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Synthesis and in vitro antitumor activity of 4(3*H*)-quinazolinone derivatives with dithiocarbamate side chains

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Abstract—A series of 4(3*H*)-quinazolinone derivatives with dithiocarbamate side chains were synthesized and tested for their in vitro antitumor activity against human myelogenous leukemia K562 cells. Among them, (3,4-dihydro-2-methyl-4-oxoquinazolin-6-yl)-methyl 4-(4-fluorophenyl)piperazine-1-carbodithioate **8q** exhibited significant inhibitory activity against K562 cells with IC_{50} value of 0.5 μM.

Structure modification of folic acid led to the discovery of a number of antifolates as efficient anticancer agents. For example, Methotrexate (**1**) (Fig. 1), an inhibitor of dihydrofolate reductase, has been used clinically for the treatment of leukemia and solid tumors in children and adults for several decades.[1] Raltitrexed (**2**)[2,3] (Fig. 1), which is an inhibitor of thymidylate synthase has been registered widely for the first-line treatment of advanced colorectal cancer. However, these so-called classical antifolates containing L-glutamic acid moiety in the molecule have shortcomings such as drug resistance, which have originated from the defective cell transport by mutation, and toxicity to the host, which is due to unnecessarily long retention inside normal cells.[4] One strategy to overcome these shortcomings is to design nonclassical lipophilic inhibitors of folate requiring enzymes by deleting or modifying L-glutamic acid component from the folate analogues.[5,6]

Recently, Brassinin (**3**)[7] (Fig. 1), a dithiocarbamate isolated from cabbage, was reported to have cancer chemopreventive activity, and its structural modification led to the design and synthesis of a potential cancer chemopreventive agent Sulforamate (**4**)[8] (Fig. 1). More recently, a

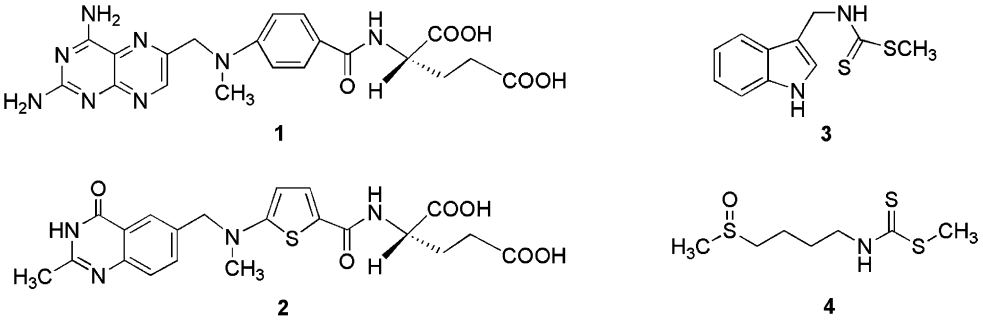

Figure 1. Structures of Methotrexate (**1**), Raltitrexed (**2**), Brassinin (**3**), and Sulforamate (**4**).

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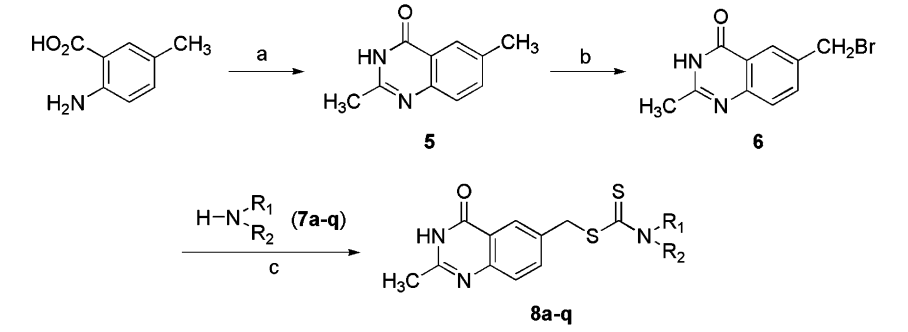
series of dithiocarbamate compounds have been synthesized and found to possess in vitro and in vivo antitumor activity.[9,10] In an effort to look for the possible nonclassical antifolates acting as antitumor agents, we were interested in the incorporation of dithiocarbamate moiety with 4(3*H*)-quinazolinone. Herein, we report the synthesis of 4(3*H*)-quinazolinone derivatives with dithiocarbamate side chains and their in vitro antitumor activity against human myelogenous leukemia K562 cells.

The procedure for the synthesis of compounds **8a**–**q** is outlined in Scheme 1. Heating 2-amino-5-methylbenzoic acid[11] with thioacetamide yielded 2,6-dimethyl-4(3*H*)-quinazolinone (**5**), which was converted to 6-bromomethyl-2-methyl-4(3*H*)-quinazolinone (**6**) by brominating with *N*-bromosuccinimide in the presence of $(PhCO)_2O_2$. According to the improved synthetic method for dithiocarbamates,[12] compounds **8a**–**q** were obtained by the reaction of 6-bromomethyl-2-methyl-4(3*H*)-quinazolinone with CS_2 and various amines (**7**) in the presence of anhydrous K_3PO_4. For the preparation of compounds **8e** and **8o**, 1-adamantylamine hydrochloride and *N*-benzylpiperazine hydrochloride was used, respectively, without previous neutralization by the tertiary amine.

The synthesized compounds **8a**–**q** were tested for their in vitro antitumor activity against human myelogenous leukemia K562 cells by MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide) assay. The assays were performed in 96-well plates as described in the literature.[13] The IC_{50} value represents the concentration, which results in a 50% decrease in cell growth after 24 h incubation.

The inhibitory activity of the synthesized compounds against K562 cells is summarized in Table 1. Methotrexate (**1**) and Brassinin (**3**) were used for comparison. The results demonstrated that compounds **8a**–**q** exhibited potent cytotoxicity and are more potent than Methotrexate and Brassinin in inhibiting K562 cell growth with IC_{50} values from 0.5 to 31.0 μM. Comparing compounds **8a**,**d** with **8b**, **8c**, and **8e**, it can be seen that compounds containing secondary amine moiety have stronger activities than those containing primary amine moiety. However, compounds containing benzyl or furfuryl primary amine moiety (**8f**,**g**) have strong activities. Furthermore, compounds **8i**,**j** have stronger activities than **8h**, also suggesting that the introduction of an aryl group into amino moiety resulted in the enhancement of anti-K562 cell activity. When the oxygen atom was introduced into the piperidine ring, the resulting compounds **8k**,**l** were more active than parent compound **8h**. As shown in Table 1, compounds **8o**, **8p**, and **8q** having aryl or arylalkyl group at the 4-position of piperazine ring have stronger activities than **8n** with methyl at 4-position of piperazine ring, moreover, introduction



—NR_1R_2	—NR_1R_2	—NR_1R_2
a —N	g —NH–CH_2–	m —N
b —N H —N	h —N	n —N —N–CH_3
c —NH	i —N CO_2Et C_6H_5	o —N —N–CH_2–
d —N $)_2$	j N	p —N —N–CH $)_2$ F
e —NH	k —N O	q —N —N F
f —NH–CH_2	l —N O	

Scheme 1. Synthesis of compounds **8a**–**q**. Reagents and conditions: (a) CH_3CSNH_2, 135–150 °C, 2 h, 73%; (b) *N*-bromosuccinimide, $(PhCO)_2O_2$, $CHCl_3$, reflux 3 h, 77%; (c) CS_2, K_3PO_4, DMF, rt, 2 h, 51–94%.

Table 1. In vitro cytotoxicity of compounds **1**–**4**, **8a**–**q** against K562 cells

Compd	IC_{50} (μM)
1	419
2	NA[a]
3	128
4	NT[b]
8a	4.4
8b	25.6
8c	31.0
8d	7.6
8e	30.6
8f	4.0
8g	2.1
8h	11.3
8i	8.7
8j	3.3
8k	7.2
8l	3.7
8m	11.0
8n	10.8
8o	7.4
8p	4.3
8q	0.5

[a] Not available.
[b] Not tested.

of fluorine atom into the aromatic ring obviously enhanced the activity and resulted in the compound **8q** possessing the strongest inhibitory activity against K562 cells.

In summary, we have designed and synthesized a series of 4(3*H*)-quinazolinone derivatives with dithiocarbamate side chains. The preliminary in vitro antitumor activity tests indicated that among the synthesized compounds, **8q**[14] exhibited significant inhibitory activity against K562 cells in culture.

Acknowledgements

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14. Selected data for compound **8q**: mp 246–248 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.2 (t, 4H, $N(CH_2)_2$), 4.13 (br s, 2H, NCH_2), 4.42 (br s, 2H, NCH_2), 4.74 (s, 2H, CH_2S), 6.94 (m, 4H, Ph–H), 7.63 (d, 1H, J = 8.5 Hz, quinazolinone 8-H), 7.82 (dd, 1H, J = 8.5 and 2.1 Hz, quinazolinone 7-H), 8.26 (d, 1H, J = 2.1 Hz, quinazolinone 5-H); ESIMS *m/z* 429.3 (MH^+). Anal. Calcd for $C_{21}H_{21}FN_4OS_2$: C 58.86%, H 4.94%, N 13.07%. Found: C 58.84%, H 4.99%, N 12.93%.