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Synthesis and antitumor activity evaluation of quinazoline derivatives bearing piperazine-1-carbodithioate moiety at C4-position

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ABSTRACT

A series of quinazoline derivatives bearing piperazine-1-carbodithioate moiety at the C4-position were synthesized using piperidine and 1-bromo-3-chloropropane as starting materials *via* eight steps. Final compounds **8a–q** and **9a–i** were evaluated for their antiproliferative activity against human lung cancer A549, breast adenocarcinoma MCF-7, and colorectal cancer HCT-116 cell lines. The results showed that fourteen of twenty-six final compounds inhibited the proliferation of three cancer cell lines with IC_{50} values less than 10 μM. When treated with a representative compound **8n**, HCT-116 cells were arrested at G0/G1 phase of the cell cycle. This provided a clue to further investigation of the mechanism of action.

Key words: Quinazoline, piperazine-1-carbodithioate, synthesis, antiproliferative activity, cell cycle.

Piperazine is a common heterocycle present in a number of well established and commercially available drugs including antitumor agents. Diversity of structural modifications on the piperazine ring has made piperazine derivatives indispensable anchors for the development of novel therapeutic agents.[1, 2] In the last decade, piperazine-1-carbodithioate and piperazine-1-carbothioyl derivatives have attracted considerable attention due to their potent biological effects, especially antitumor activity. For example, Li and co-workers synthesized 4-methyl-piperazine-1-carbodithioc acid 3-cyano-3,3-diphenylpropyl ester hydrochloride (TM208, Fig. 1), which significantly inhibited the growth of xenografted hepatocellular carcinomas and gastric carcinomas in nude mice with low toxicity.[3, 4] Recently, Liu's group synthesized a series of 1,2,3-triazole-dithiocarbamate hybrids, and found that compound **I** (Fig. 1) exhibited the most specific and robust inhibition of lysine specific demethylase 1 (LSD1) and cytotoxicity against LSD1 overexpressing gastric cancer cell lines MGC-803 and HGC-27.[5, 6] Several antitumor agents bearing piperazine-1-carbodithioate or piperazine-1-carbothioyl moiety have been developed by other researchers.[7-11]

In our previous work, 4-substituted-piperazine-1-carbodithioate moiety had been incorporated into the C6-position of 2-methyl-4-oxoquinazoline or 2,4-diaminoquinazoline to synthesize target compounds with the general formulas **II** or **III** (Fig. 1).[12, 13] Among them, compounds **IIIa–c** exhibited cytotoxicity with IC_{50} values in the range of 1.58–2.27, 1.84–3.27 and 1.47–4.68 μM, respectively, against A549, MCF-7, HeLa, HT29 and HCT-116 cells, inducing a G2/M phase arrest in HCT-116 cells.[13] Besides, we had also synthesized a series of 4-substituted-piperazine-1-carbothiohydrazide derivatives of indolin-2-one (**IV**, Fig. 1), and most of them exhibited antiproliferative activity against A549, MCF-7 and HCT-116 cell lines. Notably, it was found that removal of the piperazine moiety between the hydrazinecarbothioyl moiety and phenyl ring resulted in a decrease or loss in antiproliferative activity.[14]

On the other hand, 4-substituted-quinazolines have been identified as an important class of compounds for the development of antitumor agents, of which several 4-anilinoquinazoline kinase inhibitors have been approved for the treatment of cancer, as exemplified by Gefitinib and Erlotinib.[15, 16] Moreover, several 4-piperazinylquinazoline derivatives were reported to exhibit attractive antitumor efficacy. For instance, Tandutinib (CT53518, Fig. 1) has entered phase II clinical trials for the treatment of myeloid leukemia and advanced myelodysplasia.[17, 18] Taking inspiration from reported antitumor activities of 4-substituted-quinazoline derivatives and encouraged by our promising results of novel

compounds containing the piperazine-1-carbodithioate moiety, we attempted to introduce this moiety into the C4-position of quinazoline core to synthesize compounds **8a–q** (Scheme 1 and Table 1). The target compounds could be considered as Tandutinib analogs designed by the isosteric replacement of formamide moiety in Tandutinib with carbodithioate, which would be tested for their cytotoxicity and examined the effect on cell cycle progression.

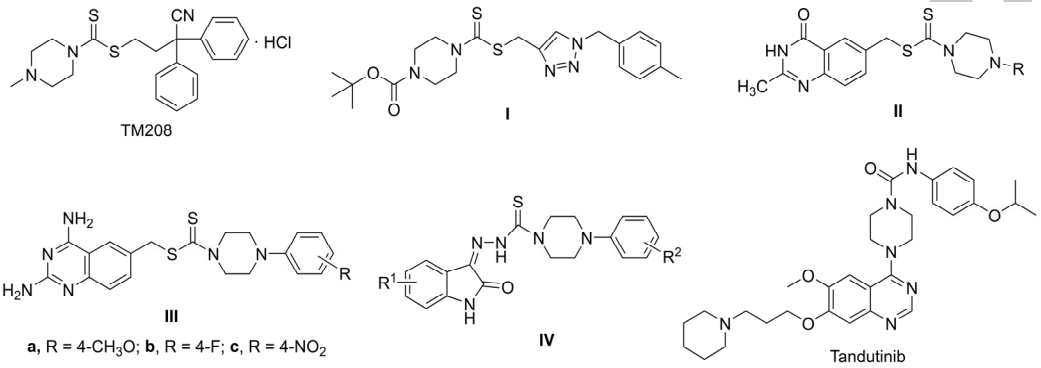
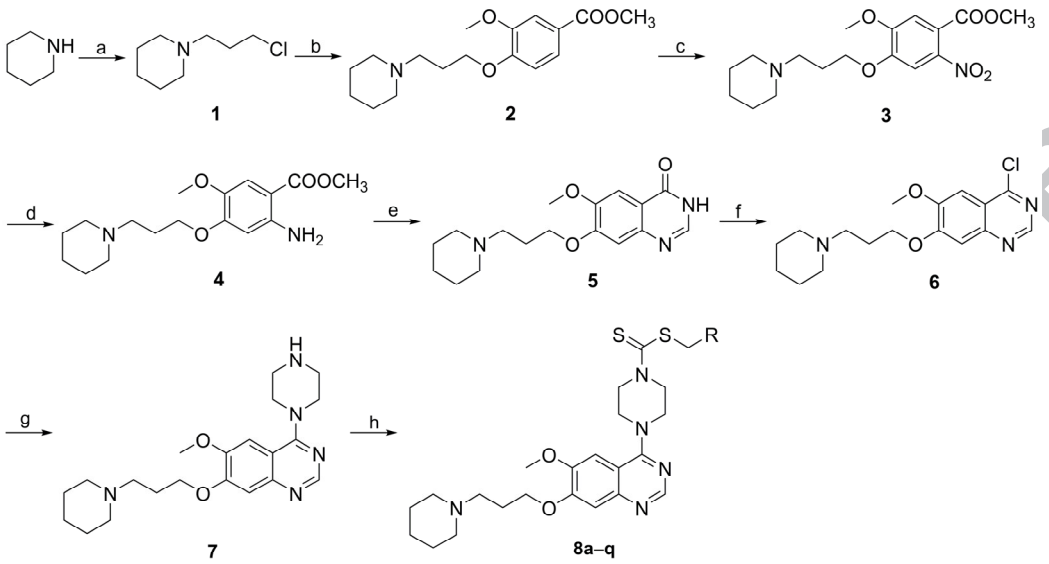


Fig. **1**. Structures of TM208, Tandutinib, and compounds **I–IV**.

The synthetic pathway of final compounds **8a–q** is outlined in Scheme 1, in which the preparation methods of intermediates **4–6** are similar to those in the synthesis of Tandutinib utilized by Knesl et al.[18] Briefly, reaction of piperidine with 1-bromo-3-chloropropane in tetrahydrofuran furnished 1-(3-chloropropyl)piperidine (**1**), which underwent etherification with methyl 4-hydroxy-3-methoxybenzoate using potassium carbonate as a base in *N*,*N*-dimethylformamide (DMF) to give methyl 3-methoxy-4-(3-(piperidin-1-yl)propoxy)benzoate (**2**). Nitration of intermediate **2** with 65% nitric acid in acetic acid, followed by the reduction with $SnCl_2 \cdot 2H_2O$ in concentrated hydrochloric acid at room temperature generated methyl 2-amino-5-methoxy-4-(3-(piperidin-1-yl)propoxy)benzoate (**4**). Cyclized product, 6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinazolin-4(3*H*)-one (**5**), was obtained by heating **4**, triethyl orthoformate and ammonium acetate in methanol for 9 h. Treatment of **5** with a mixture of phosphorus pentachloride and phosphorus oxychloride furnished 4-chloroquinazoline intermediate **6**, which was used in the next step without purificaton. Refluxing **6** with excess piperazine in isopropanol for 3 h gave 4-(piperazin-1-yl)quinazoline **7**, which reacted with carbon disulfide and the appropriate benzyl bromide, substituted-benzyl bromide, cyclohexyl bromide, or chloromethylpyridine hydrochloride in the presence of potassium phosphate to afford target

compounds **8a–q**.

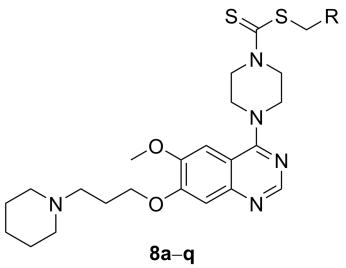


Scheme 1. Synthetic pathway of final compounds **8a–q**. Reagents and conditions: (a) 1-Bromo-3-chloropropane, K_2CO_3, THF, 45 °C, 6 h; (b) Methyl 4-hydroxy-3-methoxybenzoate, K_2CO_3, DMF, 75 °C, 6 h; (c) 65% HNO_3, AcOH, 45 °C, 6 h; (d) $SnCl_2 \cdot 2H_2O$, concentrated HCl, rt, overnight; (e) $HC(OEt)_3$, $AcONH_4$, MeOH, reflux, 9 h; (f) PCl_5, $POCl_3$, reflux, 15 h; (g) Piperazine, isopropanol, reflux, 3 h; (h) RCH_2Br/RCH_2Cl, CS_2, K_3PO_4, DMF, rt, 2 h.

The MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] cell proliferation assay was used to evaluate the antiproliferative activity of final compounds against three human cancer cell lines, namely, A549 (lung cancer), MCF-7 (breast adenocarcinoma), and HCT-116 (colorectal cancer). The inhibition of cell proliferation was determined 72 h after cells were exposed to the tested compounds at a concentration of 50 μM. The compounds with 50% or more inhibition compared with vehicle-treated cells were considered active. Inhibition of cell proliferation by these active compounds at various concentrations were further measured, and their IC_{50} (the concentration that causes 50% of cell proliferation inhibition) values were determined and summarized in Table 1. 5-Fluorouracil (5-FU), Sunitinib, and Tandutinib were used as positive controls.

Table 1.

Antiproliferative activity of compounds **8a–q** against A549, MCF-7 and HCT-116 cell lines.



8a–q

Compound	R	IC_{50}[a], μM		
		A549	MCF-7	HCT-116
8a	C_6H_5	15.67 ± 0.29	28.01 ± 0.91	28.83 ± 0.62
8b	4-$CH_3C_6H_4$	4.51 ± 0.44	3.73 ± 0.43	5.46 ± 0.49
8c	4-$CH_3OC_6H_4$	4.25 ± 0.13	4.16 ± 0.24	4.92 ± 0.77
8d	3-$CH_3OC_6H_4$	4.58 ± 0.15	3.86 ± 0.09	8.01 ± 0.42
8e	4-BrC_6H_4	4.31 ± 0.26	3.93 ± 0.66	4.52 ± 0.30
8f	4-ClC_6H_4	4.71 ± 0.22	3.94 ± 0.24	8.04 ± 0.22
8g	4-FC_6H_4	4.25 ± 0.20	4.20 ± 0.22	4.50 ± 0.36
8h	2,4-$F_2C_6H_3$	5.44 ± 0.37	4.67 ± 0.77	4.64 ± 0.21
8i	3,4-$F_2C_6H_3$	4.90 ± 0.37	3.07 ± 0.65	5.96 ± 0.10
8j	2,3,4,5,6-F_5C_6	4.58 ± 0.40	4.14 ± 0.54	4.45 ± 0.39
8k	4-$HOOCC_6H_4$	> 50	> 50	> 50
8l	4-CNC_6H_4	9.52 ± 0.53	10.07 ± 0.11	8.67 ± 0.54
8m	4-$NO_2C_6H_4$	5.36 ± 0.45	6.89 ± 0.15	5.65 ± 0.21
8n	Cyclohexyl	4.06 ± 0.22	3.99 ± 0.53	4.55 ± 0.28
8o	Pyridin-2-yl	28.78 ± 1.31	20.68 ± 0.73	20.62 ± 0.09
8p	Pyridin-3-yl	19.46 ± 1.86	22.20 ± 2.41	22.21 ± 1.43
8q	Pyridin-4-yl	> 50	> 50	> 50
5-FU		3.52 ± 0.46	32.18 ± 1.13	5.53 ± 0.90
Sunitinib		2.44 ± 0.22	6.29 ± 0.22	4.71 ± 0.32
Tandutinib		14.75 ± 0.87	14.70 ± 0.95	18.13 ± 1.06

[a] IC_{50}: The concentration that causes 50% of cell proliferation inhibition. Data are expressed as mean ± SD from triplicate determination from three independent experiments.

In view of the structural feature of 4-substituted-quinazoline-based antitumor agents,[19] especially for Tandutinib, a methoxy and a (3-(piperidin-1-yl)propoxy group were arrayed on the 6- and 7-position of the quinazoline ring, respectively, while the influence of R group in the piperazine-1-carbodithioate moiety on antiproliferative activity would be mainly investigated in this study. As shown in Table 1, compound **8a** (R = C_6H_5), which can be regarded as the parent compound, exhibited moderate antiproliferative activity with IC_{50} values in the range of 15.67–28.83 μM against

three cancer cell lines. Introduction of a methyl or a methoxy group into the 4-position of the phenyl ring of **8a** (*viz*., **8b**, R = 4-$CH_3C_6H_4$, and **8c**, R = 4-$CH_3OC_6H_4$) led to an obvious increase in antiproliferative activity against three cancer cell lines.

Similar to the compounds bearing electron-donating groups (**8b–d**), compounds bearing a moderate electron-withdrawing halogen substituent (namely, **8e–g**, R = 4-BrC_6H_4, 4-ClC_6H_4, 4-FC_6H_4) were more active than the parent compound **8a** as well. Interestingly, further introduction of a fluoro substituent into the 2- or 3-position, or four fluoro substituents into the 2, 3, 5, and 6-position of compound **8g**, resulted in compounds **8h–j** (R = 2,4-$F_2C_6H_3$, 3,4-$F_2C_6H_3$, or 2,3,4,5,6-F_5C_6) which possessed the similar activity to compound **8g**. As for the compounds bearing strong electron-withdrawing groups, however, compound **8k** (R = 4-$HOOCC_6H_4$) was inactive, while **8l** (R = 4-CNC_6H_4) and **8m** (R = 4-$NO_2C_6H_4$) were less active than compounds **8b–j**. Although compounds **8l** and **8m** were more cytotoxic against the cancer cell lines tested than the parent compound **8a**, a strong electron-withdrawing group on the phenyl ring was in general unfavorable to the antiproliferative activity, in comparison with the electron-donating groups or even moderate electron-withdrawing groups.

Replacement of the phenyl ring in the parent compound **8a** with a cyclohexyl group resulted in compound **8n**, which exhibited significant cytotoxicity with IC_{50} values of 4.06, 3.99 and 4.55 μM against A549, MCF-7 and HCT-116 cell lines, respectively. Substitution of the phenyl ring by a pyridinyl group resulted in compounds **8o–q** (R = pyridin-2-yl, pyridin-3-yl, or pyridin-4-yl). Compounds **8o** and **8q** exhibited comparable activity against A549, MCF-7 and HCT-116 cell lines to the parent compound **8a**, whereas compound **8q** was inactive at 50 μM concentration. These results indicated that a cycloalkyl group is more favorable than an electron-deficient heteroaryl group to the antiproliferative activity. Interestingly, compound **8n** exhibited comparable activities with the positive controls 5-FU and Sunitinib against A549 and HCT-116 cells, and stronger activity than 5-FU and Sunitinib against MCF-7 cells. Moreover, it was more potent than Tandutinib against all three cancer cell lines tested (4.06, 3.99, 4.55 versus 14.75, 14.70, 18.13 μM, respectively).

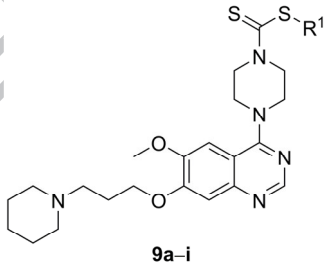
Given that compound **8n** showed decent antiproliferative potency, we replaced the cyclohexyl group in **8n** with alkyl or aliphatic heterocyclyl groups to synthesize compounds **9a–g** for further investigation of the structure-activity relationship (see Supplementary data). As shown in Table 2, replacement of the cyclohexyl group with an open-chain alkyl group (R^1 = methyl, ethyl, propyl, or

isopropyl) led to a decrease in antiproliferative activity. With reduction of the bulk of alkyl group, the antiproliferative activity decreased obviously. Similarly, compounds containing an aliphatic heterocyclyl group, *viz*., **9e** (R^1 = tetrahydrofuran-2-yl), **9f** (R^1 = 1,3-dioxolan-2-yl), **9g** (R^1 = tetrahydropyran-4-yl) exhibited weaker activity in comparison with **8n**. However, compound **9g** containing a six-membered heterocycle was more active than those containing a five-membered heterocycle (**9e**, **9f**). These results indicated that the bulk of alkyl or aliphatic heterocyclyl groups plays an important role in exerting the antiproliferative effect.

From the first series of compounds (**8a–q**), it suggests that aryl groups bearing an electron-donating substituent, but not aryl groups bearing an electron-withdrawing substituent and electron-deficient pyridinyl groups, are favorable to the antiproliferative activity. We thus synthesized two compounds containing an electron-rich heterocycle, namely, **9h** (R^1 = furan-2-yl) and **9i** (R^1 = thiophen-3-yl). As shown in Table 2, these two compounds exhibited higher antiproliferative activity than the compounds containing pyridinyl groups (**8o–q**), which was comparable with the compounds containing aryl groups with an electron-donating substituent, e.g. **8b–d**.

Table 2.

Antiproliferative activity of compounds **9a–i** against A549, MCF-7 and HCT-116 cell lines.



9a–i

Compound	R^1	IC_{50}[a], μM		
		A549	MCF-7	HCT-116
9a	CH_3	33.35 ± 1.17	27.52 ± 0.77	24.35 ± 1.52
9b	CH_3CH_2	18.36 ± 0.80	16.62 ± 0.32	10.35 ± 0.48
9c	$CH_3CH_2CH_2$	8.07 ± 0.11	10.54 ± 0.94	8.67 ± 0.65
9d	$(CH_3)_2CH$	11.27 ± 0.93	8.37 ± 0.21	10.44 ± 1.18
9e	Tetrahydrofuran-2-ylCH_2	22.80 ± 1.00	22.71 ± 0.51	19.22 ± 0.80
9f	1,3-Dioxolan-2-ylCH_2	24.62 ± 0.44	29.55 ± 1.17	23.92 ± 0.64
9g	Tetrahydropyran-4-ylCH_2	19.14 ± 0.68	18.35 ± 0.46	15.08 ± 0.40
9h	Furan-2-ylCH_2	6.58 ± 0.43	7.29 ± 0.31	7.30 ± 0.60
9i	Thiophen-3-ylCH_2	4.84 ± 0.24	5.17 ± 0.14	5.64 ± 0.22

[a] Data are expressed as mean ± SD from triplicate determination from three independent experiments.

To investigate the preliminary mechanism of action, compound **8n** was employed to examine the effect on cell cycle distribution in HCT-116 cells. HCT-116 cells were treated with compound **8n** (1 × IC_{50}, 1.25 × IC_{50}, or 1.5 × IC_{50}), 5-FU (IC_{50}), or Sunitinib (0.1 μM) for 24 h. The cells were then harvested, fixed in 70% ice-cold ethanol, stained with propidium iodide (PI), and analyzed for DNA content by flow cytometry. Three independent experiments were performed, and the results from one representative experiment were shown in Fig. 2, and the percentages of the cells in G0/G1, S, and G2/M phases were calculated and presented in Table S1 (see Supplementary data).

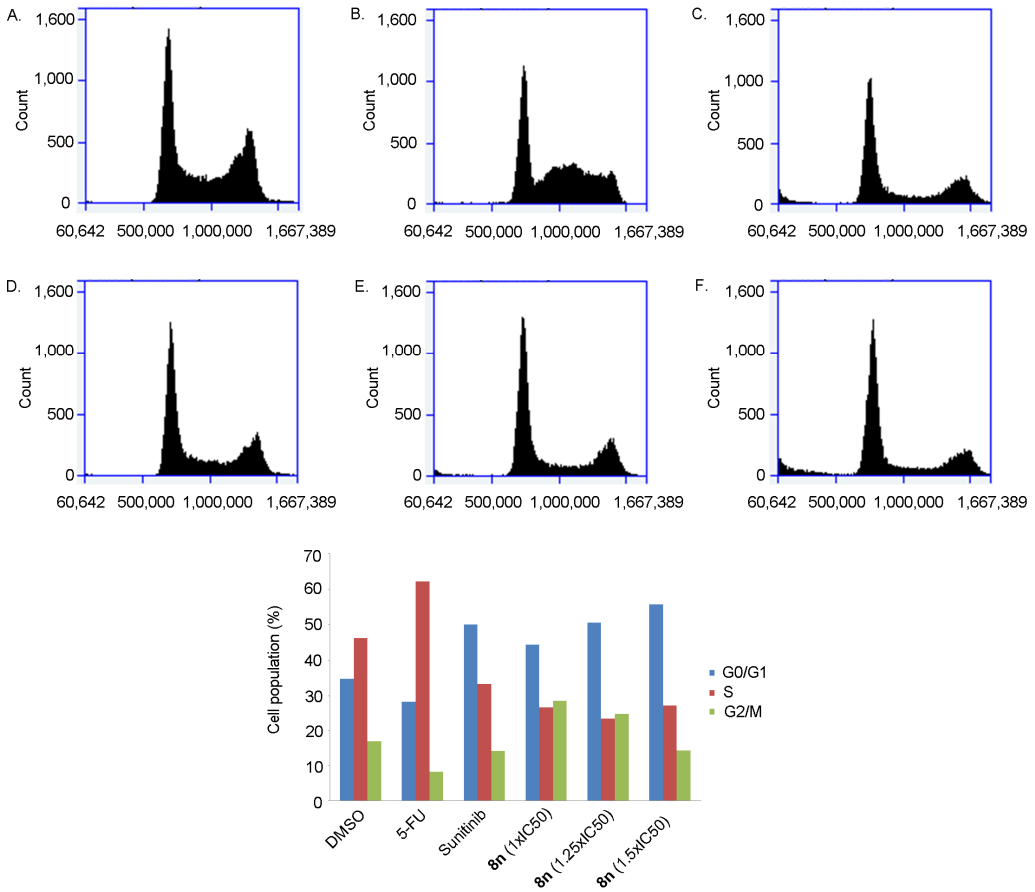


Fig. 2. Effect of compound **8n** on cell cycle progression. HCT-116 cells were treated with **8n** (1 ×, 1.25 ×, and 1.5 × IC_{50}), 5-FU, or Sunitinib for 24 h. And then, cells were harvested, fixed, stained with PI and analyzed with a flow cytometer. (A) DMSO; (B) 5-FU; (C) Sunitinib (0.1 μM); (D) **8n** (1 × IC_{50}); (E) **8n** (1.25 × IC_{50}); (F) **8n** (1.5 × IC_{50}); (G) Histogram representing effects of DMSO, 5-FU, Sunitinib, and **8n** on cell cycle progression.

5-FU is an inhibitor of thymidylate synthase (TS), blocking DNA synthesis in a variety of cancer

cells to arrest cell cycle in S-phase, while Sunitinib is an inhibitor of multiple tyrosine kinase receptors inducing G1 cell cycle arrest.[20] Our results indicated an increase of G0/G1 and a decrease of S and G2/M phase after **8n** treatment at its IC_{50} concentration for 24 h in HCT-116 cells. Moreover, a dose-dependent accumulation of G0/G1cells was observed with the concentrations increased to 1.5 times of the IC_{50} value of **8n**. Additionally, a dose-dependent increase in the sub-G1 phase was also observed after treatment with **8n** for 24 h, which was similar to the behavior of Sunitinib (Fig. 2, C and F). These properties are quite different from those of 2,4-diaminoquinazoline derivatives connecting piperazine-1-carbodithioate moiety at its C6-position (**IIIa–c**, Fig. 1),[13] which induced a G2/M phase arrest in HCT-116 cells, without an obvious sub-G1 peak in the cell cycle profile.

In conclusion, we synthesized a series of quinazoline derivatives bearing piperazine-1-carbodithioate moiety at the C4-position and tested for their antiproliferative activity against A549, MCF-7 and HCT-116 cell lines. Preliminary structure-activity relationship analysis revealed that an electron-donating or a moderate electron-withdrawing group on the phenyl ring is more favorable than a strong electron-withdrawing group to the antiproliferative activity. Replacement of the phenyl ring in the parent compound **8a** by a cyclohexyl (**8n**) or an electron-rich heterocyclyl group (**9h**, **9i**), but not an open-chain alkyl or an electron-deficient pyridinyl group, resulted in an obvious enhancement in activity. Similar to the multiple tyrosine kinase receptor inhibitor, Sunitinib, compound **8n** induced a dose-dependent arrest of HCT-116 cells at G0/G1 phase after treatment for 24 h. Therefore, our newly synthesized compounds might exert an effect on cell proliferation through the mode similar to Sunitinib, and the mechanism of action is under investigation in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at...

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Figure captions

Fig. **1**. Structures of TM208, Tandutinib, and compounds **I–IV**.

Fig. 2. Effect of compound **8n** on cell cycle progression. HCT-116 cells were treated with **8n** (1 ×, 1.25 ×, and 1.5 × IC_{50}), 5-FU, or Sunitinib for 24 h. And then, cells were harvested, fixed, stained with PI and analyzed with a flow cytometer. (A) DMSO; (B) 5-FU; (C) Sunitinib (0.1 μM); (D) **8n** (1 × IC_{50}); (E) **8n** (1.25 × IC_{50}); (F) **8n** (1.5 × IC_{50}); (G) Histogram representing effects of DMSO, 5-FU, Sunitinib, and **8n** on cell cycle progression.

Scheme 1. Synthetic pathway of final compounds **8a–q**. Reagents and conditions: (a) 1-Bromo-3-chloropropane, K_2CO_3, THF, 45 °C, 6 h; (b) Methyl 4-hydroxy-3-methoxybenzoate, K_2CO_3, DMF, 75 °C, 6 h; (c) 65% HNO_3, AcOH, 45 °C, 6 h; (d) $SnCl_2{\cdot}2H_2O$, concentrated HCl, rt, overnight; (e) $HC(OEt)_3$, $AcONH_4$, MeOH, reflux, 9 h; (f) PCl_5, $POCl_3$, reflux, 15 h; (g) Piperazine, isopropanol, reflux, 3 h; (h) RCH_2Br/RCH_2Cl, CS_2, K_3PO_4, DMF, rt, 2 h.

Graphical Abstract

Synthesis and antitumor activity evaluation of quinazoline derivatives bearing piperazine-1-carbodithioate moiety at C4-position

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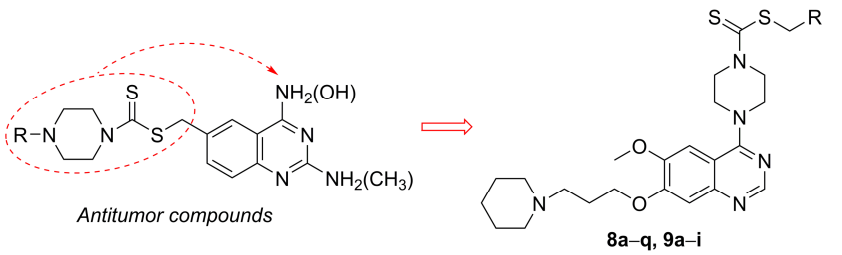

4-Quinazolinylpiperazine-1-carbodithioates **8a–q** and **9a–i** were synthesized. Most of them exhibited promising cytotoxicity, and **8n** induced a dose-dependent G0/G1 phase arrest in HCT-116 cells.