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Synthesis and bioactivity of 4-alkyl(aryl)thioquinazoline derivatives

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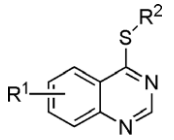
Abstract—Some S′-substituted 4-alkyl(aryl)thioquinazoline derivatives were synthesized through thioetherification reaction of 4-chloroquinazolines **2** and thiol compounds **1** refluxed in acetone in the presence of K_2CO_3. Their structures were verified by elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR. The compounds were evaluated for their anti-proliferative activities against some cancer cells in vitro by MTT method. Among them, **3c**, **3a**, **3d**, **3f**, and **3l** were highly effective against PC3 cells and **3a**–**3m** showed weak activities against Bcap37 and BGC823 cells. The IC_{50} value of **3c**, **3a**, **3d**, **3f**, and**3l** against PC3 cell was 1.8, 5.6, 8.1, 8.7, and 8.9 μM, respectively.

Overexpression of the epidermal growth factor receptor (EGFR) tyrosine kinase is associated with poor prognosis in a significant proportion of human tumors.[1,2] Compounds that inhibit EGFR autophosphorylation and concomitantly EGF-stimulated signal transduction are potentially a new class of anti-cancer drugs.[3,4] The most potent and selective EGFR inhibitors reported to date are the 4-anilino-quinazolines and related 4-anilinopyrido-[*d*]pyrimidines.[5–7] These compounds are reported to bind reversibly at the ATP binding domain of EGFR in clinical trial.[8] On the other hand, recently the synthesis and bioactivity of thioether derivatives have attracted more and more attention, among which some thioether derivatives containing quinazoline moiety with certain anti-tumor activity were reported.[9] However, in our previous work, some thioether derivatives bearing 1, 3, 4-thiadiazole and 3,4,5-trimethoxyphenyl moiety and N′-substituted benzylidene-3,4,5-trimethoxybenzohydrazide and 3-acetyl-2-substituted phenyl-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1,3,4-oxadiazole derivatives were proved to have good anti-tumor bioactivity.[10,11] As a continuation of our research for finding new anti-cancer agents, we designed a series of new 4-alkyl(aryl)thioquinazoline[12] derivatives, which were synthesized starting from gallic acid. The structures of new compounds were confirmed by spectral analysis. The anti-tumor activity of the new compounds was also evaluated by MTT method. The synthetic route to target compounds is shown in Scheme 1.

In order to optimize the reaction conditions for preparation of compounds **3**, the synthesis of **3a** was carried out under different conditions. The effects of different solvents, reaction time, the amount of K_2CO_3, and reaction temperature are summarized in Table 1. First, the effect of different organic phase was investigated. When $CHCl_3$, benzene, and toluene were used, the yields of **3a** were 17.8%, 37.1%, and 53.0%, respectively (Table



3

Scheme 1. **3a**: R^1 = H, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl; **3b**: R^1 = H, R^2 = 3-methoxyphenyl; **3c**: R^1 = 6,7,8-trimethoxyl, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl; **3d**: R^1 = 6,7,8-trimethoxyl; R^2 = 3-methoxyphenyl; **3e**: R^1 = H, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl; **3f**: R^1 = 6,7,8-trimethoxyl, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl; **3g**: R^1 = H, R^2 = allyl; **3h**: R^1 = 6-I, R^2 = Et; **3i**: R^1 = 6-I, R^2 = *n*-Pr; **3j**: R^1 = 6-I, R^2 = allyl; **3k**: R^1 = 6-I, R^2 = *n*-Bu; **3l**: R^1 = 6-I, R^2 = 5-(3,4,5-trimethoxyphenyl)-1, 3,4-oxadiazol-2-yl; **3m**: R^1 = 6-I, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl.

Keywords: Quinazoline; Thioether; Synthesis; Anti-cancer activity; Against ERK phosphorylation.
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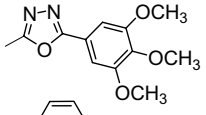
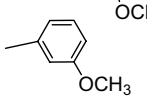
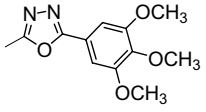
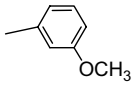
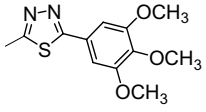
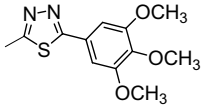
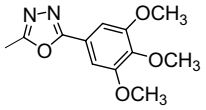
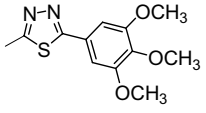
Table 1. Yields of **3a** at different reaction conditions

Entry	Solvent	Time/h	The molar ratio of K_2CO_3 to **1**	Temperature/ °C	Yield/%
1	Acetone	8.0	2:1	Reflux	76.0
2	Benzene	8.0	2:1	Reflux	37.1
3	Toluene	8.0	2:1	Reflux	53.0
4	$CHCl_3$	8.0	2:1	Reflux	17.8
5	Acetone	8.0	0.75:1	Reflux	28.0
6	Acetone	8.0	1:1	Reflux	56.1
7	Acetone	2.0	2:1	Reflux	37.0
8	Acetone	4.0	2:1	Reflux	51.8
9	Acetone	6.0	2:1	Reflux	66.8
10	Acetone	10.0	2:1	Reflux	78.8
11	Acetone	8.0	2:1	rt	20.1
12	Acetone	8.0	2:1	30	56.0
13	Acetone	10	2:1	30	67.6

1, entries 2–4). While it was found that the yield was up to 76.0% when the reaction mixture was refluxed for 8 h in acetone (Table 1, entry 1). In addition, we also examined the effects of the amount of K_2CO_3. When the molar ratio of K_2CO_3 to thio compound **1** increased from 0.75 equiv to 1 equiv, 2 equiv, **3a** could be obtained in 28.0%, 56.1%, and 76.0%, respectively (Table 1, entries 1, 5–6). For the reaction time, the yields of **3a** of 37.0%, 51.8%, 66.8%, and 76.0% were obtained in 2 h to 4 h, 6 h, and 8 h, respectively (Table 1, entries 1, 7–9). When the reaction time was prolonged further to 10 h, no significant improvement (78.8%, entry 10) was obtained, as compared to that of 8 h (76.0 %, entry 1). Also, it could be observed that the yield was significantly lower at room temperature (Table 1, entry 11). When the reaction was carried out at 30 °C, the yield was somewhat lower (56.0% after 8 h, entry 12; 67.6% after 10 h, entry 13) compared to a reflux temperature (entry 1). Hence, the best condition was selected in acetone with a molar ratio of K_2CO_3 to thiol compound **1** as 2:1 at reflux temperature for 8 h.

Table 2. Synthesis of **3a**–**3m**[13]

Entry	Compound	R_1	R_2	Yield[a] (%)
1	**3a**	H		76.0
2	**3b**	H		80.9
3	**3c**	6,7,8-Trimethoxy		67.8
4	**3d**	6,7,8-Trimethoxy		79.0
5	**3e**	H		77.5
6	**3f**	6,7,8-Trimethoxy		70.2
7	**3g**	H	Allyl	83.1
8	**3h**	6-I	Et	80.5
9	**3i**	6-I	*n*-Pr	79.2
10	**3j**	6-I	Allyl	73.1
11	**3k**	6-I	*n*-Bu	72.9
12	**3l**	6-I		71.0
13	**3m**	6-I		68.7

[a] Isolated yields.

With the optimal condition, compounds **3a**–**3m** were prepared by reaction of 4-chloroquinazoline **2** and thiol compound **1**(Table 2).

The anti-tumor activities in vitro of these compounds were evaluated against PC3, BGC823, and Bcap-37 cells by MTT method.[13] The results for title compounds **3** are summarized in Table 3.

It can be found from Table 3 that compounds **3c** (R^1 = 6,7,8-trimethoxyl, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl), **3a** (R^1 = H, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl), **3d** (R^1 = 6,7,8-trimethoxyl; R^2 = 3-methoxyphenyl), **3f** (R^1 = 6,7,8-trimethoxyl, R^2 = 5-(3,4,5-trimethoxyphenyl)-1, 3,4-thiadiazol-2-yl), and **3l** (R^1=6-I, R^2=5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl) have strong inhibitory activity against PC3 cells. The data given in Table 3 indicate that the change of substituents of the quinazoline ring affects the anti-tumor activity. When the 6,7,8-position in quinazoline ring was substituted by trimethoxyl, the compounds generally have potential anti-cancer bioactivity, such as **3c**, **3d** and **3f** with the IC_{50} value of 1.8, 8.1 and 8.7 μM against PC3 cells, respectively. Among these compounds, **3c** (R^1 = 6,7,8-trimethoxy, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2-yl) is much more active against PC3 cells than the other ones and the standard drug (PD 153035). The inhibitory activity, as could be seen from the bioassay data, is generally low for compounds **3b** (R^1 = H, R^2 = 3-methoxyphenyl), **3g** (R^1 = H, R^2 = allyl), **3h** (R^1 = 6-I, R^2 = Et), **3i** (R^1 = 6-I, R^2 = *n*-Pr), **3j** (R^1 = 6-I, R_2=allyl), and **3k** (R^1 = 6-I, R^2 = *n*-Bu). And it could be seen that compounds **3a**–**3m** have weak inhibiting activity against Bcap37 and BGC823 cells. The data given in Table 3 indicate that the changes of R^2 substituents also affect the anti-tumor activity of title compounds **3h**–**3m**. While the compound **3l** (R^1 = 6-I, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2-yl) could inhibit the proliferation of PC3 cells, with IC_{50} value of 8.9 μM, the other compounds **3g**–**3k** have relatively lower anti-tumor activities than that of **3l** (R^1 = 6-I, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl) and **3m** (R^1 = 6-I, R^2 = 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl).

Table 3. Inhibition activity (IC_{50}) of 4-alkylthio(arylthio)-quinazoline derivatives against PC3, Bcap37, and BGC823 cancer cells

Compound[a]	IC_{50}[b] (μM)		
	PC3[c]	Bcap37[d]	BGC823[e]
3a	5.6	23.4	34.5
3b	23.1	56.7	45.6
3c	1.8	20.9	39.0
3d	8.1	45.6	53.2
3e	19.0	50.9	61.2
3f	8.7	34.7	31.2
3g	48.0	67.9	78.1
3h	38.9	39.0	31.1
3i	40.9	42.1	29.0
3j	28.8	39.1	33.2
3k	32.1	31.0	29.0
3l	8.9	31.0	28.9
3m	12.2	21.0	19.0
PD 153035[f]	13.7[g]	8.9	6.9

[a] These compounds were tested as the free base.
[b] IC_{50} concentrations needed to inhibit cell growth by 50% as determined from the dose–response curve. Determination was done in three separate experiments and each was performed in triplicate.
[c] Prostate cancer.
[d] Breast cancer.
[e] Stomach cancer.
[f] The standard compound was made of comparison for activity.
[g] The value was determined by using our assay protocol.

In order to investigate further biological activities of these identified compounds, we carried out bioassay against ERK phosphorylation.[14] We were seeking to determine effects of these compounds on EGF-induced ERK1/2 phosphorylation. PC3 cells were pretreated with 20 μM of these compounds, respectively, for 30 min at 37 °C in serum-free culture media, followed by treatment with 60 ng/ml EGFR for 10 min. Then the cells were lysed and the protein samples were prepared to go through the Western blot assay. The results are shown in Figure 1. The blots, from the left to the right, are in sequence, negative control (control), positive control (EGF), and compound plus EGF (**3a** + E, **3b** + E, **3c** + E, **3d** + E, **3e** + E, **3f** + E, **3g** + E, **3h** + E, **3i** + E, **3j** + E, **3k** + E, **3l** + E, and **3m** + E). It can be seen that compounds **3a**–**3m** had no significant inhibitory effect at 20 μM on EGF-induced ERK1/2 phosphorylation in PC3 cells (Fig. 1). The results of our studies indicate that 4-alkylthio(arylthio)quinazolines (**3a**–**3m**) possess no significant inhibitory activities against EGFR, suggesting that cytotoxicity may not result from inhibiting EGFR.

In summary, we described a practical and efficient procedure for preparing 4-alkyl(aryl)thioquinazoline derivatives through thioetherification of 4-chloroquinazoline **2** and thiol compounds **1**. The reaction is experimentally simple with moderate yield. In addition, among the synthesized compounds, **3c** is highly effective against PC3

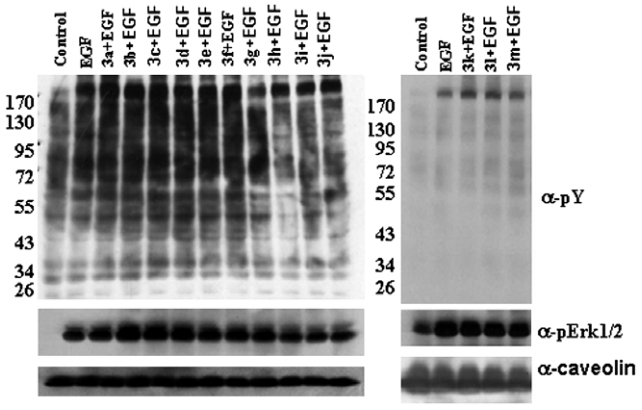


Figure 1. Inhibitory activity of compounds **3a**–**3m** against EGFR phosphorylation in PC3 cells induced by EGF. PC3 cells were cultured in 6-well plates to 100% confluence pretreated, respectively, with 20 μM compounds **3a**–**3m** for 60 min, and stimulated by EGF (60 ng/mL) for 10 min. After treatment, the cells were directly dissolved in SDS sample buffer and proteins were separated using SDS–PAGE, transferred to PVDF membrane, and blotted with anti-phosphotyrosine antibody, anti-pErk1/2 antibody or anti-caveolin antibody.

cells, **3a**, **3d**, and **3f** are moderately effective against PC3 cells. Moreover, **3a**–**3m** are weakly effective against Bcap37 and BGC823 cells. These identified 4-arylthioquinazolines containing 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole moiety can be very useful in the development of optimization strategies for cancer chemotherapy.

Acknowledgments

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12. General experimental procedure for the synthesis of 4-alkyl(aryl)thioquinazolines **3**. To the mixture of thiol compounds **1** (1.75 mmol) and 4-chloroquinazoline **2** (1.75 mmol) in acetone (10 mL) was added K_2CO_3 (3.50 mmol). The mixture was then refluxed for 8 h. After cooling, the crude product was obtained by filtration and recrystallized from ethanol to afford **3a**–**3m** as white solids. Their physicochemical properties and the spectral data can be found in supporting information.
13. MTT assay against cancer cell proliferation. All tested compounds were dissolved in DMSO (1–100 μM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density 2×10^3 cells/well/100 μL of the proper culture medium and treated with the compounds at 1–100 μM for 72 h. In parallel, the cells treated with 0.1% DMSO served as control. An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay (Roche Molecular Biochemicals, 1465-007) was performed 30 h later according to the instructions provided by Roche. This assay is based on the cellular cleavage of MTT into formazan which is soluble in cell culture medium. And the absorbance caused by formazan was measured at 595 nm with a microplate reader (Bio-Rad, model 680), which is directly proportional to the number of living cells in culture. Three types of cells were used in these assays, PC3 (prostate cancer), BGC 823 (human gastric cancer), and Bcap37 (breast cancer) cell lines, provided by ATCC and cultivated in RPMI 1640 (for PC3, BGC823, and Bcap37) supplemented with 10% fetal bovine serum. Tissue culture reagents were obtained from Gibco BRL. Skehan, P.; Storeng, R.; Scadiero, D.; Monks, A.; McMahon, I.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boy, M. R. *Natl. J. Cancer Inst.* **1990**, *82*, 1107.
14. Cell culture and protein sample preparation: PC3 cells (prostate cancer cell lines) were seeded on a 6-well plate and were incubated in RPMI 1640 medium tamine plus 10% FBS at 37 °C. After incubation for 36–48 h, the RPMI 1640 medium was removed and the cells were incubated with serum-free medium for 24 h. Then cells were treated with the compounds at the concentration of 20 μM for 60 min followed with 60 ng/mL EGF for 10 min. The plate was then placed on the ice instantaneously to quench the phosphorylation process. Medium was sucked out and cells were then rinsed with ice-cold PBS buffer twice. Then cells were treated with lysing buffer (1%NP-40, 0.1% SDS, 150 mM NaCl, 10 mM Tris–HCl, 1 mM EDTA, 0.6 mM Na_3VO_4, 10 mM NaF, 10 mM β-glycerophosphate, 1 mM DTT, 10 μg/mL leupeptin, 10 μg/ml pepstatin, and 40 μg/ml PMSF) and sample buffer, respectively, followed by immuno-blotting using P-ERK (E-4) (sc-7383, lot# J0803, Santa Cruz Biotechnology). Western blot analysis: The cell lysates prepared above were subjected to 10% SDS–PAGE and proteins were transferred to PVDF membranes (Bio-Rad). The membrane was blocked with 5% nonfat dried milk freshly made in PBS plus 0.2% Tween 20, then incubated with monoclonal antibody (anti-phosphotyrosine, anti-pErk1/2 or anti-caveolin) over night at 4 °C. Then the membrane was washed for 3×5 min with PBS plus 0.2% Tween 20. The membrane was incubated again with second antibody for 2–3 h at 25 °C, washed three times with PBS plus 0.2% Tween 20, and the signal was detected by enhanced chemical luminescence (ECL) detection system (PIERCE). Egger, D.; Bienz, K. *Mol. Biotech.* **1994**, *1*, 289.