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The *S*-Alkyl Chain Length as a Determinant of the Anti-Leukemic Activity of Cysteine Chloromethyl Ketone Compounds

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Abstract—A series of cysteine chloromethyl ketone compounds with a systematic variation of the *S*-alkyl chain length have been synthesized in order to gauge the effect of the alkyl chain length on the cytotoxicity of these compounds against human acute lymphoblastic leukemia cells. Comparable activities were observed for compounds with *S*-alkyl chains ranging from pentyl to dodecyl, with the best being undecyl ($IC_{50}=1.7$ μM) and dodecyl ($IC_{50}=2.0$ μM) against B-lineage leukemia cells and hexyl ($IC_{50}=0.7$ μM) against T-lineage leukemia cells.

We recently reported that certain cysteine chloromethyl ketone derivatives exhibit potent cytotoxicity against human acute lymphoblastic leukemia (ALL) cells.[1] Building on the result that the dodecyl derivative HI-131 (**3l**) was an exceptionally active compound against leukemia cells, we now report a follow-up structure–activity relationship (SAR) study in which the *S*-substituent was systematically varied between methyl and docosyl to determine the effect of the alkyl chain length on the cytotoxicity.

The cysteine chloromethyl ketone derivatives (**3a–s**) were synthesized by a modification of the standard literature procedure. Specifically, commercially available N-Ac-Cys-OH was *S*-alkylated by the method of Brown et al.[2] using the appropriate bromoalkane in an ammonia solution in methanol/ethyl acetate, the latter solvent being required in order to properly solvate the bromoalkane (Scheme 1). The *S*-alkylated acid **2a–s** was first converted to the diazomethyl ketone using the standard reagents (NMM, isobutyl chloroformate, diazomethane)[3] and then to the chloromethyl ketone **3a–s** by treatment with a dilute solution of HC1 in ethyl acetate. Initially we had used the standard conditions for making diazomethyl ketones, but a closer study of the dodecyl derivative (**3l**, HI-131) showed significant formation of the methyl ester as a side-product. Presumably, the mixed anhydride intermediate either could not completely form or was hydrolyzed back to the acid before the diazomethane could react with it. Carrying out the mixed anhydride formation at −78 °C should increase the stability and longevity of the mixed anhydride and improve yield. In practice, this was found to be the case and adding diazomethane directly to the solution at −78 °C without filtration led to over a 3-fold improvement in yield from 15 to 55% for the dodecyl derivative **3l** and resulted in a satisfactory yield of product for the majority of cysteine derivatives reported herein. All compounds gave satisfactory analytical and spectroscopic data.[4]

The compounds were then evaluated against two human acute lymphoblastic leukemia (ALL) cell lines, Nalm-6 (B-lineage ALL) and Molt-3 (T-lineage ALL) using standard MTT assays.[5] The IC_{50} values were determined using Graphpad Prism software, version 2.0 (San Diego, CA). An examination of the IC_{50} values revealed that the length of the alkyl chain has a profound effect on the anti-leukemic potency of this series of compounds (Table 1). Many of the compounds showed excellent cytotoxic activity against both Nalm-6 and Molt-3 cells, with the majority of compounds possessing IC_{50} values below 10 μM. Very short (methyl, ethyl) or very long (octadecyl, eicosyl, docosyl) substituents showed little or no activity. The tridecyl derivative (**3m**) showed no activity. The tetradecyl (**3n**), pentadecyl (**3o**) and hexadecyl (**3p**) derivatives had IC_{50} values ranging from 8.6 to 17.3 μM. Comparable activities were observed for compounds with a *S*-alkyl chain ranging from pentyl (**3e**) to dodecyl (**31**), all of which killed leukemic cells at low micromolar concentrations (Table 1). The most active compounds against Nalm-6 cells were the undecyl derivative (**3k**) ($IC_{50}=1.7$ μM) and our previously reported first generation lead compound, the dodecyl derivative (**3l**) ($IC_{50}=2.0$ μM). Against the

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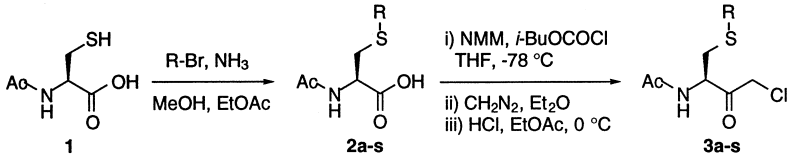
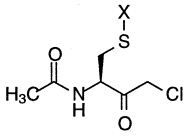


Scheme 1.

Table 1. Comparison of the effect of the *S*-alkyl chain length upon the activities of cysteine chloromethyl ketone derivatives against B-lineage (Nalm-6) and T-lineage (Molt-3) leukemia cells



No.	HI number	X	IC_{50} (μM)	
			Nalm-6 B-lineage ALL	MoIt-3 T-lineage ALL
3a	314	Methyl	30.3	80.8
3b	315	Ethyl	52.8	99.9
3c	369	Propyl	6.9	8.0
3d	363	Butyl	41.4	5.6
3e	224	Pentyl	5.8	5.4
3f	357	Hexyl	3.3	0.7
3g	263	Heptyl	4.8	2.5
3h	*352*	Octyl	5.6	4.1
31	364	Nonyl	7.3	6.7
3j	371	Decyl	4.7	3.4
3k	321	Undecyl	1.7	3.0
31	131	Dodecyl	2.0	2.3
3m	323	Tridecyl	>100	>100
3n	354	Tetradecyl	8.7	8.8
3o	225	Pentadecyl	8.9	8.6
3p	366	Hexadecyl	16.0	17.3
3q	370	Octadecyl	>100	>100
3r	226	Eicosyl	>100	>100
3s	322	Docosyl	>100	>100

T-lineage leukemia cell line, the shorter analogues showed the greater potency, with the most active compound being the hexyl derivative (**3f**) ($IC_{50} = 0.7$ μM). These compounds may be useful for treating patients with therapy-refractory or relapsed leukemia.

References and Notes

1. Perrey, D. A.; Narla, R. K.; Uckun, F. M. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 547.
2. Brown, M. J.; Milano, P. D.; Lever, D. C.; Epstein, W.; Poulter, C. D. *J. Am. Chem. Soc*. **1991**, *113*, 3176.
3. Green, G. D. J.; Shaw, E. *J. Biol. Chem*. **1981**, *256*, 1923.
4. Analytical data for selected active compounds: ***N*-Ac-*S*-hexyl-cysteine chloromethyl ketone** (**3f**, HI-357). Yellow solid (44% yield): mp 75–77 °C; ^{1}H NMR ($CDCl_3$): δ 0.87 (t, $J = 6.7$ Hz, 3H), 1.2–1.4 (m, 6H), 1.56 (m, 2H), 2.05 (s, 3H), 2.53 (t, $J = 7.4$ Hz, 2H), 2.89 (dd, $J = 6.0$, 13.9 Hz, IH), 2.96 (dd, $J =$ 5.8, 13.9 Hz, 1H), 4.35 (m, 2H), 4.89 (m, 1H), 6.36 (br d, 1H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.5, 22.9, 28.4, 29.4, 29.6, 31.3, 32.8, 47.4, 55.4, 170.1, 200.0; MS (EI): *m*/*z* 243 (M−HC1); IR (KBr) 3304, 3053, 2951, 2926, 2870, 1738, 1639, 1537, 1425, 1371, 1283 cm^{-1}. ***N*-Ac-*S*-undecyl-cysteine chloromethyl ketone** (**3k**, HI-321). Yellow solid (25% yield): mp 85–86 °C; ^{1}H NMR ($CDCl_3$): δ 0.86 (t, $J = 6.7$ Hz, 3H), 1.2–1.4 (m, 16H), 1.59 (m, 2H), 2.06 (s, 3H), 2.56 (t, $J = 7.3$ Hz, 2H), 2.89 (dd, $J = 6.3$, 13.7 Hz, 1H), 2.95 (dd, $J = 6.0$, 13.7 Hz, 1H), 4.28 (m, 2H), 4.93 (m, 1H), 6.21 (br d, 1H); ^{13}C NMR ($CDC1_3$) δ 14.1, 22.7, 22.9, 28.7, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 32.8, 32.9, 47,4, 55.4, 170.1, 200.0; MS (EI): *m*/*z* 313 (M−C1); MS (MALDI-TOF): *m*/*z* 313 (M−Cl); IR (KBr) 3305, 2918, 2850, 1738, 1651, 1537, 1466 cm^{-1}.
5. Narla, R. K.; Liu, X. P.; Myers, D. E.; Uckun, F. M. *Clin. Cancer Res*. **1998**, *4*, 1405.