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# CHEMICAL STABILITY 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.*

*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).*

**ABSTRACT** : Stability in neutral aqueous solutions of a series of nitrosourea derivatives of diamino acids was determined. Structure-activity relationships show that $N^3$-bisubstitution increased the stability of these compounds. Moreover, in $N^3$-monosubstituted and $N^3$-bisubstituted compounds, stability is : $N^1$-Methyl > $N^1$-Allyl > $N^1$-2-Chloroethyl > $N^1$-Propargyl.

The nitrosoureas are among the most potent alkylating agent known[1-3]. Moreover, the nitrosoureas, such as BCNU, CCNU and methyl-CCNU are liposoluble drugs that can penetrate the nervous central system and are useful for the treatment of brain tumors[1,4-6]. However, they are not very specific and they are toxic to normal organs and tissues.

The production of organic isocyanates during the breakdown of these products, is believed to be one of the factors which determines their toxicity[7-11]. We recently synthesize a series of products, derivatives of CCNU, that would be more stable *in vitro* and *in vivo* while remaining cytotoxic. This series of $N^3$-(substituted) derivatives of CCNU was shown to possess longer half-lives without producing organic isocyanates during their decomposition[12].

The main objective of this project was to synthesize new urea and nitrosourea derivatives of diamino acids used as carrier of the alkylating moiety that would show some selectivity for cancer cells[13]. The evaluation of the half-life of the nitrosoureas was done by an established method and the values obtained are good indicators of the chemical stability of these compounds[14,15]. As shown in Figure 1, the decomposition of these nitrosoureas followed first order kinetics over several half-lives.

We have shown in this work that the $N^3$-substitution increased the half-life of the nitrosoureas, reflecting the stability of these compounds. Moreover, this chemical modification may produce a mode of decomposition different from the usual by the nitrosoureas of the first generation (CCNU, MeCCNU, BCNU). These nitrosoureas would not produce toxic intermediates such as isocyanates shown by some researchers[12,14]. The started amino acid would be regenerated with one equivalent of $CO_2$ and water[12,16] (Figure 2).

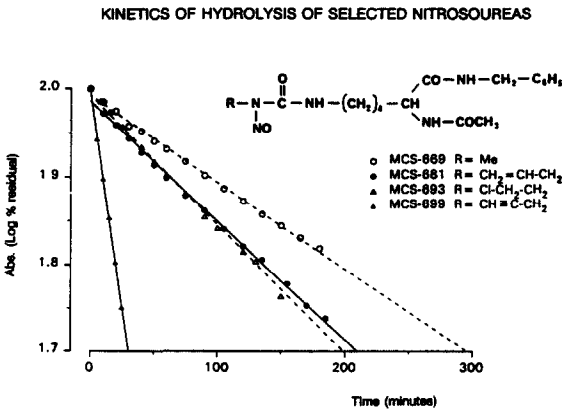



Figure 1 : Kinetics of hydrolysis of the four series of nitrosoureas

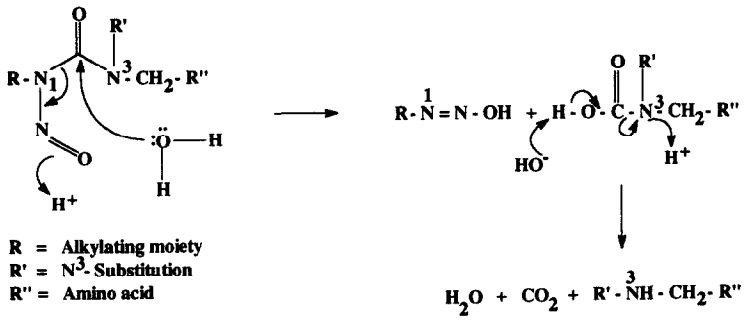
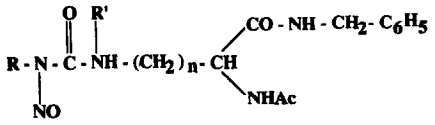



Figure 2 : General aqueous decomposition of the $N^3$-substituted nitrosoureas

As can be seen from the data in Table 1, the nitrosoureas had a better aqueous stability than CCNU (from two to many hundred times greater than that of CCNU). The data obtained for the four series of compounds show that, for the same diamino acid (Figure 1), the $N^1$-methyl nitrosoureas (MCS-669) have a longer half-lives than the $N^1$-allyl nitrosoureas (MCS-681), followed by $N^1$-2-chloroethyl nitrosoureas (MCS-693) and the $N^1$-propargyl nitrosoureas (MCS-699) respectively $N^3$-monosubstituted or $N^3$-bisubstituted (Figure 3). 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 nitrosoureas, the two series with the shortest half-lives.

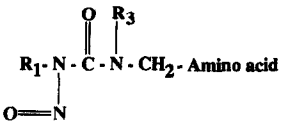
Table 1 : Half-life of the nitrosoureas




| MCS | Number | R | R' | n | λ (nm) | r² | $t_{1/2}$ (hours) |
|---|---|---|---|---|---|---|---|
| 663 | (12) | $CH_3$ | H | 3 | 393,6 | 0,999 | 6,35 |
| 664 | (29) | $CH_3$ | Pr | 3 | 364,4 | 0,739 | 980,88 |
| 665 | (30) | $CH_3$ | Bz | 3 | 379,8 | * | (∞) |
| 669 | (14) | $CH_3$ | H | 4 | 393,4 | 0,997 | 5,05 |
| 670 | (33) | $CH_3$ | Pr | 4 | 379,2 | 0,987 | 862,37 |
| 671 | (34) | $CH_3$ | Bz | 4 | 379,8 | * | (∞) |
| 675 | (16) | $CH_2$=CH-$CH_2$ | H | 3 | 397,6 | 0,998 | 2,95 |
| 676 | (37) | $CH_2$=CH-$CH_2$ | Pr | 3 | 379,5 | 0,953 | 98,17 |
| 677 | (38) | $CH_2$=CH-$CH_2$ | Bz | 3 | 375,6 | 0,769 | 378,26 |
| 681 | (18) | $CH_2$=CH-$CH_2$ | H | 4 | 397,2 | 0,996 | 3,68 |
| 682 | (41) | $CH_2$=CH-$CH_2$ | Pr | 4 | 372,0 | 0,989 | 84,16 |
| 683 | (42) | $CH_2$=CH-$CH_2$ | Bz | 4 | 373,8 | 0,832 | 358,58 |
| 687 | (20) | Cl-$CH_2$-$CH_2$ | H | 3 | 397,8 | 0,9996 | 2,77 |
| 688 | (45) | Cl-$CH_2$-$CH_2$ | Pr | 3 | 385,8 | 0,926 | 33,94 |
| 689 | (46) | Cl-$CH_2$-$CH_2$ | Bz | 3 | 387,4 | 0,970 | 107,74 |
| 693 | (22) | Cl-$CH_2$-$CH_2$ | H | 4 | 397,4 | 0,999 | 3,40 |
| 694 | (49) | Cl-$CH_2$-$CH_2$ | Pr | 4 | 379,5 | 0,942 | 84,78 |
| 695 | (50) | Cl-$CH_2$-$CH_2$ | Bz | 4 | 387,4 | 0,951 | 160,43 |
| 697 | (24) | HC≡C-$CH_2$ | H | 3 | 397,4 | 0,994 | 0,85 |
| 699 | (26) | HC≡C-$CH_2$ | H | 4 | 395,8 | 0,999 | 0,51 |
| CCNU | | | | | 397,2 | 0,998 | 1,60 |

*no hydrolysis after 723 hours (30 days)

These data are in agreement with what was previously found in our laboratories[12]. The substitution in $N^3$ increases the stability and the half-lives of the nitrosoureas. The antitumor and toxic activities of these derivatives are currently being investigated.




**Stability :**

1. $R_3$ = Pr, Bz >> $R_3$ = H
2. $R_3$ = Pr, Bz or $R_3$ = H : $R_1$ = Methyl > Allyl > 2-Chloroethyl > Propargyl

Figure 3 : Structure-Stability Relationships in the nitrosourea series

*Chemical half-life determination*: The half-life of the nitrosoureas was determined by following the decrease of absorbance in the UV of the solution incubated at 37°C (± 0,1°C) in a oscillating bath. The solutions were prepared by dissolving approximately 25 mg of each nitrosourea in 6,0 mL of absolute alcohol and 4,0 mL of phosphate buffer *p*H 7,4 ($\mu$ = 0,1). Absorbance were determined at wavelengths 364,4 nm to 397,8 nm corresponding to the absorption maxima for the respective compounds. CCNU (1-(2-chloroethyl) 3-cyclohexyl 1-nitrosourea) was used to validate our method. In our procedure, the CCNU half-life was 96 min. This value is in agreement with the published values of 53, 117 and 201 min[17-19].

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