, H. B. Gong, H. L. Zhu, Discovery of 6-substituted 4-anilinoquinazolines with dioxygenated rings as novel EGFR Tyrosine Kinase Inhibitors. *Bioorg. Med. Chem. Lett.*, **22**, 5870(2012).
10. X. Cai, H. X. Zhai, J. Wang, J. Forrester, H. Qu, L. Yin, C. J. Lai, R. Bao and C. Qian, Discovery of 7-(4-(3-Ethynylphenylamino)-7-methoxy quinazolin-6-yloxy)-N-hydroxyheptanamide (CUDC-101) as a Potent Multi-Acting HDAC, EGFR, and HER2 Inhibitor for the Treatment of Cancer. *J. Med. Chem.*, **53**, 2000(2010).
11. H. R. Ji, X. Du, Y. B. Song, X. Liu, Toxic effect and mechanisms of dibutyl phthalate. *J. Shenyang. Univ. Tech.*, **39**, 230(2017).
12. Z. Q. Cai, Z. S. Jin, D. Q. Zheng, L. Hou, G. W. Huang, J. Q. Tian, G. J. Wang, Synthesis of Several New Quinazolin-4-amines Containing p-toluenesulfonate Moiety. *J. Chem. Res.*, **40**, 573(2016).
13. H. J. Kim, H. P. Kim, Y. K. Yoon, M. S. Kim, G. S. Lee, S. W. Han, S. A. Im, T. Y. Kim, D. Y. Oh, Y. J. Bang, Antitumor Activity of HM781-36B, a pan-HER Tyrosine Kinase Inhibitor, in HER2-Amplified Breast Cancer Cells. *Anticancer Drugs*, **23**, 288(2012).
14. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Canc. Inst.*, **82**, 1107(1990).
15. M.Y. Cha, K. O. Lee, M. Kim, J. Y. Song, K. H. Lee, J. Park, Y. J. Chae,Y. H. Kim, K. H. Suh, G. S. Lee, S. B. Park, M. S. Kim, Antitumor Activity of HM781-36B, a highly Effective pan-HER inhibitor in Erlotinib-Resistant NSCLC and other EGFR-Dependent Cancer Models. *Int. J. Canc.*, **130**, 2445(2012).