Article

Synthesis of 4-Aza-cholestane Derivatives Containing Nitrosoureido Function as Antitumor Activity

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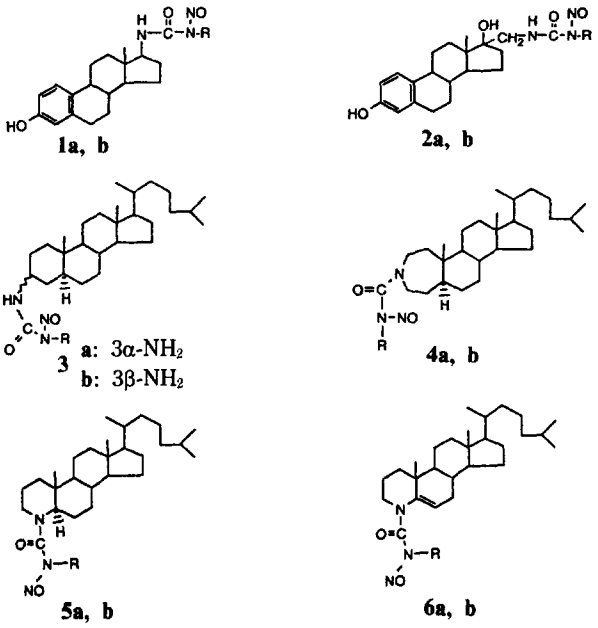
A series of 4-(2-chloroethyl)nitrosocarbamoyl- and 4-methyl nitrosocarbamoyl analogs of 4-Aza-5α-cholestane (**5a** and **5b**), 4-Aza-5-cholestene (**6a** and **6b**) were synthesizied and evaluated for their inhibitory activity against the Sarcoma 180 cell. The steroidal nitrosoureas, **5a**, **5b**, **6a** and **6b** displayed modest activity *in vitro*.

Introduction

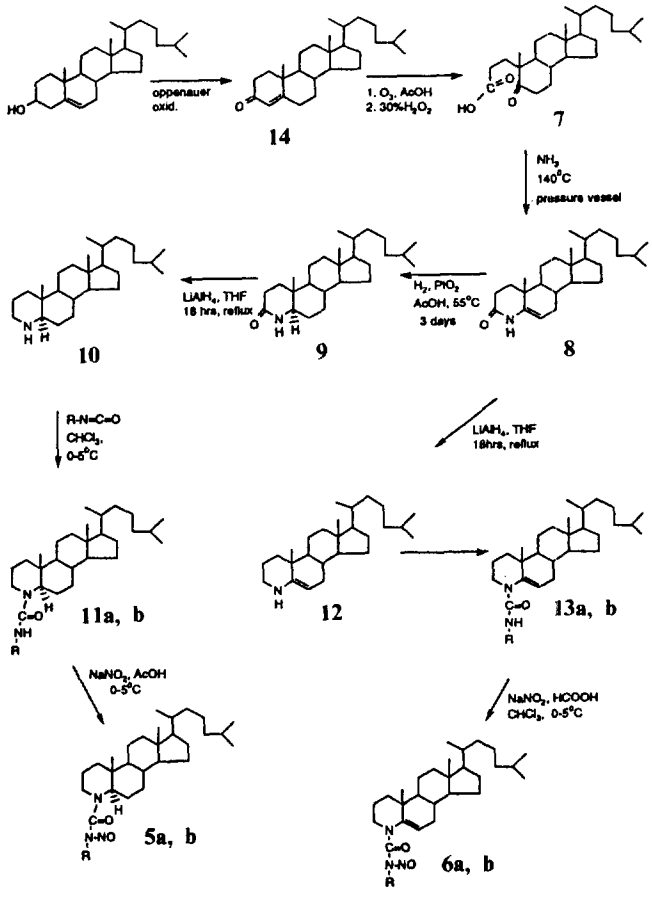
Among the cancer chemotherapeutic agents, one of the most effective cytostatic (2-chloroethyl)-nitrosoureas ($ClCH_2$-$CH_2N(NO)CONHR$) and N-methyl- N-nitrosoureas ($CH_3N(NO)$-CONHR), is the 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU)[1]. This compound, although of excellent antitumor activity, is rather toxic, and numerous analogs with modified R groups have been synthesized in order to obtain less toxic and more selective compounds. Accordingly some reports have appeared using estrogenic steroid hormones as carriers of alkylating agents in an attempt to produce antitumor agents with greater specificity of action[2]. For the same reason, several steroidal derivatives with attached nitrosourea moieties have been found to be active against selected tumor systems. These include estradiol derivatives (**1**, **2**)[3] and cholestane derivatives (**3**, **4**)[4].

In hopes of finding better therapeutic agents for antitumor activity, we report the synthesis of 4-Aza-5α-cholestane (**5a** and **5b**) and 4-Aza-5-cholestene (**6a** and **6b**) derivatives containing 2-chloroethyl- and N-methyl nitrosourea functions.



Results and Discussion

Cholesterol was used as a starting material for the synthesis of 4-(2-chloroethyl)nitrosocarbamoyl-, and 4-methylnitrosocarbamoyl-4-aza-5α-cholestane (**5a**, **5b**) and 4-(2-chloroethyl)nitrosocarbamoyl-, and 4-methylnitrosocarbamoyl-4-aza-5-cholestene (**6a**, **6b**) (Scheme 1). 5-Oxo-3,5-seco-A-norcholes-



Scheme 1.

tan-3-oic acid (**7**)[6] obtained through the Oppenauer oxidation of cholesterol, followed by ozonolysis, was reacted with concentrated aqueous ammonium hydroxide in a pressure vessel under a nitrogen atmosphere at 180℃ for 7 hours to give 4-aza-5-cholesten-3-one (**8**)[7]. Catalytic hydrogenation of the double bond of **8** was accomplished with platinum dioxide in acetic acid. The course of the reaction was monitored by the disapperance of UV absorption at 233 nm and the reduction product, 4-aza-5α-cholestan-3-one (**9**) gave a disappearance of a weak shoulder at 840 cm^{-1}, which is characteristic of a carbon-carbon double bond in this lactam.[7]

4-Aza-5α-cholestene (**10**), obtained from lithium aluminum hydride reduction was condensed with the 2-chloroethylisocyanate and methylisocyanate under anhydrous condition to afford 4-(2-chloroethyl)-carbamoyl-4-aza-5α-cholestane (**11a**) and 4-methylcarbamoyl-4-aza-5α-cholestane (**11b**), respectively. Nitrosation of the unsymmetrical 1,3-disubstituted ureas (**11a**, **11b**), can theoretically give two isomeric nitrosoureas. However, sterically bulky ureas **11a** and **11b**, made the nitrosation regioselective to yield exclusively 4-(2-chloroethyl)-nitrosocarbamoyl-4-aza-5α-cholestane (**5a**) and 4-methylnitrosocarbamoyl-4-aza-5α-cholestane (**5b**) The purity of nitrosoureas (**5a** and **5b**) has been shown by Montgomery, *et al.*[8] to be most clearly established by NMR. The spectral asymmetry of the -N(NO)CONHCH$_2$CH$_2$Cl (A_2B_2X system) group due to the NH coupling of the adjacent methylene group can be clearly distinguished from the spectral symmetry of the -NHCON(NO)CH$_2$CH$_2$Cl (A_2B_2 system) group. For the preparation of **6a** and **6b**, 4-aza-5-cholestene (**12**), obtained from lithium aluminum hydride reduction of **8**, was reacted with 2-chloroethylisocyanate and methylisocyanate under anhydrous condition to give 4-(2-chloroethyl)carbamoyl-4-aza-5-cholestene(**13a**) and 4-methylcarbamoyl-4-aza-5-cholestene (**13b**), respectively. Nitrosation of the respective **13a** and **13b** afforded expected nitrosourea compounds, **6a** and **6b** in good yields.

Antitumor activity of steroidal nitrosoureas, **5a**, **5b**, **6a** and **6b** was evaluated against Sarcoma 180 and displayed modest activity *in vitro*.

Table 1.

Compound	Yield (%)	Mp. (℃)	Mass peak (e/m)	I. R. peak (cm^{-1})
5-oxo-3,5-seco-A-norcholestan-3-oic acid (**7**)	79	153-155	404[a] 332[b]	1715(C=O; C_3, C_5)
4-aza-5-cholesten-3-one (**8**)	76	212-214	137 230 385[a,b]	1385 (N-H) 1635 (C=C; C_5) 1680 (C=0; C_3)
4-aza-5-cholesten-3-one (**9**)	63	244-246	57 232 387[a,b]	1637 (C=O; C_3)
4-aza-5α-cholestane (**10**)	62	109-111	338[b] 373[a]	
4-(2-chloroethyl)carbamoyl-4-aza-5α-cholestene (**11a**)	87	125-129	373[a] 479	3480 (N-H; urea)
4-methylcarbamoyl-4-aza-5α-cholestane (**11b**)	83	154-157	373[b] 430[a]	3480 (N-H; urea) 1637 (C=O; urea)
4-aza-5-cholestene (**12**)	72	83-85	358 371[a,b]	1460 (N-H; bending) 1650 (C=C; C_5)
4-(2-chloroethyl)carbamoyl-4-aza-5-cholestene (**13a**)	70	158-160	425[b] 476[a]	3480 (N-H; urea) 1635 (C=O; urea)
4-methylcarbamoyl-4-aza-5-cholestene (**13b**)	72	124-125	371[b] 428[a]	3480 (N-H; urea) 1635 (C=O; urea)
4-(2-chloroethyl)nitrosocarbamoyl-4-aza-5α-cholestane (**5a**)	79	112-115	373 a2[b]	1705 (C=O; urea)
4-methylnitrosocarbamoyl-4-aza-5α-cholestane (**5b**)	81	138-140	372[b] 459[a]	1705 (C=O; urea)
4-(2-chloroethyl)nitrosocarbamoyl-4-aza-5-cholestene (**6a**)	54	86-89	56[b] 490	1725 (C=O; urea) 1650 (C=C; C_5)
4-methylnitrosocarbamoyl-4-aza-5-cholestene (**6b**)	68	94-97	113[b] 426	1720 (C=O; urea)

[a] M^+ peak, [b] base peak.

Experimental Section

Melting points were determined on electrothermal capillary melting point apparatus and are uncorrected. TLC was performed on glass plates coated with aluminium oxide (silica gel 60 F254) and compounds were visualized using an UV lamp. Proton magnetic resonance spectra were obtained with Varian EM-360A spectrophotometer (solution in dimethyl-d_6-sulfoxide with tetramethylsilane as internal standard). Ultraviolet spectral data were measured with Hitachi 124 spectrometer. Pertinent data for synthesized compounds are listed in Table 1.

4-Aza-5-cholesten-3-one (8). A solution of 1.0 g (2.5 mmol) of 5-oxo-3,5-seco-A-norcholestan-3-oic acid (7) in 10 m*l* of concentrated aqueous ammonium hydroxide was heated in a pressure vessel under a nitrogen atmosphere at 135-140℃ for 8 hours. The mixture was cooled, and the solid was separated by filtration to obtain 0.7 g (76%) of white solids.

4-Aza-5α-cholestan-3-one (9). To a solution of 70 m*l* acetic acid were added 2.0 g of 8, and 0.2 g of platinum dioxide. The reaction mixture was hydrogenated at 46 psi and 55℃ for 3 days. After removing the catalyst, evaporating the solvent in vaccuo and recrystallizing the residue from acetone, 1.2 g (63%) of white solids were obtained.

4-Aza-5α-cholestane (10). To a solution of 1.0 g (2.1 mmol) of 9 in 250 m*l* of dry tetrahydrofuran was added, in small portions, 0.97 g of lithium aluminum hydride. The mixture was refluxed with stirring for 24 hours. Excess hydride was destroyed with tetrahydrofuran saturated with water. The mixture was filtered and the filtrate was evaporated *in vaccuo* to yield 0.67 g (62%) of a white solid.

4-(2-chloroethyl)carbamoyl-, and 4-methylcarbamoyl-4-aza-5α-cholestane (11a and 11b). To a solution of 10 (0.25 g, 0.7 mmol) in dry $CHCl_3$ (30 m*l*) was added 2-chloroethylisocyanate (0.4 m*l*, 5 mmol) and methylisocyanate (0.1 m*l*) for a period of 30 minutes at ice-cold water bath. The solvent was evaporated in vaccuo to an oily residue which was crystallized from methanol to give 0.29 g (87%) of white solids (**11a**), and 0.24 g (83%) of white solids (**11b**).

4-(2-chloroethyl)nitrosocarbamoyl- and 4-methylnitrosocarbamoyl-4-aza-5α-cholestane (5a and 5b). Sodium nitrate (0.5 g, 7 mmol) was added slowly to an ice-cold solution (−5℃) of **11a** (or **11b**) (0.5 g, 1 mmol) in 20 m*l* of glacial acetic acid. The reaction mixture was stirred at 0℃ for 3 hours and it was then poured into ice-water and extracted with $CHCl_3$. The extract was washed (H_2O and brine), dried ($MgSO_4$), and evaporated to oily residues which were chromatographed on alumina to afford pure white solid (**5a** and **5b**).

4-Aza-5-cholestene (12). To a solution of 1.0 g (2.1 mmol) of 8 in 80 m*l* of dry tetrahydrofuran was added, in small portions, 5 equivalent of lithium aluminum hydride. The mixture was refluxed with stirring for 24 hours. Excess hydride was destoryed with tetrahydrofuran saturated with water. The mixture was filtered and the filtrate was evaporated *in vaccuo* to yield 0.7 g (72%) of white solid.

4-(2-chloroethyl)carbamoyl- and 4-methylcarbamoyl-4-aza-5-cholestene (13a and 13b). To a solution of 12 (0.25 g, 0.67 mmol) in dry $CHCl_3$ (30 m*l*) was added 2-chloroethylisocyanate (0.02 m*l*) and methylisocyanate (0.1 m*l*) for a period of 10 minutes at ice-cold water bath. After stirring for 3 hours, the solvent was evaporated in vaccuo to on oily residue which was crystallized from methanol to give 0.25 g (70%) of white solid (**13a**) and 0.23 g (72%) of **13b**.

4-(2-chloroethyl)nitrosocarbamoyl- and 4-methylnitrosocarbamoyl-4-aza-5-cholestene (6a and 6b). Sodium nitrate (0.5 g, 7 mmol) was added slowly to an ice-cold solution of **13a** (or **13b**) (0.2 g) in 20 m*l* of 99% formic acid. The reaction mixture was stirred at 0℃ for 30 minutes and it was evaporated in vaccuo to oily residues, which were crystallized from methanol. 0.16 g (54%) of light yellow solid (**6a**) and 0.15 g (68%) of **6b**.

Antitumor Activity. Sarcoma 180 cells were cultured in RPMI 1640 medium (Gibco Laboratories) containing 10% fetal bovine serum. One$\times 10^5$ exponentially growing tumor cells were cultured for 48 hours at 37℃ in a humidified 5% CO_2 atmosphere. The number of viable cells were determined by the Dye Exclusion Method. Each experiment was performed in duplicate. Cells were tested for uptake of trypan blue according to the following procedure. Fifty microliters of the tumor cell suspension were mixed with 50 μl of 0.1% of trypan blue in physiologic salt solution. Twenty five microliters of trypan blue tumor mixture was then transferred to a microscopic slide covered with a cover slip and observed at random, and the proportion of trypan blue-stained cells was determined.

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