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Cysteine Chloromethyl and Diazomethyl Ketone Derivatives with Potent Anti-Leukemic Activity

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Abstract—A series of cysteine diazomethyl- and chloromethyl ketone derivatives has been synthesized and evaluated against human B-lineage (Nalm-6) and T-lineage (Molt-3) acute lymphoblastic leukemia cell lines. The chloromethyl ketone compounds showed potent cytotoxicity against these cell lines, with IC_{50} values in the low micromolar range. The best compounds were *N*-acetyl-*S*-dodecyl-Cys chloromethyl ketone ($IC_{50} = 2.0$ μM against Nalm-6, 2.3 μM against Molt-3) and *N*-acetyl-S-*trans*,*trans*-farnesyl-Cys chloromethyl ketone ($IC_{50} = 3.0$ μM against Nalm-6 and 1.4 μM against Molt-3).

Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer.[1–3] The outcome of ALL patients who are not cured by contemporary multimodality treatment programs is dismal.[1–5] Therefore, there is an urgent need for novel anti-leukemic agents.[4,5] In a systematic effort aimed at identifying novel anti-leukemic agents with potent cytotoxic activity against human ALL cells, we synthesized a series of 16 cysteine diazomethyl- and chloromethyl ketone derivatives.

The synthesis of the compounds is outlined in Schemes 1–3. The majority of the compounds (**3a–e**, **4a–e**, Table 1 **4f**, **4g**, Table 2) were prepared by the synthetic scheme illustrated for *N*-acetyl-*S*-farnesyl-Cys chloromethyl ketone (**4a**) (Scheme 1). The thiol group of *N*-acetyl-L-cysteine (**1**) was isoprenylated by treatment with the appropriate isoprenyl bromide in a 4 M solution of ammonia in methanol according to the method of Brown and co-workers.[6] For the preparation of *N*-acetyl-*S*-dodecyl-Cys-OH, the addition of ethyl acetate as a co-solvent was necessary to aid solvation of the 1-bromododecane. The *S*-alkylated acid (**2a**) was activated as the mixed anhydride using isobutylchloroformate and converted first to the diazomethyl ketone (**3a**) by treatment with diazomethane[7] and then to the chloromethyl ketone (**4a**) with HCl in ethyl acetate at 0 °C for 10 min.

N-Boc-*S*-farnesyl-Cys chloromethyl ketone (**4b**) was prepared by the literature procedure starting from L-cysteine.[8] However, in the case of *N*-Boc-*S*-dodecyl-Cys chloromethyl ketone (**8**), *S*-dodecyl-Cys could not be isolated. Instead, cysteine methyl ester was dodecylated in a mixture of ethyl acetate and methanol (Scheme 2) to give **6**, followed by Boc-protection and ester hydrolysis to give the acid (**7**). Conversion in turn to the diazomethyl ketone and the chloromethyl ketone proceeded as described above. The free amine (**9**) was prepared from **8** by simply deprotecting the Boc group in a saturated solution of HCl in ethyl acetate. *N*-Boc-Gly-Cys chloromethyl ketone (**13**) was also prepared from cysteine methyl ester (Scheme 3). Thus, cysteine methyl ester was first farnesylated as above to give **10** and then coupled with *N*-Boc-Gly-OH using EDC/HOBt. The ester was hydrolyzed to the acid (**11**) and the chloromethyl ketone compound (**13**) was prepared via the diazomethyl ketone precursor (**12**) (Scheme 3). All compounds gave satisfactory spectroscopic and analytical data.[9]

The cytotoxic activities of the compounds were evaluated against two acute lymphoblastic leukemia (ALL) cell lines, Nalm-6 (B-lineage ALL) and Molt-3 (T-lineage ALL) using standard MTT assays[10] and the IC_{50} values were determined using Graphpad Prism software, version 2.0 (San Diego, CA). The comparison of the IC_{50} values (Tables 1 and 2) revealed significant information regarding the structure–activity relationships affecting the anti-leukemic activity of this series of compounds. First, the chloromethyl ketone derivatives displayed greater activity than their diazo analogues. Inclusion of a dodecyl *S*-substituent (compounds **3e**, **4e**,

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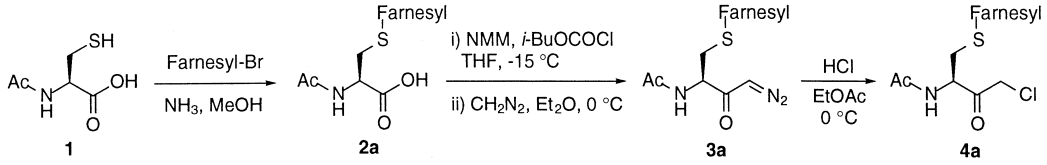
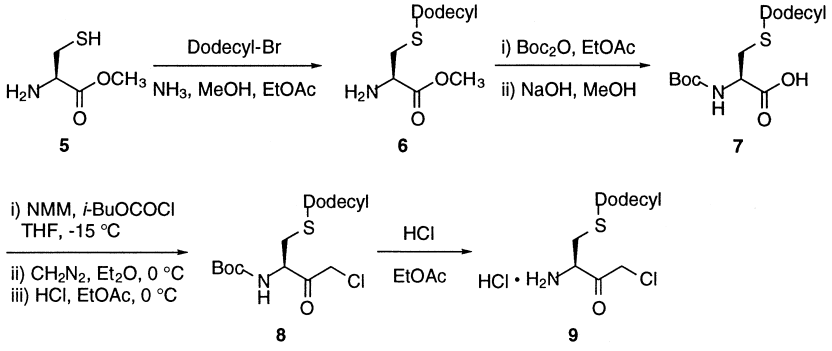
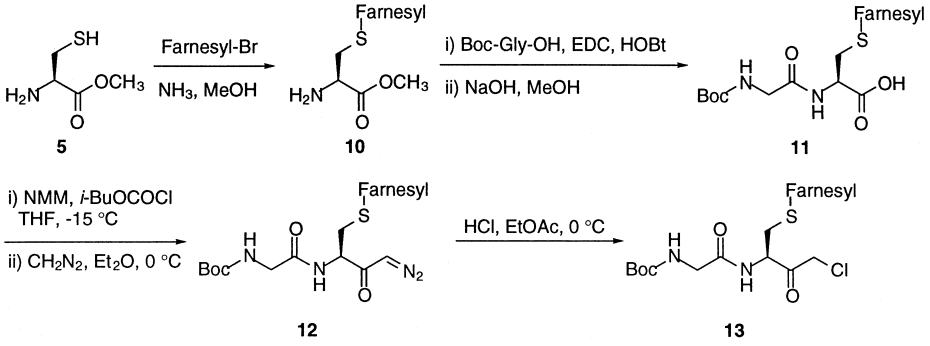


Scheme 1.

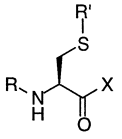


Scheme 2.



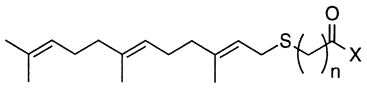
Scheme 3.

Table 1. Structure and activities of *S*-alkyl cysteine diazo and chloromethyl ketone derivatives against Nalm-6 (B-lineage ALL) and Molt-3 (T-lineage ALL) cell lines.



No.	HI Number	R	R′	X	IC_{50} (μM)	
					Nalm-6 B-lineage ALL	Molt-3 T-lineage ALL
12	401	Boc-Gly	*trans*,*trans*-Farnesyl	$CH{=}N_2$	51.3	84.5
3b	82	Boc	*trans*,*trans*-Farnesyl	$CH{=}N_2$	49.8	50.1
3a	367	Ac	*trans*,*trans*-Farnesyl	$CH{=}N_2$	30.3	32.2
3c	122	Ac	*trans*-Geranyl	$CH{=}N_2$	>100	>100
3d	123	Ac	3-Methyl-2-butenyl	$CH{=}N_2$	>100	>100
3e	348	Ac	Dodecyl	$CH{-}N_2$	15.4	22.9
13	130	Boc-Gly	*trans*,*trans*-Farnesyl	$CH_2{-}Cl$	12.9	17.5
4b	124	Boc	*trans*,*trans*-Farnesyl	$CH_2{-}Cl$	10.7	7.7
4a	368	Ac	*trans*,*trans*-Farnesyl	$CH_2{-}Cl$	3.0	1.4
4c	127	Ac	*trans*-Geranyl	$CH_2{-}Cl$	>100	>100
4d	128	Ac	3-Methyl-2-butenyl	$CH_2{-}Cl$	12.6	7.9
4e	131	Ac	Dodecyl	$CH_2{-}Cl$	2.0	2.3
8	129	Boc	Dodecyl	$CH_2{-}Cl$	15.1	15.5
9	252	H.HCl	Dodecyl	$CH_2{-}Cl$	17.7	12.5

Table 2. Structure and activities of farnesylthio methyl ketone derivatives against Nalm-6 and Molt-3 cell lines



No.	HI Number	n	X	IC_{50} (μM)	
				Nalm-6 B-lineage ALL	Molt-3 T-lineage ALL
4f	126	1	CH_2-Cl	84.3	>100
4g	125	2	CH_2-Cl	40.7	35.5

8, **9**) yielded compounds with excellent cytotoxic activities whose IC_{50} values ranged from 2.0 to 17.7 μM against Nalm-6 cells and from 2.3 to 22.9 μM against Molt-3 cells. The presence of a *trans*,*trans*-farnesyl substituent was also associated with excellent activty, but such compounds were at best equal to and generally less potent than their dodecyl analogues. Surprisingly, compound **4c** with a C-10 geranyl unit lacked anti-leukemic activity, whereas both compound **4d** with a C-5 prenyl unit and compound **4a** with a C-15 farnesyl unit were highly cytotoxic against Nalm-6 and Molt-3 leukemia cells (Table 1). Of the various N-terminal substituents examined, the *N*-acetyl group was preferred, with both larger (Boc; **3b**, **4b**, **8**) and smaller (H; **9**) derivatives showing lower activity. Table 2 shows that non-cysteine derivatives derived from the appropriate mercapto-carboxylic acid were not very active which demonstrates that inclusion of the amido functionality is important for anti-leukemic activity.

The p53-deficient Nalm-6 cell line was previously shown to be resistant to multiple chemotherapeutic agents, including alkylating agents, steroids, topoisomerase I inhibitors, topoisomerase II inhibitors, vincristine and taxol.[11–13] Therefore, the exquisite sensitivity of Nalm-6 cells to cysteine chloromethyl ketone derivatives is quite encouraging. Further development of the potent lead compounds, *N*-acetyl-S-dodecyl-Cys chloromethyl ketone (**4e**, HI-131) and *N*-acetyl-*S*-*trans*,*trans*-farnesyl-Cys chloromethyl ketone (**4a**, HI-368) may lead to the establishment of effective salvage programs for therapy-refractory acute lymphoblastic leukemia patients.

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9. Analytical data for selected active compounds: ***N*-Ac-*S*-*trans*-*trans*-farnesyl-Cys chloromethyl ketone (HI-368) (4a).** mp: 59–61 °C; ^{1}H NMR ($CDCl_3$): δ 1.56 (s, 6H), 1.64 (s, 6H), 2.01 (s, 3H), 2.04 (m, 8H), 2.78 (dd, J = 6.6, 13.9 Hz, 1H), 2.90 (dd, J = 5.8, 13.9 Hz, 1H), 3.14 (m, 2H), 4.31 (s, 2H), 4.85 (m, 1H), 5.05 (t, J = 6.9 Hz, 2H), 5.17 (t, J = 7.7 Hz, 1H), 6.36 (br, 1H); ^{13}C NMR ($CDCl_3$): δ 199.9, 170.0, 140.5, 135.4, 131.3, 124.2, 123.5, 119.2, 60.4, 55.3, 47.3, 39.7, 39.6, 31.9, 29.9, 26.7, 26.4, 25.7, 22.9, 17.7, 16.2, 16.0; IR (KBr): 3300, 3061, 2926, 2852, 2825, 1738, 1633, 1541, 1448, 1421, 1371, 1286, 1078 cm^{-1}; MS (EI): m/z 363 (M - HCl). ***N*-Ac-*S*-(3-methyl-2-butenyl)-Cys chloromethyl ketone (HI-128) (4d).** ^{1}H NMR ($CDCl_3$): δ 1.68 (s, 3H), 1.76 (s, 3H), 2.06 (s, 3H), 2.83 (dd, J = 6.5, 13.8 Hz, 1H), 2.95 (dd, J = 6.0, 13.8 Hz, 1H), 3.17 (m, 2H), 4.34 (m, 2H), 4.89 (q, J = 6.3 Hz, 1H), 5.20 (t, J = 7.8 Hz, 1H), 6.32 (d, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 199.8, 169.9, 136.8, 119.4, 55.4, 47.3, 32.1, 30.2, 25.8, 23.0, 17.9; IR (KBr): 3302, 3060, 2976, 2958, 2927, 2853, 1738, 1635, 1541, 1423, 1371, 1286, 1216, 1078 cm^{-1}; MS (EI): m/z 227 (M - Cl). ***N*-Ac-*S*-dodecyl-Cys chloromethyl ketone (HI-131) (4e).** mp: 73–74 °C; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.7 Hz, 3H), 1.26 (m, 18H), 1.57 (m, 2H), 2.06 (s, 3H), 2.54 (t, J = 7.3 Hz, 2H), 2.88 (dd, J = 6.3, 13.9 Hz, 1H), 2.99 (dd, J = 6.0, 13.9 Hz, 1H), 4.35 (m, 2H), 4.91 (m, 1H), 6.31 (br, 1H); ^{13}C NMR ($CDCl_3$): δ 195.2, 165.3, 50.8, 42.7, 29.6, 28.4, 28.2, 27.3, 25.1, 25.0, 24.9, 24.83, 24.8, 24.7, 24.5, 24.1, 18.3, 18.1, 9.5; IR (KBr): 3302, 2924, 2854, 1738, 1660, 1537, 1456, 1377, 1261, 1165, 1095, 1040 cm^{-1}; MS (MALDI-TOF): m/z 364.9 (M + 1), 328.9 (M-Cl).
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