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Synthesis and bioactivities of 6,7,8-trimethoxy-*N*-aryl-4-aminoquinazoline derivatives

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Abstract—A series of 4-aminoquinazoline derivatives is prepared by the nucleophilic substitution reaction of 6,7,8-trimethoxy-4-chloroquinazoline and aryl amine. The structures of the compounds are confirmed by elemental analysis, IR, and ^{1}H NMR spectral data. The compounds are also evaluated for their ability to inhibit tumor cells PC3, A431, Bcap-37, and BGC823 by MTT assays. Among them, **6b** and **6e** are found as potent inhibitors, with IC_{50} values ranging from 5.8 to 9.8 μM, in vitro assay.

1. Introduction

Many components of mitogenic signaling pathways in normal and neoplastic cells have been identified. These include the large family of protein kinases, which function as components of signal transduction pathways, playing a central role in diverse biological processes, such as control of cell growth, metabolism, differentiation, and apoptosis.[1–3] The development of selective protein kinase inhibitors that can block or modulate diseases caused by abnormalities in these signaling pathways is widely considered as a promising approach for drug development. Due to their deregulation in human cancers, protein kinases, such as EGF, PDGF, belonging to the epidermal growth factor-receptor family are considered as prime targets for the development of selective inhibitors. Therefore, tyrosine kinases are expected to be promising targets for cancer chemotherapy.[4,5] The development of specific inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase has received wide attention, and several elaborations of the fundamental 4-anilinoquinazoline pharmacophore have now been reported as potent and selective inhibitor of this class of enzymes.[6–8] With growing interest in quinazoline compounds, their synthesis and biological studies have assumed enormous importance. Certain compounds containing 3,4,5,-trimethoxyphenyl moiety have been reported to be biologically active, for example, Combretastatin A-4 (**CA-4**) is an excellent substance for antitumor activity, which was first isolated from the South African bush willow tree *Combretum caffrum*. Due to its structural simplicity and potent cytotoxicity, many synthetic analogues of CA-4 have been developed.[9–11] The existing study has revealed that the group of 3,4,5-trimethoxyphenyl is a crucial pharmacophore for antitumor activities of CA-4 analogues.[12] 4-alkyl(aryl)thio-quinazoline derivatives reported by us earlier were shown to have antitumor effects.[13] and that 4-anilinoquinazoline pharmacophore has been shown to be effective inhibitors of EGF and PDGF.[14,15] We reasoned that the compounds containing both active groups might generate a new group of biologically active compounds.

Therefore, in continuation to our research for finding new anticancer agents, we have synthesized new quinazoline compounds (**6a**–**6s**) with the methoxy (OCH_3) groups present in the phenyl ring. Starting from gallic acid, the synthetic route to 6,7,8-tri-methoxy-*N*-aryl-4-aminoquinazoline derivatives is shown in Schemes 1 and 2. The structure of title compounds is characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. Following the reported method,[16,17] the synthetic method under microwave irradiation is rapid and high yielding. While in classical method, an yield of 19.9–66.2% is

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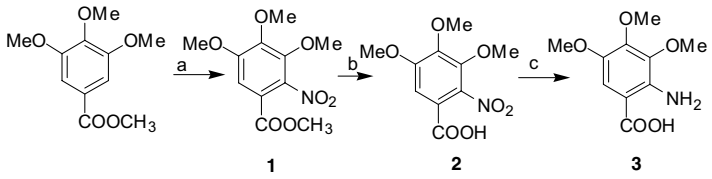
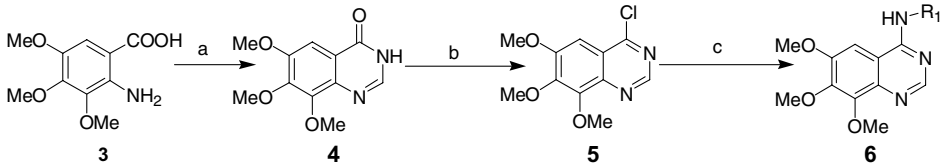


Scheme 1. Synthesis of 2-amino-3,4,5,-trimethoxybenzoic acid. Reagents and conditions: (a) nitric acid, 40–50 °C, 1–2 h; (b) 0.8 mol/L sodium hydroxide, 95% ethanol, 45–50 °C, 1–2 h; 35% HCl, pH 2–3; (c) Sn, HCl, 20 °C, 4–5 h, then 80 °C, 20 min.



Scheme 2. Synthesis of *N*-arylsubstituted-4-aminoquinazoline derivatives. Reagents and conditions: (a) formamide, 120–140 °C, 5–6 h; (b) $POCl_3$, DMF, 100–105 °C, 2–3 h; (c) aryl amine, 2-propanol, MW, 50 W, 80 °C, 10 min.

achieved in 4–24 h, a considerable improvement in yield (55.6–92.5%) is noticed in just 10 min under microwave condition.

The compounds were evaluated for their antiproliferative activities against PC3, A431, Bcap-37, and BGC823 cells hyperplasia in vitro by MTT method. Among them, **6a** and **6d** were found highly effective against PC3 cells and **6e** showed moderate activities against Bcap37 and BGC823 cells. The IC_{50} values of potent inhibitors **6b** and **6e** against PC3 cells were 9.8, 8.9 μM, respectively.

2. Chemistry

Compound **3** was synthesized from gallic acid as described in Scheme 1. O-methylation of the phenol with dimethyl sulfate followed by esterification using methanol produced methyl 3,4,5-trimethoxybenzoate. Reaction of the ester with concentrated 70% nitric acid in acetic acid at 40–50 °C gave the methyl 2-nitro-3,4,5-trimethoxybenzoate **1.** Hydrolysis of this nitro ester followed by reduction of the nitro group by tin dichloride at 80 °C generated 2-amino-3,4,5-trimethoxybenzoic acid. Cyclization of the latter by formamide and subsequent chlorination by phosphorus oxychloride provided 4-chloro-6,7,8-trimethoxyquinazoline (Scheme 2). The desired products (**6a**–**6s**) were then obtained under microwave irradiation by a nucleophilic substitution reaction involving an aryl amine and the chloro-quinazoline. The results of microwave reaction are compared with those obtained under classical reactions (Table 1).

It can be seen that with one step microwave assisted approach, not only is the reaction time significantly reduced from 4–24 h to 10 min, but the yields are also considerably improved (55.6–92.5% with microwave against 19.9–66.2% with classical method).

In order to optimize the reaction parameters, we selected compound **6f** and subjected it to further study under different conditions. The results are shown in Table 2. Without microwave irradiation (Table 1) compound **6f** could be obtained in 63.4% after 12 h. When the reaction was carried out under microwave irradiation at 80 °C, the yield of **6f** was increased to 80.0% in 5 min and to 86.0% in 10 min. With longer reaction time (Table 2, entry 3), slight lowering in the yield was observed and might be attributed to the formation of some byproduct. On increasing the microwave power from 40 to 50, 80, and 100 W, 70.9%, 86.0%, 88.0%, and 87.2% yields were obtained, respectively (Table 2, entries 2 and 4–6). No significant-improvement was thus noticed at higher power settings when compared to that at 50 W. When the reaction temperature was increased

Table 1. Reaction conditions used for the microwave assisted synthesis of **6a**–**6s**

Product	Microwave method[b]		Classical method[c]	
	Reaction time (min)	Yield[a] (%)	Reaction time (h)	Yield [a] (%)
6a	10	80.9	4	51.7
6b	10	78.0	5	46.8
6c	10	80.1	5	61.2
6d	10	59.0	5	19.9
6e	10	89.0	5	65.6
6f	10	86.0	12	63.4
6g	10	92.5	12	36.1
6h	10	82.3	12	55.4
6i	10	79.0	24	63.8
6j	10	89.6	12	66.2
6k	10	89.9	8	64.4
6l	10	75.4	12	54.3
6m	10	58.6	12	34.5
6n	10	55.6	24	32.7
6o	10	77.7	24	64.0
6p	10	80.3	5	41.9
6q	10	79.5	8	58.0
6r	10	78.8	24	48.9
6s	10	80.2	24	61.4

[a] Yields of isolated products.
[b] Reaction conditions: *i*-PrOH, reflux under MW (50 W power).
[c] Reaction conditions: *i*-PrOH, reflux temperature.

Table 2. Different conditions used for the microwave assisted synthesis of **6f**

Entry	Reaction time (min)	Power (W)	Reaction temperature (°C)	Yield[a] (%)
1	5	50	80	80.0
2	10	50	80	86.0
3	20	50	80	84.5
4	10	40	80	70.9
5	10	80	80	88.0
6	10	100	80	87.2
7	10	50	30	56.9
8	10	50	50	77.0
9	10	50	70	80.9

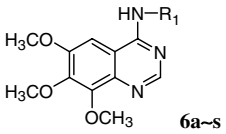
[a] Yields of isolated products. Each reaction was repeated three times and the average yield was recorded.

from 30 °C to 50 °C, 70 °C, and 80 °C, **6f** was obtained in 56.9%, 77.0%, 80.9%, and 86.0% yields, respectively (Table 2, entry 2 and entries 7–9). So, under optimal condition, when reacted with the appropriate amine and 4-chloro-6,7,8-trimethoxyquinazoline, **6f** can be obtained in 86.0% yield with a microwave irradiation at 50 W for 10 min at 80 °C.

3. Anticancer activity

Some potential compounds for treatment of cancer have been identified by studying their anti-cancer activities on 3 human tumor cells. Series of the test compounds including **6a–6s** and their commercial analogue

Table 3. Growth inhibition of selected cell lines



6a~s

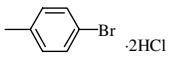
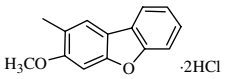
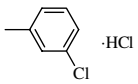
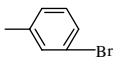
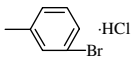
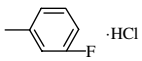
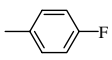
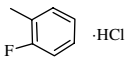
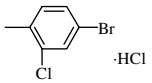
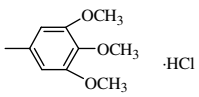
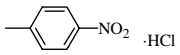
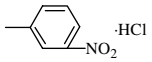
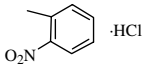
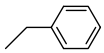
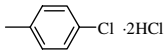
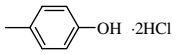
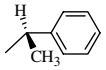
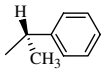
Compound[a]	R_1	IC_{50}[b] (μM)			
		Bcap-37[c]	PC3[d]	A431[e]	BGC823[f]
6a		31.2	23.4	42.1	23.8
6b		8.1	9.8	9.0	9.9
6c		21.0	19.0	32.1	19.0
6d		21.0	12.6	11.0	12.1
6e		12.3	8.9	7.6	5.8
6f		22.1	25.3	29.7	28.9
6g		21.0	35.6	29.7	29.9
6h		28.7	24.5	29.0	39.6
6i		63.2	18.8	72.1	36.7
6j		89.0	21.0	51.5	31.0
6k		76.5	30.0	65.4	23.1

Table 3 (*continued*)

Compound[a]	R_1	IC_{50}[b] (μM)			
		Bcap-37[c]	PC3[d]	A431[e]	BGC823[f]
6l		28.9	25.5	45.2	32.2
6m		31.1	24.4	37.7	39.0
6n		29.9	31.2	31.0	21.0
6o		65.4	36.7	21.2	37.7
6p		52.2	31.0	19.0	34.4
6q		45.5	20.1	22.2	35.4
6r		23.2	18.8	21.0	12.0
6s		12.9	10.0	12.0	11.9
PD 153035[g]		13.7[h]	8.9	6.9[i]	16.8

[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. Determined by three separate experiments and each was performed in triplicate.
[c] Breast cancer.
[d] Prostate cancer.
[e] Uterus cancer.
[f] Stomach cancer.
[g] The standard compound used for comparison of activity.
[h] The value was determined by using our assay protocol.
[i] The IC_{50} value of 3.0 μM for A431 cells reported by Ras et al.[19]

PD153035 [6,7-dimethoxy-*N*-(3-bromophenyl)-4-aminoquinazoline][18] were evaluated for their anti-proliferation activities against four types of human cancer cell lines, breast cancer, prostate cancer, uterus cancer, and stomach cancer cells (Table 3).

As shown in Table 3, the nature of group attached at the N-atom present at the side chain of 4 position of quinazoline ring greatly influences the anti-tumor properties of these compounds. The compounds **6a**, **6c**, **6f**–**6q**, and **6r** showed weak to moderate anti-tumor activities against all the tested tumor cells. Compounds **6b**, **6e**, and **6s** bearing 2-methoxydibenzofuran-3-yl, 3-bromophenyl, (*S*)-α-methylbenzyl at the 4-position in quinazoline show best anti-tumor activities with average IC_{50} ranging from 5.8 to 12.9 μM over all the cancer cell lines. Remarkably, these values are comparable to the average IC_{50} of PD 153035 (6.9–16.8 μM). It is clear that the cell growth inhibition bioactivity of some title compounds, such as **6b**, and **6e**, is better than their commercial analogue PD153035. For example, the structure of **6b** and PD153035 is totally similar except that PD153035 contains 6,7-dimethoxy substitution while **6b** is 6,7,8-trimethoxy derivative, and the cell growth inhibition activity of **6b** is better than that of PD153035. An interesting observation noted was that in case of 6,7,8-trimethoxy-*N*-(3-bromophenyl)-4-aminoquinazoline, the hydrochloride salt of the amine (**6e**) was more effective than the free amine (**6d**). Compound **6d** has the average IC_{50}values ranging from 12.1 to 21.0 μM which are considerably lower than those of compound **6e**. This finding is thought to be beneficial for further SAR studies of quinazoline analogues.

In order to evaluate biological activity of these identified compounds, we carried out several ERK phosphorylation experiments. First, we sought to determine effects of these compounds on (epidermal growth factor) EGF-induced ERK1/2 phosphorylation. PC3 cells were pretreated with 20 μM of these compounds, respectively, for 60 min at 37 °C in media with serum, followed by treatment with 40 ng/mL EGF for 10 min. The result

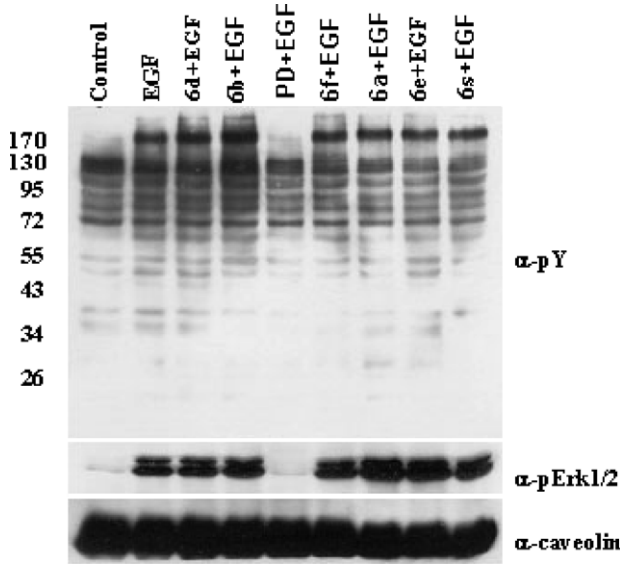


Figure 1. Effect of quinazoline derivatives on phosphorylation of EGFR and ERK 1/2 triggered by EGF.

is shown in Figure 1. The blot was in sequence, negative control (control), positive control (EGF), and compound plus EGF (**6d+E**, **6b+E**, **PD153035+E**, **6f+E**, **6a+E**, **6e+E**, and **6s+E**). It can be seen that the compounds **6a**, **6b**, **6d**, **6e**, **6f**, and **6s** at 20 μM had no significant inhibitory effect on EGF-induced ERK1/2 phosphorylation in PC3 cells. Results in Figure 1 indicate that **PD153035**at 20 μM blocked ERK1/2 phosphorylation in PC3 cells induced by EGF.

The preliminary bioassay demonstrated that these compounds possess no significant inhibitory activities against EGFR, while the 4-anilinoquinazolines usually do, suggesting that cytotoxicity may not result from inhibiting EGFR and there is probably a new mechanism which accounts for their activity.

PC3 cells cultured in 12-well were pretreated with quinazoline derivatives (20μM) for 60 min followed by treatment with EGF (60 ng/mL for 10 min). Cells were harvested, resuspended in 2× sample buffer (Tris–HCl, pH 7.4, 4% SDS, glycerine, glycerol 20%, bromophenol blue 1%, and β-mercaptoethanol 5%), sonicated, and denatured at 90 °C water for 5 min. Proteins were separated on 10% SDS–PAGE and subjected to Western blot analyses. Antibodies used for Western blot analyses were anti-phosphotyrosine, anti-pErk1/2, and anti-caveolin.

4. Conclusion

In summary, the current method offers a novel approach for the preparation of new *N*-aryl-4-aminoquinazoline derivatives **6a**–**6s** under microwave irradiation. The synthesis from gallic acid is rapid and provides higher yield as compared to the classical method. All compounds are fully characterized by spectroscopic methods. The compounds **6b** and **6e** were found to be potent inhibitors of tumor cells, with IC_{50} values ranging from 5.8 to 9.8 μg/mL in vitro assay.

5. Experimental

5.1. Analysis and instruments

Unless otherwise stated, all common reagents and solvents were used as obtained from commercial suppliers without further purification. All melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and are not corrected. The infrared spectra were recorded on a Bruker VECTOR22 spectrometer in KBr disks. ^{1}H NMR (solvent DMSO-d_6) and ^{13}C NMR spectra (solvent DMSO-d_6) were recorded on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. D_2O exchange was used to confirm the assignment of NH proton signals. Elemental analysis was performed by an Elementar Vario-III CHN analyzer. Microwave reactions were performed on a variable power Focused Microwave Synthesis, Discover™ LabMate equipped with a high sensitivity IR sensor for temperature control and measurement.

5.2. Preparation of methyl 2-nitro-3,4,5-trimethoxybenzoate (1)

To a three-necked 100 mL round-bottom flask equipped with a magnetic stirrer bar were added methyl 3,4,5-trimethoxybenzoate[20] and 35% acetic acid (10 mL). The solution was stirred at 10–30 °C on an ice bath and 70% concentrated nitric acid (2.5 mL) was added dropwise over a period of 30 min. The mixture was diluted with cold water (60 mL), and the resulting precipitate was filtered and washed well with water, dried to give compound as pale yellow solid (0.61 g), yield, 25.4%; mp 62–63 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.83 (s, 3H, $COOCH_3$), 3.94 (t, 9H, $3OCH_3$, J = 25 Hz), 7.34 (s, 1H, Ph-H); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 53.7, 57.1, 61.6, 63.1, 109.2, 117.7, 139.6, 145.5, 146.2, 154.7, 163.3; IR (KBr): 866.0, 1112.9, 1232.5, 1344.4, 1496.8, 1544.9, 1579.7, 2846.9, 2962.7, 3012.8 cm^{-1}; Anal. Calcd for $C_{11}H_{13}NO_7$: C, 48.71; H, 4.83; N, 5.16. Found: C, 48.47; H, 5.02; N, 4.81.

5.3. Preparation of 2-nitro-3,4,5-trimethoxybenzoic acid (2)

To a 250 mL three-necked-round-bottom flask equipped with a magnetic stirrer were added methyl 2-nitro-3,4,5-trimethoxybenzoate (14.0 g, 50 mmol) and 0.8 mol/L sodium hydroxide solution (120 mL, 100 mmol) in 95% ethanol (60 mL). The mixture was stirred at 45–50 °C for 1–2 h and the course of the reaction was followed by TLC (eluent: petroleum/ethyl acetate, 1:1, v:v). After the completion of the reaction, the mixture was cooled to room temperature and acidified with 35% HCl solution until a pH of 2–3 was obtained. The resulting precipitate was filtered and washed with water, dried to give compound as pale yellow solid (1.2 g), yield, 92.3%; mp 160–161 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.88 (t, 9H, $3OCH_3$, J = 25 Hz), 7.32 (s, 1H, Ph-H); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.0, 61.5, 63.1, 109.3, 118.8, 139.8, 145.3, 145.8, 154.5, 164.1; IR (KBr): 725.2, 1116.8, 1246.0, 1340.5, 1496.8,

1546.9, 1577.8, 1695.4, 2850.8, 3107.3 cm^{-1}; Anal. Calcd for $C_{10}H_{11}NO_7$: C, 46.70; H, 4.31; N, 5.45. Found: C, 46.77; H, 4.14; N, 5.32.

5.4. Preparation of 2-amino-3,4,5-trimethoxybenzoic acid (3)

To a 100 mL three-necked-round-bottom flask equipped with a magnetic stirrer was added Sn powder (5.3 g, 45 mmol) in concentrated HCl (18 mL) and stirred at 20 °C for 4–5 h until a homogeneous solution was obtained. 2-Nitro-3,4,5-trimethoxybenzoic acid (2.6 g, 10 mmol) was then added and the resulting mixture was heated at 80 °C for 20 min. Concentrated HCl (5 mL) solution was added and again stirred for 10 min. The mixture was cooled to 0–10 °C and white solid was filtered and washed with concentrated HCl. The solid was then neutralized with 10% K_2CO_3 solution until a pH of 9–10 was obtained. The resulting mixture was stirred for 1–2 h and filtered to give a gray solid. The solid was again treated with 10% K_2CO_3solution, acidified with 30% HOAc solution to adjust the pH in the range 2–3. The resulting precipitate was filtered and washed with water, dried to give compound as white solid (1.4 g), yield, 61.7%; mp126–128 °C;^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.82 (t, 9H, $3OCH_3$ J = 25 Hz), 3.45 (s, 2H, NH_2), 7.07 (s, 1H, Ph-H); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.6, 60.6, 60.9, 104.5, 110.9, 140.4, 141.6, 142.9, 147.6, 169.4; IR (KBr): 744.5, 1149.6, 1273.0, 1458.2, 1575.8, 1670.4, 3200.0, 3379.3, 3487.3, 3379.3 cm^{-1}; Anal. Calcd for $C_{10}H_{13}NO_5$: C, 52.86; H, 5.77; N, 6.16. Found: C, 52.74: H, 5.43; N, 6.02.

5.5. Preparation of 6,7,8-trimethoxyquinazoline-4-one (4)

To a 100 mL three-necked-round-bottom flask equipped with a magnetic stirrer were added 2-amino-3,4,5-trimethoxybenzoic acid (1.1 g, 5 mmol) and formamide (2 mL). The resulting solution was refluxed at 120–140 °C for 5–6 h, then cooled to 100 °C. Water (10 mL) was added dropwise, cooled to 60 °C, and again another 10 mL of water was added in one shot. The reaction mixture was brought to room temperature, filtered to give a gray solid (0.9 g), which was recrystallized from anhydrous ethanol to finally afford a white solid (0.3 g), yield, 25.4%; mp 220–222 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.88 (t, 9H, $3OCH_3$, J = 25 Hz), 7.35 (s, 1H, H-5 of quinazolone), 8.01 (s, 1H, H-2 of quinazolone),12.21 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.4, 61.4, 62.4, 101.7, 119.2, 139.0, 143.3, 147.7, 148.3, 152.7, 160.6; IR (KBr): 798.5, 1126.4, 1286.5, 1473.6, 1612.5, 1664.6, 3163.3, 3200.0, 3304.1 cm^{-1}; Anal. Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.86. Found: C, 56.48; H, 5.18; N,12.04.

5.6. Preparation of 4-chloro-6,7,8-trimethoxyquinazoline (5)

A mixture of 6,7,8-trimethoxyquinazoline-4-one (0.6 g, 2.5 mmol) in $POCl_3$(25 mL) and DMF (0.12 mL) was stirred under reflux at 100–105 °C for 2–3 h. The solvent was then removed under reduced pressure. The residue was added into chloroform (20 mL). The mixture was poured into ice water (30 g), neutralized with saturated $NaHCO_3$ solution to a pH of 4–5, and filtered. The mother liquor was extracted with chloroform (2× 20 mL), and the combined organic layers were dried over anhydrous magnesium sulfate and filtered again. After removal of the chloroform under reduced pressure, the gray solid was recrystallized from petroleum ether to afford a brown solid (0.4 g), yield, 62.5%; mp100–103 °C;^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.93 (s, 1H, H-2 of quinazoline), 7.29 (s, 1H, H-5 of quinazoline), 4.01 (t, 9H, $3OCH_3$, J = 25 Hz); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.3, 61.1, 62.2, 98.6, 120.1, 142.7, 146.3, 148.1, 150.9, 154.3, 158.9; IR (KBr): 786.9, 1136.1, 1246.0, 1467.8, 1602.9, 2845.0, 2945.3, 3099.6 cm^{-1}; Anal. Calcd for $C_{11}H_{12}N_2O_4$: C, 51.88; H, 4.35; N, 11.00. Found: C, 52.02; H, 4.21; N, 10.94.

5.7. General procedure for 6,7,8-trimethoxy-*N*-aryl-4-aminoquinazolines (6a–6s) (microwave method)

A mixture of 4-chloro-6,7,8-trimethoxyquinazoline (4.0 mmol) and aryl amine (4.0 mmol) in 2-propanol (10 mL) was stirred for three minutes, then the mixture was irradiated in the microwave oven at 50 W for 10 min at 80 °C. Upon completion of the reaction, as monitored by TLC, the solvent was removed under reduced pressure and the residue was washed with water, filtered off, and purified by silica gel column chromatography (petroleum ether/ethyl acetate, 5:1, v:v) to give the title compounds.

5.7.1. General procedure for 6,7,8-trimethoxy-*N*-aryl-4-amino quinazolines (6a–6s) (classical method). A solution of 4-chloroquinazoline (4.0 mmol) and aryl amine (4.0 mmol) in 2-propanol (100 mL) was stirred under reflux for 4–24 h. The work-up was carried out as described for the microwave method.

5.7.2. 6,7,8-Trimethoxy-*N*-(4-bromophenyl)-4-aminoquinazoline hydrochloride (6a). Pale yellow needles, yield, 80.9%; mp170–173 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, $3OCH_3$, J = 15 Hz), 7.71 (s, 4H, Ph-H), 8.14 (s, 1H, H-5 of quinazoline) 8.76 (s, 1H, H-2 of quinazoline), 11.53 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.8, 61.9, 62.7, 100.6, 109.9, 127.3, 132.2, 136.5, 142.1, 148.2, 149.6, 154.5, 158.9; IR (KBr): 804.3, 1128.4, 1288.5, 1489.1, 1627.9, 2847.0, 2947.2, 3010.9, 3200.0, 3400 cm^{-1}; Anal. Calcd for $C_{17}H_{18}BrCl_2N_3O_3$: C, 44.09; H, 3.92; N 9.07. Found: C, 43.82; H, 4.08; N, 9.04.

5.7.3. 6,7,8-Trimethoxy-*N*-(2-methoxydibenzofurane-3-yl)-4-aminoquinazoline hydrochloride (6b). Pale yellow needles; yield, 78.0%; mp160–162 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.04 (t, 12H, $4OCH_3$, J = 55 Hz), 7.47 (t, 1H, H-6 of dibenzofurane, J = 10 Hz), 7.55 (t, 1H, H-7 of dibenzofurane, J = 15 Hz), 7.72 (d, 1H, H-8 of dibenzofurane, J = 7.45 Hz), 7.88 (s, 1H, H-4 of dibenzofurane), 8.03 (s, 1H, H-1 of dibenzofurane), 8.09 (s, 1H, H-5 of quinazoline), 8.22 (d, 1H, H-5 of dibenzofurane, J = 7.45 Hz), 8.68 (s, 1H, H-2 of quinazoline), 11.45 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.3,

56.9, 61.3, 62.0, 99.8, 103.6, 108.6, 111.4, 111.7, 121.3, 123.1, 123.3, 123.5, 127.7, 141.4, 145.3, 147.7, 148.9, 150.9, 154.0, 156.3; IR (KBr): 763.8, 1124.5, 1286.5, 1473.6, 1626.0, 2850.8, 2949.2, 3010.9, 3200.0, 3600 cm^{-1}; Anal. Calcd for $C_{25}H_{27}Cl_2N_3O_5$: C, 57.70; H, 5.23; N, 8.07. Found: C, 57.50; H, 5.03; N, 8.16.

5.7.4. 6,7,8-Trimethoxy-*N*-(3-chlorophenyl)-4-aminoquinazoline hydrochloride (6c). Pale yellow needles, yield, 80.1%, mp 172 °C (dec); ^1H NMR (DMSO-d_6, 500 MHz) δ: 4.02 (t, 9H, $3OCH_3$, J = 15 Hz), 7.41 (d, 1H, H-6 of Ph-H, J = 9.15), 7.53 (t, 1H, H-5 of Ph-H, J = 5 Hz), 7.71 (d, 1H, H-4 of Ph-H, J = 9.2 Hz), 7.90 (s, 1H, H-2 of Ph-H), 8.81 (s, 1H, H-2 of quinazoline), 11.43 (s, 1H, NH);^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.1, 61.2, 62.0, 99.8, 109.3, 123.0, 124.2, 126.2, 130.4, 132.8, 138.1, 147.6, 149.2, 153.9, 158.4; IR (KBr): 785.0, 1126.4, 1288.5, 1487.1, 2846.9, 2947.2, 3012.8, 3012.8, 3600.0 cm^{-1}. Anal. Calcd for $C_{17}H_{17}Cl_2N_3O_3$: C, 53.42; H, 4.48; N, 10.99. Found: C, 53.49; H, 4.60; N, 11.00.

5.7.5. 6,7,8-Trimethoxy-*N*-(3-bromophenyl)-4-aminoquinazoline (6d). Pale yellow needles, yield, 59.0%, mp126–127 °C; ^1H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, $3OCH_3$, J = 15 Hz), 7.46 (t, 1H, H-5 of Ph-H, J = 20.0 Hz),11.51 (s, 1H, NH), 7.54 (d, 1H, H-6 of Ph-H, J = 8.05 Hz), 7.76 (d, 1H, H-4 of Ph-H, J = 8.05 Hz), 8.02 (s, 1H, H-5 of quinazoline), 8.13 (d, J = 4.0 Hz, 1H, H-2 of Ph-H), 8.81 (s, 1H, H-2 of quinazoline); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.7, 61.9, 62.7, 100.6, 109.9, 121.8, 124.1, 127.7, 129.7, 131.3, 138.8, 148.2, 149.8, 154.5,159.0; IR (KBr): 788.9, 1126.4, 1288.5, 1487.1, 1627.9, 2852.7, 2941.4, 3010.9, 3307.9 cm^{-1}; Anal. calcd for $C_{17}H_{16}BrN_3O_3$: C, 52.32; H, 4.13; N, 10.77. Found: C, 52.49; H, 4.00; N, 10.90.

5.7.6. 6,7,8-Trimethoxy-*N*-(3-bromophenyl)-4-aminoquinazoline hydrochloride (6e). Pale yellow needles, yield, 89.0%; mp 180 °C (dec); ^1H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, $3OCH_3$, J = 5 Hz), 7.46 (t, 1H, H-5 of Ph-H, J = 15 Hz), 7.54 (d, 1H, H-6 of Ph-H, J = 8.0 Hz), 7.77 (d, 1H, H-4 of Ph-H, J = 8.0 Hz), 8.03 (s, 1H, H-2, of Ph-H), 8.20 (s, 1H, H-5 of quinazoline), 8.81 (s, 1H, H-2 of quinazoline),11.62 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz) δ 57.2, 61.3, 62.1, 100.2, 109.3, 121.2, 123.6, 127.1, 129.1, 130.7, 138.8, 141.5, 147.7, 149.1, 154.0, 158.5; IR (KBr): 786.9, 1126.4, 1286.5, 1487.1, 1626.0, 2848.9, 2945.3, 3010.9, 3600.0 cm^{-1}; Anal. Calcd for $C_{17}H_{17}BrClN_3O_3$: C, 47.85; H, 4.02; N, 9.85. Found: C, 47.80; H, 4.09; N, 9.75.

5.7.7. 6,7,8-Trimethoxy-*N*-(3-fluorophenyl)-4-aminoquinazoline hydrochloride (6f). Pale yellow needles, yield, 86.0%; mp 232–234 °C; ^1H NMR (DMSO-d_6, 500 MHz): δ 4.03 (t, 9H, $3OCH_3$, J = 15 Hz), 7.20 (t, 1H, H-2 of Ph-H, J = 8.0 Hz), 7.54 (q, 1H, H-5 of Ph-H, J = 15 Hz), 7.73 (d, 1H, H-6 of Ph-H, J = 8.0 Hz), 7.73 (d, 1H, H-4 of Ph-H, J = 10.9 Hz), 8.21 (s, 1H, H-5 of quinazoline), 8.81 (s, 1H, H-2 of quinazoline), 11.63 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz) δ 57.8, 61.9, 62.7, 100.7, 109.9, 112.3, 112.5, 121.1, 130.9, 131.0, 148.3, 149.7, 154.6, 159.0, 161.4; IR (KBr): 1134.1, 1288.5, 1489.1, 1627.9, 2852.6, 2951.1, 3012.8, 3419.8 cm^{-1}; Anal. Calcd for $C_{17}H_{17}ClFN_3O_3$: C, 55.82; H, 4.68; N, 11.49. Found: C, 55.77; H, 4.60; N, 11.33.

5.7.8. 6,7,8-Trimethoxy-*N*-(3-fluorophenyl)-4-aminoquinazoline (6g). Pale yellow needles, yield, 92.5%; mp 252–254 °C, ^1H NMR (DMSO-d_6, 500 MHz): δ 3.93 (t, 9H, $3OCH_3$, J = 40 Hz), 6.96 (t, 1H, H-2 of Ph-H, J = 8.0 Hz), 7.43 (q, 1H, H-5 of Ph-H, J = 5 Hz), 7.63 (d, 1H, H-6 of Ph-H, J = 8.0 Hz), 7.71 (s, 1H, H-5 of quinazoline), 7.88 (d, 1H, H-4 of Ph-H, J = 12.1 Hz), 8.56 (s, 1H, H-2 of quinazoline), 9.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.2, 60.7, 61.6, 97.6, 108.4, 109.6, 117.4, 129.6, 129.7, 146.0, 151.4, 156.1, 160.7, 162.7; IR (KBr): 1118.7, 1271.1, 1454.3, 1618.3, 2833.4, 2933.7, 3074.5 cm^{-1}; Anal. Calcd for $C_{17}H_{16}FN_3O_3$: C, 62.00; H, 4.90; N, 12.76. Found: C, 61.97; H, 4.85; N, 12.59.

5.7.9. 6,7,8-Trimethoxy-*N*-(4-fluorophenyl)-4-aminoquinazoline (6h). Pale yellow needles, yield, 82.3%; mp 184–186 °C; ^1H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, $3OCH_3$, J = 15 Hz), 7.34 (t, 2H, H-2, 6 of Ph-H, J = 20 Hz), 7.71–7.74 (m, 2H, H-3, 5 of Ph-H), 8.17 (s, 1H, H-5 of quinazoline), 8.73 (s, 1H, H-2 of quinazoline), 11.61 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.8, 61.8, 62.7, 100.7, 109.7, 116.1, 116.2, 127.6, 127.7, 133.3, 142.1, 148.1, 149.7, 154.5, 159.1, 159.8, 161.7; IR (KBr): 788.9, 1126.4, 1282.7, 1487.1, 1626.0, 2856.6, 2947.2, 3016.7, 3356.1 cm^{-1}; Anal. Calcd for $C_{17}H_{16}FN_3O_3$: C, 62.00; H, 4.90; N, 12.76. Found: C, 61.99; H, 4.75; N, 12.69.

5.7.10. 6,7,8-Trimethoxy-*N*-(2-fluorophenyl)-4-aminoquinazoline hydrochloride (6i). Pale yellow needles, yield, 79.0%, m.p.194 °C (dec); ^1H NMR (DMSO-d_6, 500 MHz) δ: 4.03 (t, 9H, $3OCH_3$, J = 15 Hz), 7.36-7.56 (m, 4H, H-3,4,5, 6 of Ph-H), 8.23 (s, 1H, H-5 of quinazoline), 8.72 (s, 1H, H-2 of quinazoline), 11.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.1, 61.2, 62.0, 100.2, 108.9, 116.2, 116.3, 128.6, 129.3, 141.7, 147.7, 149.2, 153.9, 155.9, 157.8, 159.3; IR (KBr): 759.9, 1010.7, 1126.4, 1257.6, 1487.1, 1629.9, 2854.7, 2945.3, 3363.9, 3600.1 cm^{-1}; Anal.Calcd for $C_{17}H_{17}ClFN_3O_3$: C, 55.82; H, 4.68; N, 11.49. Found: C, 55.66; H, 4.86; N, 11.31.

5.7.11. 6,7,8-Trimethoxy-*N*-(2-chloro-4-bromophenyl)-4-aminoquinazoline hydrochloride (6j). White needles, yield, 89.6%; mp 178 °C (dec); ^1H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, $3OCH_3$, J = 5 Hz), 7.54 (d, 1H, H-6 of Ph-H, J = 8.6 Hz), 7.74 (d, 1H, H-5 of Ph-H, J = 8.6 Hz), 8.00 (d, 1H, H-3 of Ph-H, J = 2.3 Hz), 8.04 (s, 1H, H-5 of quinazoline), 8.71 (s, 1H, quinazoline H-2); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 62.3, 66.7, 67.4, 104.9, 105.1, 114.1, 126.4, 136.6, 137.7, 138.8, 147.2, 153.3, 154.4, 159.8, 164.8; IR (KBr): 1126.4, 1286.5, 1487.1, 1626.0, 2848.9, 2945.3, 3014.7, 3365.8 cm^{-1}; Anal. Calcd for $C_{17}H_{16}BrCl_2N_3O_3$: C, 44.28; H, 3.50; N, 9.11. Found: C, 43.96; H, 3.96; N, 9.01.

5.7.12. 6,7,8-Trimethoxy-*N*-(3,4,5-trimethoxyphenyl)-4-aminoquinazoline hydrochloride (6k). Yellow needles, yield, 89.9%; mp 195 °C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.71 (s, 3H, 4-CH_3 of Ph-H), 3.81 (s, 6H, 3, 5–2CH_3 of Ph-H), 4.02 (t, 9H, 3OCH_3, J = 20 Hz), 11.52 (s, 1H, NH), 7.09 (s, 2H, H-1,6 of Ph-H), 8.17 (s, 1H, H-5 of quinazoline), 8.75 (s, 1H, H-2 of quinazoline); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.6, 57.8, 60.7, 61.2, 62.7, 100.8, 103.5, 109.7, 132.8, 136.6, 148.1, 149.6, 153.3, 154.5, 159.0; IR (KBr): 1130.3, 1284.6, 1456.3, 1633.7, 2839.2, 2947.2, 3419.8 cm^{-1}; Anal. Calcd for $C_{20}H_{24}ClN_3O_6$: C, 54.86; H, 5.52; N, 9.60. Found: C, 54.80; H, 5.51; N, 9.50.

5.7.13. 6,7,8-Trimethoxy-*N*-(4-nitrophenyl)-4-aminoquinazoline hydrochloride (6l). Pale yellow needles, yield, 75.4%; mp 212 °C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 4.02 (t, 9H, 3OCH_3, J = 20 Hz), 8.06 (s, 1H, H-5 of quinazoline), 8.15 (d, 2H, 2, 6-H of Ph-H, J = 8.6 Hz), 8.36 (d, 2H, 3, 5-H of Ph-H, J = 8.6 Hz), 8.82 (s, 1H, H-2 of quinazoline), 11.21 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 56.9, 61.2, 62.0, 99.3, 110.5, 123.2, 124.4, 143.9, 147.4, 149.7, 153.6, 157.8; IR (KBr): 1130.3, 1284.6, 1340.5, 1487.1, 1514.1, 1627.9, 2839.0, 2951.1, 3010.9, 3365.8 cm^{-1}; Anal. Calcd for $C_{17}H_{17}ClN_4O_5$: C, 51.98; H, 4.36; N, 14.26. Found: C, 51.63; H, 4.72; N, 14.12.

5.7.14. 6,7,8-Trimethoxy-*N*-(3-nitrophenyl)-4-aminoquinazoline hydrochloride (6m). Pale yellow needles, yield, 58.6%; mp 204–206 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.03 (t, 9H, 3OCH_3, J = 25 Hz), 7.79 (t, 1H, 5-H of Ph-H, J = 15 Hz), 8.18 (d, 1H, 6-H of Ph-H, J = 9.7 Hz), 11.84 (s, 1H, NH), 8.26 (s, 1H, H-5 of quinazoline), 8.29 (d, 1H, 4-H of Ph-H, J = 9.2 Hz), 8.72 (s, 1H, 2-H of Ph-H), 8.86 (s, 1H, H-2 of quinazoline); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.9, 61.9, 62.7, 100.8, 110.1, 121.3, 130.7, 131.2, 138.6, 142.3, 148.3, 149.8, 154.6, 159.1; IR (KBr): 1120.6, 1284.6, 1346.3, 1485.2, 1469.8, 1631.8, 2843.1, 2947.2, 3014.7, 3419.8 cm^{-1}; Anal. Calcd for $C_{17}H_{17}ClN_4O_5$: C, 51.98; H, 4.36; N, 14.26. Found: C, 51.71; H, 4.55; N, 14.32.

5.7.15. 6,7,8-Trimethoxy-*N*-(2-nitrophenyl)-4-aminoquinazoline hydrochloride (6n). White needles, yield, 55.6%; mp 210 °C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, 3OCH_3, J = 20 Hz), 7.65 (t, 1H, 5-H of Ph-H, J = 7.8 Hz), 7.77 (d, 1H, 6-H of Ph-H, J = 7.5 Hz), 7.90 (t, 1H, 4-H of Ph-H, J = 9.2 Hz,), 8.17 (d, 1H, 3-H of Ph-H, J = 7.5 Hz), 8.18 (s, 1H, H-5 of quinazoline), 8.66 (s, 1H, H-2 of quinazoline), 12.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.1, 61.2, 62.0, 99.9, 109.3, 125.2, 128.1, 128.9, 134.3, 144.8, 146.3, 147.8, 149.3, 153.9, 158.7; IR (KBr): 792.7, 1130.3, 1286.5, 1355.9, 1458.2, 1489.0, 1642.0, 2845.0, 2945.3, 3018.6, 3200.2 cm^{-1}; Anal. Calcd for $C_{17}H_{17}ClN_4O_5$: C, 51.98; H, 4.36; N, 14.26. Found; C, 52.28:, H, 4.57; N, 14.29.

5.7.16. 6,7,8-Trimethoxy-*N*-benzyl-4-aminoquinazoline hydrochloride (6o). White needles, yield, 77.7%; mp173–175 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.87 (t, 9H, 3OCH_3, J = 50 Hz), 4.79 (d, 2H, CH_2, J = 5.7 Hz,), 7.23 (t, 1H, 4-H of Ph-H, J = 7.8 Hz), 7.31–7.37 (m, 4H, 2, 3, 5, 6-H of Ph-H), 7.54 (s, 1H, H-5 of quinazoline), 8.36 (s, 1H, H-2 of quinazoline), 8.60 (t, 1H, NH, J = 5.7 Hz); ^{13}C NMR (DMSO-d_6, 500 MHz) δ 42.1, 56.7, 61.4, 62.2, 98.5, 111.7, 127.7, 128.8, 140.2, 140.9, 146.3, 147.6, 152.2, 153.1, 159.1 cm^{-1}; Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.56; H, 5.95; N, 12.93.

5.7.17. 6,7,8-Trimethoxy-*N*-(4-chlorophenyl)-4-aminoquinazoline hydrochloride (6p). Pale yellow needles, yield, 80.3%; mp175 °C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.02 (t, 9H, 3OCH_3, J = 20 Hz), 7.57 (d, 2H, 2, 6-H of Ph-H, J = 8.6 Hz), 7.77 (d, 2H, 3,5-H of Ph-H, J = 8.6 Hz), 8.23 (s, 1H, H-5 of quinazoline), 8.75 (s, 1H, H-2 of quinazoline), 11.57 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.2, 61.2, 62.0, 100.2, 109.9, 126.4, 128.6, 135.8, 147.5, 149.3, 153.7, 158.4; IR (KBr): 806.3, 1130.3, 1286.5, 1471.7, 1627.9, 2852.7, 2947.2, 3010.9, 3419.8 cm^{-1}; Anal. Calcd for $C_{17}H_{18}Cl_3N_3O_3$: C,48.77; H, 4.33; N, 10.04. Found: C, 48.53; H, 4.93; N, 9.97.

5.7.18. 6,7,8-Trimethoxy-*N*-(4-hydroxyphenyl)-4-aminoquinazoline hydrochloride (6q). Pale yellow needles, yield, 79.5%; mp 153 °C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.00 (t, 9H, 3OCH_3, J = 15 Hz), 6.87 (d, 2H, 2, 6-H of Ph-H, J = 8.6 Hz), 7.43 (d, 2H, 3, 5-H of Ph-H, J = 8.6 Hz), 8.15 (s, 1H, H-5 of quinazoline), 8.66 (s, 1H, H-2 of quinazoline), 9.75 (s, 1H, OH), 11.42 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 57.1, 61.2, 61.9, 100.2, 109.1, 115.2, 126.3, 127.6, 141.6, 147.3, 149.1, 153.7, 156.1, 158.3; IR (KBr): 1128.4, 1273.0, 1473.6, 1626.0, 2854.7, 2949.2, 3030.2, 3226.9, 3419.8, 3600.0 cm^{-1}; Anal. Calcd for $C_{17}H_{19}Cl_2N_3O_4$: C, 51.01; H, 4.78; N, 10.50. Found: C, 51.24; H, 5.14; N, 10.52.

5.7.19. 6,7,8-Trimethoxy-(*R*)-*N*-(4-α-methylbenzyl)-4-aminoquinazoline hydrochloride (6r). White needles, yield, 78.8%; mp 207–209 °C; $[\alpha]_{22}^{D}$ −213.0°(c = 0.01 mol/L, EtOH), ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.60 (d, 3H, CH_3, J = 7.5 Hz), 3.87 (t, 9H, 3OCH_3, J = 45 Hz), 5.62 (d, 1H, CH, J = 7.45 Hz), 7.21 (t, 1H, 4-H of Ph-H, J = 7.5 Hz), 7.32 (t, 2H, 3, 5-H of Ph-H, J = 7.5 Hz), 7.42 (d, 2H, 2, 6-H of Ph-H, J = 7.5 Hz), 7.64 (s, 1H, H-5 of quinazoline), 8.22 (d, 1H, NH, J = 7.45 Hz), 8.31 (s, 1H, H-2 of quinazoline); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 22.9, 49.6, 56.9, 61.4, 62.2, 98.7, 111.6, 126.6, 127.1, 128.8, 140.9, 145.4, 146.3, 147.6, 152.2, 153.0, 158.3; IR (KBr): 702.1, 1120.6, 1280.7, 1477.5, 1612.5, 2831.5, 2831.5, 2933.7, 3026.3, 3261.6 cm^{-1}; Anal. Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.31; H, 5.97; N, 12.23.

5.7.20. 6,7,8-Trimethoxy-(*S*)-*N*-(4-α-methylbenzyl)-4-aminoquinazoline hydrochloride (6s). White needles, yield, 80.2%; mp 205–206 °C; $[\alpha]_{22}^{D}$ +208.0 (c = 0.01 mol/L, EtOH), ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.60 (d, 3H, CH_3, J = 6.9 Hz), 3.96 (t, 9H, 3OCH_3, J = 5 Hz), 5.62 (d, 1H, CH, J = 7.45 Hz), 7.21 (t, 1H, 4-H of Ph-H, J = 7.45 Hz), 7.32 (t, 2H, 3, 5-H of Ph-H,

J = 7.45 Hz), 7.42 (d, 2H, 2, 6-H of Ph-H, J = 7.45 Hz), 7.64 (s, 1H, H-5 of quinazoline), 8.22 (d, 1H, NH, J = 7.45 Hz), 8.31 (s, 1H, H-2 of quinazoline); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 22.9, 49.6, 56.9, 61.4, 62.2, 98.7, 111.6, 126.6, 127.1, 128.8, 140.9, 145.4, 146.3, 147.6, 152.2, 153.0, 158.3; IR (KBr): 700.2, 1130.3, 1311.3, 1311.6, 1477.5, 1612.5, 2831.5, 2935.7, 2972.3, 3028.2, 3242.3 cm^{-1}. Anal. Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38; Found: C, 67.43; H, 6.23; N, 12.37.

5.8. 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 2×103 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-diphenyl tetrazoliumbromide] 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, A431(uterus cancer), PC3 (prostate cancer), BGC 823 (human gastric cancer), and Bcap37 (breast cancer) cell lines, provided by ATCC and cultivated in RPMI 1640 (for PC3, A431, BGC823, and Bcap37) supplemented with 10% fetal bovine serum. Tissue culture reagents were obtained from Gibco BRL.[21]

5.9. 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 20 μM concentration for 60 min followed by 60 ng/mL EGF for 10 min. The plate was then instantaneously placed on the ice 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 lysis 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 immunoblotting using P-ERK (E-4) (sc-7383, lot# J0803, Stanta Cruz Biotechnology).

5.9.1. 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-phosphotyrosin, anti-pErk1/2 or anti-caveolin) overnight at 4 °C. The membrane was then washed for 3× 5 min with PBS plus 0.2% Tween 20. It 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).[22]

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