Synthesis of 3-Substituted 7-(3,3-Dimethyl-1-triazeno)-10-methylphenothiazines as Potential Antitumor Agents

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A series of 3-substituted (chloro, bromo, fluoro or methyl) 7-(3,3-dimethyl-1-triazeno)-10-methylphenothiazines were synthesized as potential antitumor agents. Treatment of *p*-substituted anilines with ammonium thiocyanate in the presence of bromine gave 6-substituted 2-aminobenzthiazoles which, after methylation with methyl iodide were hydrolyzed in 50% potassium hydroxide to give 5-substituted 2-methylaminothiophenols in moderate yield. Condensation of methylaminothiophenols with 3,4-dichloronitrobenzene in ethanol under an atomsphere of nitrogen gave adducts which were cyclized in dimethylformamide under the catalysis of copper and cuprous iodide to give 3-substituted 7-nitro-10-methylphenothiazines. The nitro group was reduced to the amino function with stannous chloride. Diazotization of the amines followed by coupling with dimethylamine gave the corresponding triazenes.

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Phenothiazine derivatives are well known for their central nervous system (CNS) activity (1). Several studies also showed that some phenothiazines produce antitumor effects (2-5). Notably, chlorpromazine (CPZ) and 7-hydroxychloropromazine (70H-CPZ) were found to inhibit the growth of mouse B-16 melanoma at 19 mg/kg/day, the growth of the Harding-Passey melanoma was also inhibited to a similar degree by CPZ (2). Although the mechanism of antitumor action of CPZ against B-16 has not been ascertained, the preferential concentration of CPZ in melanoma may have toxic effects on the metabolism of the melanocyte.

We reported previously the synthesis of a series of halogenated (chloro, bromo or iodo) 4-(3,3-dimethyl-1-triazeno)quinolines as potential antitumor agents (6). 8-Chloro-4-(3,3-dimethyl-1-triazeno)quinoline produces significant antitumor activity against both P388 and L1210 leukemia with % T/C of 165 to 172. The other chloro, bromo, or iodo analogs all possess marginal activity against P388. The encouraging antineoplastic activity of halogenated 4-(3,3-dimethyl-1-triazeno)quinolines and their high melanin binding affinity *plus* the reports of antitumor activity of chlorpromazine led us to synthesize a series of 3-substituted 7-(3,3-dimethyl-1-triazeno)phenothiazine as potential antitumor agents.

The preparation of 3-substituted 7-(3,3-dimethyl-1-triazeno)phenothiazines is illustrated in the Scheme. Procedures used by Mital (7) and Brewster (8) for the preparation of 5-substituted aminothiophenols were adapted for the preparation of the key intermediates, 5-substituted 2-methylaminothiophenols. Thus, treatment of *p*-substituted anilines (I, X = Cl, Br, F, or CH_3) with ammonium thiocyanate and bromine gave good yields of 6-substituted 2-aminobenzthiazoles II. Methylation of II with methyl iodide gave selectively the hydrobromide salts of 6-substituted 2-imino-3-methylbenzthiazoles III in yields of 50-70%. Hydrolytic cleavage of compounds III with 50% aqueous potassium hydroxide afforded 5-substituted 2-methylaminothiophenols IV in moderate to poor yields. The time required for complete hydrolysis of benzthiazoles III depends on the nature of substituents; analogs with electron donating substituents (methyl) required much longer time than those with electron withdrawing function (fluoro, chloro and bromo). In addition to compounds IV formation of 6-substituted 2-oxo-3-methylbenzthiazoles (V) was also observed as the hydrolysis products.

5-Substituted 2-methylaminothiophenols IV were condensed (9,10) smoothly with 3,4-dichloronitrobenzene in ethanol under nitrogen to give good yields of the sulfide adducts VI. The possibility that the condensation products were *N*-adducts was ruled out by the fact that all adducts (VI) showed NH stretching peak (3400 cm^{-1}) in ir and a doublet (J = 4.5 Hz) for -N-CH_3 protons around 2.87 ppm in nmr. Cyclization of the condensation products VI to nitrophenothiazines in boiling dimethylformamide was catalyzed by cuprous iodide and copper (9,10). Good yields of compounds VI and VII were obtained only when the reactions were carried out under nitrogen throughout the reactions. Reduction of the nitro group VII to the corresponding amino function VIII was best achieved by a mild reducing agent, stannous chloride reagent (11). Diazotization (sodium nitrate) (6) of the amines VIII in fluoboric acid at salted ice bath temperature (−5 to 0°) followed by coupling with dimethylamine gave the desired final products IX in 40-60% yield.

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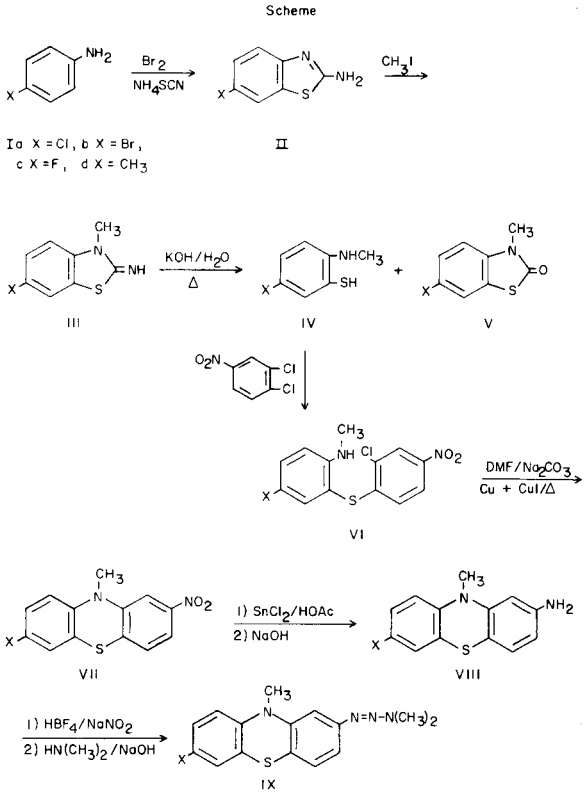
Scheme



EXPERIMENTAL

All melting points were taken on a calibrated Thomas-Hoover capillary melting point apparatus. Analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Infrared spectra were taken on a Perkin-Elmer Infrared Model 727B and nmr spectra were obtained with a Varian T-60A spectrometer using tetramethylsilane (TMS) as an internal standard.

6-Substituted 2-Aminobenzthiazoles (II).

The *p*-substituted aniline (I, 0.1 mole) and ammonium thiocyanate (0.2 mole) in 150 ml of glacial acetic acid were cooled in an ice bath and stirred mechanically. To the solution, bromine (0.2 mole) in 25 ml. of glacial acetic acid was added dropwise at such a rate to keep the temperature below 10° throughout the addition. Stirring was continued for additional thirty minutes after the bromine addition. The precipitate of the benzthiazole hydrobromide was collected, dissolved in hot water and basified with a saturated sodium carbonate solution. The free substituted benzthiazole was collected, washed with water and dried under vacuum. Recrystallization from the appropriate solvent gave 6-substituted 2-aminobenzthiazole (II; X = F, Cl, Br, or CH_3) in good yield. Physical properties of II are listed in Table I.

6-Substituted 2-Imino-3-methylbenzthiazoles (III).

A mixture of 6-substituted 2-aminobenzthiazole (0.1 mole) and methyl iodide (0.1 mole) was boiled in 50 ml of absolute ethyl alcohol for 12 to 15 hours. The precipitated hydroiodide salt of the 6-substituted 2-imino-3-methylbenzthiazole (III, X = F, Cl, Br, or CH_3) was collected, dissolved in hot water and basified with a saturated sodium carbonate solution to give the 3-methylbenzthiazole (III) which was collected, washed with water and dried under vacuum. Recrystallization from the appropriate solvent gave the desired compounds in good yields. The physical properties of III are listed in Table I.

5-Substituted 2-Methylaminothiophenols (IV).

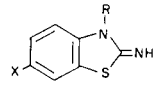
The title compounds were obtained in moderate yields through hydrolytic cleavage of the 6-substituted 2-imino-3-methylbenzthiazole (III). The hydrolysis was achieved by refluxing III with a 50% aqueous potassium hydroxide solution for 36 hours to 3 days. The solution was diluted with water and the *p*H of the solution was adjusted to *p*H 6 with 5*N* acetic acid. The aqueous solution was extracted several times with ethyl acetate and the solvent was removed *in vacuo* to give the 5-substituted 2-methylaminothiophenol (IV, X = F, Cl, Br, or CH_3) in moderate yield. The product was used for reactions without further purification.

Condensation of 5-Substituted 2-Methylaminothiophenol (V) with 3,4-Dichloronitrobenzene.

A mixture of 3,4-dichloronitrobenzene (0.1 mole) and IV (0.1 mole) in absolute ethanol was heated to 60° under a slow stream of nitrogen. To the clear solution, an aqueous solution of sodium carbonate was added over a period of 15-20 minutes. The whole reaction mixture was then heated under reflux for 8 to 10 hours. The completion of the reaction was monitored by thin layer chromatography. The solvent was removed under reduced pressure and the residue was suspended in water and extracted several times with ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under reduced pressure. Recrystallization of the residue from the appropriate solvent gave the condensation product, substituted diphenylsulfide (VI) in good yields.

Table I

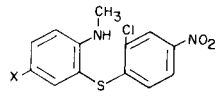
Substituted 2-Aminobenzthiazole



Compound No.	R	X	M.p. °C	Yield %	Recrystallization Solvent	Molecular Formula	Analyses Calculated C	H	N	Found C	H	N
IIa	H	Cl	197-199	96	50% ethanol	$C_7H_5ClN_2S$	45.62	2.72	15.22	45.60	2.78	15.17
IIb	H	Br	213-214	92	benzene/methanol	$C_7H_5BrN_2S$	36.68	2.18	12.23	36.74	2.24	12.23
IIc	H	F	181-183	85	ethanol	$C_7H_5FN_2S$	50.00	2.98	16.67	49.93	3.02	16.63
IId	H	CH_3	131-132	81	benzene	$C_8H_8N_2S$	58.54	4.88	17.07	58.52	4.91	17.06
IIIa	CH_3	Cl	88-89	93	50% ethanol	$C_8H_7ClN_2S$	48.48	3.54	14.14	48.43	3.56	14.08
IIIb	CH_3	Br	108-109	63	ligroin/ethyl acetate	$C_8H_7BrN_2S$	39.51	2.88	11.52	39.58	2.94	11.52
IIIc	CH_3	F	78-79	77	THF	$C_8H_7FN_2S$	52.75	3.85	15.39	52.79	3.89	15.38
IIId	CH_3	CH_3	>250	76	water	$C_9H_{10}N_2S \cdot HI$	35.29	3.59	9.15	35.36	3.60	9.02

Table II

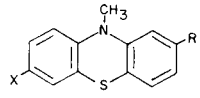
Substituted Diphenylsulfide



Compound No.	X	M.p. °C	Yield %	Recrystallization Solvent	Molecular Formula	Analyses Calculated C	H	N	Found C	H	N
VIa	Cl	143-144	78	methanol	$C_{13}H_{10}Cl_2N_2O_2S$	47.56	3.05	8.54	47.48	3.10	8.52
VIb	Br	140-141	82	ethyl acetate	$C_{13}H_{10}BrClN_2O_2S$	41.82	2.68	7.51	41.78	2.71	7.49
VIc	F	108-109	80	methanol	$C_{13}H_{10}ClFN_2O_2S$	50.00	3.21	8.97	49.95	3.22	8.95
VId	CH_3	140-142	75	methanol	$C_{14}H_{13}ClN_2O_2S$	54.55	4.22	9.09	54.45	4.25	9.07

Table III

Substituted 10-Methylphenothiazine



Compound No.	R	X	M.p. °C	Yield %	Recrystallization Solvent	Molecular Formula	Analyses Calculated C	H	N	Found C	H	N
VIIa	NO_2	Cl	192-193	90	methanol/ethyl acetate	$C_{13}H_9ClN_2O_2S$	53.43	3.08	9.59	53.37	3.14	9.58
VIIb	NO_2	Br	191-192	85	methanol/ethyl acetate	$C_{13}H_9BrN_2O_2S \cdot \frac{1}{4}C_6H_6$	48.67	2.94	7.83	48.91	2.88	7.92
VIIc	NO_2	F	162-163	96	methanol/ethyl acetate	$C_{13}H_9FN_2O_2S$	56.52	3.26	10.15	56.44	3.26	10.13
VIId	NO_2	CH_3	149-150	86	methanol/ethyl acetate	$C_{14}H_{12}N_2O_2S$	61.76	4.41	10.29	61.78	4.44	10.28
IXa	$-N=N-N(CH_3)_2$	Cl	160-162	38	ligroine/ethyl acetate	$C_{15}H_{15}ClN_4S \cdot H_2O$	53.57	5.06	16.67	53.82	4.59	16.76
IXb	$-N=N-N(CH_3)_2$	Br	162-164	63	ligroine/ethyl acetate	$C_{15}H_{15}BrN_4S \cdot H_2O$	47.24	4.46	14.70	47.45	4.08	14.60
IXc	$-N=N-N(CH_3)_2$	F	191-193	54	ligroine/ethyl acetate	$C_{15}H_{15}FN_4S \cdot H_2O$	56.25	5.31	17.50	56.52	4.75	17.54
IXd	$-N=N-N(CH_3)_2$	CH_3	125-127	49	ligroine/ethyl acetate	$C_{16}H_{18}N_4S \cdot 1.5H_2O$	59.08	6.31	17.23	59.41	5.93	17.33

Physical properties of compound VI are listed in Table II.

3-Substituted 7-Nitro-10-methylphenothiazines.

A mixture of VI (0.016 mole), copper powder (0.21 g), cuprous iodide (0.61 g) and sodium carbonate (1.7 g) was refluxed in dimethylformamide (150 ml) under a slow stream of nitrogen for 10 to 12 hours. After cooling, the reaction mixture was poured slowly into a large excess of water with vigorous stirring. The precipitate was collected, washed with water, dried and suspended in ethyl acetate (500 ml). The insoluble substance was removed by filtration and the filtrate was evaporated to dryness. Crystallization of the residue from the appropriate solvent gave the cyclized product, VII, in good yield. The physical properties of these cyclization products are listed in Table III.

Preparation of 3-Substituted 7-(3,3-Dimethyl-1-triazeno)-10-methylphenothiazines (IX).

A mixture of stannous chloride (4.5 g), acetic anhydride (4.0 g) and acetic acid sufficient to make 20 ml was cooled in an ice bath with stirring. The suspension was saturated with hydrogen chloride gas until a clear solution was obtained. To the clear solution, 1.0 g of the nitro compound VII was added and stirred at room temperature until the suspension became colorless. A few drops of water was added to decompose the excess stannous chloride. The reaction mixture was cooled again in an ice bath and stirred for 1 hour. The precipitate was collected, suspended in water and basified with a 40% sodium hydroxide solution. The free amine was collected, washed with water and dried *in vacuo* to give the 3-substituted 7-amino-10-methylphenothiazine VIII in good yield. The products were used for further reaction without purification. The amino compound (VIII, 1.0 g) in 15 ml of 48% fluoboric acid was cooled in a salted ice bath to $-5°$. To the stirred suspension, an aqueous solution of sodium nitrite (1.0 g in 2 ml of water) was added dropwise over a period of 1 hour. After the addition of sodium nitrite solution, the reaction mixture was allowed to stir at ice cold temperature for an additional hour. To the solution, dimethylamine (40%, 3 ml) was added dropwise and stirred for additional 30 minutes. The reaction mixture was then basified by the dropwise addition of 40% sodium hydroxide (8.0 g in 20 ml of water). The whole reaction mixture was again stirred for another 30 minutes, diluted with an equal volume of water and extracted with ethyl acetate. The ethyl acetate extracts were combined, washed with water, dried over sodium sulfate and evaporated to dryness under reduced pressure. The crude triazenes were purified by florisil column chromatography. Crystallization from the appropriate solvents gave the final products IX in 40-60% yields. The physical properties of these compounds are listed in Table III.

Acknowledgment.

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