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Dithiocarbamic acid esters as anticancer agent. Part 1: 4-Substituted-piperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl esters

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Abstract—A variety of 4-N atom substituted derivatives were synthesized and evaluated for their in vitro anticancer activities using 4-methylpiperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl ester **4** as lead compound. Among them, compound **6a** without any substituent on 4-N atom (R^1 = H) was found to be the most active anticancer agent with IC_{50} = 5.3 μM against HL-60 and IC_{50} = 11.5 μM against Bel-7402, respectively. Increase in the polarity and/or introduction of suitable acyl groups at the 4-N atom of the lead compound **4** are favorable for the improvement of activity.

Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21st century. Because it is difficult to discover novel agents that selectively kill tumor cells or inhibit their proliferation without the general toxicity, the use of traditional cancer chemotherapy is still very limited.

Dithiocarbamic acid ester is a common class of organic molecules. They exhibit a variety of valuable biological effects, including antibacterial activity,[1,2] antifungal activity,[3] the ability to chelate heavy metals,[4,5] and to function as NO scavengers.[6] Recently, it was found by Hirschelman's group that (4-methanesulfinyl-butyl)-dithiocarbamic acid methyl ester (sulforamate **1**, Fig. 1) and 5-oxohexyl dithiocarbamic acid methyl ester (oxomate **2**, Fig. 1) are potent phase II enzyme inducers which could be used as cancer chemopreventive agents.[7–9] Another group from Italy also found that the metal complex of dithiocarbamic acid esters exhibited anticancer activity. For example, the platinum complexes have similar activity but less toxicity than the cisplatin.[10–12] However, little systematic research has been reported about anticancer activity of this class of compounds, although compound RWJ-025856 (**3**) was unexpectedly found to have attenuating effects on tumor necrosis factor α (TNFα)-induced apoptosis in murine fibrosarcoma WEHI 164 cells.[13]

We have developed a convenient one-pot method for the synthesis of dithiocarbamic acid ester and have prepared

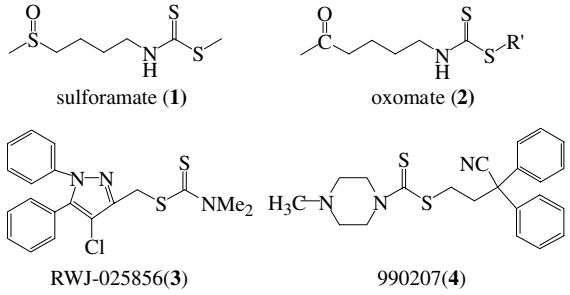

Figure 1. Structures of compounds **1**–**4**.

Keywords: Dithiocarbamic acid ester; Anticancer activity; 4-Substituted-piperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl ester; Synthesis.
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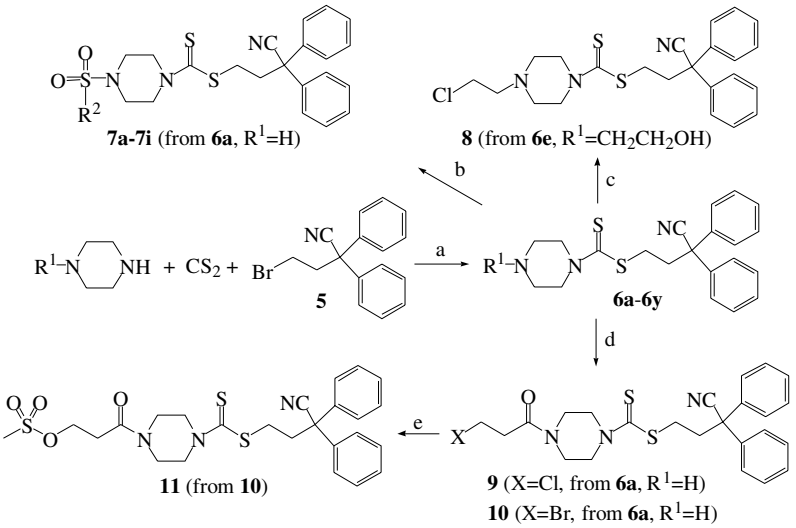
many compounds using this method.[14–16] Random screening of those compounds led to the discovery of several compounds possessing significant antitumor activity.[17,18] One of the best compounds is 4-methyl-piperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl ester (**4**, Fig. 1) with 79% and 75% inhibition rates against HL-60 and Bel-7402 cell lines at 33 μM in vitro, respectively. A further in vivo test of its hydrochloride salt (**4·HCl**), which has better solubility, indicated that the inhibition rates against tumor growth of sarcoma 180 (S_{180}), hepatocyte carcinoma 22 (H_{22}), and implanted human gastric carcinoma in nude mice were 46.4–59.6% ($P < 0.01$), 39.3–51.6% ($P < 0.05 \sim 0.01$), and 18.1–59.0% ($P < 0.01$) at different doses from 50 to 200 mg/kg, respectively. More importantly, **4·HCl** showed very low toxicity. Taking it orally at a dose of 10 g/kg continuously for 10 days, the rats are neither dead nor damage of organs observed by visual examination. Furthermore, the body weight of tested group is similar to that of control group.[19]

To the best of our knowledge, dithiocarbamic acid ester **4** represents a new kind of compound with a novel structure, significant anticancer activity, and very low toxicity. Encouraged by this result, we selected compound **4** as a lead compound to further explore the structure–activity relationships (SAR) with the aim of optimizing potency and anticancer activity. In this article, we focus our attention on the optimization of substituents at the 4-N atom of piperazine in the lead compound **4**.

The synthetic routes of all the target compounds are shown in Scheme 1. According to our improved and well-established method,[20] a variety of 1-N-substituted piperazines, which were commercially available or prepared by standard methods, were reacted with carbon disulfide and 3-cyano-3,3-diphenyl-propyl bromide **5** in the presence of anhydrous potassium phosphate at room temperature to afford the corresponding dithiocarbamic acid esters **6a**–**6y** in high yields. Direct sulfonation of **6a** ($R^1 = H$) with methylsulfonyl chloride and various substituted phenylsulfonyl chloride under basic conditions afforded 4-methylsulfonylpiperazine-1-carbodithioic acid 3-cyano-3,3-diphenyl-propyl ester **7a** and 4-phenylsulfonylpiperazine-1-carbodithioic acids 3-cyano-3,3-diphenyl-propyl esters **7b**–**7i**. The hydroxylethyl analgoue **6e** ($R^1 = HOCH_2CH_2$–) was reacted with thionyl chloride in dichloromethane at room temperature to give the corresponding chloride product **8**. The direct N-acylation of **6a** with β-chloropropionyl chloride provided the β-chloropropionyl derivative **9**. Using β-bromopropionyl chloride under identical conditions gave the desired β-bromopropionyl derivative **10**. The compound **11** was synthesized from the reaction of **10** with silver methanesulfonate[21] in refluxing acetonitrile for 4.5 h in 58% yield.

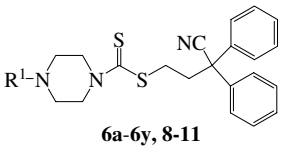
All of the synthesized compounds were screened for the preliminary in vitro anticancer activity against six different cell lines: a human promyelocyticfina leukemic cell line (HL-60), a human hepatocellular carcinoma cell line (Bel-7402), a human gastric carcinoma cell line (BGC-823), a human cervical carcinoma cell line (HeLa), a human prostatic carcinoma cell line (PC 3MIE8), and a human breast carcinoma cell line (MDA-MB-435), at four different concentrations. Because most of compounds showed inhibition activity only on HL-60 and Bel-7402 cells at concentration of >33 μM, we herein merely listed biological results at concentration of 33 μM in Table 1 in order to discuss the SAR.[22–24] Meantime, the IC_{50} values for the most potent compounds, **4**, **6a**, and **6e** were tested.

As seen in Table 1, in order to obtain the diversity of 4-N substituted derivatives of **4**, substituents such as alkyl, aryl, heteroaryl, sulfonyl benzyl, and benzoyl groups were chosen to replace the 4-N methyl group of compound **4**. Although most of the compounds showed weak or no activity, it is interesting to note that the compound **6a** without a substituent on 4-N atom ($R^1 = H$)

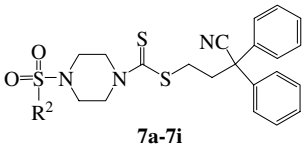


Scheme 1. Reagents and conditions: (a) K_3PO_4, acetone, rt; (b) R^2SO_2Cl, Et_3N, CH_2Cl_2, rt; (c) $SOCl_2$, CH_2Cl_2, reflux, 2 h, 79%; (d) XCH_2CH_2COCl, $NaHCO_3$, CH_2Cl_2, reflux, 0.5 h, 57% for **9**, 73% for **10**; (e) CH_3SO_3Ag, CH_3CN, reflux, 4.5 h, 58%.

Table 1. The biological activities of compounds **6a**–**y**, **7a**–**i**, and **8**–**11** at a concentration of 3.3×10^{-5} μM



6a-6y, 8-11



7a-7i

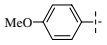
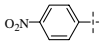
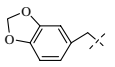
Compound	R^1 or R^2	Inhibition rate against HL-60 (%)	Inhibition rate against Bel-7402 (%)
4	CH_3–	79.0 (9.9[a])	75.2 (27.1[a])
6a	H–	98.7 (5.3[a])	95.5 (11.5[a])
6b	C_2H_5–	−7.81	4.97
6c	*n*-C_4H_9–	−13.5	11.0
6d	*n*-C_6H_{13}–	2.57	12.7
6e	$HO(CH_2)_2$–	92.5 (24.5[a])	57.7 (11.7[a])
6f		10.5	0
6g		0	4.3
6h		0	15.9
6i		8.5	26.3
6j		0	0
6k		4.2	0
6l		0.7	23.3
6m		0	14.6
6n		4.8	0
6o		8.5	14.9
6p		9.8	15.5
6q		0	0
6r		7.8	2.2
6s		31.8	22.6
6t		24.2	33.2
6u	C_6H_5 CH_2–	12.5	4.7
6v		0	16.6

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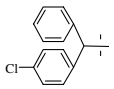
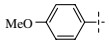
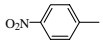
Table 1 (*continued*)

Compound	R^1 or R^2	Inhibition rate against HL-60 (%)	Inhibition rate against Bel-7402 (%)
6w		2.7	18.0
6x		30.4	17.7
6y		0	35.0
7a		24.9	13.4
7b		0	0
7c		1.5	5.4
7d		0	0.2
7e		0	5.5
7f		0	0
7g		0	0
7h		0	0
7i		0	9.7
8	$ClCH_2CH_2$–	53.6	28.7
9	$Cl(CH_2)_2CO$–	43.3	22.0
10	$Br(CH_2)_2CO$–	82.2	23.4
11	$MeSO_3CH_2CH_2CO$–	0	1.09

[a] IC_{50} value.

exhibited better activities (98.7% against HL-60, IC_{50} = 5.3 μM; 95.5% against Bel-7402, IC_{50} = 11.5 μM) than those of the lead compound **4** (R^1 = Me, 79.0% against HL-60, IC_{50} = 9.9 μM; 75.2% against Bel-7402, IC_{50} = 27.1 μM). This result demonstrates that the introduction of bulky substituent is unfavorable for the improvement of activity.

Comparing compound **6b** (R^1 = $-C_2H_5$) with **6e** (R^1 = $-CH_2CH_2OH$), it was found that despite only slight differences between their structures, their activities were completely different. Compound **6b** showed no activity, while **6e** showed inhibition rates of 92.5% against HL-60 and 57.7% against Bel-7402. Based on the above results and the activity data of compound **6a**, we consider that the polarity of the molecule might be a very important factor affecting the activity.

Introduction of heteroaryl groups (**6s**, R^1 = pyridyl; **6t**, R^1 = pyrimidinyl) led to a significant decrease of activity

compared with the lead compound **4**. However, their activities are obviously higher than those of the aryl substituted derivatives (**6f**–**6r**). This result suggested that it is possible to improve the activity by introducing suitable heteroaryl groups into the 4-N atom of the lead compound **4**. Furthermore, the activity improvement of **6s** and **6t** may be related to the increase in polarity of molecule caused by introduction of heteroaryl group. The activity results of compounds **6x** (R^1 = furoyl, 30.4% against HL-60) and **6y** (R^1 = ethoxycarbonyl, 35.0% against Bel-7402) also support the above suggestions.

Because the furoyl substituted analogue **6x** showed better activity than other substituted analogues, we speculate that replacement of the 4-N-Me of the lead compound **4** by acyl groups with anticancer action would improve the pharmacological properties. Therefore, 3-chloropropionyl, 3-bromopropionyl, and 3-methanesulfonyloxy-propionyl were selected as substituents to obtain the compounds **9**–**11**. At the same time, as the analogue of compound **6e**, the chloroethyl substituted compound **8** was also prepared. As shown in Table 1, except for compound **11**, all of the compounds **8**–**10** exhibited higher activities than that of the compound **6x**. Most importantly, the 3-bromopropionyl derivative **10** showed 82.2% inhibition rate against HL-60. This result demonstrates that it is possible to improve the anticancer activity by introducing suitable acyl groups at the 4-N atom of the lead compound **4**.

In summary, we have synthesized various 4-N atom substituted derivatives of the lead compound **4** and tested their preliminary in vitro anticancer activities. Compound **6a** without substituent on the 4-N atom was found to be more potent than the lead compound **4**. Increase in the polarity and/or introduction of suitable acyl groups at 4-N atom of the lead compound **4** are favorable to improve the activity. These results provide promising information for further development of potent inhibitors. Further optimization results for the lead compound **4** will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.085.

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centrations was added to wells, and RPMI-1640 medium in control cells, then cells were incubated for 48 h. HL-60 cells were assayed by MTT, and the Bel-7402 cells were assayed by SRB. The absorbance of each well was measured using a microculture plate reader at 570 nm (MTT) and 540 nm (SRB).

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