

Preparation of N'-Substituted Anilino-N-Methyl-N'-Nitrosoureas as Candidate Antitumor Agents

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Various N'-substituted anilino-N-methyl-N'-nitrosoureas (2a-n) were easily prepared from the reaction of substituted phenylhydrazines (3-, 4-CH₃, 3-, 4-OCH₃, 3-, 4-F, 3-, 4-Cl, 4-Br, 2-, 3-, 4-NO₂, 2, 4-(NO₂)₂) with methyl isocyanate, followed by the nitrosation with 99% HCOOH and dry sodium nitrite powder. Surprisingly, of these series of analogues, the anilino-nitrosoureas substituted with electron-withdrawing nitro groups (2k-n) showed significantly low ED₅₀ values of 1.4~3.4 µg/ml. In addition, none of these compounds substituted with electron-donating groups exhibited cytotoxicities.

Key words: N'-substituted anilino-N-methylurea, N'-substituted anilino-N-methyl-N'-nitrosourea, nitrosation of urea, antitumor activity, L1210 cell, ED₅₀

INTRODUCTION

A large number of nitrosoureas have been synthesized and evaluated for antitumor activity against leukemia. Several nitrosoureas, including N,N'-bis(2-chloroethyl)-N-nitrosourea (BCNU, carmustine), N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea (CCNU, lomustine) and N-(2-chloroethyl)-N'-(trans-4-methylcyclohexyl)-N-nitrosourea (Me-CCNU, semustine), are currently in clinical use (Wheeler, 1976). These compounds, although of excellent antitumor activity, are rather toxic, and numerous analogues with modified groups have been prepared in order to obtain less toxic and more selective compounds (Prestayko *et al.*, 1981).

Previously, we examined the activity of several steroidal nitrosoureas such as (Kim *et al.*, 1983a, 1985, 1986, 1993a, b) amino acid-attached nitrosoureas (Kim *et al.*, 1983c, b, 1984), acyclonucleoside nitrosoureas (Kim *et al.*, 1994b) and substituted aryl nitrosoureas (Kim *et al.*, 1991a, b, 1992a, b, 1994a). Based on the antitumor activity of these compounds, we have synthesized the potential methyl nitrosourea group which has been incorporated into the phenylhydrazines substituted with either electron-donating or electron-withdrawing groups, and we report here the antitumor activity of a series of 14 kinds of N'-substituted anilino-N-methyl-N'-nitrosoureas (1a-n).

MATERIALS AND METHODS

Melting points were determined on an electrothermal capillary melting point apparatus and are uncorrected. TLC was performed on glass plates coated with silicone oxide (silica gel 60F₂₅₄) and compounds were visualized using a UV lamp. Proton nuclear magnetic resonance spectra were obtained with a Varian EM-360 A spectrophotometer and Varian Gemini 200MHz (solution in dimethylsulfoxide-d₆ with tetrametylsilane as internal standard). Ultraviolet spectral data were measured with a Hitachi 124 spectrometer. The organic solvents and chemicals were obtained from commercial products and purified by the appropriate methods before use. Pertinent data for synthesized compounds are listed in Table I and II.

General Procedure for the Synthesis of N'-Substituted-Anilino-N-Methylureas (Table I, 1a-n)

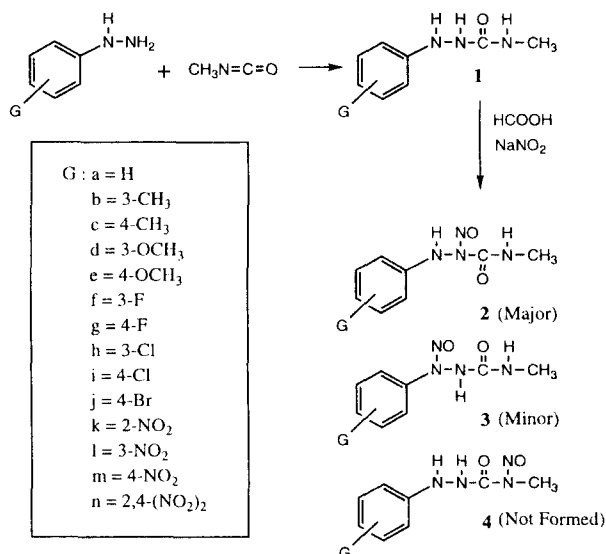
To a solution of phenylhydrazine or substituted phenylhydrazine (0.03 mol) in anhydrous ether (225 ml) was added methyl isocyanate (0.033 mol) by a syringe in small portions, under a nitrogen atmosphere, and the reaction mixture was stirred for 30 min at 0°C. The solvent was removed by evaporation to give a solid. Crystallization from an appropriate solvent afforded an analytically pure solid.

General Procedure for the Synthesis of N'-Substituted Anilino-N-Methyl-N-Nitrosoureas (Table II, 2a-n)

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Table I. N'-Substituted Anilino-N-Methylurea (1a-n)

Comp.	G	mp (°C)	Recryst Solvent	Yield (%)	IR(cm ⁻¹)		Nmr(δ)				
					N-H	C=O	CH ₃	N ₁ H	N ₂ H	N ₃ H	G-φ
a	H	135	C ₆ H ₆	80	3440	1650	2.5~2.6	6.3	7.6	7.8	6.5~7.3
b	3-CH ₃	187	toluene	77	3435	1650	2.5~2.7	6.3	7.4	7.6	6.5~7.1
c	4-CH ₃	156~158	toluene	75	3410	1630	2.5~2.6	6.0	7.0	7.3	6.5~6.9
d	3-OCH ₃	121~127	EtOAc	55	3435	1645	2.5~2.7	6.2	7.3	7.5	6.5~7.0
e	4-OCH ₃	220~221	C ₆ H ₆	56	3425	1660	2.52	6.40	7.31	8.40	6.7~7.3
f	3-F	120	EtOH	66	3440	1640	2.5~2.6	6.1	7.7	7.8	6.4~7.2
g	4-F	146	C ₆ H ₆	78	3450	1660	2.5~2.6	6.0	7.2	7.5	6.6~6.9
h	3-Cl	136	toluene	70	3350	1645	2.7~2.8	6.0	7.4	7.5	6.5~7.2
i	4-Cl	172~173	toluene	72	3430	1630	2.4~2.6	6.3	7.6	7.7	6.5~7.2
j	4-Br	179~182	toluene	51	3410	1645	2.4~2.6	6.3	7.7	7.8	6.5~7.3
k	2-NO ₂	225~226	EtOH	82	3300	1640	2.52	6.50	7.95	8.89	6.25~8.12
l	3-NO ₂	186~187	EtOH	61	3370	1680	2.55	6.45	7.95	8.10	6.90~7.6
m	4-NO ₂	243~244	EtOH	66	3340	1630	2.68	6.53	8.78	8.18	8.08
n	2,4-(NO ₂) ₂	114	CCl ₄	88	3350	1620	2.0~2.1	6.70	8.15	9.10	7.8~8.9



A solution of N'-substituted anilino-N-methylurea, **1 a-n** (0.122 mol) in 99% HCOOH (30 ml) was cooled to 0~5°C and treated with dry NaNO₂(0.146 mol) in small portions. The reaction mixture was stirred for 1 hr at 0~5°C, diluted with H₂O (40 ml) and stirred for an additional 2 hrs at 0~5°C. The reaction mixture was poured into an ice-H₂O mixture (300 ml) and extracted with CHCl₃ (2×370 ml). The combined organic layers were washed with H₂O (2×300 ml) dried (Na₂SO₄), filtered and evaporated under reduced pressure to give a solid. Crystallization from an appropriate solvent gave a pure solid.

Evaluation of Antitumor Activity (Kim *et al.*, 1983, 1984, 1985).

Following the NCI manual, (Cell Culturem, 1972), the ED₅₀ value of methyl-CCNU as a positive control

compound, was determined to check the reliability of our procedures. The growth ratio for each dose of the testing substance, Y, was calculated by the following $[(T-C_0)/(C-C_0)] \times 100 = Y(\%)$ where T=mean cell count for each dose of testing substance after 48 hours incubation; C=mean cell count for control after 48 hours incubation; C₀=mean cell count at the start of incubation. When Y values were plotted against dose of methyl-CCNU semi-logarithmically, a straight line could be obtained. Using the straight line, a concentration of methyl-CCNU which could inhibit the growth of murine leukemic lymphoblast L1210 cells by 50% (ED₅₀) was estimated to be 1.7 μg/ml. The values of ED₅₀ for the synthesized N'-substituted anilino-N-methyl-N'-nitrosoareas which inhibit the growth of cells to the level of 50% of the untreated control were then determined by the procedure described in the NCI manual. Under the NCI protocol, ED₅₀ values of the synthesized compounds, **2a-n** are shown in Table II.

RESULTS AND DISCUSSION

As shown in experimental section, a series of reactions of substituted phenylhydrazines (3-, 4-CH₃, 3-, 4-OCH₃, 3-, 4-F, 3-, 4-Cl, 4-Br, 2-, 3-, 4-NO₂, 2, 4-(NO₂)₂) with methylisocyanate afforded the corresponding N'-substituted-anilino-N-methylureas (**1a-n**) in good yields. Nitrosation of the unsymmetrical anilinoareas (**1a-n**) can theoretically give three types of regioisomeric nitrosoareas; N'-substituted anilino-N-methyl-N'-nitrosoareas (**2a-n**), N'-substituted anilino-N'-nitroso-N-methylureas (**3a-n**) and N'-substituted anilino-N-methyl-N-nitrosoareas (**4a-n**). However, Montgomery and coworkers (Montgomery *et al.*, 1975) have shown that the regioselectivity of nitrosation using an anhydrous

Table II. N'-Substituted Anilino-N-Methyl-N'-Nitrosoarea (**2a-n**)

Comp.	G	mp (°C)	Recryst Solvent	Yield (%)	IR(cm ⁻¹)		Nmr(δ)			ED ₅₀
					N=O	C=O	CH ₃	NH	G-φ	
a	H	66	C ₆ H ₆	17	1480	1710	2.8~2.9	8.2	7.4~2.9	22~44
b	3-CH ₃	80~85	C ₆ H ₆	22	1500	1705	2.8~3.0	8.5	7.4~7.8	11.5
c	4-CH ₃	92~97	EtOH	17	1500	1700	2.8~3.1	8.4	7.3~7.8	7~11
d	3-OCH ₃	110~112	EtOH	21	1500	1700	2.8~2.9	8.0	7.1~7.8	9~16
e	4-OCH ₃	83~84	EtOAc	18	1500	1690	2.75	8.20	7.0~7.75	<32
f	3-F	106~108	EtOAc	18	1495	1705	2.8~3.0	8.1	7.1~7.8	4.5
g	4-F	78	C ₆ H ₆	23	1500	1705	2.8~3.0	7.9	7.0~8.0	7.5
h	3-Cl	95~97	C ₆ H ₆	28	1495	1715	2.8~3.0	8.1	7.4~7.9	9.1
i	4-Cl	138	EtOH	37	1500	1650	2.9~3.0	8.3	7.6~7.9	7.8
j	4-Br	98~99	EtOAc	20	1500	1700	2.7~2.9	8.3	7.5~7.8	17~23
k	2-NO ₂	121~122	EtOH	57	1500	1700	2.8	8.50	7.3~8.15	1.4
l	3-NO ₂	124~125	EtOAc	58	1500	1705	2.8	8.55	7.67~8.7	3.4
m	4-NO ₂	169~170	EtOAc	51	1505	1700	2.78	8.48	7.88~8.27	1.8
n	2,4-(NO ₂) ₂	181~183	EtOH	23	1505	1715	2.8	8.51	7.9~8.34	3.1

The concentration of the reference compound, Me-CCNU inhibits the growth of L1210 cell. (ED₅₀ 1.7 µg/ml)

formic acid medium is influenced by steric factors. Therefore, the nitrosation of unsymmetrical N'-substituted anilino-N-methylurea, **1a-n**, with 99% HCOOH and dry sodium nitrite powder would be expected to yield predominantly N'-substituted anilino-N-methyl-N-nitrosoarea **4**. However, nitrosation by the above conventional method gave the nitrosoarea with no evidence of the formation of the desired regioisomer **4**. The regioselectivity in the nitrosation of **1a-n** was examined using NaNO₂ and various 4 acids (diluted HCL, HCOOH, CH₃COOH, CF₃COOH) (Kim et al., 1991a, b). In all cases, the desired **4** was not observed to be a major product. The nitrosated products gave two spots (9.5: 0.5 ratio) with strikingly different elution mobility on TLC. The two compounds were separated by column chromatography using silica gel as the adsorbant. The structural assignments of the major product were based on analysis of IR and NMR spectra (Table II). The IR spectra of the nitrosated compound with the higher R_f value showed a band at 1480~1530 cm⁻¹, indicating the presence of a nitroso group, and the sharp absorption at 1690~1730 cm⁻¹ was characteristic of the shift to a higher wavenumber of the carbonyl absorption caused by the strong inductive effect of nitroso group of the ureido function (Montgomery et al., 1979).

The NMR spectrum of -N(NO)CONHCH₃ showed the NH coupling of the adjacent methyl group as a doublet centered at δ2.7~3.0, and therefore it can be clearly distinguished from the -NHCON(NO)CH₃ spectra in the desired compound **4**. The examination of the NH proton in the -N(NO)CONHCH₃ system showed the extreme downfield shift at δ7.9~8.5 (2.1 ppm downfield compared with -NHCONH-CH₃) due to the strong hydrogen bonding with the strongly elec-

tron-withdrawing electronegative nitroso group. These spectral data are consistent with the correct assignment of the structure, N'al-substituted anilino-N-methyl-N'-nitrosoareas (**2a-n**) as a major product (9.5:0.5) in nitrosating the N'-substituted anilino-N-methylureas (**1a-n**) and the other trace compound probably **3a-n**, were not fully characterized due to their instability.

Antitumor activities (Kim et al., 1983, 1984, 1985) of 14 N'-substituted anilino-N-methyl-N'-nitrosoareas (Table II, **2a-n**) were evaluated using murine leukemic lymphoblast L1210 cells and the assay results are shown in Table II. In these tests, N'-(trans-4-methylcyclohexyl)-N-(2-chloroethyl)-N-nitrosoarea (Me-CCNU, semustine) was used as the reference compound. The concentration of methyl-CCNU which would inhibit the growth of L1210 cells by 50% (ED₅₀) was estimated as 1.7 µg/ml. Examination of the data in Table II showed that the anilino group substituted with nitro groups (**2k-n**) was found to be about as active as methyl-CCNU. It should be noted that the compounds we have reported here are not originally designed cytotoxic N'-substituted anilino-N-methyl-N-nitrosoarea analogs **4a-n**. Nevertheless, these compounds **2a-n** showed the cytotoxic effect. Presently, further work is in progress on the regioselective synthesis of nitrosoareas **4a-n** of the substituted anilino nitrosoarea analogs.

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