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SYNTHESIS AND *IN VITRO* ANTI-HIV AND ANTITUMOR ACTIVITY OF NEW 2-CHLOROETHYL THIOCARBAMATES

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ABSTRACT : A series of new 2-chloroethyl thiocarbamates was synthesized and tested in the *in vitro* US-NCI primary anti-HIV and antitumor drug screens. Compound (3) demonstrated some anti-HIV and antitumor activity. This compound was cytotoxic in 3/6 leukemia cell lines tested but had no activity in 50 other cell lines tested. Compound (3) may serve as lead compound for new potential more specific anticancer agents.

The 2-chloroethylnitrosoureas are among the most active anticancer drugs in many experimental leukemia and solid tumors.[1,2,3] Their clinical activity has been established for a broad spectrum of human malignancies, including lymphomas, melanomas, acute lymphocytic leukemias, multiple myelomas, gliomas and gastrointestinal neoplasms.[2,4,5]

Recently, different research groups, including ours, have reported that 2-chloroethylureas, methylureas and propargylureas had anticancer activity thereby showing that the N^1-nitroso group of the nitrosoureas was not essential to the *in vitro* anticancer activity of these compounds (Figure 1).[6-8] Moreover, a few of these ureas were shown to have some specificity towards tumor cell lines.[6]

Cl-CH_2CH_2-N^1(R)-CO-N^3H-R'	Cl-CH_2CH_2-N(R)-CO-S-R'
R = H **2-chloroethylureas**	R = H **2-chloroethylthiocarbamates**
R = NO **2-chloroethylnitrosoureas**	R = NO **2-chloroethylnitrosothiocarbamates**

R' = Transporting moiety

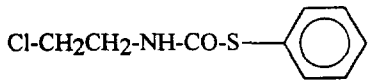
Figure 1

The goal of this exploratory study was to investigate the effect on antitumor activity and specificity of a bioisosteric replacement of the amino N^3 group of the ureas. In this work, we have produced 2-chloroethyl thiocarbamates derivatives and we have evaluated the biological activity of these compounds. We report here the synthesis and the *in vitro* anti-HIV and antitumor activity of a series of new 2-chloroethyl thiocarbamates.

MATERIALS AND METHODS - The compounds illustrated in Figure 2 were synthesized in the Medicinal Chemistry laboratories of the Faculty of Pharmacy of the University of Montreal. Elemental analyses were performed by the Guelph Chemical Lab. and are within $\pm 0.4\%$ of theorical values. ^{1}H NMR spectra were determined on a Varian VXR-300 spectrophotometer using the deuterated solvent ($CDCl_3$) as internal standard.

$Cl\text{-}CH_2CH_2\text{-}NH\text{-}CO\text{-}S\text{-}CH_2CH_2\text{-}OH$

(1)- MCS-798



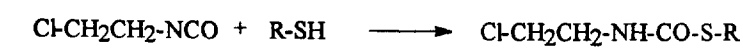
(2)- MCS-799

$Cl\text{-}CH_2CH_2\text{-}NH\text{-}CO\text{-}S\text{-}CH_2\text{-}COO\text{-}CH_2CH_3$

(3)- MCS-800

Figure 2

General procedure for the synthesis of the thiocarbamates (Figure 3): To a cooled (0-5 °C) solution of the sulfydryl derivative (0.1 mol) in 100 mL of anhydrous ether was slowly added 0.1 mol (10.55 g) of 2-chloroethyl isocyanate. The solution was stirred for 8 hours on an ice bath. The solution was then concentrated *in vacuo* over a warm bath .



2-chloroethylisocyanate **(1) - (3)**

Figure 3

(1)- 1-(2-hydroxyethyl)-3-(2-chloroethyl) thiocarbamate (MCS-798; NSC-641086): Yield: 17.31 g (94%) colorless liquid, stench!; ^1H NMR ($CDCl_3$) δ 5.15 (1H, m, NH), 4.22 (2H, m, CH_2), 3.62 (2H, t, CH_2), 3.52 (2H, m, CH_2), 2.74 (2H, m, CH_2), 1.78 (1H, s, OH); Anal. $C_5H_{10}ClNO_2S$ (C, H, N).

(2)- 1-phenyl-3-(2-chloroethyl) thiocarbamate (MCS-799) [4930-24-9]: Yield: 19.84 g (92%) colorless liquid, stench!; ^1H NMR ($CDCl_3$) δ 7.23 (5H, m, phenyl), 3.63 (4H, m, CH_2CH_2), 1.22 (1H, t, NH); $C_9H_{10}ClNOS$ (C, H, N).

(3)- Ethyl 2-[3-(2-chloroethyl) thiocarbamate]acetate (MCS-800; NSC-641087): Yield: 19.86 g (88%) colorless liquid, stench!; ^1H NMR ($CDCl_3$) δ 5.15 (1H, m, NH), 4.22 (2H, m, CH_2), 3.62 (2H, t, CH_2), 3.52 (2H, m, CH_2), 2.74 (2H, m, CH_2), 1.78 (1H, s, OH); $C_7H_{12}ClNO_3S$ (C, H, N).

Anti-HIV drug screen and Antitumor drug screen: The compounds were tested by the US-NCI as previously described.[6,9]

RESULTS AND DISCUSSION - Figure 3 illustrates the pathways used for the synthesis of the thiocarbamates. The reaction between the sulfydryl derivatives and 2-chloroethyl isocyanate produced the 2-chloroethyl thiocarbamates (**1-3**) in very good yields.

The determination of the anti-HIV and antiproliferative activities of compounds (**1-3**) were performed according to a published method by the US-NCI.[10] All the compound tested showed only very weak *in vitro* anti-HIV activity and only compound (**3**) showed weak antiproliferative activity in the non-infected T4 lymphocyte cell line (CEM-IW) tested (data not shown).

These results can be compared to a series of methyl- and propargylureas that we have previoulsy published.[9] Although the carrier groups of the methyl- and propargylureas were different (i.e., amino acids), these compounds had more anti-HIV and significantly more antiproliferative activity in the same *in vitro* anti-HIV model. Hence, the bioisosteric replacement of the amino N^3 group seems to reduced the anti-HIV and antiproliferative activity in this model.

The compounds were also tested in the US-NCI primary antitumor drug screen.[11-13] Based on a disease oriented strategy, the screen currently incorporates 60 human cell lines representing lung cancers, colon cancers, leukemias, ovarian cancers, melanomas, brain tumors, and renal cancers.[11,13] The hypothesis proposed in this screening is that exploitable mechanisms of histiospecific drug cytotoxicity exist and that such differences can be detected in cell lines adapted to long-term tissue culture.[11,12] This approach could lead to the discovery of new drugs to treat the major cancers.

The results of the *in vitro* anticancer activity in leukemia cell lines are shown in Table 1. Compound (**3**) was cytotoxic in 3/6 leukemia cell lines tested. The level of activity of compound (**3**) is comparable to other nitrosoureas (i.e. BCNU, CCNU, MeCCNU) tested in the same conditions (data not shown). However, all

Table 1 - *In vitro* anticancer activity expressed as GI_{50} (M) in 6 leukemia cell lines.

	(1) - MCS-798	(2) - MCS-799	(3) - MCS-800
CCRF-CEM	> 1 X 10^{-4}*	N.A.	7.22 X 10^{-6}
HL-60 (TB)	> 1 X 10^{-4}	N.A.	2.23 x 10^{-5}
K-562	> 1 X 10^{-4}	N.A.	2.90 X 10^{-5}
MOLT-4	> 1 X 10^{-4}	N.A.	> 1 X 10^{-4}
RPMI-8226	> 1 X 10^{-4}	N.A.	> 1 X 10^{-4}
SR	> 1 X 10^{-4}	N.A.	> 1 X 10^{-4}

* This value represent the maximum concentration used in this assay. The GI_{50} value was not reached at that concentration.

compounds tested were inactive in the 11 NSCLC, 2 SCLC, 9 colon cancers, 8 CNS cancers, 9 melanoma, 6 ovarian cancers, and 9 renal cancers cell lines.

Compound's (**3**) specific cytotoxicity against selected leukemia cell lines as compared to its lack of cytotoxicity in all other cell lines tested is noteworthy. This selectivity should be further explored and compound (**3**) should serve as lead compound for new potential more specific anticancer agents.

CONCLUSION - In this exploratory study, we synthesized and tested the *in vitro* anti-HIV and anticancer activity of a series of new 2-chloroethyl thiocarbamates derivatives. We have shown that these compounds are devoided of *in vitro* anti-HIV activity and have only weak antiproliferative activity against a non-infected T4 lymphocyte cell line. In the *in vitro* anticancer model, compound (**3**) was cytotoxic against 3/6 leukemia cell lines. However, it was not cytotoxic in the 54 other cell lines tested. This cell line specificity should be further explore. Compound (**3**) may serve as lead compound for new potential anticancer agents.

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