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Synthesis and Anti-HIV Activity of New Urea and Nitrosourea Derivatives of Diamino Acids

Hélène Dulude*, Romano Salvador and Gilles Gallant

Medicinal Chemistry Laboratory, Faculty of Pharmacy, University of Montreal, Box 6128, Station A, Montreal, Quebec, Canada, H3C 3J7

Abstract—A series of N^1-methyl, N^1-allyl, N^1-(2-chloroethyl) and N^1-propargyl urea and nitrosourea derivatives of diamino acids (L-ornithine and L-lysine) was synthesized and was shown to have weak activity in counteracting the cytopathic effects of the HIV-1 on a T_4 lymphocyte cell line (CEM-IW). However, selected compounds may possess some immunomodulatory activity.

Introduction

The nitrosoureas are among the most potent alkylating agents.[1–3] Some twenty-five years ago, BCNU (1,3-bis-(2-chloroethyl)-1-nitrosourea) was shown to possess antiviral activity *in vitro* and *in vivo* in animal models against many viruses.[4–7] The antiviral mechanism was unknown but it was proposed that BCNU had a specific antiviral activity or some action on the host immunological system.[4–7]

Recently, workers in our laboratory have shown that a new series of urea and nitrosourea derivatives of diamino acids was devoid of anti-HIV activity.[8] The lack of activity was thought to be caused by the short chemical half-life of these products.[8]

In order to evaluate the anti-HIV activity of ureas and nitrosoureas with longer half-lives, we have synthesized four series of urea and nitrosourea derivatives of diamino acids. Two specifically blocked diamino acids were coupled with four different alkylating moieties in order to evaluate their relative biological activities. New N^1-methyl, N^1-allyl, N^1-(2-chloroethyl) and N^1-propargyl ureas and nitrosoureas resulted from this synthetic program. Our laboratories chose diamino acids with the *L* configuration since they are less toxic and produce nitrosoureas with good biological activity (anticancer activity in animal[9,10] and in *in vitro* models[11]). Moreover, L-lysine *per se* possesses an inhibitory effect on the growth of some types of tumor cells.[12,13]

Chemistry

The starting diamino acids selectively blocked at the proper functional groups (Table 1) were prepared according to modifications of published methods (Fig. 1).[14–21] The ureas and nitrosoureas (Table 2 and Table 3) were prepared according to Figures 2 and 3. The active carbamates and nitrosocarbamates were synthesized according to a method previously published by our group.[8]

All capillary melting points were determined using a Büchi 535 melting point apparatus and are reported uncorrected. Elemental analyses were performed by Guelph Chemical Laboratories Ltd and are within ±0.4% of theoretical values. IR Spectra were determined on a Perkin-Elmer 710A or a Perkin-Elmer 257 spectrophotometer, and ^{1}H NMR spectra were determined on a Varian VXR-300 spectrophotometer using the deuterated solvent (DMSO-d_6) as internal standard. Chiral integrity was assessed by optical rotation on an Atago Polax-D polarimeter. UV-vis spectra were recorded on a Hitachi U-2000 spectrophotometer (Tables 1, 2 and 3).

In Vitro Anti-HIV and Anti-Proliferative Activities

The determination of the anti-HIV and anti-proliferative activities of the compounds (**1–50**) was performed according to a published NCI method.[22] This *in vitro* procedure is designed to detect agents acting at any of the stages of the HIV-1 reproductive cycle. The compounds were dissolved in DMSO (< 0.25%) then diluted 1:100 in cell culture medium before preparing serial half-$\log_{10}$ dilutions. T_4 lymphocytes (CEM-IW cell line) were added, and after a brief interval cell-free HIV-1 (IIIb) was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. The culture was incubated at 37 °C in a 5% CO_2 atmosphere for 6 days.[22]

*Address for correspondence: Hélène Dulude B.Pharm. Ph.D., Bristol-Myers Squibb, 2365 Côte-de-Liesse, Montréal (Québec), Canada H4N 2M7 (Tel. 514-333-4884, FAX 514-331-8880)

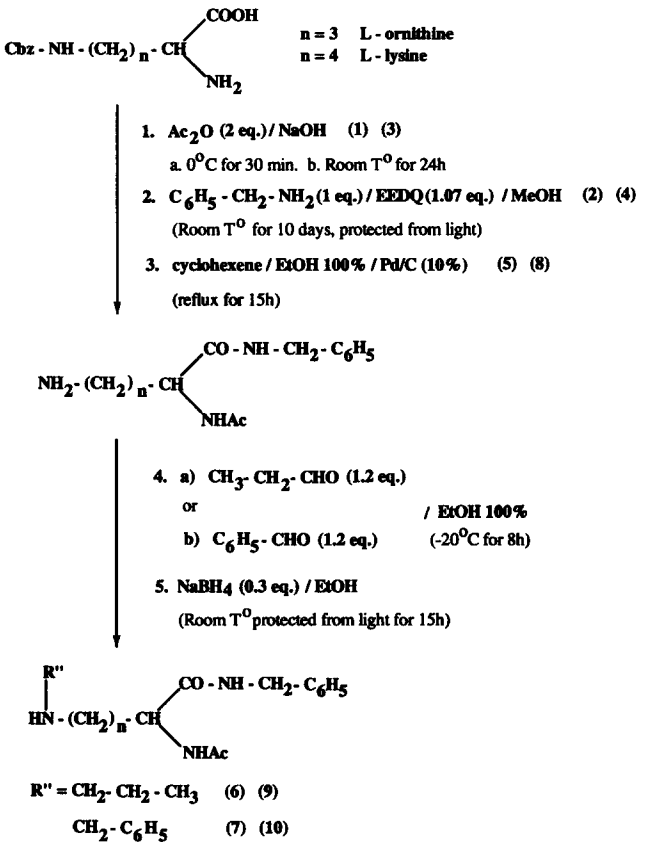
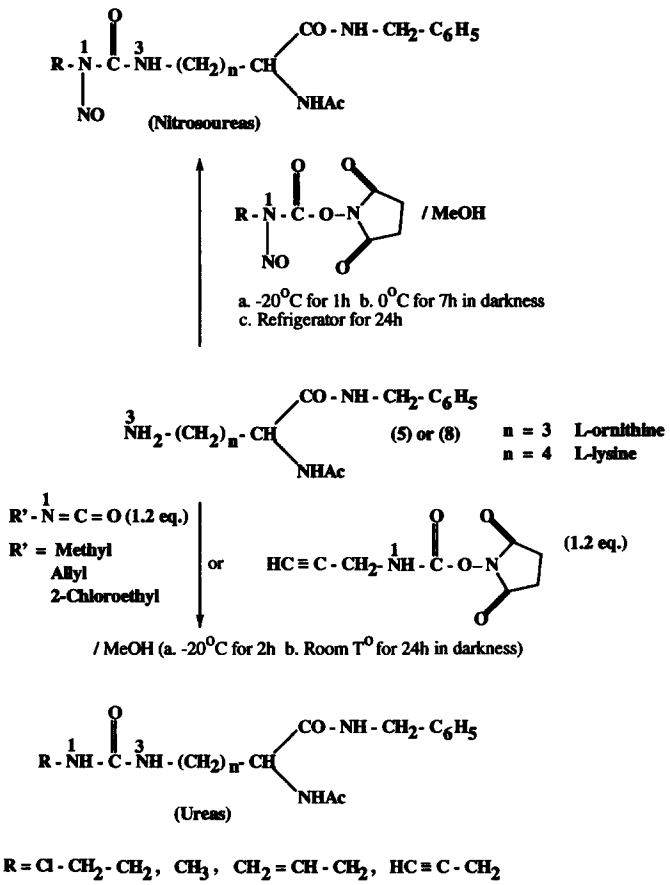


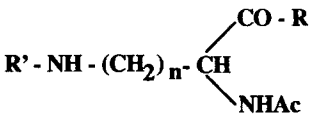
Figure 1. Synthesis of the selectively blocked diamino acids.



R = Cl - CH_2 - CH_2, CH_3, CH_2 = CH - CH_2, HC ≡ C - CH_2

Figure 2. Synthesis of ureas and nitrosoureas unsubstituted at N^3.

Table 1. Selectively blocked diamino acids



MCS #	Number	n	R	R'	Yield (%)	M.P. (°C)	^{1}H NMR δ (DMSO-d_6)	$[\alpha]_D^{oC}$ (M, MeOH)	ANAL.
650	(1)	3	a	a'	100	-	5.00 1.83 (2s); 8.11 (d); 2.98 (q); 7.32 4.125 1.48 (3m)	$[\alpha]_D^{26}$ = –4.49° (0.1)	C, H, N ($C_{15}H_{20}N_2O_5$)
651	(2)	3	b	a'	67	191.6-192.5	5.00 1.84 (2s); 8.02 4.26 (2d); 8.42 (t); 2.98 (q); 7.30 4.21 1.50 (3m)	$[\alpha]_D^{25.5}$ = –5.97° (0.08)	C, H, N ($C_{22}H_{27}N_3O_4$)
652	(3)	4	a	a'	97	97.1-104.4	5.00 1.83 (s); 8.09 (d); 2.97 (q); 7.32 4.11 1.46 (3m)	$[\alpha]_D^{27}$ = –1.38° (0.09)	C, H, N ($C_{16}H_{22}N_2O_5$)
653	(4)	4	b	a'	67	172.2-172.6	5.00 1.84 (2s); 8.00 4.26 (2d); 8.43 (t); 2.95 (q); 7.30 4.21 1.40 (3m)	$[\alpha]_D^{28.5}$ = –17.03° (0.07)	C, H, N ($C_{23}H_{29}N_3O_4$)
654	(5)	3	b	b'	100	-	1.84 (s); 8.07 4.26 (2d); 8.47 (t); 7.28 4.21 2.49 1.51 (4m)	$[\alpha]_D^{27}$ = –21.60° (0.1)	C, H, N ($C_{14}H_{21}N_3O_2$)
655	(6)	3	b	c'	96	-	1.84 (s); 8.06 4.26 (2d); 8.45 0.84 (2t); 7.29 4.22 2.43 1.51 (4m)	$[\alpha]_D^{27.5}$ = 0.55° (0.1)	C, H, N ($C_{17}H_{27}N_3O_2$)
656	(7)	3	b	d'	98	-	3.67 1.84 (2s); 8.04 4.25 (2d); 8.43 (t); 2.47 (q); 7.25 4.21 1.57 (3m)	$[\alpha]_D^{27.5}$ = 4.86° (0.08)	C, H, N ($C_{21}H_{27}N_3O_2$)
657	(8)	4	b	b'	100	-	1.84 (s); 8.05 4.26 (2d); 8.47 (t); 7.27 4.21 2.49 1.45 (4m)	$[\alpha]_D^{27.5}$ = –5.94° (0.1)	C, H, N ($C_{15}H_{23}N_3O_2$)
658	(9)	4	b	c'	96	-	1.84 (s); 8.00 4.26 (2d); 8.44 0.84 (2t); 7.25 4.20 2.43 1.47 (4m)	$[\alpha]_D^{28}$ = 5.27° (0.1)	C, H, N ($C_{18}H_{29}N_3O_2$)
659	(10)	4	b	d'	88	-	3.66 1.84 (2s); 8.00 4.26 (2d); 8.44 2.43 (2t); 7.27 4.22 1.49 (3m)	$[\alpha]_D^{28}$ = 2.57° (0.1)	C, H, N ($C_{22}H_{29}N_3O_2$)

R: a = OH, b = NH–CH_2–C_6H_5
R': a' = Cbz, b' = H, c' = Pr, d' = Bz

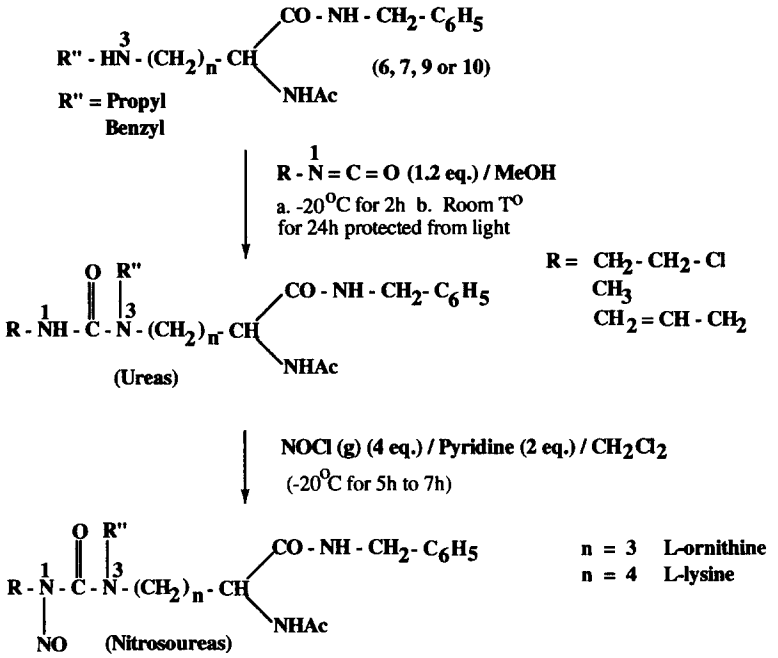
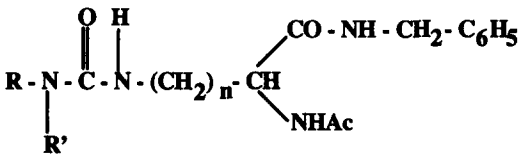


Figure 3. Synthesis of ureas and nitrosoureas substituted at N^3.

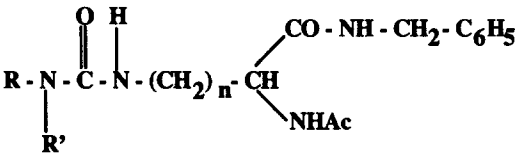
Table 2. Ureas and nitrosoureas unsubstituted at N^3



MCS #	Number	n	R	R'	Yield (%)	M.P. (°C)	IR cm^{-1} (NNO)	^1H NMR (DMSO-d_6)	$[\alpha]_D^{oC}$ (M, MeOH)	ANAL.
660	(11)	3	a	a'	59	215.3–217.0	-	1.84 (s); 8.02 4.26 2.50 (3d); 8.41 5.86 (2t); 5.64 2.94 (2q); 7.26 4.21 1.44 (3m)	$[\alpha]_D^{28}$ = 3.43° (0.09)	C, H, N ($C_{16}H_{24}N_4O_3$)
663	(12)	3	a	b'	46	150.6–153.4	1525	3.08 1.84 (2s); 8.05 4.27 (2d); 8.77 8.43 (2t); 3.27 (q); 7.27 4.27 1.55 (3m)	$[\alpha]_D^{26}$ = –4.20° (0.08)	C, H, N ($C_{16}H_{23}N_5O_4$)
666	(13)	4	a	a'	77	210.6–211.5	-	1.84 (s); 7.99 4.25 2.51 (3d); 8.42 5.82 (2t); 5.64 2.92 (2q); 7.26 4.20 1.41 (3m)	$[\alpha]_D^{27}$ = 6.94° (0.09)	C, H, N ($C_{17}H_{26}N_4O_3$)
669	(14)	4	a	b'	86	226.1–231.9	1525	3.07 1.83 (2s); 8.00 4.25 (2d); 8.73 8.42 (2t); 3.23 (q); 7.26 4.20 1.47 (3m)	$[\alpha]_D^{27.5}$ = –4.04° (0.08)	C, H, N ($C_{17}H_{25}N_5O_4$)
672	(15)	3	b	a'	67	209.3–211.3	-	1.84 (s); 8.02 4.25 (2d); 8.41 3.59 (2t); 2.96 (q); 7.28 5.90 5.78 5.03 4.25 (5m)	$[\alpha]_D^{24}$ = 7.55° (0.1)	C, H, N ($C_{18}H_{26}N_4O_3$)
675	(16)	3	b	b'	42	125.7–127.3	1525	1.84 (s); 8.04 4.33 4.28 (3d); 8.78 8.43 (2t); 3.26 (q); 7.26 5.61 4.95 4.28 1.65 (5m)	$[\alpha]_D^{24}$ = –4.11° (0.08)	C, H, N ($C_{18}H_{25}N_5O_4$)
678	(17)	4	b	a'	63	179.6–181.3	-	1.84 (s); 7.99 4.25 (2d); 8.42 5.89 5.85 3.59 (4t); 2.94 (q); 7.27 5.78 5.03 4.20 1.40 (5m)	$[\alpha]_D^{27}$ = 3.66° (0.09)	C, H, N ($C_{19}H_{28}N_4O_3$)
681	(18)	4	b	b'	71	140.8–143.3	1525	1.83 (s); 8.00 4.33 4.27 (3d); 8.75 8.42 (2t); 3.26 (q); 7.26 5.61 4.96 4.20 1.47 (5m)	$[\alpha]_D^{27.5}$ = –10.32° (0.08)	C, H, N ($C_{19}H_{27}N_5O_4$)
684	(19)	3	c	a'	78	124.4–139.4	-	1.84 (s); 8.03 4.26 (2d); 8.42 (t); 6.10 3.28 2.96 (3q); 7.26 4.26 3.55 1.50 (4m)	$[\alpha]_D^{26.5}$ = 6.38° (0.09)	C, H, N ($C_{17}H_{25}N_4O_3Cl$)
687	(20)	3	c	b'	88	-	1525	1.86 (s); 8.06 4.28 (2d); 8.79 8.44 (2t); 3.29 (q); 7.27 4.28 4.06 3.61 1.63 (5m)	$[\alpha]_D^{24.5}$ = 6.24° (0.08)	C, H, N ($C_{17}H_{24}N_5O_4Cl$)

R: a = CH_3, b = CH_2=CH–CH_2, c = Cl–CH_2–CH_2, d = HC≡C–CH_2
R': a' = H, b' = NO

Table 2 (continued). Ureas and nitrosoureas unsubstituted at N^3



MCS #	Number	n	R	R'	Yield (%)	M.P. (°C)	IR cm^{-1} (NNO)	^1H NMR (DMSO-d_6)	$[\alpha]_D^{oC}$ (M, MeOH)	ANAL.
690	(21)	4	c	a'	44	133.5–137.7	-	1.85 (s); 8.00 4.26 (d); 8.43 6.09 6.03 3.55 (4t); 3.30 2.94 (2q); 7.26 4.20 1.41 (3m)	$[\alpha]_D^{24.5}$= 5.34° (0.08)	C, H, N ($C_{18}H_{27}N_4O_3Cl$)
693	(22)	4	c	b'	71	88.6–100.4	1525	1.84 (s); 8.01 4.26 (2d); 8.77 8.43 4.09 3.61 (4t); 3.25 (q); 7.29 4.22 1.48 (3m)	$[\alpha]_D^{23.5}$= –3.35° (0.07)	C, H, N ($C_{18}H_{26}N_5O_4Cl$)
696	(23)	3	d	a'	63	160.1–169.8	-	1.84 (s); 8.05 4.25 (2d); 8.44 6.15 6.02 3.03 (4t); 2.97 (q); 7.26 4.25 3.75 1.46 (4m)	$[\alpha]_D^{26.5}$= 7.18° (0.09)	C, H, N ($C_{18}H_{24}N_4O_3$)
697	(24)	3	d	b'	68	-	1525	1.84 (s); 8.06 4.26 (d); 8.86 8.44 (2t); 3.27 (q); 7.25 4.45 4.26 3.34 1.61 (5m)	$[\alpha]_D^{26.5}$= –3.31° (0.08)	C, H, N ($C_{18}H_{23}N_5O_4$)
698	(25)	4	d	a'	46	184.7–190.8	-	3.02 1.84 (2s); 8.01 4.22 3.76 (3d); 8.43 6.12 5.95 (3t); 4.22 2.93 (2q); 7.26 1.42 (2m)	$[\alpha]_D^{27}$= –3.99° (0.08)	C, H, N ($C_{19}H_{26}N_4O_3$)
699	(26)	4	d	b'	57	110.3–117.0	1525	1.84 (s); 8.02 4.23 (2d); 8.84 8.43 (2t); 3.25 (q); 7.26 4.46 4.23 3.37 1.45 (5m)	$[\alpha]_D^{27}$= –3.15° (0.08)	C, H, N ($C_{19}H_{25}N_5O_4$)

R: a = CH_3, b = CH_2=CH–CH_2, c = Cl–CH_2–CH_2, d = HC≡C–CH_2
R': a' = H, b' = NO

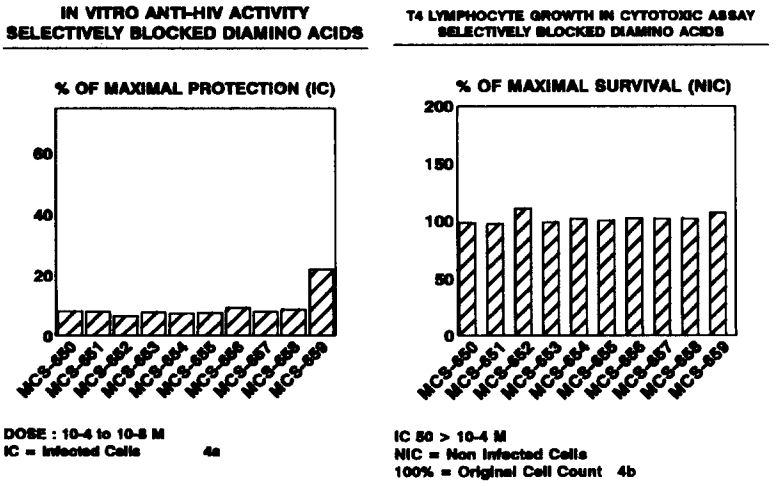
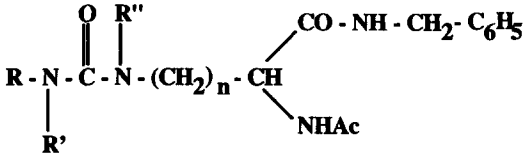


Figure 4. *In vitro* anti-HIV and cytotoxic activities of the selectively blocked diamino acids.

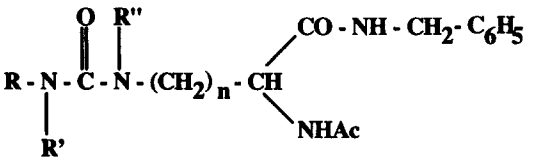
Table 3. Ureas and nitrosoureas substituted at N^3



MCS -	Number	n	R	R'	R"	Yield (%)	IR cm^{-1} (NNO)	^1H NMR (DMSO-d_6)	$[\alpha]_D^{°C}$ (M, MeOH)	ANAL.
661	(27)	3	a	a'	a"	57	-	1.85 (s); 8.03 4.27 2.53 (3d); 8.45 3.10 3.01 0.78 (4t); 6.05 (q); 7.27 4.24 1.45 (3m)	$[\alpha]_D^{28.5}$ = 4.22° (0.09)	C, H, N ($C_{19}H_{30}N_4O_3$)
662	(28)	3	a	a'	b"	80	-	4.36 1.85 (2s); 8.01 4.27 2.57 (3d); 8.44 3.08 (2t); 6.27 (q); 7.25 4.22 1.47 (3m)	$[\alpha]_D^{27}$ = –3.82° (0.08)	C, H, N ($C_{23}H_{30}N_4O_3$)
664	(29)	3	a	b'	a"	78	1435	3.04 1.85 (2s); 8.05 4.26 (2d); 8.46 0.82 (2t); 7.27 4.26 3.30 1.57 (4m)	$[\alpha]_D^{24.5}$ = –3.10° (0.09)	C, H, N ($C_{19}H_{29}N_5O_4$)
665	(30)	3	a	b'	b"	91	1435	4.63 3.01 1.84 (3s); 8.03 4.25 (2d); 8.44 3.35 (2t); 6.79 4.21 1.54 (3m)	$[\alpha]_D^{25.5}$ = 5.77° (0.07)	C, H, N ($C_{23}H_{29}N_5O_4$)
667	(31)	4	a	a'	a"	54	-	1.84 (s); 8.00 2.53 4.26 (3d); 8.45 0.79 (2t); 6.04 (q); 7.29 4.22 3.04 1.40 (4m)	$[\alpha]_D^{27.5}$ = 5.81° (0.08)	C, H, N ($C_{20}H_{32}N_4O_3$)
668	(32)	4	a	a'	b"	88	-	4.39 1.84 (2s); 7.99 4.25 2.58 (3d); 8.44 3.02 (2t); 6.29 (q); 7.26 4.21 1.40 (3m)	$[\alpha]_D^{28}$ = 5.14° (0.07)	C, H, N ($C_{24}H_{32}N_4O_3$)
670	(33)	4	a	b'	a"	87	1435	3.05 1.84 (2s); 8.00 4.26 (2d); 0.83 8.44 (2t); 7.27 4.23 3.29 1.42 (4m)	$[\alpha]_D^{28}$ = –2.72° (0.07)	C, H, N ($C_{20}H_{31}N_5O_4$)
671	(34)	4	a	b'	b"	88	1435	4.64 3.03 1.84 (3s); 7.99 4.25 (2d); 8.43 3.29 (2t); 7.27 4.20 1.41 (3m)	$[\alpha]_D^{28}$ = –4.75° (0.07)	C, H, N ($C_{24}H_{31}N_5O_4$)
673	(35)	3	b	a'	a"	63	-	1.84 (s); 8.44 8.03 4.26 (3d); 6.33 3.61 0.79 (3t); 7.27 5.78 5.02 4.26 3.08 1.50 (6m)	$[\alpha]_D^{25}$ = 7.72° (0.08)	C, H, N ($C_{21}H_{32}N_4O_3$)
674	(36)	3	b	a'	b"	81	-	4.40 1.85 (2s); 8.02 4.27 (2d); 8.44 6.52 3.68 3.13 (4t); 7.27 5.80 5.06 4.27 1.49 (5m)	$[\alpha]_D^{26.5}$ = 6.48° (0.07)	C, H, N ($C_{25}H_{32}N_4O_3$)
676	(37)	3	b	b'	a"	58	1435	1.84 (s); 8.05 4.26 (2d); 8.46 0.81 (2t); 7.26 5.63 5.10 4.29 3.25 1.58 (6m)	$[\alpha]_D^{25.5}$ = –6.39° (0.08)	C, H, N ($C_{21}H_{31}N_5O_4$)
677	(38)	3	b	b'	b"	79	1435	4.63 1.84 (2s); 8.03 4.28 (2d); 8.43 3.36 (2t); 7.27 5.56 5.07 4.28 1.55 (5m)	$[\alpha]_D^{25.5}$ = –7.90° (0.07)	C, H, N ($C_{25}H_{31}N_5O_4$)
679	(39)	4	b	a'	a"	74	-	1.84 (s); 8.00 4.26 (2d); 8.44 6.32 3.62 0.79 (4t); 7.28 5.79 5.01 4.21 3.07 1.47 (6m)	$[\alpha]_D^{28}$ = 5.56° (0.09)	C, H, N ($C_{22}H_{34}N_4O_3$)
680	(40)	4	b	a'	b"	88	-	4.42 1.84 (2s); 7.99 4.25 (2d); 8.43 6.55 3.68 3.06 (4t); 7.26 5.80 5.04 4.21 1.42 (5m)	$[\alpha]_D^{28.5}$ = 7.64° (0.07)	C, H, N ($C_{26}H_{34}N_4O_3$)

R: a = CH_3, b = CH_2=CH–CH_2, c = Cl–CH_2–CH_2
R': a' = H, b' = NO
R": a" = CH_2–CH_2–CH_3, b" = CH_2–C_6H_5

Table 3 (continued). Ureas and nitrosoureas substituted at N^3



MCS-	Number	n	R	R'	R"	Yield (%)	IR cm^{-1} (NNO)	^1H NMR (DMSO-d_6)	$[\alpha]_D^{oC}$ (M, MeOH)	ANAL.
682	(41)	4	b	b'	a"	75	1435	1.84 (s); 8.01 4.26 (2d); 8.44 0.83 (2t); 7.26 5.63 5.08 4.25 3.29 1.46 (6m)	$[\alpha]_D^{28}$ = 2.54° (0.07)	C, H, N ($C_{22}H_{33}N_5O_4$)
683	(42)	4	b	b'	b"	71	1435	4.66 1.83 (2s); 7.99 (d); 8.42 3.29 (2t); 7.28 5.56 5.08 4.25 4.25 1.46 (6m)	$[\alpha]_D^{28.5}$ = –7.15° (0.06)	C, H, N ($C_{26}H_{33}N_5O_4$)
685	(43)	3	c	a'	a"	64	-	1.87 (s); 8.18 4.25 (2d); 8.56 4.76 (2t); 7.28 4.25 3.55 3.30 1.53 0.83 (6m)	$[\alpha]_D^{27}$ = 4.42° (0.08)	C, H, N ($C_{20}H_{31}N_4O_5Cl$)
686	(44)	3	c	a'	b"	80	-	4.39 1.86 (2s); 8.07 4.27 (2d); 8.47 4.82 3.09 (3t); 7.30 4.27 3.57 3.36 1.52 (5m)	$[\alpha]_D^{27}$ = 4.71° (0.06)	C, H, N ($C_{24}H_{31}N_4O_5Cl$)
688	(45)	3	c	b'	a"	46	1435	1.85 (s); 8.06 4.27 (2d); 8.47 (t); 7.27 4.27 4.03 3.66 3.32 1.59 0.83 (7m)	$[\alpha]_D^{25}$ = 4.51° (0.07)	C, H, N ($C_{20}H_{30}N_5O_4Cl$)
689	(46)	3	c	b'	b"	46	1435	4.65 1.84 (2s); 8.03 4.19 (2d); 8.43 (t); 7.23 4.19 4.05 3.61 3.29 1.45 (6m)	$[\alpha]_D^{26}$ = 8.21° (0.07)	C, H, N ($C_{24}H_{30}N_5O_4Cl$)
691	(47)	4	c	a'	a"	77	-	1.85 (s); 8.08 4.26 (2d); 8.52 4.76 (2t); 7.27 4.26 3.56 3.29 1.54 0.83 (6m)	$[\alpha]_D^{25}$ = 3.18° (0.07)	C, H, N ($C_{21}H_{33}N_4O_5Cl$)
692	(48)	4	c	a'	b"	66	-	4.41 1.84 (2s); 8.03 4.22 (d); 8.48 4.83 3.04 (3t); 7.30 4.22 3.58 3.29 1.42 (5m)	$[\alpha]_D^{25.5}$ = 6.04° (0.06)	C, H, N ($C_{25}H_{33}N_4O_5Cl$)
694	(49)	4	c	b'	a"	46	1435	1.84 (s); 8.01 4.27 (2d); 8.43 (t); 7.28 4.27 4.04 3.64 3.30 1.36 0.84 (7m)	$[\alpha]_D^{24.5}$ = 4.04° (0.07)	C, H, N ($C_{21}H_{32}N_5O_4Cl$)
695	(50)	4	c	b'	b"	40	1435	4.68 1.83 (2s); 7.99 4.25 (2d); 8.43 3.67 3.27 (3t); 7.29 4.25 4.06 1.38 (4m)	$[\alpha]_D^{25.5}$ = –3.00° (0.07)	C, H, N ($C_{25}H_{32}N_5O_4Cl$)

R: a = CH_3, b = CH_2=CH–CH_2, c = Cl–CH_2–CH_2
R': a' = H, b' = NO
R": a" = CH_2–CH_2–CH_3, b" = CH_2–C_6H_5

The tetrazolium salt XTT was added to all wells, and cultures were incubated to allow formazan colour development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity.

Drug-treated virus-infected cells were compared with drug-treated non-infected cells, and with other appropriate controls on the same plate. Data were reviewed in comparison with other tests done at the same time and a determination of activity was made. Agents that interact with virions, cells, or virus gene-products, interfering with viral activities, will protect cells from cytolysis. All tests are compared with at least one positive (e.g. AZT-treated) control, done simultaneously under identical conditions.[22]

The results of the *in vitro* and anti-proliferative activity are shown in Figures 4 to 6. The data in Figures 4a and 5a–5d, show the percent of maximal protection for each compound in infected cells or the anti-HIV activity *in*

vitro of the compounds (a compound must have, at least, a 50% protection level to be considered active).

Many compounds showed only weak activity in counteracting the cytopathic effects of the HIV-1 on CEM-IW cells: the ten selectively blocked diamino acids (Fig. 4a) and the N^1-methyl and N^1-allyl ureas and nitrosoureas (Figs 5a and 5b). N^1-2-Chloroethyl derivatives showed weak activity, however these were the most active compounds in counteracting the cytopathic effects of the HIV-1 on this cell line. The highest value is for compound MCS-689 (56% protection, Fig. 5c). The four N^1-propargyl derivatives showed weak activity, comparable to that of the N^1-2-chloroethyl series in this model, the highest value being for compound MCS-696 (58%, Fig. 5d).

An analysis of variance (ANOVA) of the results, using the Systat program (Systat Inc., Evanston, IL, U.S.A.) ($P < 0.05$) followed by *a posteriori* analysis with Bonferroni correction for multiple comparisons,[23] showed that the activity in counteracting the cytopathic effects of the HIV-1 on CEM-IW cells was highest for the N^1-propargyl and the N^1-2-chloroethyl series than the selectively blocked diamino acids, the N^1-methyl and N^1-allyl series.

The data in Figures 4b and 6a–d show the percent of maximal survival of uninfected cells in the presence of the compound or the intrinsic cytotoxicity of the compounds (100% of maximal survival = original cell count). The majority of the compounds showed only weak cytotoxicity in uninfected T_4 cells ($IC_{50} > 10^{-4}$ M) (the N^1-propargyl ureas and nitrosoureas showed some cytotoxicity but only at high concentrations). In fact, the majority possess a tendency to stimulate the growth of the T_4 lymphocytes uninfected by the HIV-1 (only at low concentration for the N^1-propargyl ureas and nitrosoureas; at high concentration, the nitrosoureas can stimulate the growth of the T_4 lymphocytes infected by the virus). Hence, these compounds may possess an immunomodulatory activity that could be verified only in *in vivo* models.

An analysis of variance (ANOVA) of the results, using the Systat program (Systat Inc., Evanston, IL, U.S.A.) ($P < 0.05$) followed by *a posteriori* analysis with Bonferroni correction for multiple comparisons,[23] showed that the immunomodularity activity was higher for the N^1-propargyl (Fig. 6d) and the N^1-allyl series (Fig. 6b) followed by the N^1-2-chloroethyl series (Fig. 6c) and finally the selectively blocked diamino acids (Fig. 4b) with the N^1-methyl series (Fig. 6a).

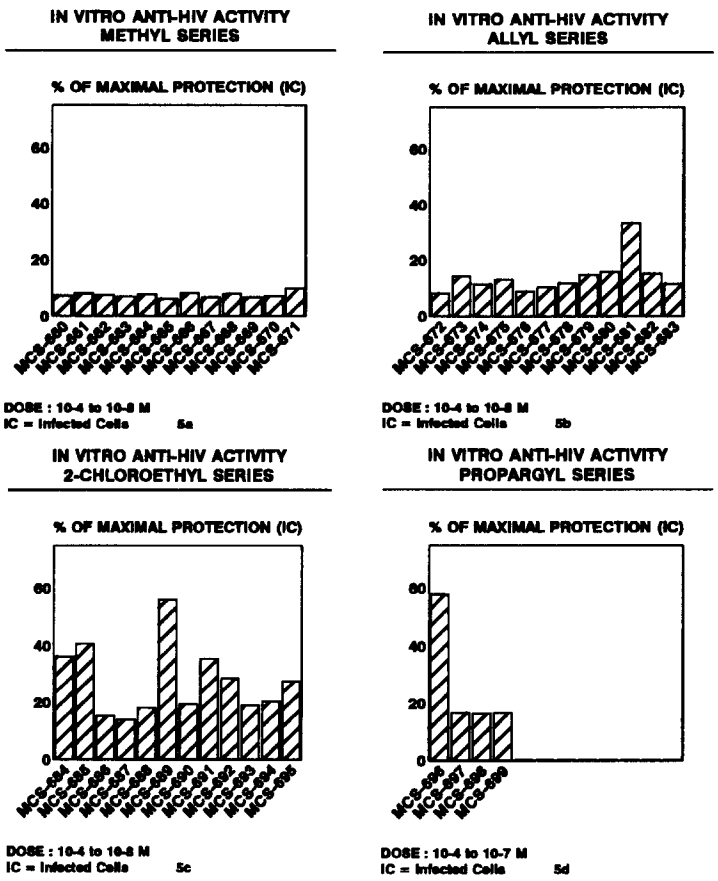


Figure 5. *In vitro* anti-HIV activity of the four series of ureas and nitrosoureas.

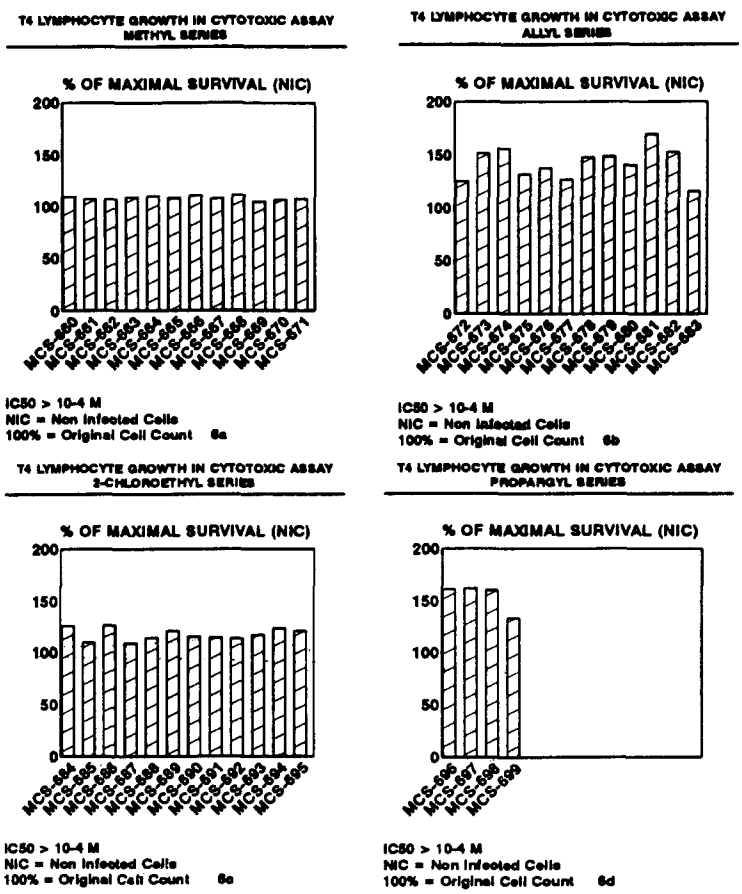


Figure 6. *In vitro* cytotoxic activity of the four series of ureas and nitrosoureas.

In conclusion, all agents tested have shown weak activity in counteracting the cytopathic effects of the HIV-1 on a T_4 lymphocyte cell line (CEM-IW); however, all the compounds were not cytotoxic in the above cell line. The lack of activity and cytotoxicity of our compounds could, in part, be explained by their extremely long chemical half-life (from two to many hundred times greater than that of CCNU) indicating pronounced stability thus depriving the compounds of possible active functionalities.[24] Surprisingly, the *N*-allyl and *N*-propargyl ureas and nitrosoureas may possess some immunomodulatory activity. The latter compounds stimulated the proliferation of T_4 lymphocytes uninfected by HIV without producing the same effect on the same cell line infected by HIV. This activity was not expected since the nitrosoureas are known to be immunosuppressive. It would be appropriate to further explore the immunomodulatory activity in *in vivo* models. This type of product may be of interest in adjuvant therapy of AIDS. Also, it will be interesting to look at the data obtained in an *in vitro* anticancer screening panel.

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References

1. Johnston, T. P.; McCaleb, G. S.; Opliger, P. S.; Montgomery, J. A. *J. Med. Chem.* **1966**, *9*, 892.

2. Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1975**, *18*, 104.

3. Schabel, Jr F. M. *Cancer Treat. Rep.* **1976**, *60*, 665.

4. Sidwell, R. W.; Dixon, G. J.; Sellers, S. M.; Schabel, Jr F. M. *Appl. Microbiol.* **1965**, *13*, 579.

5. Sidwell, R. W.; Dixon, G. J.; Sellers, S. M.; Schabel, Jr F. M. *Cancer Chemother. Rep.* **1966**, *50*, 299.

6. Sidwell, R. W.; Arnett, G.; Dixon, G. J. *Appl. Microbiol.* **1966**, *14*, 405.

7. Sidwell, R. W.; Dixon, G. J.; Sellers, S. M.; Schabel, Jr F. M. *Appl. Microbiol.* **1968**, *16*, 370.

8. Gallant, G.; Salvador, R.; Dulude, H. *Antiviral Chem. Chemother.* **1991**, *2*, 313.

9. Rodriguez, M.; Imbach, J. L.; Martinez, J. *J. Med. Chem.* **1984**, *27*, 1222.

10. Suami, T.; Kato, T.; Takino, H.; Hisamatsu, T. *J. Med. Chem.* **1982**, *25*, 829.

11. Gallant, G.; Salvador, R.; Dulude, H. *Anticancer Res.* **1993**, *13*, 133.

12. Choy, Y. M.; Kwok, T. T.; Fung, K. P.; Lee, C. Y. *Am. J. Chin. Med.*, **1983**, *11*, 69.

13. Choy, Y. M.; Fung, K. P. *Chemotherapy*, **1989**, *35*, 119.

14. Benoiton, L. *Can. J. Chem.*, **1964**, *42*, 2043.

15. Benoiton, N.L. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins. A Survey of Recent Developments*, Vol. 5, pp. 163–211, Weinstein, B., Ed.; Marcel de Ker Inc.; New York, 1978.

16. McIntire, F. C. *J. Am. Chem. Soc.* **1947**, *69*, 1377.

17. Montero, J. L.; Leydet, A.; Messiez-Munoz, A.; Dewynter, G.; Imbach, J. L. *Eur. J. Med. Chem. Chim. Ther.* **1984**, *19*, 512.

18. Quitt, P.; Hellerbach, J.; Vogler, K. *Helv. Chim. Acta* **1963**, *32*, 327.

19. Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651.

20. Neuberger, A.; Sanger, F. *Biochem. J.* **1943**, *37*, 515.

21. Yamamoto, H.; Hayakawa, T. *Biopolymers* **1972**, *11*, 1259.

22. Weislow, O. W.; Kiser, R.; Fine, D.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl Cancer Inst.* **1989**, *81*, 577.

23. Wallenstein, S.; Zucker, C. L.; Fleiss, J. L. *Circ. Res.* **1980**, *47*, 1.

24. Dulude, H.; Salvador, R.; Gallant, G. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2697.

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