http://dx.doi.org/10.5012/bkcs.2014.35.12.3489

Synthesis of Dithiolopyrrolone Derivatives and Their Leukocyte-Increasing Activities

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Received June 27, 2014, Accepted August 11, 2014

In search of new antileukopenia agents, twenty dithiolopyrrolone derivatives were synthesized and evaluated for their leukocyte-increasing activities in normal mice. Among the synthesized compounds **4-23**, compounds **5** and **6** showed significant leukocyte-increasing activity ($p < 0.01$), and compounds **4**, **9** and **16** had a moderate effect ($p < 0.05$). Compound **5** also displayed stronger leukocyte-increasing activity than that of the positive recombinant human granulocyte colony stimulating factor (rhG-CSF). Above all, compound **5** would be a potential antileukopenia agent which deserved further research.

Key Words : Dithiolopyrrolone derivatives, Leukocyte-increasing activity, Leukopenia, Antileukopenia, rhG-CSF

Introduction

White blood cell (WBC) is an important component of the host defence against bacteria, viruses, fungi and parasites. The normal adult total WBC count is 4×10^9 cells/L to 10×10^9 cells/L, and the count below 4×10^9 cells/L is considered leukopenia.[1] Leukopenia is a common disease process that has a myriad of etiologies.[2] Chemotherapy is the most common cause of drug-related leukopenia, also non-chemotherapy drugs is another cause.[3] For the treatment of the leukopenia, the cytokines, such as recombinant human granulocyte colony stimulating factor (rhG-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) have been widely used in clinic.[4-6] However, several factors have limited their practical application, for example, rhG-CSF is only used in the case the patient faces 30% risk to develop febrile neutropenia.[7] Therefore, searching for agents with novel structural frameworks to overcome the disadvantages of the existing antileukopenia drugs has become more urgent than ever before.

Dithiolopyrrolones have shown a wide range of biological activities in against various gram-positive and gram-negative bacteria, yeasts, fungi and amoeboid parasites.[8-14] Dithiolopyrrolones have also been reported to have anticancer activity,[15-17] and shown potent membrane stabilization and platelet anti-aggregate activities.[18] We first reported that ZL-004 (Figure 1) exhibited antileukopenia activity.[19] In our previous study, the amino group at the C-6 position of ZL-004 was modified into carbamic ester derivatives, we synthesized a series of substituted 6-amino-4-(2,4-dimethoxyphenyl)-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones and evaluated for their antileukopenia activity.[20] However, the *in vivo* biological activities of ZL-004 and its analogues depend on many parameters, including bioavailability. We found that ZL-004 and its analogues exhibited antileukopenia activity but their low solubility makes it be utilized difficultly. Ureas

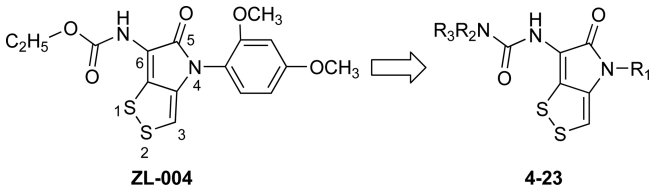


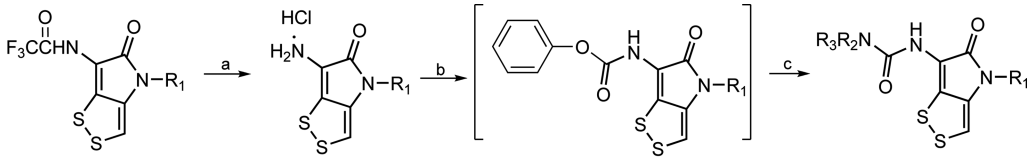
Figure 1. The structures of compound ZL-004 and target compounds (**4-23**).

are functional groups which have been found in many natural and artificial (macro) drug molecules.[21] Introducing urea functionality in compounds is amenable for altering physicochemical properties of the resultant drugs, and thus potentially increasing their *in vivo* activity.[22]

In this work, we mainly focused on the modification at N-4 and C-6 positions of ZL-004 and the structure–activity relationships (SARs) of dithiolopyrrolone derivatives. The amino group at the C-6 position of ZL-004 was modified into urea derivatives, and the modification at N-4 position of ZL-004 was also to examine the influence on leukocyte-increasing activity (Figure 1). Herein, a series of new dithiolopyrrolone derivatives (**4-23**) were designed, synthesized, and evaluated for their leukocyte-increasing activities.

Results and Discussion

Chemistry. Targeted compounds **4-23** were obtained in a three-step synthesis as shown in Scheme 1. Initially, the deprotection of trifluoroacetyl groups on amino group of substituted dithiolopyrrolone derivatives **1a-c** by hydrolysis with concentrated hydrochloric acid gave **2a-c** as hydrochloride salt.[16,20] The crude product was used directly in the next step. Treatment of **2a-c** with phenyl chloroformate in presence of triethylamine to get intermediates **3a-c**.[23] Then the corresponding amines (R_3R_2NH) were reacted with intermediates **3a-c** to provide **4-23**.[24-26] The structures of all



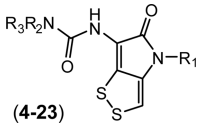
1a: R_1= 2,4-dimethoxyphenyl **2a**: R_1= 2,4-dimethoxyphenyl **3a**: R_1= 2,4-dimethoxyphenyl
1b: R_1= 4-methoxyphenyl **2b**: R_1= 4-methoxyphenyl **3b**: R_1= 4-methoxyphenyl
1c: R_1= benzyl **2c**: R_1= benzyl **3c**: R_1= benzyl

Scheme 1. Synthetic route for the preparation of dithiolopyrrolone derivatives (**4-23**). Reagents and conditions: (a) HCl (conc.), CH_3OH, reflux, 3 h, 50-60%; (b) phenyl chloroformate, Et_3N, THF, rt, 3 h; (c) amines, CH_2Cl_2, rt, 2 h, 30-50% (two steps).

newly synthesized compounds were confirmed by ESI-MS, ^{1}H NMR and elemental analysis. The preparation condition is mild and all reagents are commercially available.

Leukocyte-Increasing Activity. The synthesized compounds were evaluated for their *in vivo* leukocyte-increasing activities according to a reported method,[19,20] rhG-CSF was taken as a positive control drug in the assays. The results, expressed as WBC count values and percentage of neutrophil (NE%) of total WBC, were summarized in Table 1. As shown in Table 1, some of the prepared compounds displayed leukocyte-increasing activities in normal mice. The WBC count and NE% of total WBC were increased signifi-

Table 1. Effects of series of test compounds on WBC count of normal mice peripheral blood and NE% of total WBC (WBC unit: × 10^3 cells/μL)



(**4-23**)

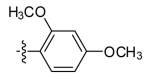
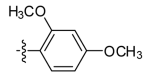
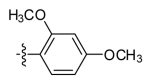
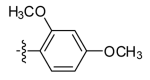
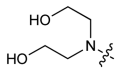
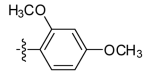
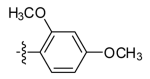
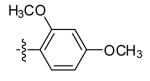
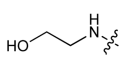
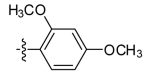
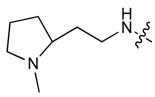
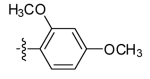
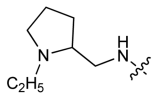
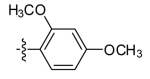
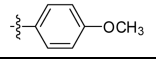
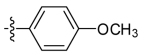
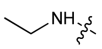
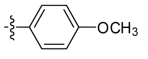
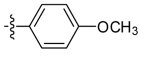
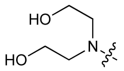
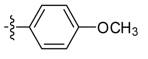
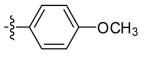
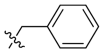
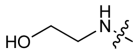
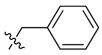
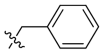
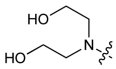
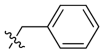
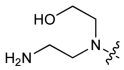
Compd.	R_1	R_3R_2N-	Day 0		Day 3		Day 5	
			WBC	NE%	WBC	NE%	WBC	NE%
4			7.4 ± 1.6	25.2 ± 2.8	9.8 ± 1.0	34.2 ± 3.8	11.4 ± 1.7*	50.2 ± 4.9*
5			7.0 ± 1.1	25.2 ± 1.9	16.8 ± 2.4**	57.2 ± 5.4**	19.5 ± 3.3**	67.2 ± 6.3**
6			6.8 ± 1.3	20.2 ± 1.6	15.6 ± 3.0**	55.2 ± 5.8**	16.9 ± 2.4**	63.2 ± 5.9**
7			6.2 ± 0.6	23.2 ± 2.4	9.2 ± 1.9	33.2 ± 3.9	10.7 ± 1.9	32.2 ± 3.1
8			6.2 ± 0.7	25.2 ± 2.1	7.2 ± 0.5	29.2 ± 2.0	6.6 ± 0.9	28.2 ± 2.7
9			5.8 ± 0.6	24.2 ± 2.0	11.4 ± 6.8*	47.2 ± 5.0*	12.4 ± 6.8*	48.2 ± 5.3*
10			8.2 ± 1.6	29.2 ± 2.7	8.4 ± 1.3	27.2 ± 3.4	8.6 ± 1.4	30.2 ± 3.5
11			5.6 ± 0.6	33.2 ± 2.7	9.8 ± 1.8	36.2 ± 2.9	10.8 ± 1.9	39.2 ± 4.6
12			5.6 ± 0.6	31.2 ± 1.8	5.4 ± 1.1	29.2 ± 2.8	7.2 ± 1.3	30.2 ± 2.9
13			7.2 ± 1.2	26.2 ± 2.4	7.6 ± 1.1	30.2 ± 3.6	8.8 ± 1.3	29.2 ± 3.8
14			7.6 ± 0.9	27.2 ± 2.4	8.6 ± 1.6	28.2 ± 2.9	6.9 ± 1.5	27.2 ± 2.1

Table 1. Contiued

Compd.	R_1	R_3R_2N-	Day 0		Day 3		Day 5	
			WBC	NE%	WBC	NE%	WBC	NE%
15			7.7 ± 0.9	32.2 ± 3.6	8.8 ± 1.8	35.2 ± 4.0	9.2 ± 1.6	39.2 ± 4.5
16			8.4 ± 1.6	30.2 ± 2.9	9.6 ± 1.8	31.2 ± 4.1	13.2 ± 1.9*	49.2 ± 5.5*
17			5.9 ± 0.6	28.2 ± 2.2	10.1 ± 2.3	38.2 ± 4.2	10.6 ± 2.5	40.2 ± 4.0
18			7.5 ± 0.7	30.2 ± 3.0	8.2 ± 1.5	31.2 ± 3.3	7.1 ± 1.7	28.4 ± 2.9
19			8.9 ± 1.5	33.2 ± 3.1	10.9 ± 2.4	33.7 ± 3.1	10.6 ± 2.5	30.2 ± 2.9
20			6.6 ± 1.0	24.2 ± 2.0	8.8 ± 1.7	25.2 ± 1.9	8.4 ± 1.6	26.2 ± 2.8
21			6.9 ± 0.8	27.2 ± 2.1	6.3 ± 1.4	25.2 ± 1.8	7.2 ± 1.3	27.2 ± 2.9
22			6.9 ± 1.0	33.2 ± 2.8	8.1 ± 1.3	33.9 ± 3.4	8.4 ± 1.6	35.0 ± 3.1
23			6.5 ± 1.0	30.2 ± 2.6	9.5 ± 1.6	33.2 ± 3.3	9.0 ± 1.8	32.2 ± 3.1
rhG-CSF			6.7 ± 0.9	28.2 ± 2.3	15.5 ± 2.9**	54.2 ± 5.4**	18.8 ± 2.7**	65.2 ± 6.4**
Blank control			6.7 ± 0.6	25.2 ± 2.7	6.6 ± 0.8	27.2 ± 3.0	6.6 ± 0.8	33.2 ± 3.2

Data are shown as the mean values ± S.D (n = 5). Study day 0 is pre-treatment; study days 3 and 5 are 24 hours after 3 and 5 daily administration respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, *vs*. blank control group.

cantly in **5** and **6** groups ($p < 0.01$) after administration for 5 days, especially the WBC count in the **5** group mice increased to $(19.5 \pm 3.3) \times 10^3$ cells/μL, and the NE% of total WBC in the **5** group mice increased to (67.2 ± 6.3)%. Compared with rhG-CSF, compound **5** and **6** also exhibited stronger or comparable activity. Three compounds (**4**, **9**, **16**) had a moderate effect ($p < 0.05$). It is evident that when the 2,4-dimethoxyphenyl was replaced with 4-methoxyphenyl at N-4 position as in compounds **14-19**, a decreased in leukocyte-increasing potency was observed. However, the compounds bearing benzyl group at R_1 (**20**, **21**, **22**, and **23**) were almost devoid of activity. These results suggest that introduction of 2,4-dimethoxyphenyl group to N-4 position is favorable. For R_3R_2N compounds, ethylamino group and diethylamino group were better for the activity, on the other hand, corresponding methylamino, bis (2-hydroethyl)amino, pyrrolyl, piperidyl, 2-hydroxyethylamino analogs (**4**, **7**, **8**, **9**, **10**) showed weak leukocyte-increasing activity, and the other heterocyclic derivatives (**11**, **12**, **13**) also did not display obvious leukocyte-increasing activity.

Experimental Section

Chemistry. All starting materials were commercially available at analytical grade and used as purchased without further purification. Reaction progress was monitored by thin layer chromatography (TLC), performed on precoated silica GF254 plates (0.2 mm, Chemical Industry Institute, Yantai, China). Column chromatography was performed on precoated silica gel 60 F254 plates and visualization on TLC was achieved by UV light (254 and 354 nm). Melting points were determined on WRS-21 melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on INOVA 400 (400 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are expressed in ppm relative to TMS, and coupling constants (*J*) are expressed in hertz (Hz). Mass spectra were recorded on an Agilent 1946B ESI-MS instrument. Elemental analyses were performed by Atlantic Microlab (Atlanta, GA, USA). The Synthetic route for the preparation of dithiolopyrrolone derivatives is summarized in Scheme 1.

General Procedure for Synthesis of Intermediates 2a-c. The intermediates **2a-c** were prepared by the method according to the literature reported.[16,20] In this study, substituted dithiolopyrrolone derivatives **1a-c** (10.0 mmol) were dissolved in methanol (40 mL), then to the solution was added concentrated hydrochloric acid (10 mL). The reaction was heated to 90 °C for 3 h, then the hot solution was filtered, the filtrate was cooled to 0 °C and stirred overnight to get compounds **2a-c** (yield: 50-60%). The product was used directly in the next step.

General Procedure for Synthesis of Target Molecules 4-23. A mixture of compounds **2a-c** (6.0 mmol), triethylamine (780 mg, 7.6 mmol) and phenyl chlorocarbonate (967 mg, 6.2 mmol) was dissolved in THF (80 mL) at room temperature. The mixture was stirred for 3 h. The solvent

was concentrated under reduced pressure to obtain the residue, and the dichloromethane (80 mL) was added. The organic portions were washed with brine and dried over anhydrous sodium sulfate to get the solution of intermediates **3a-c** in dichloromethane. To the solution of intermediates **3a-c** in dichloromethane, a solution of the corresponding amines (12.0 mmol) in THF (20 mL) was added dropwise. The mixture was stirred for 2 h at room temperature. The organic layer was washed with brine, dried over anhydrous sodium sulfate, evaporated and purified by flash column chromatography on silica with methanol/chloroform to yield compounds **4-23** (yield: 30-50%). The spectral data of **4-23** were described as follows:

1-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-3-methylurea (4). Yellow solid, yield 45%, mp 248-250 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.78 (3H, s), 3.76 (3H, s), 3.84 (3H, s), 6.20 (1H, s), 6.31-6.58 (2H, m), 7.14 (1H, d, J = 8.4 Hz), 7.59 (1H, s), 8.21 (1H, s); ESI-MS *m/z*: 388.11 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{15}H_{15}N_3O_4S_2$: C 49.30, H 4.14, N 11.50; Found: C 49.38, H 4.06, N 11.55.

1-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-3-ethylurea (5). Yellow solid, yield 50%, mp 236-238 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (3H, t, J = 7.2 Hz), 3.43 (2H, q, J = 7.2 Hz), 3.78 (3H, s), 3.86 (3H, s), 6.26 (1H, s), 6.55-6.60 (2H, m), 6.69 (1H, s), 7.20 (1H, d, J = 8.4 Hz); ESI-MS *m/z*: 402.10 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{16}H_{17}N_3O_4S_2$: C 50.64, H 4.52, N 11.07; Found: C 50.54, H 4.59, N 11.01.

3-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-1,1-diethylurea (6). Yellow solid, yield 35%, mp 180-183 °C; ^{1}H NMR (400 MHz, CDC1$_3$) δ 1.25 (6H, t, J = 7.6 Hz), 3.39 (4H, q, J = 7.6 Hz), 3.78 (3H, s), 3.86 (3H, s), 6.26 (1H, s), 6.55-6.60 (2H, m), 6.69 (1H, s), 7.20 (1H, d, J = 8.4 Hz); ESI-MS *m/z:* 408.02 $(M+H)^+$; Elem. Anal. Calcd. For $C_{18}H_{21}N_3O_4S_2$: C 53.05, H 5.19, N 10.31; Found: C 53.18, H 5.07, N 10.25.

3-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-1,1-bis(2-hydroxyethyl)urea (7). Yellow solid, yield 33%, mp 190-192 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.35-3.40 (4H, m), 3.58-3.65 (4H, m), 3.72 (3H, s), 3.82 (3H, s), 5.14 (2H, s), 6.59-6.72 (3H, m), 7.14-7.18 (1H, m), 8.79 (1H, s); ESI-MS *m/z:* 462.07 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{18}H_{21}N_3O_6S_2$: C, 49.19; H, 4.82; N, 9.56; Found: C, 49.28; H, 4.74; N, 9.64.

***N*-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)pyrrolidine-1-carboxamide (8).** Yellow solid, yield 35%, mp 198-200 °C; ^{1}H NMR (400 MHz, CDC1$_3$) δ 1.94-1.98 (4H, m), 3.46 (4H, d, J = 6.4 Hz), 3.76 (3H, s), 3.84 (3H, s), 6.24 (1H, s), 6.49-6.58 (2H, m), 7.16 (1H, d, J = 8.8 Hz); ESI-MS *m/z*: 428.15 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{18}H_{19}N_3O_4S_2$: C 53.32, H 4.72, N 10.36; Found: C 53.45, H 4.77, N 10.27.

***N*-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)piperidine-1-carboxamide (9).** Yellow solid, yield 40%, mp 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.48-1.56 (6H, m), 3.39-3.41 (4H, m), 3.72 (3H, s), 3.82 (3H, s), 6.61-6.69 (2H, m), 6.74 (1H, s), 7.17 (1H, d, J = 8.8 Hz), 8.05 (1H, s); ESI-MS *m/z*: 442.13 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{19}H_{21}N_3O_4S_2$: C 54.40, H 5.05, N 10.02; Found: C 54.52, H 5.11, N 9.97.

1-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-3-(2-hydroxyethyl)urea (10). Yellow solid, yield 33%, mp 226-228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.17 (2H, t, J = 6.0 Hz), 3.45 (2H, t, J = 6.0 Hz), 3.78 (3H, s), 3.81 (3H, s), 4.61 (1H, s), 6.51 (1H, s), 6.57-6.7 (3H, m), 7.14 (1H, d, J = 8.4 Hz); ESI-MS *m/z*: 418.10 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{16}H_{17}N_3O_5S_2$: C, 48.60; H, 4.33; N, 10.63; Found: C, 48.45; H, 4.19; N, 10.75.

1-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-3-(2-(1-methylpyrrolidin-2-yl)-ethyl)urea (11). Yellow solid, yield 31%, mp 200-202 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (2H, m), 1.63 (2H, m), 1.74 (2H, m), 2.14 (2H, m), 2.49 (2H, m), 3.79 (3H, s), 3.82 (3H, s), 6.58 (1H, s), 6.61-6.73 (2H, m), 7.20 (2H, d, J = 8.0 Hz), 8.26 (1H, s); ESI-MS *m/z*: 463.24 $(M+H)^+$; Elem. Anal. Calcd. For $C_{21}H_{26}N_4O_4S_2$: C, 54.52; H, 5.67; N, 12.11; Found: C, 54.42; H, 5.78; N, 12.24.

1-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-3-((1-ethylpyrrolidin-2-yl)methyl)urea (12). Yellow solid, yield 34%, mp 195-197 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, t, J = 8.0 Hz), 1.49-1.80 (4H, m), 2.05-2.20 (4H, m), 2.79 (1H, m), 2.97-3.05 (2H, m), 3.74 (3H, s), 3.82 (3H, s), 6.80 (1H, s), 6.85-6.95 (2H, m), 7.20 (1H, d, J = 8.0 Hz), 8.49 (1H, s); ESI-MS *m/z*: 463.07 $(M+H)^+$; Elem. Anal. Calcd. For $C_{21}H_{26}N_4O_4S_2$: C, 54.52; H, 5.67; N, 12.11; Found: C, 54.66; H, 5.50; N, 12.19.

***N*-(4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)piperazine-1-carboxamide (13).** Yellow solid, yield 30%, mp 180-182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.17 (4H, m), 3.74-3.79 (7H, m), 3.88 (3H, s), 6.24 (1H,s), 6.56 (1H, d, J = 8.8 Hz), 7.14 (1H, d, J = 8.8 Hz), 8.17 (1H, s); ESI-MS *m/z*: 421.13 $(M+H)^+$; Elem. Anal. Calcd. For $C_{18}H_{20}N_4O_4S_2$: C, 51.41; H, 4.79; N, 13.32; O, 15.22; S, 15.25; Found: C, 51.48; H, 4.82; N, 13.26.

1-(4-(4-Methoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo-[4,3-*b*]pyrrol-6-yl)-3-methylurea (14). Yellow solid, yield 41%, mp 280-282 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (3H, s), 3.80 (3H, s), 6.64 (1H, s), 6.94 (1H, s), 7.02-7.06 (2H, m), 7.22-7.34 (2H, m), 8.36 (1H, s); ESI-MS *m/z:* 358.11 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{14}H_{13}N_3O_3S_2$: C 50.13, H 3.91, N 12.53; Found: C 50.02, H 3.95, N 12.44.

1-Ethyl-3-(4-(4-methoxyphenyl)-5-oxo-4,5-dihydro-[1,2]-dithiolo[4,3-*b*]pyrrol-6-yl)urea (15). Yellow solid, yield 30%, mp 240-242 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (3H, t, J = 6.8 Hz), 3.02 (2H, q, J = 6.8 Hz), 3.87 (3H, s), 6.08 (1H, s), 6.60 (1H, s), 7.02-7.08 (2H, m), 7.28-7.34 (2H, m), 8.49 (1H, s); ESI-MS *m/z*: 372.13 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{15}H_{15}N_3O_3S_2$: C 51.56, H 4.33, N 12.03; Found: C 51.64, H 4.26, N 12.09.

1,1-Diethyl-3-(4-(4-methoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)urea (16). Yellow solid, yield 42%, mp 170-172 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (6H, t, J = 7.2 Hz), 3.38 (4H, q, J = 7.2 Hz), 3.85 (3H, s),

6.54 (1H, s), 6.65 (1H, s), 6.98-7.00 (2H, m), 7.31-7.43 (2H, m); ESI-MS *m/z*: 400.13 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{17}H_{19}N_3O_3S_2$: C 54.09, H 5.07, N 11.13; Found: C 54.16, H 5.01, N 11.21.

1,1-Bis(2-hydroxyethyl)-3-(4-(4-methoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)urea (17). Yellow solid, yield 45%, mp 165-167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.39-3.42 (4H, m), 3.52-3.57 (4H, m), 3.80 (3H, s), 5.20 (2H, s), 6.86 (1H, s), 6.98-7.00 (2H, m), 7.32-7.34 (2H, m), 8.79 (1H, s); ESI-MS *m/z*: 432.14 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{17}H_{19}N_3O_5S_2$: C, 49.86; H, 4.68; N, 10.26; Found: C, 49.77; H, 4.70; N, 10.21.

***N*-(4-(4-Methoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo-[4,3-*b*]pyrrol-6-yl)pyrrolidine-1-carboxamide (18).** Yellow solid, yield 35%, mp 198-200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86-1.96 (4H, m), 3.48 (4H, t, *J* = 6.0 Hz), 3.84 (3H, s), 6.50-6.54 (2H, m), 6.98-7.00 (2H, m), 7.28-7.33 (2H, m), ESI-MS *m/z*: 398.10 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{17}H_{17}N_3O_3S_2$: C 54.38, H 4.56, N 11.19; Found: C 54.46, H 4.47, N 11.27.

***N*-(4-(4-Methoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo-[4,3-*b*]pyrrol-6-yl)piperidine-1-carboxamide (19).** Yellow solid, yield 38%, mp 182-184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58-1.65 (6H, m), 3.45-3.48 (4H, m), 3.84 (3H, s), 6.54 (1H, s), 6.74 (1H, s), 6.98-7.00 (2H, m), 7.28-7.33 (2H, m), ESI-MS *m/z*: 412.13 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{18}H_{19}N_3O_3S_2$: C 55.51, H 4.92, N 10.79; Found: C 55.61, H 4.83, N 10.85.

1-(4-Benzyl-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-3-(2-hydroxyethyl)urea (20). Yellow solid, yield 35%, mp 145-147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20 (2H, t, *J* = 5.2 Hz), 3.49 (2H, t, *J* = 5.6 Hz), 4.88 (2H, s), 6.57 (1H, s), 7.16-7.26 (5H, m), 8.34 (1H, s); ESI-MS *m/z*: 372.06 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03; Found: C, 51.50; H, 4.28; N, 12.13.

***N*-(4-Benzyl-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)pyrrolidine-1-carboxamide (21).** Yellow solid, yield 35%, mp 220-222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.84 (4H, m), 3.36 (4H, t, *J* = 6.4 Hz), 4.95 (2H, s), 7.12 (1H, s), 7.22-7.34 (5H, m), 8.80 (1H, s); ESI-MS *m/z*: 382.06 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.77; N, 11.69; Found: C, 56.89; H, 4.70; N, 11.61.

3-(4-Benzyl-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-1,1-bis(2-hydroxyethyl)urea (22). Yellow solid, yield 42%, mp 165-167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.27-3.41 (4H, m), 3.59-3.75 (4H, m), 4.92 (2H, s), 7.12 (1H, s), 7.22-7.35 (5H, m), 8.80 (1H, s); ESI-MS *m/z*: 416.04 $(M+Na)^+$; Elem. Anal. Calcd. For $C_{17}H_{19}N_3O_4S_2$: C, 51.89; H, 4.87; N, 10.68; Found: C, 51.80; H, 4.83; N, 10.78.

1-(2-Aminoethyl)-3-(4-benzyl-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-1-(2-hydroxyethyl)urea (23). Yellow solid, yield 42%, mp 167-169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60-2.82 (4H, m), 3.12-3.17 (2H, m), 3.29-3.47 (2H, m),4.93 (2H, s), 6.77 (1H, s), 7.08 (1H, s), 7.21-7.39 (5H, m); ESI-MS *m/z*: 393.06 $(M+H)^+$; Elem. Anal. Calcd. For $C_{17}H_{20}N_4O_3S_2$: C, 52.02; H, 5.14; N, 14.27; Found: C, 52.11; H, 5.10; N, 14.35.

Leukocyte-Increasing Activity. The experiments were performed according to established procedures with some modifications. In the method, the BALB/c mice weighing between 18 and 20 g were used for the study. The mice were randomly divided into test compounds group, positive control group and blank control group, 5 mice in each group. Test compounds were dissolved in tween 80 suspensions. The synthesized compounds **4-23** were considered as test. Test compounds group mice were received the compounds **4-23** respectively each at a dose of 10 mg/kg body weight by i.g. once daily, positive control group mice were injected with rhG-CSF at the dose levels of 22.5 μg/kg body weight by s.c. once daily, and blank control group mice were received 0.5 mL 0.5% CMC-Na solution by i.g. once daily. All the mice were successively treated for 5 days. Eye-bleed samples were taken from each group at day 0, 3 and 5, respectively. Followed by measuring the total leukocyte by HEMAVET 950 animal hematology analyzer as described previously,[19,20] and the leukocyte-increasing activities of test compounds were evaluated.

Conclusion

In summary, twenty novel dithiolopyrrolone derivatives were designed and synthesized, and all the synthesized compounds were evaluated for their leukocyte-increasing activities in normal mice. Some test compounds with structural modifications exhibited promising leukocyte-increasing activity and the SARs have also been studied. The consequences demonstrated that compounds with 2,4-dimethoxyphenyl group at N-4 position and ethylamino group at R_3R_2N region of the molecule show better activities. The most potent compound **5** which might be utilized for the development of new candidate for treatment of leukocytopenia.

Acknowledgments. This work was supported by a grant from the Ministry of Science and Technology of China 'New Drug Innovation 2009X09313-006'.

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