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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-ARYL-4-AMINOQUINAZOLINES AS ANTITUMOR AGENTS

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Abstract

Quinazoline is an important pharmacophore, showing many biological activities. Some novel 2-aryl-4-aminoquinazolines were designed from 3-arylisoquinoline-1-amines using bioisostere approach. The 4-aminoquinazoline derivatives were prepared from corresponding quinazolinones in 3 steps. The cytotoxicity of these compounds was evaluated against 4 cancer cell lines including human epidermic carcinoma (KB), hepatocellular carcinoma (Hep-G2), human lung carcinoma (LU-1) and human breast carcinoma (MCF-7). Quinazolineamine compound **4a** showed good antitumor activity on KB cell line.

Keywords. Anticancer, 3-arylisoquinolinamine, 2-aryl-4-aminoquinazolines.

1. INTRODUCTION

Cancer is a group of diseases characterized by aberrations in cellular growth, proliferation, and survival pathways, resulting in uncontrolled expansion of cancer cells and tumor formation. These diseases represent one of the most severe health problems worldwide. Surpassing heart diseases,it is taking the position number one killer due to various worldwidefactors. Although major advances have been made in the chemotherapeutic management of patients, the discovering and development of new anticancer agents remains critically important [1, 2].

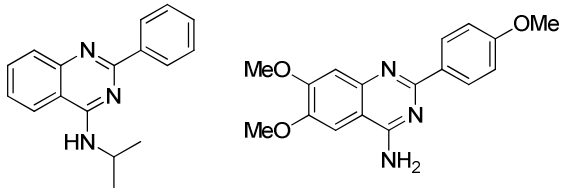


Figure 1: Representative anticancer 2-aryl-4-aminoquinazolines

Quinazoline derivatives including 2-aryl-4-aminoquinazolines possess diverse biological activities such as anticancer, antibacterial, anti-hypertensive effects [3-6].We have designed and synthesized some 4-amino-2-phenyl-quinazolinesfrom 3-arylisoquinoline-1-aminesas antitumor agents using bioisostere approach [7]. In our continuous efforts to develop new antitumor agents, we design and prepare some novel 2-aryl-4-aminoquinazoline derivatives [8, 9]. We expected that 2-aryl-4-aminoquinazoline compounds would exhibit potent anticancer activity.

2. MATERIALS AND METHODS

All chemicals were used as received from commercial sources without further purification.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 (500 MHz, ^{1}H; 125 MHz ^{13}C) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) or the internal solvent signal of deuterated solvents (^{13}C and ^{1}H). Multiplicities are indicated by s (singlet), d (doublet), m (multiplet) and dd (double doublet). Coupling constants *J* are reported in Hertz. The mass spectra were recorded on an Agilent 1260 LC/MS instrument using electrospray ionization (ESI) method. For thin layer chromatography, analytical TLC plates (70-230 mesh silica gel (Merck) were used. Visualization was accomplished with UV (254 nm).

*General experimental procedure of compound **2a-2d***

Reaction mixture of compound **1a-d** and excess $POCl_3$ was heated at 100 ^oC. After reaction was over, $POCl_3$ was removed by vaccum, cold water was added and mixture was extracted with ethyl acetate. The combined organic extracts was washed with brine, dried over Na_2SO_4 and then concentrated.

4-Chloro-6-methyl-2-(naphthalen-1-yl)quinazoline (2a)

Yield: 71 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.80 (d, *J* = 8.5 Hz, 1H); 8.22 (dd, *J* = 7.5 Hz, *J* = 1.5 Hz, 1H); 8.09 (s, 1H); 8.07 (d, *J* = 8.5 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H); 7.92 (d, *J* = 7.5 Hz, 1H), 7.82 (dd, *J* = 7.5 Hz, *J* = 1.5 Hz, 1H), 7.63-7.52 (m, 3H), 2.54 (s, 3H).

4-Chloro-6-methyl-2-(*o*-tolyl)quinazoline (2b)

Yield: 62 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.00-7.96 (m, 3H); 7.74 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H); 7.38-7.30 (m, 3H), 2.65 (s, 3H), 2.58 (s, 3H).

4-Chloro-6-methyl-2-(*m*-tolyl)quinazoline (2c)

Yield: 57 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.38-8.35 (m, 2H); 8.00 (s, 1H); 7.98 (d, *J* = 8.5 Hz,1H); 7.75 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H); 7.41 (t, *J* = 8 Hz,1H); 7.32 (t, *J* = 7.5 Hz,1H); 2.59 (s, 3H), 2.48 (s, 3H).

4-Chloro-2-(3-methoxyphenyl)-6-methylquinazoline (2d)

Yield: 53%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.17 (dd, *J* = 8 Hz, *J* = 1 Hz, 1H); 8.12 (t, 1H); 8.00 (s, 1H); 7.98 (d, *J* = 7.5 Hz,1H); 7.75 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H); 7.42 (t, *J* = 8 Hz,1H); 7.05 (m, 1H); 3.94 (s, 3H), 2.59 (s, 3H).

*General experimental procedure of compound **3a-3d***

Reaction mixture of compound **2a-d** and sodium azide in DMF was stirred at room temparature for 3 hours. After reaction was over, water was added and mixture was extracted with ethyl acetate. The combined organic extracts was washed with brine, dried over Na_2SO_4 and then concentrated.The residue was purified by column chromatography to give compound **3a-d**.

4-Azido-6-methyl-2-(naphthalen-1-yl)quinazoline (3a)

Yield: 80 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.57 (s, 1H); 8.15 (d, *J* = 7.5 Hz, 1H); 8.13 (d, *J* = 7.5 Hz, 1H); 8.08 (dd, *J* = 7.0 Hz, *J* = 1.0 Hz, 1H); 8.00 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.5 Hz, 1H); 7.92 (d, *J* = 7.5 Hz, 1H), 7.82 (dd, *J* = 8.0 Hz, *J* = 1.5 Hz, 1H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.58 (td, *J* = 7.5 Hz, *J* = 1.0 Hz, 1H); 7.52 (td, *J* = 7.0 Hz, *J* = 1.0 Hz, 1H); 2.70 (s, 3H).

4-Azido-6-methyl-2-(*o*-tolyl)quinazoline (3b)

Yield: 85 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.53 (s, 1H); 8.08 (d, *J* = 8 Hz,1H), 7.80 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H); 7.72 (d, *J* = 7.5 Hz,1H), 7.54 (td, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H), 7.45-7.41 (m, 2H), 2.67 (s, 3H), 2.36 (s, 3H).

4-Azido-6-methyl-2-(*m*-tolyl)quinazoline (3c)

Yield: 72 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.45-8.37 (m, 3H); 8.02 (m, 1H); 7.73 (m,1H); 7.52-7.43 (m,2H); 2.63 (s, 3H), 2.52 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$)δ(ppm): 149.8, 143.5, 141.4, 140.2, 138.5, 135.1, 133.1, 130.6, 130.4, 128.6, 128.6, 127.4, 123.9, 114.5, 21.7, 21.5.

4-Azido-2-(3-methoxyphenyl)-6-methylquinazoline (3d)

Yield: 70%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.47 (s, 1H); 8.24 (d, *J* = 8.0 Hz,1H); 8.16 (t, 1H); 8.07 (d, *J* = 8.5 Hz,1H); 7.76 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H); 7.53 (t, *J* = 8 Hz,1H); 7.19 (dd, *J* = 8.5 Hz, *J* = 2.5 Hz, 1H); 3.95 (s, 3H), 2.64 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$)δ(ppm): 159.7, 149.9, 143.1, 141.1, 140.4, 135.2, 131.6, 129.8, 128.7, 124.0, 122.7, 118.6, 115.0, 114.6, 55.5, 21.7.

*General experimental procedure of compound **4a-4d***

Reaction mixture of compound **3a-d** and 10% Pd/C(5% w/w) in ethanol was stirred at room temparature for overnight. After reaction was over, mixture was filtered to remove the catalyst and the filtrate was concentrated. The residue was purified by column chromatography to give compound **4a-d**.

6-Methyl-2-(naphthalen-1-yl)quinazoline-4-amine (4a)

Yield: 78 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.48 (m, 1H); 7.95-7.86 (m, 4H), 7.61-7.44 (m, 5H); 6.11 (s, 2H); 2.49 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 162.3, 161.0, 148.9, 137.3, 136.0, 135.2, 134.0, 131.1, 129.4, 128.4, 128.2,

128.0, 126.3,126.2, 125.7, 125.2, 120.6, 112.5, 21.6. ESI-MS m/z: 286.0 $[M+H]^+$.

6-Methyl-2-(*o*-tolyl)quinazolin-4-amine (4b)

Yield: 67%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.85 (s, 1H); 7.71 (d, *J* = 8.5 Hz,1H), 7.61 (d, *J* = 8.5 Hz, *J* = 1.5 Hz,1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.28 (dd, *J* = 8.5 Hz, *J* = 1.0 Hz,1H), 7.22 (m,1H); 2.47 (s, 3H), 2.35 (s, 3H).^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 160.9, 160.3, 149.3, 138.6, 137.9, 135.6, 135.1, 130.7, 128.7, 128.6, 128.2, 125.4, 120.6, 112.8, 21.6, 21.5.ESI-MS m/z: 248.0 $[M-H]^+$.

6-Methyl-2-(*m*-tolyl)quinazolin-4-amine (4c)

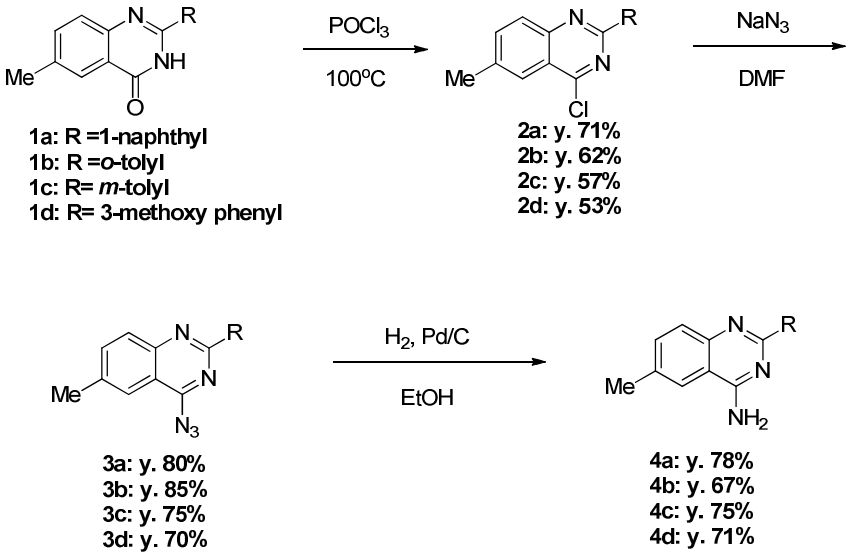
Yield: 75%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.29 (s, 1H); 8.27 (d, *J* = 7.5 Hz,1H); 7.85 (d, *J* = 8.5 Hz,1H); 7.59 (dd, *J* = 8.5 Hz, *J* = 1.5 Hz, 1H); 7.48 (s, 3H), 7.37 (t, *J* = 7.5 Hz,1H); 7.27 (d, *J* = 7.5 Hz,1H); 2.50 (s, 3H), 2.45 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 160.9, 160.3, 149.3, 138.6, 137.9, 135.6, 135.1, 130.7, 128.7, 128.6, 128.2, 125.4, 120.6, 112.8, 21.6, 21.5. ESI-MS m/z: 248.0 $[M-H]^+$.

2-(3-Methoxyphenyl)-6-methylquinazolin-4-amine (4d)

Yield: 70 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.08-8.03 (m, 2H), 7.85 (d, *J* = 8.5 Hz,1H); 7.59 (dd, *J* = 8.0 Hz, *J* = 1.5 Hz, 1H); 7.4 (s, 1H); 7.38 (t, *J* = 8 Hz,1H); 7.01 (m, 1H); 3.91 (s, 3H), 2.50 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 160.9, 159.8, 159.7, 149.2, 140.0, 135.8, 135.2, 134.4, 129.3, 128.5, 120.7, 120.7, 116.5, 112.9, 55.3, 21.6. ESI-MS m/z: 266.1 $[M+H]^+$.

Cytotoxicity assay

Compounds **4a**-**d** were assayed for *in vitro* cytotoxicity in a panel of human tumor cell lines at the Institute of Chemistry, VAST, according to procedures described by Boyd [10].The cell lines included **KB** (*Human epidermic carcinoma*), **Hep G2** (*Hepatocellular carcinoma*), **LU-1** (*Human lung carcinoma*), and **MCF-7** (*Human breast carcinoma*). The cytotoxic effects of each compound were obtained as IC_{50} values, which represent the compound concentrations required to cause 50% inhibition.



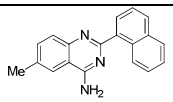
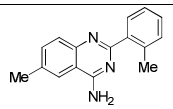
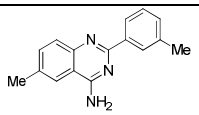
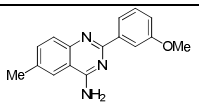
Scheme 1: Synthetic route of 2-aryl-4-aminoquinazolines

3. RESULTS AND DISCUSSION

2-Aryl-quinazoline compounds were prepared from corresponding 2-aryl-quinazolinones [11]in 3 steps. Amide group of quinazolinones **1a**-**d** were converted to imine chloride by reaction with $POCl_3$in moderate yields (53-75 %). The imine compounds **2a**-**d** are quite reactive so they can be used without purification, only a small amount was purified to confirm the structures. The compound **2a**-**d** was reacted with sodium azide to give compound **3a**-**d** in good yields (70-80 %). The azide group was easily reduced to amine using hydrogenation reaction with Pd/C catalyst in EtOH [3]. The desired compounds were obtained in good yield (67-78 %). The structures of intermediates and target compound **4a**-**d** were confirmed by NMR and ESI-MS spectroscopic data.

All of the prepared 2-aryl-4-quinazolinones were evaluated for cytotoxicity against 4 human cancer cell lines including human epidermic carcinoma (KB), hepatocellular carcinoma (Hep-G2), human lung carcinoma (LU-1) and human breast carcinoma (MCF-7) using Boyd's procedure [10]. As shown in table 1,compound **4a** with naphthalene group showed the best cytotoxicity of 6.5 µg/mL against KB cell line. Compound **4b** exhibited moderate activity (18.48 µg/mL) while quinazolineamines **4c** and **4d** were almost inactive. It seems that the substituents at 2-position (methyl group) of 2-aryl ring is beneficial for activity. In term of selectivity, compound **4a** showed selective affect on KB cell line with IC = 6.5 µg/mL, 4-20 times stronger than on other cell lines.

Table 1: In vitro Cytotoxicity of 2-aryl-4-aminoquinazolines

Compound	Structure	IC_{50}, µg/mL			
		KB	Hep-G2	LU-1	MCF-7
4a		6.5	24.2	94.12	>128
4b		18.48	42.67	80	77.6
4c		59.43	67.3	104	>128
4d		97.03	>128	>128	>128
Ellipticine		**0.31**	**0.35**	**0.45**	**0.53**

4. CONCLUSION

In conclusion, 4 new 2-aryl-4-aminoquinazolines were prepared and evaluated for cytotoxicity against 4 human cancer cell lines (KB, Hep-G2, LU-1 and MCF-7). Compound **4a** (2-naphthyl 7-methyl-4-aminoquinazoline) showed good anticancer activity at micromol concentration and showed selective effect on KB cell line. Further study on antitumor quinazoline derivatives are under investigation and will be reported elsewhere.

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