Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol 4, No 19, pp. 2353-2358, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00339-4

SYNTHESIS, CHEMICAL HALF-LIFE AND DECOMPOSITION OF NEW N^3-(SUBSTITUTED) DERIVATIVES OF CCNU

Gilles Gallant*, Romano Salvador and Hélène Dulude, *Medicinal Chemistry Laboratory, Faculty of Pharmacy, University of Montreal,Box 6128, Station A, Montreal, Quebec, Canada, H3C 3J7.*

*New address and address for correspondence : Gilles Gallant B.Pharm. Ph.D., *Bristol-Myers Squibb, 2365 Côte-de-Liesse, Montréal (Québec), Canada H4N 2M7 (Tel. 514-333-2019, FAX 514-331-8880).*

ABSTRACT : A series of new N^3-(substituted) derivatives of CCNU was synthesized with the aim of producing more stable compounds with increased cytotoxicity. The nitrosoureas produced in this study were much more stable in aqueous medium than CCNU (15 to 100 times longer half-lives). The decomposition of these nitrosoureas seems to be different in that they produce the original amine plus other decomposition products.

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]

CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea), synthesized by Johnston *et al.*,[6] has been used to treat human cancers since the mid seventies. It has a short *in vitro* chemical half-life of approximately 2 hours and an even shorter *in vivo* chemical half-life of less than 15 minutes.[2,7,8] CCNU is quite labile in aqueous media, having a $t_{0.95}$ of 50 minutes at room temperature.[9] The toxicity of CCNU, chiefly thrombocytopenia and leucopenia, is one of the major factors limiting its use against cancer.[10] The production of organic isocyanates during the breakdown of the 2-chloroethyl-nitrosoureas, is believed to be one of the factors which determines their toxicity.[10,11,12]

The goal of this study was to produce derivatives of CCNU that would be more stable *in vitro* and *in vivo* while remaining cytotoxic. We undertook a systematic study of the activity and the toxicity of N^3-(substituted) derivatives of CCNU. These compounds were shown to possess a longer chemical half-life and should not produce organic isocyanates during their decomposition, making them possibly less toxic.

We report here the synthesis and some physico-chemical properties of a series of new N^3-(substituted) derivatives of CCNU. The coumpounds have been submitted to the US-NCI primary antitumor drug screen. The antitumor activity and toxicity of these derivatives will be published later.

MATERIALS AND METHODS

Capillary melting points were determined on a Büchi 535 melting point apparatus and are reported uncorrected. Elemental analyses were performed by the Guelph Chemical Laboratories and are within $\pm 0.4\%$ of theorical values. IR spectra were determined on a Perkin-Elmer 710A spectrophotometer and ^{1}H NMR

spectra were determined on a Varian VXR-300 spectrophotometer using the deutarated solvent ($CDCl_3$) as internal standard. UV-VIS spectra were recorded on a Hitachi U-2000 spectrophotometer. Refraction index were determined on a Bausch & Lomb refractometer at 20 °C.

General procedure for the synthesis of the ureas (Table 1): To a cooled (0-5 °C) solution of the amine (0.1 mol) in the appropriate solvent was slowly added 0.1 mol (10.55 g) of 2-chloroethyl isocyanate. The solution was stirred for 3 to 4 hours on an ice bath. The solution was filtered and the white solid was dried overnight over P_2O_5. A recrystallisation was sometimes necessary.

Table 1 - Characteristics of the ureas.

$ClCH_2CH_2$-NH-CO-N(*c*He)R					
	R	**YIELD (%)**	**M.P. (°C)**	**^{1}H NMR (CH_2CH_2Cl)**	**ANAL.**
(1)	-Me	44	89.6-91.1	δ 3.61 (m)	C, H, N ($C_{10}H_{19}ClN_2O$)
(2)	-Et	74	89.5-90.1	3.64 (m)	C, H, N ($C_{11}H_{21}ClN_2O$)
(3)	-Pr	58	86.4-87.7	3.54 (m)	C, H, N, ($C_{12}H_{23}ClN_2O$)
(4)	-*i*Pr	78	112.2-113.4	4.72 3.96 (2t)	C, H, N, ($C_{12}H_{23}ClN_2O$)
(5)	-*i*Bu	63	73.2-76.5	3.66 (m)	C, H, N, ($C_{13}H_{25}ClN_2O$)
(6)	-*t*Bu	53	73.7-77.1	3.63 (m)	C, H, N, ($C_{13}H_{25}ClN_2O$)
(7)	-*c*Pr	31	144.1-149.3	3.65 (m)	C, H, N, ($C_{12}H_{21}ClN_2O$)
(8)	-*c*Pe	35	89.9-91.1	4.72 3.98 (2t)	C, H, N, ($C_{14}H_{25}ClN_2O$)
(9)	-*c*He	54	87.2-90 0	4.71 3.96 (2t)	C, H, N, ($C_{15}H_{27}ClN_2O$)
(10)	-CH_2CH_2OH	62	80.9-83.6	3.67 3.57 (2t)	C, H, N, ($C_{11}H_{21}ClN_2O_2$)
(11)	-$(CH_2)_3OH$	83	148.0-151.5	3.84 (m)	C, H, N, ($C_{12}H_{23}ClN_2O_2$)
(12)	-$CH_2CHOHCH_3$	63	95.5-96.1	3.59 3.48 (2m)	C, H, N, ($C_{12}H_{23}ClN_2O_2$)
(13)	-CH_2CHCH_2	34	78.9-80.5	3.53 (m)	C, H, N, ($C_{12}H_{21}ClN_2O$)
(14)	-CH_2CCH	28	92.4-95.4	3.66 (m)	C, H, N, ($C_{12}H_{19}ClN_2O$)

General procedure for the synthesis of the nitrosoureas (Table 2): To a cooled (-20 to -10 °C) solution of the amine (0.005 mol) in the appropriate solvent was slowly added 0.005 mol (1.34 g) of N-(2-Chloroethyl)-N-Nitrosocarbamic acid N'-Hydroxysuccinimide ester. The solution was stirred for 7 to 8 hours at low (-10 to 0 °C) temperature. $CHCl_3$ was added and the solution was then extracted with a 5% HCl solution, water, a saturated $NaHCO_3$ solution and finally water. The solution was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford the crude nitrosourea.

Chemical half-life determination: The chemical half-life of the N-Nitroso compounds were determined by following the change of absorbance of solution during incubation at 37 °C. The solutions were prepared by

dissolving the compounds in absolute ethanol and then adding 19 volumes of phosphate buffer, *p*H = 7.4. Absorbances were determined at wavelenghts near 400 nm corresponding to the absorption maxima for the respective compounds.

Table 2 - Characteristics of the nitrosoureas.

$ClCH_2CH_2$-N(NO)-CO-N(*c*He)R					
	R	YIELD (%)	IR (NNO) (cm_{-1})	^1H NMR (CH_2CH_2Cl)	ANAL.
(15)	-Me	91	1480	δ 4.13 3.67 (2t)	C, H, N ($C_{10}H_{18}ClN_3O_2$)
(16)	-Et	93	1500	4.12 3.67 (2t)	C, H, N ($C_{11}H_{20}ClN_3O_2$)
(17)	-Pr	84	1450	3.76 (m)	C, H, N, ($C_{12}H_{22}ClN_3O_2$)
(18)	-*i*Pr	64	1450	4.74 4.05 (2t)	C, H, N, ($C_{12}H_{22}ClN_3O_2$)
(19)	-*i*Bu	76	1450	4.10 3.60 (2t)	C, H, N, ($C_{13}H_{24}ClN_3O_2$)
(20)	-*t*Bu	71	1460	4.35 3.90 (2t)	C, H, N, ($C_{13}H_{24}ClN_3O_2$)
(21)	-*c*Pr	68	1450	4.11 3.58 (2t)	C, H, N, ($C_{12}H_{20}ClN_3O_2$)
(22)	-*c*Pe	42	1460	4.75 3.98 (2t)	C, H, N, ($C_{14}H_{24}ClN_3O_2$)
(23)	-*c*He	71	1450	4.73 3.98 (2t)	C, H, N, ($C_{15}H_{26}ClN_3O_2$)
(24)	$-CH_2CH_2OH$	66	1430	3.68 3.52 (2t)	C, H, N, ($C_{11}H_{20}ClN_3O_3$)
(25)	$-(CH_2)_3OH$	50	1450	3.71 3.54 (2t)	C, H, N, ($C_{12}H_{22}ClN_3O_3$)
(26)	$-CH_2CHOHCH_3$	51	1420	3.59 3.06 (2t)	C, H, N, ($C_{12}H_{22}ClN_3O_3$)
(27)	$-CH_2CHCH_2$	58	1420	3.60 3.49 (2m)	C, H, N, ($C_{12}H_{20}ClN_3O_2$)
(28)	$-CH_2CCH$	72	1420	4.13 4.10 (2t)	C, H, N, ($C_{12}H_{18}ClN_3O_2$)

Partition coefficient determination: The apparent partition coefficients of the ureas and the nitrosoureas were determined in a 1-octanol phosphate buffer (*p*H 7.4, μ = 0.5) system. The phosphate buffer and 1-octanol solutions were mutually saturated at 20 °C before use. The compounds were dissolved in the 1-octanol phase and the octanol-buffer mixtures were shaken for 3 hours at 37 °C to reach distribution equilibrium. The phases were separated by centrifugation and, for the nitrosoureas, the absorbance of each phase were determined at wavelenghts near 400 nm corresponding to the absorption maxima for the respective compounds. For the ureas, the refraction indexes were determined at 20 °C. The concentrations of the respective compounds were determined from a calibration curve previously prepared using five different concentrations (correlation coefficient 0.95 - 0.99). The partition coefficients were calculated as Log P.[13]

Decomposition of compound (16): A solution of 0.0068 mol/L of compound (16) in a mixture of ethanol and phosphate buffer (pH 7.4, μ = 0.5) was kept at 37 °C for more than 600 hours (26 days). The solution was then concentrated *in vacuo*, and the residue was developed on a Polygram SIL G/UV_{254} silica gel by 17:3:1

(v/v) n-butanol-acetic acid-water. The thin layer chromatography was sprayed with ninhydrin to visualize the spots.

RESULTS AND DISCUSSION

Figure 1 illustrates the pathways used for the synthesis of the ureas and the nitrosoureas. The reaction between the N-(substituted)-cyclohexylamines (**29**) and 2-chloroethyl isocyanate (**30**) produced the 2-chloroethylureas (**1-14**) in good yields. The nitrosation of these ureas with sodium nitrite in formic acid did not produce acceptable yields. Steric hindrance could be one of the factors involved. We decided to prepare the nitrosoureas by the nucleophilic attack of the N-(susbtituted)-cyclohexylamines (**29**) on the active nitrosocarbamate (**31**).[14] The yields of these reactions were generally very good.

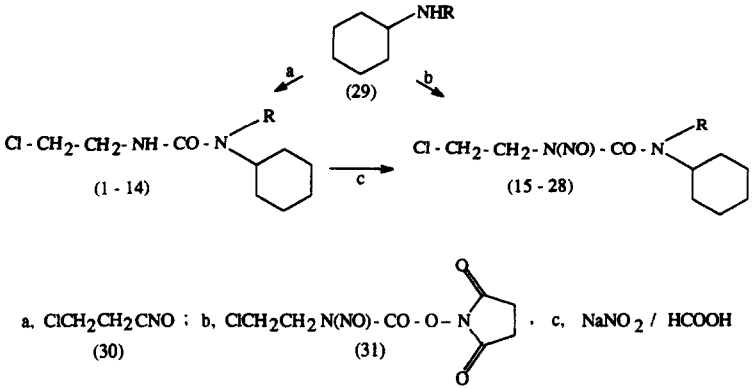

Figure 1 - Synthesis of the ureas and nitrosoureas.

As shown in Figure 2, the decomposition of these nitrosoureas followed first order kinetics over several half-lives. The values of the chemical half-lifves of the nitrosoureas are shown in Table 3. In our hands, the half-life of CCNU was 125 $\pm$ 20 minutes (2.09 $\pm$ 0.26 hours). This value is in agreement with the value of 117 minutes published by Heal *et al.*[8]

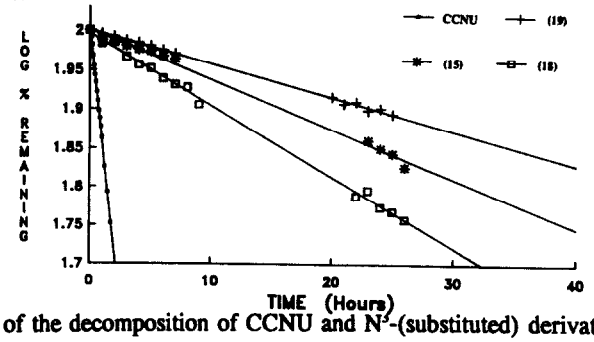

Figure 2 - First-order plots of the decomposition of CCNU and N^2-(substituted) derivatives of CCNU.

Table 3 - Physico-chemical properties of the ureas and nitrosoureas.

UREAS		NITROSOUREAS		
	Log P		Log P	$t_{1/2}$ (HOURS)
(1)	1.41	(15)	0.97	47.8
(2)	0.84	(16)	1.38	131.7
(3)	2.49	(17)	1.91	69.8
(4)	2.36	(18)	1.78	32.9
(5)	1.93	(19)	2.23	70.2
(6)	3.03	(20)	2.46	N.A.
(7)	3.20	(21)	1.63	63.0
(8)	3.34	(22)	2.77	206.0
(9)	4.03	(23)	3.46	62.6
(10)	-0.04	(24)	-0.61	37.6
(11)	0.50	(25)	-0.07	N.A.
(12)	0.37	(26)	-0.20	43.3
(13)	1.48	(27)	0.91	29.1
(14)	0.15	(28)	-0.42	76.8
		CCNU	2.83	2.1

As can be seen from these data, the N^3 substitution produces much more stable derivatives than CCNU in an aqueous medium. In fact, the half-lives of these derivatives were 15 to 100 times longer than CCNU. The structure and the half-life relationship in this series could not be understood in mechanistic term.

It has already been shown by Hansch *et al.*[15] that the lipophilic character of the nitrosoureas is the most important parameter determining their antitumor activity. In this publication, Hansch *et al.* also pointed out that the more hydrophilic neutral congeners might yield analogues with a better therapeutic index.[15] Based on the Log P values shown in Table 3, compounds (15), (24), (25), (26), (27) and (28) should be the most interesting nitrosoureas in this work.

Among the recently developed potential anticancer agents, some ureas, precursors of the nitrosoureas, were shown to have anticancer activity[16,17,18] contrary to what was originally believed.[3,19] Again, based on the Log P values shown in Table 3, the most interesting ureas should be compounds (2), (10), (11), (12) and (14).

Finally, the decomposition of compound (16) during more than 600 hours in a buffered solution (*p*H 7.4) gave the starting amine (Rf = 0.296) as one of the ninhydrin positive decomposition products. This demonstrates that the decomposition of compound (16) is following a different decomposition path than the non-N^3-(substituted) nitrosoureas. However, the thin layer chromatography also gave three other ninhydrin positive

decomposition products (Rf = 0.188, 0.114 and 0.068). These products are not the starting nitrosourea (**16**) (Rf = 0.160) nor is it the urea (**2**) (Rf = 0.772). The determination of the structure of these three less polar products is in progress in our laboratory.

CONCLUSION

We synthesized a series of N^3-(substituted) derivatives of CCNU in order to assess their chemical stability and toxicity and to verify whether they remained active against cancer. These derivatives were much more stable in aqueous medium than CCNU as shown by their 15 to 100 times longer half-life compared to CCNU. The decomposition of these derivatives seems to be different in that they produce the original amine plus three other decomposition products. The determination of the structure of these products is currently being investigated in our laboratory. Finally, based on the Log P values shown in Table 3, the most interesting nitrosoureas in this study should be compounds (**16**), (**25**), (**26**), (**27**), (**28**) and (**29**). The antitumor and toxic activities of these derivatives are currently being investigated.

AKNOWLEDGEMENT

Partial financial support by the Cancer Research Society Inc, is gratefully acknowledged.

REFERENCES AND NOTES:

1. Schabel FM. *Cancer Treat Rep*, **1976**, *60*, 665-698.
2. Mitchell EP.; Schein PS. *Cancer Treat Rep*, **1986**, *70*, 31-41.
3. Johnston TP.; Montgomery JA. *Cancer Treat Rep*, **1986**, *70*, 13-30.
4. Wasserman TH.; Slavik M.; Carter SK. *Cancer Treat Rev*, **1974**, *1*, 131-151.
5. Wasserman TH.; Slavik M.; Carter SK. *Cancer Treat Rev*, **1974**, *1*, 251-269.
6. Johnston TP.; McCaleb GS.; Opliger PS.; Montgomery JA. *J Med Chem*, **1966**, *9*, 892-911.
7. Oliverio VT. *Cancer Treat Rev*, **1976**, *60*, 703-707.
8. Heal JM.; Fox P.; Schein PS. *Biochem Pharmacol*, **1979**, *28*, 1301-1306.
9. Bosanquet AG. *Cancer Chemother Pharmacol*, **1985**, *14*, 83-95.
10. Hilton J.; Maldarelli F.; Sargent S. *Biochem Pharmacol*, **1978**, *27*, 1359-1363.
11. Gibson NW.; Hickman JA. *Biochem Pharmacol*, **1982**, *31*, 2795-2800.
12. Ali-Osman F.; Giblin J.; Berger M.; Murphy MJ.; Rosenblum ML. *Cancer Res*, **1985**, *45*, 4185-4191.
13. Leo A.; Hansch C.; Elkins D. *Chem Rev*, **1971**, *71*, 525-616.
14. Gallant G.; Salvador R.; Dulude H. *Antiviral Chem. Chemother.* **1991**, *2*, 313-319.
15. Hansch C.; Leo A.; Schmidt C.; Jow PYC. *J Med Chem*, **1980**, *23*, 1095-1101.
16. Gaudreault RC.; Lacroix J.; Pagé M.; Joly LP. *J Pharm Sci*, **1988**, *77*, 185-187.
17. Lacroix J.; Gaudreault RC.; Pagé M.; Joly LP. *Anticancer Res*, **1988**, *8*, 595-598.
18. Pagé M.; Gaudreault RC.; Lacroix J.; Joly LP. *Anticancer Res*, **1988**, *8*, 1093.
19. Goldin A.; Venditti JM.; Mead JAR.; Glynn JP. *Cancer Chemother Rep*, **1964**, *40*, 57-74.

(*Received in USA* 5 *August* 1994; *accepted* 31 *August* 1994)