Synthesis and anticancer activities of diquinazoline diselenides compounds

Gang Liu[1,*], YinJiu Huang[2,*], Kun Cao[1], ChunNuan Ji[1], Lin Sun[1], ShengGuang Xu[1], XiGuang Liu[1]

[1]School of Chemistry and Materials Science, Ludong University, Yantai, 264025, China.

[2]Department of Bioscience, Bengbu Medical College, Bengbu, 233030, China

Síntesis y actividades anticancerígenas de los compuestos de diselenuro de diquinazolina

Síntesi i activitats anticancerígens dels compostos de diselenuro de diquinazolina

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RESUMEN

Se han diseñado y sintetizado una serie de compuestos nuevos de diselenuro de diquinazolina con 4-cloroquinazolina y diselenuro de sodio. Sus estructuras se han confirmado mediante IR, ^{1}H NMR, ^{13}C NMR, y análisis elementales. La actividad antitumoral de los nuevos compuestos se evaluaba con el método MTT. Los compuestos 1a, 1c, 1h y 1i mostraban actividad contra las células MDA-MB-435, A549, MDA-MB-231, SiHa, y HeLa. Además, en comparación con los fármacos anticancerígenos comerciales Gefitinib, Oxaliplatino, Taxol, 10-Hidroxi camptotecina y clorhidrato de Epirubicina, el producto 1a ejercía mejores efectos antitumorales en las correspondientes líneas celulares en una concentración 10 μM.

Palabras clave: Quinazolina; diselenuro de diquinazolina; síntesis; actividades anticancerígenas; método MTT.

SUMMARY

A series of novel diquinazoline diselenide compounds was designed and synthesized with substituted 4-chloroquinazoline and sodium diselenide. Their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and elemental analyses. The antitumor activity of the new compounds was evaluated by MTT method. Compound 1a, 1c, 1h and 1i were found to have activities against MDA-MB-435, A549, MDA-MB-231, SiHa, and HeLa cells. Moreover, compared with the commercial anticancer drugs Gefitinib, Oxaliplatin, Taxol, 10-Hydroxycamptothecin, and Epirubicin Hydrochloride, 1a exerted better antitumor effects on corresponding cell lines at 10 μM.

Keywords: Quinazoline; diquinazoline diselenide; synthesis; anticancer activities; MTT method.

RESUM

S'han dissenyat i sintetitzat una sèrie de compostos nous de diselenuro de diquinazolina amb 4-cloroquinazolina i diselenuro de sodi. S'han confirmat les seves estructures mitjançant IR, ^{1}H NMR, ^{13}C NMR, i anàlisis elementals. L'activitat antitumoral dels nous compostos s'avaluava amb el mètode MTT. Els compostos 1a, 1c, 1h y 1i mostraven activitat contra les cèl·lules MDA-MB-435, A549, MDA-MB-231, SiHa, i HeLa. A més, en comparació amb els fàrmacs anticancerígens comercials Gefitinib, Oxaliplatino, Taxol, 10-Hidroxi camptotecina i clorhidrat de Epirubicina, el producte 1a exercia millors efectes antitumorals en les corresponents línies cel·lulars en una concentració 10 μM.

Paraules clau: Quinazolina; diselenuro de diquinazolina; síntesi; activitats anticancerígens; mètode MTT.

Corresponding author: [1]*iuhuang1973@163.com, [2]*shdliugang@163.com

INTRODUCTION

Quinazoline compounds are a series of small-molecule drugs with good EGFR inhibition activity.[1] Some examples are Iressa (ZD 1839), Tarceva (OSI-774), Gleevec (STI571), and CI-1033. Selenide compounds especially heterocyclic containing-selenium compounds exert multi-targeted anticancer effects through various mechanisms.[2,3] Some selenide drugs that are currently undergoing clinical studies include Ebselen[4], which has anti-inflammatory and antioxidant properties, and Selenazofurin[5], which has antiviral and antitumor properties. As part of our ongoing research on heterocyclic compounds, we were particularly interested in 4-substituted quinazolines from O, N and S atom[6-8]. Their activities are found to vary, but anticancer activity is not significantly reduced. According to bio-electronic line principle, we design and synthesize a series of novel diquinazoline diselenide compounds considering the multi-mechanism and multi-targeting anticancer effects of organoselenium compounds, together with the good inhibitory activity of EGFR quinazoline compounds. In this paper, a series of diquinazoline diselenide compounds was prepared with substituted 4-chloroquinazoline and sodium diselenide (Figure 1). The structures of the new compounds were confirmed by elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR. The antitumor activities of the new compounds were also evaluated by MTT method. Among them, 1a, 1c, 1h and 1i showed good activities against MDA-MB-435, A549, MDA-MB-231, SiHa, and HeLa cells. The inhibition activity of 1a against MDA-MB-435, A549, MDA-MB-231, SiHa, and HeLa cells was 99.7%, 81.6%, 93.1%, 90.6%, and 94.2% at 10 μM concentration.

RESULTS AND DISCUSSION

The synthesis of intermediates 3a, 3b, 3c, 3d, 3h and 3i from anthranilic acid or substituted anthranilic acid reacted with formamide. The synthesis of intermediates 3e and 3j from anthranilic acid (2a) or substituted anthranilic acid reacted with urea. The synthesis of intermediate 3f from anthranilic acid reacted with acetic anhydride, and then reacted with ammonium acetate. The synthesis of intermediates 3g needs three steps from 2a. Firstly anthranilic acid reacted with benzoyl chloride, and then reacted with aqua ammonia, finally reacted with sodium hydroxide. In chlorination step, synthesis of 4e, 4g and 4j must needed phosphorus oxychloride catalyzing by dimethylformamide. The others intermediates needed thionyl chloride catalyzing by dimethylformamide. R_1-R_3 expresses atom or radical see in Table 1.

Unfortunately, the yields of the synthesis of diselenide compounds were not ideal below in 50%. Some products were insoluble in ethanol during the reaction. It's as a by-product after the analysis, most probably were quinazoline diselenide sodium compounds. Based on literature[9], the yields of synthesis of dibenzyl diselenides by using benzyl chloride or benzyl bromide as starting materials were very good. But using 4-chloroquinazoline compounds as starting materials, the yields of synthesis of diquinazoline diselenide compounds were not achieve the desired goals. The yields remained low changing the reaction conditions, which itself quinazoline ring large size had an important effect. The novel diquinazoline diselenide compounds were the symmetrical structure of compounds. Based on analysis of ^{1}H NMR and ^{13}C NMR spectrogram, H and C chemical shift had the same position in both quinazoline groups.

MDA-MB-435, A549, MDA-MB-231, SiHa, and HeLa cell lines were used for anticancer drug discovery screening through MTT assay. Table 2 showed that after 72 h of treatment, 1a, 1b, 1d, 1f, and 1h showed considerable anticancer activities for the above cell lines at 10 μM and even at 1 μM *in vitro* compared with the DMSO negative control. Furthermore, compared with the commercial anticancer drugs Gefitinib (a small molecular-targeted anticancer drug), Oxaliplatin (a traditional chemotherapeutic drug), Taxol (a traditional chemotherapeutic drug), 10-Hydroxycamptothecin (one kind of plant anticancer drug), and Epirubicin Hydrochloride (an antibiotics anticancer drug), 1a showed better antitumor effects on corresponding cell lines at 10 μM (Table 2). These result suggested that 1a was a potential antitumor agent.

Figure 1 *Synthesis of diquinazolin-4-yl diselenide compounds*

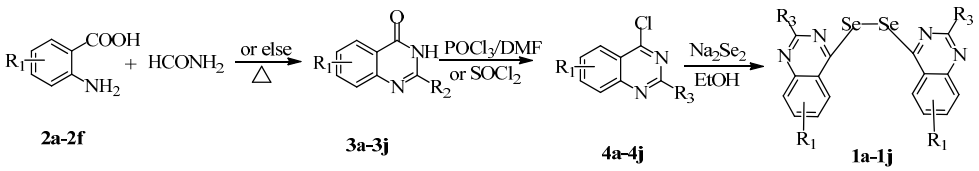

Table 1 *atom or radical of R_1, R_2 and R_3*

Substituent Group	Title compound 1a-1j and Intermediates 4a-4j									
	1a, 4a	1b, 4b	1c, 4c	1d, 4d	1e, 4e	1f, 4f	1g, 4g	1h, 4h	1i, 4i	1j, 4j
R_1	H	6-Cl	6-Br	6-I	H	H	H	6,8-2Cl	6,8-2Br	6-Cl
R_3	H	H	H	H	Cl	CH_3	Ph	H	H	Cl

Substituent Group	Intermediates 3a-3j									
	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
R_1	H	6-Cl	6-Br	6-I	H	H	H	6,8-2Cl	6,8-2Br	6-Cl
R_2	H	H	H	H	OH	CH_3	Ph	H	H	OH

Substituent Group	Intermediates 2a-2f					
	2a	2b	2c	2d	2e	2f
R_1	H	6-Cl	6-Br	6-I	6,8-2Cl	6,8-2Br

Table 2 *Effect of title compounds 1a-1j against cell viability of different tumor cell lines*

Compound	Inhibitory ratio (%)					
	MDA-MB-435		A549		MDA-MB-231	
	1 μM	10 μM	1μM	10μM	1 μM	10μM
1a	-0.16 ±1.59	99.69±0.18*	5.54 ±2.38	81.64 ±2.90*	5.27 ±3.25	93.11 ±1.58*
1b	6.32 ±5.95	51.1 ±3.21*	-20.92±4.68*	20.07 ±6.00*	4.32 ±6.95	16.9 ±5.33*
1c	-1.41 ±2.68	63.67±1.23*	-36.78 ±5.68*	28.66 ±1.52*	-19.91±11.71*	34.46±23.88*
1d	-0.22 ±3.25	42.3 ±4.56*	-7.68 ±5.10	38.37 ±3.51*	-6.18 ±6.29	7.60 ±2.89
1e	-0.22 ±3.25	4.23 ±4.56	-5.16 ±4.67	10.82 ±4.90	-29.33 ±14.31*	-7.56 ±6.47
1f	-10.83±3.87	3.40 ±2.59	-6.35 ±4.38	45.66 ±1.92*	-6.34 ±7.02	4.01 ±1.96
1g	4.73 ±5.29	3.12 ±6.96	3.44 ±2.71	16.15 ±3.23*	-3.67 ±5.25	8.21 ±2.61
1h	9.93 ±4.56	59.07±3.95*	27.77 ±2.06*	34.4 ±2.03*	-12.75 ±7.72*	15.94 ±5.06*
1i	-1.83 ±3.87	43.4 ±2.59*	2.71 ±6.14	17.86 ±5.68*	-1.89 ±6.48	-4.44 ±3.97
1j	6.32 ±5.95	-1.10 ±3.21	10.02 ±3.99	-4.61 ±3.50	10.35 ±3.69*	11.4 ±1.07*
Oxaliplatin	14.36±2.90*	48.73±4.76*	16.48 ±3.46*	39.76 ±2.71*	10.22 ±6.75	45.39 ±0.12*
Gefitinib	10.98 ±7.59	70.48±5.23*	-6.04 ±9.53	83.3 ±9.53*	9.73 ±8.04	67.52 ±4.14*
10-Hydroxy camptothecin	95.49±1.13*	99.15±0.20*	60.98 ±1.62*	77.94 ±1.19*	NT[a]	NT[a]
Taxol	95.6 ±0.75*	92.32±1.65*	88.35 ±0.92*	100 ±0.68*	NT[a]	NT[a]
Epirubicin Hydrochloride	27.03±3.02*	95.95±1.09*	50.95 ±1.79*	100 ±0.21*	50.9 ±13.44*	95.28 ±4.22*

Compound	Inhibitory ratio (%)			
	SiHa		HeLa	
	1μM	10μM	1μM	10μM
1a	27.82 ±6.56*	90.57 ±5.73*	23.13 ±2.07	94.24 ±2.10*
1b	5.45 ±1.16	34.06 ±8.77*	5.15 ±5.46	28.67 ±6.52*
1c	40.66 ±5.42*	68.58 ±7.98*	1.55 ±4.80	17.15 ±7.74
1d	9.38 ±1.28*	22.59 ±1.89*	-23.41 ±9.56	8.40 ±6.69
1e	7.50 ±3.49	21.15 ±3.87*	2.86 ±6.72	12.84 ±4.38
1f	5.45 ±1.16	34.06 ±8.77*	-11.37 ±5.36	0.34 ±5.13
1g	7.5 ±3.49	21.15 ±3.87*	-0.54 ±2.99	-2.38 ±3.85
1h	19.34 ±3.96*	23.26 ±4.78*	5.86 ±4.07	18.14 ±3.22
1i	20.55 ±8.94*	36.92 ±6.12*	-20.59 ±8.57	0
1j	15.72 ±11.41	64.45 ±7.44*	2.49 ±7.37	-18.35 ±7.24
Oxaliplatin	34.22 ±5.56*	50.86 ±6.66*	-0.45 ±6.35	35.63 ±4.71*
Gefitinib	NT[a]	NT[a]	NT[a]	NT[a]
10-Hydroxy camptothecin	37.39 ±2.39*	75.31 ±0.35*	NT[a]	NT[a]
Taxol	77.54 ±1.90*	99.08 ±0.20*	NT[a]	NT[a]
Epirubicin Hydrochloride	73.14 ±8.16*	99.77 ±0.37*	NT[a]	NT[a]

*Compared with DMSO control, compound 1a-1j, Gefitinib or Oxaliplatin treatment of 72 h showed statistically significant inhibition ($P<0.05$) against cell growth.

[a] NT mean not tested.

EXPERIMENTAL SECTION

All melting points of the products were determined on a MP120 digital melting point tester (Jinan Haineng Instruments, Ltd. Co., China) and are not corrected. The infrared spectra were recorded on a MAGANA-IR550(series II)spectrometer in KBr disks. ^{1}H and ^{13}C-NMR spectra were recorded on a spectrometer BRUKER AVANCE III 600 MHz (600 and 150 MHz respectively) at room temperature in DMSO-d_6 using TMS as internal standard. Elemental analysis was performed by an Elementar Vario EL III CHN analyzer. The mass spectra were taken on a Agilent 6210 spectrometer.

The following compounds were prepared as described in the literature. *4-chloroquinazoline* (4a): white needle crystal, yield 54.5%, mp 94-95 °C (mp 96.5-97.5 °C[10]); *4,6-dichloroquinazoline* (4b): white solid, yield 62.5%, mp 152-154 °C (mp 154-155 °C[11]); *6*-bromo-*4-chloroquinazoline* (4c): yellowish solid, yield 34.2%, mp 162-164 °C (mp 164-166 °C[12]); *4-chloro-6*-iodo*quinazoline* (4d): orange-yellow solid, yield 33.1%, mp 188-191 °C (mp 193-195°C[13]); *2,4-dichloro quinazoline* (4e)*:* yield 80.2%, mp 118-121 °C (mp 119.5 °C[14])*; 4-chloro-2-methylquinazoline* (4f)*:* yield 18.5%, mp 98.9-101.3 °C (mp 86-88 °C[15])*; 4-chloro-2-phenylquinazoline* (4g)*:* yellowish solid, yield 87.3%, mp 127.0-128.0 °C (mp 124 °C[16])*; 4,6,8-trichloroquinazoline* (4h)*:* yellowwish solid, yield 15.3%, mp 145.5-147.5 °C (mp 139-140 °C[17])*; 6,8-di*bromo-*4-chloroquinazoline* (4i): orange-yellow solid, yield 66.8%, mp 159.0-159.3 °C (mp 189-190 °C[13]); 2*,4,6-trichloroquinazoline* (4j)*:* yellowish solid, yield 75.5%, mp 121-124 °C (mp 131 °C[18]). All the other reagents used in the experiment were analytical reagents without any modification.

General procedure for preparation diquinazolin-4-yl diselenide compounds 1a-1j

4-Chloroquinazoline or substituted 4-chloroquinazoline (5.0 mmol) was added to an ethanolic sodium diselenide solution 0.51 g (2.5 mmol) in 1 h. The solution was heated with reflux for 8–24 h, and reaction completion was monitored by TLC. After cooling and acidification using glacial acetic acid (pH 5), the solvents were removed *in vacuo* and recrystallized from DMF-H_2O to give the desired products 1a–1j.

1,2-bis(quinazolin-4-yl)diselane (1a): orange-red solid, yield 44.2%, mp 220.7-222.0 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3140.8, 3055.6 (Ar-H), 1617.2-1466.4 (quinazoline skeleton). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.66 (2H, s, H-2, 2' Ar), 7.76 (2H, d, *J* = 8.4, H-8, 8' Ar), 7.99 (2H, d, *J* = 8.4, H-5, 5' Ar), 8.21 (2H, t, *J* = 7.8, H-6, 6' Ar), 8.61 (2H, t, *J* = 7.8, H-7, 7' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 127.6 (2C, C-5, 5' Ar), 129.5 (2C, C-6, 6' Ar), 132.4 (2C, C-8, 8' Ar), 136.3 (2C, C-7, 7' Ar), 143.9 (2C, C-10, 10' Ar), 144.7 (2C, C-9, 9' Ar), 154.2 (2C, C-2, 2' Ar), 162.8 (2C, C-4, 4' Ar). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 420.0 $[M+2]^+$ (56), 417.9 $[M]^+$ (48), 327.7 $[M-C_6H_4N]^+$ (100), 301.7 $[M-C_6H_4N-CN]^+$ (33), 274.8 $[M-C_6H_4N-2CN]^+$ (88), 207.4 $[M-H-C_8H_5N-{}_2Se]^+$ (80), 120.2 $[M-C_8H_5N_2Se-C_6H_4N]^+$ (22). Anal. calcd for $C_{16}H_{10}N_4Se_2$: C 46.17, H 2.42, N 13.46; found C 46.42, H 2.53, N 13.31.

1,2-bis(6-chloroquinazolin-4-yl)diselane (1b): orange--red solid, yield 29.0%, mp 255.2-257.9 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3083.3 (Ar-H), 1615.4-1450.9 (quinazoline skeleton), 647.7 (C-Cl). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.78 (2H, d, *J* = 8.4, H-7, 7' Ar), 8.00 (2H, d, *J* = 8.4, H-8, 8' Ar), 8.20 (2H, s, H-5, 5' Ar), 8.55 (2H, s, H-2, 2' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 130.9 (2C, C-5, 5' Ar), 131.3 (2C, C-10, 10' Ar), 133.6 (2C, C-8, 8' Ar), 133.9 (2C, C-7, 7' Ar), 136.1 (2C, C-6, 6' Ar), 142.6 (2C, C-9, 9' Ar), 145.2 (2C, C-2, 2' Ar), 186.9 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_8Cl_2N_4Se_2$: C 39.62, H 1.66, N 11.55; found C 39.50, H 1.45, N 11.38.

1,2-bis(6-bromoquinazolin-4-yl)diselane (1c): orange-red solid, yield 40.8%, mp 225.9-227.3 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3017.6 (Ar-H), 1617.4-1454.8 (quinazoline skeleton), 559.1 (C-Br). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.71 (2H, d, *J* = 9.0, H-8, 8' Ar), 8.12 (2H, dd, $J_{7,8}$ = 9.0, $J_{5,7}$ = 1.8, H-7, 7' Ar), 8.22 (2H, s, H-2, 2' Ar), 8.71 (2H, d, *J* = 1.8, H-5, 5' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 122.4 (2C, C-6, 6' Ar), 131.4 (2C, C-10, 10' Ar), 133.9 (2C, C-5, 5' Ar), 134.1 (2C, C-8, 8' Ar), 138.8 (2C, C-7, 7' Ar), 142.9 (2C, C-9, 9' Ar), 145.3 (2C, C-2, 2' Ar), 186.5 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_8Br_2N_4Se_2$: C 33.48, H 1.40, N 9.76; found C 33.25, H 1.28, N 9.98.

1,2-bis(6-iodoquinazolin-4-yl)diselane (1d): orange-red solid, yield 29.3%, mp 225.9-227.3 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3013.1 (Ar-H), 1615.7-1450.7 (quinazoline skeleton), 490.0 (C-I). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.53 (2H, d, *J* = 8.4, H-8, 8' Ar), 8.20 (2H, s, H-5, 5' Ar), 8.25 (2H, d, *J* = 7.8, H-7, 7' Ar), 8.91 (2H, s, H-2, 2' Ar). ^{13}C NMR spectrum (150 MHz, DMSO--d_6), δ, ppm (*J*, Hz): 95.6 (2C, C-6, 6' Ar), 131.0 (2C, C-10, 10' Ar), 134.2 (2C, C-5, 5' Ar), 140.4 (2C, C-8, 8' Ar), 143.2 (2C, C-7, 7' Ar), 144.3 (2C, C-9, 9' Ar), 145.3 (2C, C-2, 2' Ar), 186.5 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_8I_2N_4Se_2$: C 28.77, H 1.21, N 8.39; found C 28.64, H 1.09, N 8.55.

1,2-bis(2-chloroquinazolin-4-yl)diselane (1e): yellow--green solid, yield 9.5%, mp 134.2-136.0 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3079.0 (Ar-H), 1682.2-1469.2 (quinazoline skeleton). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.18 (2H, t, *J* =7.8, H-6, 6' Ar), 7.38 (2H, t, *J* = 7.8, H-7, 7' Ar), 7.46 (2H, d, *J* =7.8, H-5, 5' Ar), 7.89 (2H, d, *J* = 7.8, H-8, 8' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 114.8 (2C, C-10, 10' Ar), 116.6 (2C, C-5, 5' Ar), 122.8 (2C, H-8, 8' Ar), 127.3 (2C, C-6, 6' Ar), 135.4 (2C, C-7, 7' Ar), 141.0 (2C, H-9, 9' Ar), 159.3 (2C, C-2, 2' Ar), 172.5 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_8Cl_2N_4Se_2$: C 39.62, H 1.66, N 11.55; found C 39.66, H 1.51, N 11.44.

1,2-bis(2-methylquinazolin-4-yl)diselane (1f): nacarat solid, yield 6.3%, mp 135.4-137.5 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3074.7 (Ar-H), 2924.7 (ν_{asCH3}), 2854.8 (ν_{sCH3}), 1684.2-1468.1 (quinazoline skeleton). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.34 (6H, s, $2CH_3$), 7.65 (2H, t, *J* =7.8, H-6, 6' Ar), 7.84 (2H, d, *J* =7.8, H-5, 5' Ar), 7.89 (2H, t, *J* =7.8, H-7, 7' Ar), 8.19 (2H, d, *J* =7.8, H-8, 8' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 24.8 (2C, $2CH_3$), 123.4 (2C, C-10, 10' Ar), 126.5 (2C, C-5, 5' Ar), 128.9 (2C, C-6, 6' Ar), 129.1 (2C, C-8, 8' Ar), 135.3 (2C, C-7, 7' Ar), 147.7 (2C, C-9, 9' Ar), 160.6 (2C, C-4, 4' Ar), 161.4 (2C, C-2, 2' Ar). Anal. calcd for $C_{18}H_{14}N_4Se_2$: C 48.66, H 3.18, N 12.61; found C 48.38, H 2.95, N 12.48.

1,2-bis(2-phenylquinazolin-4-yl)diselane (1g): orange solid, yield 11.6%, mp 235.1-238.1 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3066.7 (Ar-H), 1631.9-1455.4 (quinazoline skeleton). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.52-7.61 (8H, m, H-3, 3', 4, 4', 5, 5' Ph + H-6, 6' quinazoline), 7.75 (2H, d, *J* = 8.4, H-5, 5' Ar), 7.85 (2H, t, *J* = 8.4, H-7,7' Ar), 8.17-8.21 (6H, m, H-8, 8' quinazoline + H-2, 2', 6, 6' Ph). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 121.5 (2C, C-10, 10' Ar), 126.3 (2C, C-5, 5' Ar), 127.1 (2C, C-6, 6' Ar), 128.0 (2C, C-8, 8' Ar), 128.2 (2C, C-4, 4' Ph), 129.1 (4C, C-2, 2', 6, 6' Ph), 131.8 (4C, C-3, 3', 5, 5' Ph), 133.2 (2C, C-7, 7' Ar), 135.1 (2C, C-1, 1' Ph), 149.2 (2C, C-9, 9' Ar), 152.8 (2C, C-4, 4' Ar), 162.7 (2C, C-2, 2' Ar). Anal. calcd for $C_{28}H_{18}N_4Se_2$: C 59.17, H 3.19, N 9.86; found C 59.38, H 2.99, N 9.73.

1,2-bis(6,8-dichloroquinazolin-4-yl)diselane (1h): orange solid, yield 30.5%, mp 261.5-263.8 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3082.9 (Ar-H), 1607.9-1470.9 (quinazoline skeleton), 656.6 (C-Cl). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.10 (2H, s, H-5, 5' Ar), 8.27 (2H, s, H-7, 7' Ar), 8.90 (2H, s, H-2, 2' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 118.1 (2C, C-5, 5' Ar), 122.0 (2C, C-10, 10' Ar), 131.2 (2C, C-7, 7' Ar), 133.4 (2C, C-6, 6' Ar), 134.3 (2C, C-8, 8' Ar), 155.8 (2C, C-9, 9' Ar), 166.1 (2C, C-2, 2' Ar), 173.0 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_6Cl_4N_4Se_2$: C 34.69, H 1.09, N 10.11; found C 34.90, H 0.91, N 10.13.

1,2-bis(6,8-dibromoquinazolin-4-yl)diselane (1i): orange-red solid, yield 9.5%, mp 124.2-125.3 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3079.6 (Ar-H), 1600.2-1465.1 (quinazoline skeleton), 632.7 (C-Br). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.28 (2H, s, H-5, 5' Ar), 8.51 (2H, s, H-7, 7' Ar), 8.92 (2H, s, H-2, 2' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 118.5 (2C, C-6, 6' Ar), 120.1 (2C, C-10, 10' Ar), 124.4 (2C, C-5, 5' Ar), 125.8 (2C, C-8, 8' Ar), 139.9 (2C, C-7, 7' Ar), 147.4 (2C, C-9, 9' Ar), 155.9 (2C, C-2, 2' Ar), 165.9 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_6Br_4N_4Se_2$: C 26.26, H 0.83, N 7.66; found C 26.11, H 0.98, N 7.50.

1,2-bis(2,6-dichloroquinazolin-4-yl)diselane (1j): pale yellow solid, yield 18.3 %, mp 133.2-135.5 ºC. IR spectrum (thin layer), ν, cm^{-1}: 3071.8 (Ar-H), 1694.1-1464.6 (quinazoline skeleton), 619.4 (C-Cl). ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.67 (2H, d, *J* = 8.4, H-7, 7' Ar), 7.78 (2H, d, *J* =8.4, H-8, 8' Ar), 7.81 (2H, s, H-5, 5' Ar). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 118.0 (2C, C-10, 10' Ar), 118.9 (2C, C-5, 5' Ar), 126.4 (2C, C-8, 8' Ar), 129.4 (2C, C-7, 7' Ar), 135.9

(2C, C-6, 6' Ar), 150.5 (2C, C-9, 9' Ar), 162.2 (2C, C-2, 2' Ar), 172.9 (2C, C-4, 4' Ar). Anal. calcd for $C_{16}H_6Cl_4N_4Se_2$: C 34.69, H 1.09, N 10.11; found C 34.52, H 1.11, N 9.89.

MTT assay [19]

MDA-MB-435, A549, MDA-MB-231, SiHa, or HeLa cells were grown in 96-well plates (3,000 cells per well) and maintained in 10 % FBS/RPMI 1640 medium at 37 °C for 24 h. After cell attachment, the tested cells were treated with either DMSO alone as negative control or varying concentrations of tested drug. After 72 h of treatments, the inhibition effects of title compounds on tested cells were measured using an ELISA plate reader (BioTek-Synergy HT). The experiment was done three times independently. As a result, the inhibition rates of title compounds against tested tumor cells were measured using the following formula.

$$\text{Inhibition rate}\,(\%) = (1 - \frac{\text{experiment value}}{\text{negative control value}}) \times 100\%$$

CONCLUSIONS

A series of diquinazoline diselenide compounds was prepared with substituted 4-chloroquinazoline and sodium diselenide. Based on all findings, we concluded that the title compounds possessed wide-spectrum anticancer activity in MDA-MB-435, A549, MDA-MB-231, SiHa, and HeLa cell lines. Furthermore, compared with the commercial anticancer drugs Gefitinib, Oxaliplatin, Taxol, 10-Hydroxycamptothecin, and Epirubicin Hydrochloride, 1a also exerted better antitumor effects on the corresponding cell lines at 10 μM.

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