Synthesis of Substituted 6-Amino-4-(2,4-dimethoxyphenyl)-[1,2]dithiolo-[4,3-*b*]pyrrol-5-ones and Their Raising Leukocyte Count Activities

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The design and synthesis of a series of substituted 6-amino-4-(2,4-dimethoxyphenyl)-[1,2]dithiolo[4,3-*b*]-pyrrol-5-ones are described. All the synthesized compounds were evaluated for raising leukocyte count activities in normal mice. Four compounds (8a, b, d, h) exhibited raising leukocyte count activities close or higher than positive control recombinant human granulocyte colony stimulating factor (rhG-CSF), and some (8e–g, k, p, r) had a moderate effect. Among them, the most potent compound 8a was evaluated for its antileukopenia activity in cyclophosphamide (CTX) treated mice. Interestingly, 8a exhibited significant antileukopenia activity as compared to rhG-CSF. The results suggest that this kind of compounds might be utilized for the development of new candidate for treatment of leukocytopenia.

Key words dithiolopyrrolone; raising leukocyte count; antileukopenia; rhG-CSF

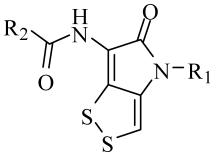
Dithiolopyrrolones are a class of natural products that possess the 4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-one skeleton, which were isolated from the bacteria *Streptomyces* sp. in the 1940s[1] and from other organisms since that time.[2–4] This group of compounds includes thiolutin, aureothricin, isobutyropyrrothin, holomycin, xenorhabdins and thiomarinols A–G[5–9] (Fig. 1). Dithiolopyrrolones are reported with diverse biological activities including against various Gram-positive and Gram-negative bacteria, yeasts, fungi and amoeboid parasites.[10–12] Dithiolopyrrolones have also been reported to have anticancer activity.[13,14]

The modifications of the dithiolopyrrolones have been extensively reported in literature.[15–21] However, all the modifications were intended to improve the antibacterial or anticancer activity. In our previous work, we first observed that ZL-004 (Fig. 2) exhibited antileukopenia activity,[22] which can significantly raise the white blood cells (WBC) count in normal mice and chemotherapy-induced mice. The mechanism of ZL-004[23] is to act on the mouse bone marrow causing proliferation and differentiation, the elevation of WBC is due to an increase in the absolute neutrophil count, which could promote the recovery of hemopoiesis. Also, it can raise WBC count in a dose-dependent manner without significant changes in erythrocyte, platelet counts or hemoglobin concentrations.

In order to better understand the structure–activity relationship (SAR) and discover novel antileukopenia agents with higher potency and good safety profiles, we embarked on design and synthesis of analogues of ZL-004. In this study, we reported the practical synthesis, the SAR, the raising leukocyte count activity in normal mice and the antileukopenia activity in CTX-treated mice of the newly synthesized dithiolopyrrolones.[24,25]

Results and Discussion

Chemistry The synthesis of the target compounds was accomplished by the procedures shown in Chart 1. 1,3-Bis(*tert*-butylthio)propan-2-one was prepared from 1,3-dichloroacetone in the presence of potassium carbonate in acetone, which was coupled with 2,4-dimethoxyanile in toluene utilizing *p*-toluenesulfonic acid as catalyst. The reaction mixture was refluxed for 5h, and then the mixture was allowed to cool to −5°C, oxayl chlorite was added to the reaction mixture to generate the compound **3** in 75% yield. Ammonization of **3** by *in situ* synthesis of ammonium butanoate in a reflux toluene solution generated **4** in 73% yield. Protection of the amino group of **4** with trifluoroacetic anhydride in dichloromethane gave **5** in 95% yield, which was complexed with mercuric acetate in trifluoroacetic acid, and then pure hydrogen sulfide gas was bubbled through the reaction solution to obtain cyclization product **6**. The deprotection of trifluoroacetyl groups on amino group by hydrolysis with concentrated hydrochloric acid was carried out in methanol at 100°C then transformed to **7** as hydrochloride salt. Then condensation of **7** and chloroformate derivatives to give the ZL-004 and **8a**–**d**; condensation of compound **7** and triphosgene in presence of tetrahydrofuran (THF), followed by concentrated hydrochloric acid hydrolysis to form **8e**, or the corresponding alcohols were reacted with triphosgene in presence of THF then condensed



a: R_1=Me, R_2=Me, Thiolutin
b: R_1=Me, R_2=Et, Aureothricin
c: R_1=Me, R_2=i-Pr, Isobutyropyrrothin
d: R_1=H, R_2=Me, Holomycin

Fig. 1. Chemical Structures of Some of the Naturally Occurring Dithiolopyrrolones

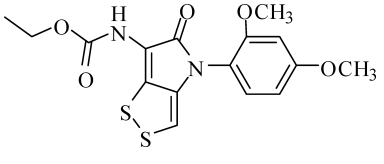


Fig. 2. Chemical Structure of ZL-004

The authors declare no conflict of interest.

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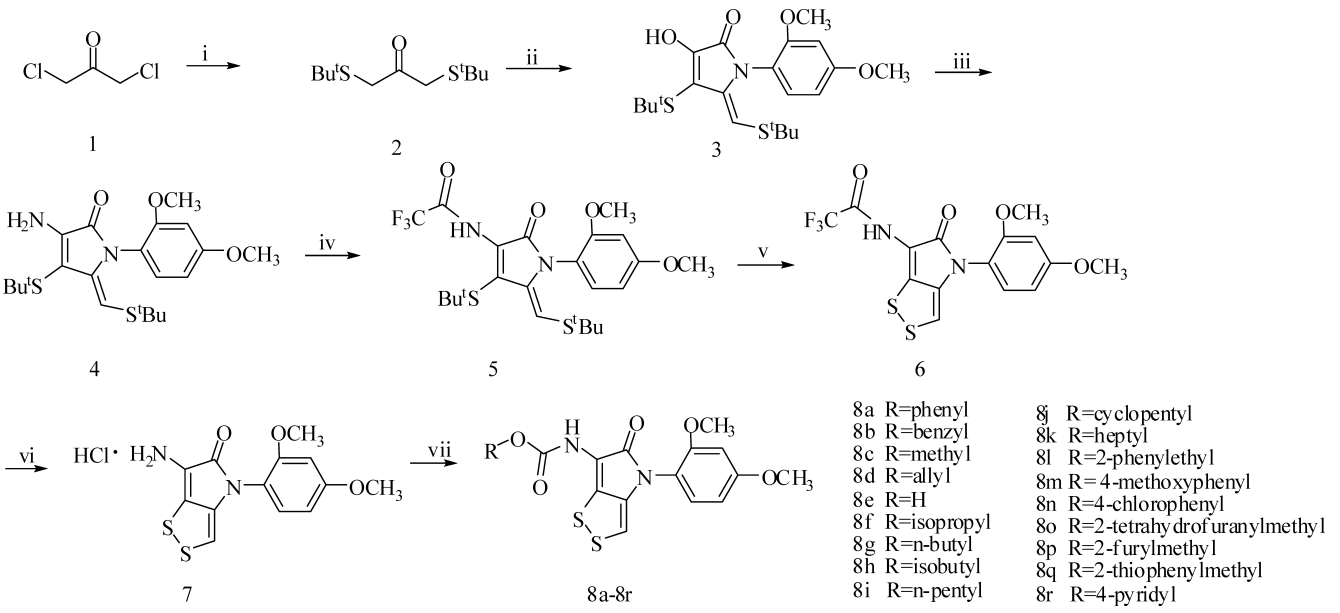

Reagents and conditions: (i) *tert*-butyl mercaptan, K_2CO_3, acetone, 0°C–rt; (ii) 2,4-dimethoxylphenylamine, TsOH, toluene, reflux, then oxalyl chloride, Et_3N, 0°C–rt; (iii) ammonia, *n*-butanoic acid, toluene, reflux, 3 h; (iv) trifluoroacetic anhydride, CH_2Cl_2, rt; (v) $Hg(OAc)_2$, TFA, then H_2S, rt; (vi) HCl (conc.), CH_3OH, reflux, 3 h; (vii) ROCOCl, Et_3N, THF, 0°C–rt; or R-OH, triphosgene, Et_3N, THF.

Chart 1. Synthesis of the Substituted 6-Amino-4-(2,4-dimethoxyphenyl)-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones

with **7** to provide **8f**–**r**. All the newly synthesized compounds were characterized with ^{1}H-NMR, MS and elemental analysis.

Effects of Test Compounds in Normal Mice We reported here the *in vivo* effects of series of test compounds on WBC count of normal mice peripheral blood and percentage of neutrophil (NE%) of total WBC in Table 1. Recombinant human granulocyte colony stimulating factor (rhG-CSF) was used as positive control in our assay systems.[26,27] The results of the WBC count and the NE% of total WBC in normal mice test (Table 1) showed that after 5 d administration, the WBC count in the **8a** group mice increased from $(7.9\pm1.0)\times10^3$ cells·μL to $(24.9\pm3.1)\times10^3$ cells·μL, and the NE% of total WBC in the **8a** group mice were higher than that in the blank control group mice. Significant differences of both WBC count and NE% of total WBC were found between the **8a** group and the blank control group ($p<0.01$) after administration for 5 d, suggesting that the phenyl derivative **8a** displayed significant raising leukocyte count activity in the normal mice. To investigate substituent effects on the phenyl group, electron donating methoxy group and electron withdrawing chloro atom were introduced on the phenyl ring at the C4 position which provided compounds **8m** and **n**. However, both of these two compounds displayed a declined activity. These results suggest that the introduction of either electron donating group or electron withdrawing group to the phenyl ring at the C4 position is unfavorable. When the phenyl group of compound **8a** was replaced with benzyl group as in compound **8b** ($p<0.01$), a slight decrease in raising leukocyte count potency was observed. Among the alkyl derivatives, **8d** and **h** could also displayed raising leukocyte count activity ($p<0.01$), but in a less extent. On the other hand, corresponding isopropyl, *n*-butyl, *n*-pentyl, cycolpentyl and *n*-heptyl analogs (**8f**, **g**, **i**–**k**) had a moderate effect ($p<0.05$). Compound **8e** also had a moderate effect ($p<0.05$). While compound **8l**, in which the phenyl group was replaced with 2-phenylethyl group, showed weak raising leukocyte count activity. Moreover, replacement of phenyl moiety of compound **8a** with 2-tetrahydrofuranylmethyl group, 2-furylmethyl group, 2-thiophenylmethyl group and 4-pyridyl group to afford aromatic heterocyclic compounds **8o**–**r**, which were less active than rhG-CSF and ZL-004. Methyl derivative **8c** was almost devoid of raising leukocyte count activity. It is noteworthy that no significant differences of both the WBC count and the NE% of total WBC were found on day 5 between the four most potent compound (**8a**, **b**, **d**, **h**) and ZL-004 or rhG-CSF group ($p>0.05$). These facts implied that the alkyl groups containing from 2 to about 4 carbon atoms, in particular ethyl, vinyl, isobutyl seem to be favorable. All of the data analyzed above indicated that the phenyl group might be most favorable. Therefore, phenyl derivative **8a** was selected for further evaluation.

Effects of Compound 8a in CTX-Treated Mice In order to further investigate the effect of test compounds in chemotherapy-induced leucopenia, compound **8a** was selected to evaluate its antileukopenia activity in CTX-treated mice (Table 2). Significant differences of both WBC count and NE% of total WBC were observed after **8d** administration between the **8a** group and the CTX model group ($p<0.01$), but no significant difference was found between **8a** group and ZL-004 or rhG-CSF group ($p>0.05$), suggesting that the compound **8a** displayed significant antileukopenia activity in the mice model of leukopeania.

Conclusion

In this study, eighteen substituted 6-amino-4-(2,4-dimethoxyphenyl)-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones were designed and synthesized, and all the newly synthesized compounds were evaluated for their raising leukocyte count activity in normal mice. Among these synthetic compounds, compound **8a**, **b**, **d** and **h** showed significant raising leukocyte count activity as comparable to blank control. Furthermore, compound **8a** was evaluated in CTX-treated mice which showed significant antileukopenia activity. These results suggest that

Table 1. Effects of Series of Test Compounds on WBC Count of Normal Mice Peripheral Blood and NE% of Total WBC (WBC Unit: $\times 10^3 \cdot \mu L$)

Group/Study day	Day 0		Day 3		Day 5	
	WBC	NE%	WBC	NE%	WBC	NE%
8a	7.9±1.0	33.7±2.9	18.2±2.7**	67.4±7.1**	24.9±3.1**	72.1±7.5**
8b	7.8±1.3	32.5±3.1	14.6±1.7*	54.1±6.0*	17.4±2.7**	68.3±6.5**
8c	8.1±1.2	31.6±2.7	8.8±1.3	35.5±3.8	8.6±1.4	36.6±4.4
8d	8.9±1.2	31.6±2.6	17.8±2.3**	60.0±6.1**	18.7±2.8**	65.5±7.0**
8e	7.8±0.9	19.9±2.3	12.5±1.7	56.1±5.3	13.1±1.7*	48.4±6.7*
8f	8.1±1.2	23.3±3.0	11.9±2.0	38.6±4.9	12.5±1.5*	50.0±6.0*
8g	6.4±0.9	25.6±2.8	12.7±2.1	39.6±5.0	13.3±1.8*	52.8±4.9*
8h	8.1±0.9	20.1±2.6	12.9±1.5*	52.1±4.9*	16.8±1.9**	68.6±7.4**
8i	8.8±1.2	27.7±2.6	11.5±1.6	46.6±5.1	14.9±1.6*	51.8±5.2*
8j	6.8±0.8	20.8±2.2	11.6±1.6	23.4±3.0	13.0±1.9*	50.4±3.8*
8k	7.5±1.0	20.8±2.4	11.6±1.5	25.6±2.9	13.3±1.8*	55.8±3.4*
8l	5.1±0.6	30.0±2.9	9.6±1.4	38.7±4.1	11.2±2.0	42.4±4.5
8m	8.9±1.3	20.8±1.9	10.2±1.5	24.3±2.3	10.2±1.6	32.3±3.6
8n	6.4±0.6	23.2±2.4	8.7±1.3	28.9±2.7	8.9±1.5	36.9±4.1
8o	6.9±0.9	24.4±2.3	9.3±1.6	28.9±3.1	10.9±1.7	35.3±7.0
8p	9.0±1.5	34.0±3.5	11.5±1.7	38.9±3.8	12.0±2.1	41.0±4.6
8q	5.2±0.4	29.8±3.7	9.9±1.4	36.5±3.7	11.2±2.0	39.6±5.2
8r	6.9±0.8	31.2±3.5	10.0±1.6	38.9±4.2	12.1±1.5	43.7±5.3
ZL-004	7.6±1.2	31.5±3.6	13.4±1.8*	46.3±6.0*	18.9±2.7**	78.2±7.4**
rhG-CSF	6.7±1.1	25.4±3.0	17.5±2.8**	57.8±6.5**	19.2±2.0**	60.6±5.4**
Blank control	8.0±1.4	21.7±2.6	8.4±1.4	26.6±3.2	8.2±1.4	35.8±3.3

Data are mean±S.D. for 8 mice per group. Study day 0 is pre-treatment; study days 3 and 5 are 24 h after 3 and 5 daily administration, respectively. *$p<0.05$, **$p<0.01$, *vs.* blank control group.

Table 2. Effects of Compound **8a** on WBC Count of CTX Model Mice Peripheral Blood and NE% of Total WBC (WBC Unit: $\times 10^3 \cdot \mu L$)

Group/Study day	Day 0		Day 4		Day 6		Day 8	
	WBC	NE%	WBC	NE%	WBC	NE%	WBC	NE%
CTX model	5.8±0.7	28.4±2.7	1.2±0.2	5.3±0.4	1.3±0.1	6.7±0.8	5.7±0.7	28.3±5.0
8a	5.9±0.6	27.3±2.6	1.2±0.1	5.5±0.3	2.7±0.3*	18.7±5.0*	18.4±2.4**	76.8±8.6**
ZL-004	5.8±0.5	28.6±2.0	1.1±0.1	4.3±0.4	1.4±0.2	6.2±4.4	15.3±2.1**	77.9±9.0**
rhG-CSF	5.9±0.8	30.4±3.1	1.1±0.2	4.6±0.5	1.8±0.2	6.7±3.5	15.4±2.2**	59.7±6.6**
Blank control	6.0±0.7	26.8±2.7	6.2±0.8**	26.5±3.5**	6.4±0.7**	28.6±3.6**	6.3±0.8	34.7±4.5

Data are mean±S.D. for 8 mice per group. Study day 0 is pre-treatment; study days 4, 6 and 8 are 24 h after 4, 6 and 8 daily administration, respectively. *$p<0.05$, **$p<0.01$, *vs.* CTX model group.

compound **8a** might be utilized as potential candidate for further development of novel agent for the treatment of leukocytopenia, and further investigation of this kind of compounds may be of interest.

Experimental

Chemistry Melting points were determined on a Yanaco MP-S3T micro-melting point apparatus and uncorrected. Mass spectra were determined on a HP5989A mass spectrometer. ^{1}H-NMR spectra were obtained on INOVA 400 (400 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are in ppm relative to TMS, and coupling constants (J) are expressed in hertz (Hz). Elemental analyses were obtained with a Carlo Erba EA 1108 instrument. Column chromatography was performed with Silica Gel Geduran Si 60 (Qindao Ocean Chemical Company, China). All the solvents were commercially available materials of reagent grade.

1,3-Bis(*tert*-butylthio)acetone (2) To a mixture of K_2CO_3 (304.2 g, 2.2 mol) and *tert*-butyl mercaptan (95 g, 1.1 mol) in acetone (1.0 L) at 0°C under the atmosphere of N_2, was added 1,3-dichloroacetone (63.5 g, 0.5 mol) dropwise and the mixture was stirred for 16 h at room temperature. The resulting solution was filtered, and the solvent were removed *in vacuo*. The residue was distillated under reduced pressure (10 mmHg) to afford compound **2** (107.6 g, 92.1%) as pale yellow oil.

4-*tert*-Butyl Mercapto-5-tertbutyl Mercapto Methenyl-1-(2,4-dimethoxyphenyl)-3-hydroxy-1,5-dihydropyrrolidone (3) A mixture of compound **2** (105.8 g, 0.45 mol), 2,4-dimethoxylaniline (68.9 g, 0.45 mol) and *p*-toluenesulfonic acid (8.6 g, 0.05 mol) in toluene (1.2 L) was heated for 8 h under reflux. The mixture was allowed to cool to −5°C, oxalyl chloride (57.2 g, 0.45 mol) was added dropwise and followed by the dropwise addition of a solution of triethylamine (91 g, 0.9 mol) in toluene (100 mL). The reaction mixture was allowed to warm to room temperature and was stirred for another 12 h. The reaction solution was concentrated *in vacuo* and water (1.0 L) was added to the residue, the aqueous phase was extracted with CH_2Cl_2 (3×500 mL). The combined organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvent were removed *in vacuo*. The residue was recrystallized from isoproanol to give compound **3** (128 g, 67.4%) as pale yellow solid. mp 165–166°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.26 (9H, s), 1.37 (9H, s), 3.72 (3H, s), 3.83 (3H, s), 6.42 (1H,

s), 6.50–6.52 (2H, m), 7.11 (1H, d, *J*=8.4 Hz), 7.6 (1H, br s). Electrospray ionization (ESI)-MS *m/z*: 424.18 $[M+H]^+$.

4-*tert*-Butyl Mercapto-5-*tert*-butyl Mercapto Methenyl-1-(2,4-dimethoxyphenyl)-3-amino-1,5-dihydropyrrolidone (4) A solution of *n*-butanoic acid (140 mL) and toluene (20 mL) was heated to 120°C and then pure ammonia gas was bubbled through the solution for 5 h. To the reaction mixture was added a solution of compound **3** (10.2 g, 0.24 mol) in toluene (20 mL). After the addition was completed, the pure ammonia gas was continued to bubble through the solution for 2 h. After the reaction was completed, the reaction mixture was poured into 2 mol/L aqueous NaOH (450 mL) at 0°C, the aqueous phase was extracted with CH_2Cl_2 (2×200 mL). The combined organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvent were removed *in vacuo*. The residue was recrystallized from ethyl acetate/*n*-hexane to afford compound **4** (8.14 g, 80%) as pale yellow solid. mp 141–143°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.22–1.38 (18H, m), 3.79 (3H, s), 3.87 (3H, s), 6.49–6.54 (2H, m), 6.88 (1H, s), 7.13 (1H, d, *J*=8.4 Hz), 8.24 (2H, br s). ESI-MS *m/z*: 423.19 $[M+H]^+$.

4-*tert*-Butyl Mercapto-5-*tert*-butyl Mercapto Methenyl-1-(2,4-dimethoxyphenyl)-3-trifluoro Acetamido-1,5-dihydropyrrolidone (5) To a solution of compound **4** (5 g, 11.8 mol) in CH_2Cl_2 (20 mL) was added the trifluoroacetic anhydride (3.7 g, 17.8 mmol), and the mixture was stirred for 1 h at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was recrystallized from *n*-hexane to afford title compound **5** (5.8 g, 95%) as pale yellow solid. mp 180–182°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.24 (9H, s), 1.37 (9H, s), 3.72 (3H, s), 3.82 (3H, s), 6.10 (1H, s), 6.48–6.51 (2H, m), 7.10 (1H, d, *J*=8.4 Hz). ESI-MS *m/z*: 519.16 $[M+H]^+$.

***N*-[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]-dithiolo[4,3-*b*]pyrrol-6-yl]-2,2,2-trifluoroacetamide (6)** A mixture of compound **5** (5.8 g, 11.2 mmol) and mercuric acetate (3.2 g, 11.2 mmol) was dissolved in TFA (20 mL) at room temperature under the atmosphere of N_2, the reaction mixture was stirred for 1 h. The resulting mixture was concentrated *in vacuo*, the residue was dissolved in the CH_3CN (100 mL). Then hydrogen sulfide gas was bubbled through the reaction solution for 3 h. The reaction mixture was then degassed of excess hydrogen sulfide gas *via* bubbling N_2 through the reaction mixture for 1 h. The reaction solution was concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2 (200 mL). The slurry was filtered through diatomaceous earth and the filtrate was concentrated *in vacuo* to obtain the crude product, which was recrystallized from methanol to give title compound **6** (2.85 g, 63%) as pale yellow solid. mp 188–189°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.72 (3H, s), 3.82 (3H, s), 6.61–6.74 (2H, m), 6.81 (1H, s), 7.18 (1H, d, *J*=8.8 Hz). ESI-MS *m/z*: 405.02 $[M+H]^+$.

6-Amino-4-(2,4-dimethoxyphenyl)-4*H*-[1,2]dithiolehetero-cyclicpentene[4,3-*b*]-5-pyrrolone Hydrochloride (7) To a solution of compound **6** (1.9 g, 4.7 mmol) in methanol (20 mL) was added concentrated hydrochloric acid (5 mL). The reaction mixture was refluxed for 3 h, then the hot solution was filtered, the filtrate was cooled to 0°C and was stirred for 12 h to get green crystalline solid **7** (1.3 g, 80%), mp 230–232°C. The product was used directly in the next step.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]-dithiolo[4,3-*b*]-pyrrol-6-yl]carbamic Acid Ethyl Ester (ZL-004) To a solution of compound **7** (300 mg, 0.9 mmol) and triethylamine (181 mg, 1.8 mmol) in THF (20 mL) at room temperature was added the ethyl chlorocarbonate (194 mg, 1.8 mmol). After the addition was completed, the mixture was stirred for 1 h. The reaction solution was concentrated *in vacuo* and the residue was dissolved in water (100 mL), the aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic layer was washed with brine and dried over $MgSO_4$, filtered, evaporated, and purified by flash column chromatography to get compound ZL-004 (228 mg, 67.0%) as pale yellow solid, mp 208–210°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.25 (3H, t, *J*=8.0 Hz), 3.74 (3H, s), 3.84 (3H, s), 4.17 (2H, q, *J*=8.0 Hz), 6.62–6.76 (3H, m), 7.72 (1H, d, *J*=8.4 Hz), 9.31 (1H, br s). ESI-MS *m/z*: 403.06 $[M+Na]^+$. *Anal.* Calcd for $C_{16}H_{16}N_2O_5S_2$: C, 50.51; H, 4.24; N, 7.36; O, 21.03; S, 16.86. Found: C, 50.43; H, 4.20; N, 7.45; O, 21.10; S, 16.82.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Phenyl Ester (8a) Compound **8a** was synthesized as similar procedure described for ZL-004 from **7** using phenyl chlorocarbonate in place of ethyl chlorocarbonate: Yield 90.9%, mp 204–206°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.76 (3H, s), 3.83 (3H, s), 6.33 (1H,s), 6.53–6.58 (2H, m), 7.18–7.25 (4H,m), 7.36–7.41 (2H, m). ESI-MS *m/z*: 429.06 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{16}N_2O_5S_2$: C, 56.06; H, 3.76; N, 6.54; O, 18.67; S, 14.97. Found: C, 56.13; H, 3.81; N, 6.44; O, 18.60; S, 15.02.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Benzyl Ester (8b) Compound **8b** was synthesized as similar procedure described for ZL-004 from **7** using benzyl chlorocarbonate in place of ethyl chlorocarbonate: Yield 85.5%, mp 165–166°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.76 (3H, s), 3.83 (3H, s), 4.31 (2H, s), 6.60–6.62 (2H, m), 6.73 (1H, s), 7.12–7.29 (2H, m), 7.32–7.36 (4H, m), 9.39 (1H, s). ESI-MS *m/z*: 465.08 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{18}N_2O_5S_2$: C, 57.00; H, 4.10; N, 6.33; O, 18.08; S, 14.49. Found: C, 57.13; H, 4.15; N, 6.25; O, 18.01; S, 14.46.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]-carbamic Acid Methyl Ester (8c) Compound **8c** was synthesized as similar procedure described for ZL-004 from **7** using methyl chlorocarbonate in place of ethyl chlorocarbonate: Yield 80.2%, mp 186–188°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.68 (3H, s), 3.75 (3H, s), 3.82 (3H, s), 5.82 (3H, s), 6.37–6.80 (3H, m), 7.20 (1H, d), 9.44 (1H, br s). ESI-MS *m/z*: 389.14 $[M+Na]^+$. *Anal.* Calcd for $C_{15}H_{14}N_2O_5S_2$: C, 49.17; H, 3.85; N, 7.65; O, 21.83; S, 17.50. Found: C, 49.25; H, 3.77; N, 7.60; O, 21.73; S, 17.65.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Allyl Ester (8d) Compound **8d** was synthesized as ZL-004 from **7** using allyl chlorocarbonate in place of ethyl chlorocarbonate: Yield 82.3%, mp 210–212°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 3.81 (3H, s), 4.61 (2H, d, *J*=4.4 Hz), 5.20–5.23 (1H, m), 5.35–5.39 (1H, m), 5.95 (1H, m), 6.28 (1H, s), 6.51–6.55 (2H, m), 7.15 (1H, d), 9.48 (1H, br s). ESI-MS *m/z*: 415.08 $[M+Na]^+$. *Anal.* Calcd for $C_{17}H_{16}N_2O_5S_2$: C, 52.03; H, 4.11; N, 7.14; O, 20.38; S, 16.34. Found: C, 51.96; H, 4.14; N, 7.19; O, 20.29; S, 16.42.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid (8e) To a solution of compound **7** (400 mg, 1.16 mmol) in THF (20 mL) was added a solution of triphosgene (234 mg, 0.8 mmol) in THF (3 mL) at

0°C. After the addition was completed, the reaction mixture was allowed to warm to room temperature and was stirred for 1 h. The solution was concentrated *in vacuo* and THF (5 mL) was added to the residue, then concentrated hydrochloric acid (5 mL) was added to the reaction mixture and stirred for 30 min. The reaction solution was concentrated *in vacuo* and water (30 mL) was added, the aqueous phase was extracted with CH_2Cl_2 (3×15 mL). The combined organic layer was washed with brine and dried over $MgSO_4$, filtered, evaporated, and purified by flash column chromatography to get compound **8e** (337 mg, 83%) as pale yellow solid. mp 245-248°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 3.82 (3H, s), 6.24 (1H, s), 6.60–6.73 (3H, m), 7.19 (1H, d, J=8.4 Hz), 8.31 (1H, s). ESI-MS m/z: 374.05 $[M+Na]^+$. *Anal.* Calcd for $C_{14}H_{12}N_2O_5S_2$: C, 47.72; H, 3.43; N, 7.95; O, 22.70; S, 18.20. Found: C, 47.65; H, 3.40; N, 7.99; O, 22.64; S, 18.32.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Isopropyl Ester (8f) The solution of isopropanol (36 mg, 0.6 mmol) and triethylamine (61 mg, 0.6 mmol) in THF (20 mL) was stirred and allowed to cool to 0°C, followed by the dropwise addition of a solution of compound of triphosgene (180 mg, 0.6 mmol) in THF (5 mL). The reaction mixture was allowed to warm to room temperature and was stirred for 30 min. The reaction mixture was added the compound **7** (300 mg, 0.9 mmol) and was stirred at room temperature for 2 h. The reaction solution was concentrated *in vacuo* and water (30 mL) was added, the aqueous phase was extracted with CH_2Cl_2 (3×15 mL). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered, evaporated, and purified by column chromatography to get title compound **8f** (283 mg, 80%) as pale yellow solid, mp 230–232°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.25 (6H, d, J=6.0 Hz), 3.73 (3H, s), 3.82 (3H, s), 4.88–4.91 (1H, m), 6.61–6.64 (1H, m), 6.74–6.75 (2H, m), 7.19 (1H, d, J=8.8 Hz), 9.17 (1H, brs). ESI-MS m/z: 417.09 $[M+Na]^+$. *Anal.* Calcd for $C_{17}H_{18}N_2O_5S_2$: C, 51.76; H, 4.60; N, 7.10; O, 20.28; S, 16.26. Found: C, 51.89; H, 4.55; N, 7.17; O, 20.19; S, 16.20.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid *n*-Butyl Ester (8g) Compound **8g** was synthesized as similar procedure described for **8f** from **7** using *n*-butyl alcohol in place of isopropanol: Yield 75%, mp 177–178°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.91 (3H, t, J=7.2 Hz), 1.35–1.40 (2H, m), 1.56–1.63 (2H, m), 3.73 (3H, s), 3.82 (3H, s), 4.11 (2H, t, J=6.4 Hz), 6.60–6.75 (3H, m), 7.19 (1H, d, J=8.8 Hz), 9.31 (1H, brs). ESI-MS m/z: 431.09 $[M+Na]^+$. *Anal.* Calcd for $C_{18}H_{20}N_2O_5S_2$: C, 52.92; H, 4.93; N, 6.86; O,19.58; S, 15.70. Found: 52.84; H, 4.97; N, 6.90; O, 19.51; S, 15.78.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Isobutyl Ester (8h) Compound **8h** was synthesized as similar procedure described for **8f** from **7** using isobutyl alcohol in place of isopropanol: Yield 68%, mp 226–227°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.92 (6H, d, J=7.2 Hz), 1.91 (1H, m), 3.74 (3H, s), 3.82 (3H, s), 3.89 (2H, d), 6.60–6.75 (3H, m), 7.19 (1H, d, J=8.8 Hz), 9.35 (1H, s). ESI-MS m/z: 431.09 $[M+Na]^+$. *Anal.* Calcd for $C_{18}H_{20}N_2O_5S_2$: C, 52.92; H, 4.93; N, 6.86; O, 19.58; S, 15.70. Found: C, 52.99; H, 4.89; N, 6.91; O, 19.48; S, 15.73.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid *n*-Pentyl Ester (8i) Compound **8i** was synthesized as similar procedure described for **8f** from **7** using *n*-pentanol in place of isopropanol: Yield 63.3%, mp 178–179°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.89 (3H, t, J=7.6 Hz), 1.34–1.36 (4H, m), 1.61 (2H, m), 3.74 (3H, s), 3.83 (3H, s), 4.09 (2H, t, J=7.2 Hz), 6.61–6.74 (3H, m), 7.20 (1H, d, J=8.4 Hz), 9.33 (1H, s). ESI-MS m/z: 445.11 $[M+Na]^+$. *Anal.* Calcd. for $C_{19}H_{22}N_2O_5S_2$: C, 54.01; H, 5.25; N, 6.63; O, 18.93; S, 15.18. Found: C, 54.10; H, 5.26; N, 6.58; O, 18.99; S, 15.07.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Cyclopentyl Ester (8j) Compound **8j** was synthesized as similar procedure described for **8f** from **7** using cyclopentanol in place of isopropanol: Yield 67.5%, mp 228–230°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.56–1.69 (6H, m), 1.84–1.86 (2H, m), 3.72 (3H, s), 3.82 (3H, s), 5.07–5.08 (1H, m), 6.61–6.74 (3H, m), 7.19 (1H, d, J=8.8 Hz), 9.16 (1H, s). ESI-MS m/z: 443.11 $[M+Na]^+$. *Anal.* Calcd for $C_{19}H_{20}N_2O_5S_2$: C, 54.27; H, 4.79; N, 6.66; O, 19.02; S, 15.25. Found: C, 54.33; H, 4.82; N, 6.71; O, 18.95; S, 15.19.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Heptyl Ester (8k) Compound **8k** was synthesized as similar procedure described for **8f** from **7** using *n*-heptanol in place of isopropanol: Yield 81.5%, mp 144–146°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.89 (3H, t, J=6.6 Hz), 1.3–1.34 (8H, m), 1.65–1.68 (2H, m), 3.75 (3H, s), 3.83 (3H, s), 4.17 (2H, t, J=6.4 Hz), 6.27 (1H, s), 6.53–6.57 (2H, m), 6.88 (1H, s), 7.17 (1H, d, J=8.8 Hz). ESI-MS m/z: 473.18 $[M+Na]^+$. *Anal.* Calcd for $C_{21}H_{26}N_2O_5S_2$: C, 55.98; H, 5.82; N, 6.22; O, 17.75; S, 14.23. Found: C, 55.89; H, 5.86; N, 6.19; O, 17.79; S, 14.27.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid 2-Phenylethyl Ester (8l) Compound **8l** was synthesized as similar procedure described for **8f** from **7** using 2-phenylethanol in place of isopropanol: Yield 68.5%, mp 200–203°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.94 (2H, t, J=6.8 Hz), 3.73 (3H, s), 3.83 (3H, s), 4.30 (2H, t, J=6.8 Hz), 6.61–6.63 (1H, m), 6.73–6.76 (2H, m), 7.18–7.31 (5H, m), 9.41 (1H, brs). ESI-MS m/z: 479.07 $[M+Na]^+$. *Anal.* Calcd for $C_{22}H_{20}N_2O_5S_2$: C, 57.88; H, 4.42; N, 6.14; O, 17.52; S, 14.05. Found: C, 57.95; H, 4.47; N, 6.08; O, 17.46; S, 14.04.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid 4-Methoxyphenyl Ester (8m) Compound **8m** was synthesized as similar procedure described for **8f** from **7** using *p*-methoxyphenol in place of isopropanol: Yield 65%, mp 204–206°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 3.74 (3H, s), 3.83 (3H, s), 6.62–6.64 (1H, m), 6.75–6.76 (1H, m), 6.82 (1H, s), 6.96 (2H, d, J=9.2 Hz), 7.12 (2H, d, J=8.8 Hz), 7.21 (1H, d, J=9.2 Hz), 9.99 (1H, brs). ESI-MS m/z: 481.02 $[M+Na]^+$. *Anal.* Calcd for $C_{21}H_{18}N_2O_6S_2$: C, 55.01; H, 3.96; N, 6.11; O, 20.94; S, 13.99. Found: C, 55.12; H, 3.99; N, 6.03; O, 20.88 S, 13.98.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid 4-Chlorophenyl Ester (8n) Compound **8n** was synthesized as similar procedure described for **8f** from **7** using *p*-chlorophenol in place of isopropanol: Yield 70%, mp 234–236°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.75 (3H, s), 3.84 (3H, s), 6.48–6.70 (5H, m), 7.19 (1H, d, J=8.8 Hz), 7.66 (1H, s), 9.53 (1H, brs). ESI-MS m/z: 485.03 $[M+Na]^+$. *Anal.* Calcd for $C_{20}H_{15}ClN_2O_5S_2$: C, 51.89; H, 3.27; Cl, 7.66; N, 6.05; O, 17.28; S, 13.85 . Found: C, 51.84; H,

3.31; Cl, 7.71; N, 6.07; O, 17.33; S, 13.74.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Tetrahydrofuran-2-ylmethyl Ester (8o) Compound **8o** was synthesized as similar procedure described for **8f** from **7** using tetrahydrofurfuryl alcohol in place of isopropanol: Yield 60.5%, mp 190–191°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.72 (3H, s), 3.82 (3H, s), 5.14 (2H, s), 6.47–6.78 (5H, m), 7.19 (1H, d, J=8.8 Hz), 7.68 (1H, s), 9.53 (1H, s). ESI-MS m/z: 459.07 $[M+Na]^+$. *Anal.* Calcd for $C_{19}H_{16}N_2O_6S_2$: C, 52.77; H, 3.73; N, 6.48; O, 22.20; S, 14.83. Found: C, 52.68; H, 3.77; N, 6.51; O, 22.17; S, 14.87.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Furan-2-ylmethyl Ester (8p) Compound **8p** was synthesized as similar procedure described for **8f** from **7** using 2-furanmethanol in place of isopropanol: Yield 62.3%, mp 156–158°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.60–1.63 (1H, m), 1.79–1.95 (3H, m), 3.62–3.68 (1H, m), 3.73–3.8 (4H, s), 3.87 (3H, s), 4.04–4.11 (3H, m), 6.21–6.76 (3H, m), 7.18 (1H, d, J=8.4 Hz), 9.45 (1H, br s). ESI-MS m/z: 455.08 $[M+Na]^+$. *Anal.* Calcd for $C_{19}H_{20}N_2O_6S_2$: C, 52.28; H, 4.62; N, 6.42; O, 21.99; S, 14.69. Found: C, 52.38; H, 4.63; N, 6.47; O, 21.91; S, 14.61.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Thiophen-2-ylmethyl Ester (8q) Compound **8q** was synthesized as similar procedure described for **8f** from **7** using 2-thiophenemethanol in place of isopropanol: Yield 65.8%, mp 225–226°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 3.83 (3H, s), 5.34 (2H, s), 6.61–6.75 (4H, m), 7.03 (1H, s), 7.19 (1H, d, J=8.4 Hz), 7.57 (1H, d, J=7.6 Hz), 9.56 (1H, br s). ESI-MS m/z: 449.02 $[M+H]^+$. *Anal.* Calcd for $C_{19}H_{16}N_2O_5S_3$: C, 50.88; H, 3.60; N, 6.25; O, 17.84; S, 21.45. Found: C, 50.95; H, 3.63; N, 6.19; O, 17.80; S, 21.43.

[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]carbamic Acid Pyridine-4-yl Ester (8r) Compound **8r** was synthesized as similar procedure described for **8f** from **7** using 4-hydroxypyridine in place of isopropanol: Yield 72.4%, mp 176–178°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.73 (3H, s), 3.82 (3H, s), 6.62–6.85 (3H, m), 7.21–7.23 (1H, m), 7.47–7.50 (1H, m), 7.7–7.73 (1H, m), 8.47–8.5 (2H, m), 10.30 (1H, s). ESI-MS m/z: 430.05 $[M+H]^+$. *Anal.* Calcd for $C_{19}H_{15}N_3O_5S_2$: C, 53.14; H, 3.52; N, 9.78; O, 18.63; S, 14.93. Found: C, 53.19; H, 3.50; N, 9.80; O, 18.59; S, 14.92.

Raising Leukocyte Count Activities in Normal Mice The raising leukocyte count activities in normal mice of all the synthesized compounds were performed according to the modified procedure described by Sun *et al.*[23] The BALB/c mice were randomly divided into blank control group, positive control group and dithiolopyrrolone series of compounds group, 8 mice in each group. Dithiolopyrrolone series of compounds were suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) containing less than 4% Tween 80. rhG-CSF group mice were injected with rhG-CSF (22.5 μg/kg) subcutaneously once daily. Dithiolopyrrolone series of compounds group mice were administered ig (20 mg/kg) 0.5 mL once daily, and blank control group mice were administered ig an equivalent of 0.5% CMC-Na solution once daily. All the mice were successively treated for 5 d. Peripheral blood samples were obtained from the retro-orbital venous plexus with ethylenediaminetetraacetic acid (EDTA) anticoagulant. Eye-bleed samples were taken from each group at day 0, 3 and 5, respectively. The total leukocyte were counted on a HEMAVET 950 animal hematology analyzer, and the effects of dithiolopyrrolone series of compounds group on peripheral blood leukocytes of normal mice were evaluated.

Antileukopenia Activity in CTX-Treated Mice Antileukopenia activity in CTX-treated mice were performed according to the method described in literature.[28,29] Compound **8a** was selected to evaluate its antileukopenia activity in CTX-treated mice. Forty BALB/c mice were randomly divided into blank control group, positive control group, dithiolopyrrolone series of compounds group and CTX model group, 8 mice in each group. Blank control group mice were not treated in any way. The other four groups of mice were treated with CTX by daily subcutaneous injection at a dose of 100 mg/kg for up to 3 d. Routine blood tests of mice in each group were performed on an animal hematology analyzer. There was significant difference between the CTX-treated group and the blank control group ($p<0.01$), indicating that the models of CTX-induced hematopoietic dysfunction in mice were successfully established. Following that, rhG-CSF group mice were injected with rhG-CSF (22.5 μg/kg) subcutaneously once daily. Dithiolopyrrolone series of compounds group mice were administered with compounds ZL-004 and **8a** (20 mg/kg) through gavaging once a day (0.5 mL). The mice of CTX model group and blank control group were treated with 5% CMC-Na solution (0.5 mL) daily. All the mice were treated successively for 8 d. Peripheral blood specimens were sampled from each group at days 0, 4, 6 and 8, respectively. The total leukocyte were counted on a HEMAVET950 animal hematology analyzer, and the effect of compound **8a** on peripheral blood leukocytes of CTX-treated mice was evaluated.

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