of acetone was added, and, after cooling the solution to −5 °C, 15 drops of Et_3N were added with stirring and the mixture was kept at 4 °C for 24 h. It was then evaporated to a syrup and 100 mL of benzene was added; after reducing the volume to 10 mL, the solution was applied to a dry silica gel column (3 × 100 cm), and the product was eluted with a mixture of benzene–acetone (9:1): yield 855 mg (13%); mp 117–121 °C dec; MS *m/e* 131 (M^+); UV λ_{max} (pH 7.0) 307 nm (ϵ 7729); λ_{max} (pH 6.0) 272 nm (ϵ 5947), 308 (ϵ 3144) sh; λ_{max} (pH 5.0) 270 nm (ϵ 7729).

5-Methyl-1,3-oxazine-2,6(3*H*)-dione. A. 3-Ethoxycarbonylamino-2-methylacrylic acid[18] (17.3 g) was added to hot (75–80 °C) polyphosphoric acid (170 g, 82–84% P_2O_5) with stirring. The mixture was stirred at 75–80 °C for a total of 45 min, poured into 100 mL of ice–water, and stirred to a smooth slurry. The precipitate was filtered, washed with cold water, and dried over P_2O_5: yield 8.5 g; mp 129–131 °C. The product was recrystallized from ethyl acetate: yield 6.2 g (56%); mp 134–135.5 °C; UV λ_{max} (EtOH) 271 nm (ϵ 6500); NMR (Me_2SO-d_6) δ 1.83 (d, 3, J_{CH_3-4} = 1.5 Hz), 7.56 (q, 1, J_{4-CH_3} = 1.5 Hz, H-4).

B. A cold (0 °C) 5.25% solution (30 mL) of NaOCl containing 1 g of NaOH was added to a cold (0 °C) suspension of citraconimide (2.22 g) in 8 mL of H_2O. The mixture was stirred at 0 °C for 5 h, and cold dilute H_2SO_4 was used to adjust the pH to ~3. The precipitated product was filtered, washed with water, and dried, yield 1.78 g (72%).

Biological. The procedures for determining the growth-inhibitory capacity of the compounds and for carrying out the inhibition analyses have been published previously.[19,20]

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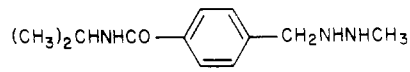
A Structural Modification Study of Procarbazine

Louis T. Weinstock and C. C. Cheng*[1]

Midwest Research Institute, Kansas City, Missouri 64110. *Received August 24, 1978*

Eight analogues of the antineoplastic compound procarbazine were prepared by varying one portion of the molecule, keeping either the methylhydrazinomethyl or the *N*-(1-methylethyl)benzamido portion of procarbazine intact. Preliminary screening results indicated that none of the analogues tested in leukemias L1210 and P388 were as active as the original compound.

N-(1-Methylethyl)-α-(2-methylhydrazino)-*p*-toluamide (procarbazine, **1**), a methylhydrazine derivative synthesized



1

in 1963,[2a] has demonstrated pronounced tumor-inhibitory effects,[2b] has a marked influence on the growth of several transplantable tumors,[3] causes chromosome breakage in mouse cancer cells,[4] inhibits rat prostatic 5α-reductase and araginase activity,[5] and has been studied clinically, either singly or in combination.[6-16] Procarbazine is particularly useful in patients with Hodgkin's disease and non-Hodgkin's lymphoma. Metabolism and mechanism of action studies of this unique agent have also been reported.[17-23]

Procarbazine was found to be carcinogenic[24] and to produce cardiovascular[25] and severe CNS toxicity in patients with hepatic metastasis.[11] The latter toxicity could be related to the procarbazine acting either as an inhibitor of monoamine oxidase[6,26,27] or through depletion of the cofactor pyridoxal phosphate.[28] A structural modification study of this agent has therefore been conducted in this laboratory in order to uncover more desirable compounds. Compounds **2**–**7** represent our preliminary selection of structural variations wherein the methylhydrazinomethyl moiety of **1** is kept intact with the modification of the amide portion or varying the hydrazine unit but leaving the rest of the molecule unaltered.

Chemistry. The pyrimidine derivative **2** was prepared by the condensation of 5-(chloromethyl)uracil[29] and *N*-acetyl-*N*-methylhydrazine,[30] followed by removal of the protecting group. The thiosemicarbazone **3** was obtained from 4-[(2-methylhydrazino)methyl]benzaldehyde.[31]

The benzyloxy isostere of procarbazine (**4**) was prepared as follows. Condensation of 4-(bromomethyl)-*N*-(1-methylethyl)benzamide (**8**) with *N*-[(benzyloxy)carbon-

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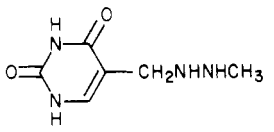

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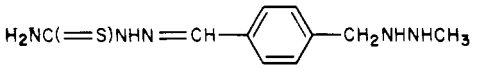

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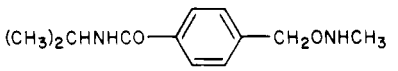

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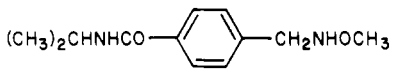

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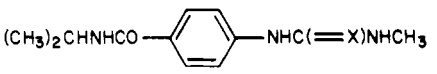
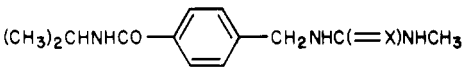
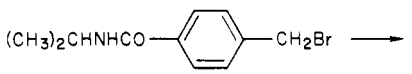


6a, X = O
b, X = S



7a, X = O
b, X = S



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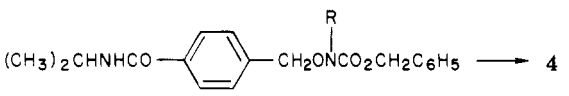
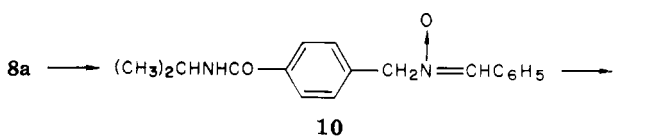


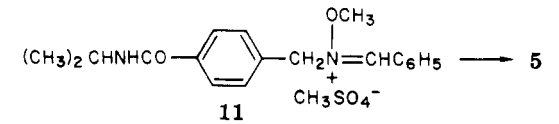
9a, R = H
b, R = CH_3

yl]hydroxylamine[32] in 70% aqueous EtOH gave a near quantitative yield of benzyl [[4-[[(1-methylethyl)-amino]carbonyl]phenyl]methoxy]carbamate (**9a**). (When the same reaction was carried out in absolute EtOH, a mixture of **9a** and the N,O-dialkylated product was formed.) Methylation of **9a** with CH_3I yielded the corresponding *N*-methyl derivative **9b**. Treatment of the latter with ethanolic HCl gave **4** as a HCl salt.

Synthesis of the methoxy isostere of procarbazine, **5**, was accomplished by the following route. Treatment of the benzyl bromide **8** with *anti*-benzaldoxime[33] gave the nitrone **10**. Gerjovich and Smathers[34] reported that nitrones could be O-methylated with $(CH_3)_2SO_4$ in refluxing $C_6H_5CH_3$. However, the expected product **11** could not be obtained from **10** by following the reported reaction conditions. The methylation was later realized with an excess amount of $(CH_3)_2SO_4$ and using CH_3CN as the reaction solvent. The unstable methylated intermediate **11** was not isolated but was converted to the product **5** by



10



11

acid hydrolysis.

The urea (**6a**) and the thiourea (**6b**) derivatives were prepared by treatment of 4-amino-*N*-(1-methylethyl)-benzamide[35] with methyl isocyanate and methyl isothiocyanate, respectively. In a similar manner, compounds **7a** and **7b** were prepared from 4-(aminoethyl)-*N*-(1-methylethyl)benzamide. The latter was readily obtained by hydrogenation of the corresponding 4-cyano derivative[36a] in the presence of Raney nickel.

Biological Activity and Discussion. Preliminary screening results of the aforementioned compounds and their intermediates indicated that marginal in vivo activity was shown by the urea derivative **6a** against leukemia L1210 (T/C = 132 at 50 mg/kg) and by the nitrone **10** against leukemia P388 (T/C = 153 at 800 mg/kg)[36b] and the confirmed in vitro activity of the benzyl bromide **8** against the cell culture of human epidermoid carcinoma of the nasopharynx (KB); other compounds were inactive against these tumor strains. Our initial attempt to uncover more desirable analogues of procarbazine was, thus, not realized. The strictness in structural modification of procarbazine may be comparable to that of methylglyoxal bis(guanylhydrazone).[37]

Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.04% of the theoretical values.

5-[(2-Methylhydrazino)methyl]-2,4(1*H*,3*H*)-pyrimidinedione (2). To a solution of 8 g (0.05 mol) of 5-chlorouracil[29] in 125 mL of $HCONMe_2$ was added 8.8 g (0.1 mol) of *N*-acetyl-*N*-methylhydrazine. The mixture was heated at 70 °C for 2 h and evaporated to dryness in vacuo. To the residue was added 125 mL of 2 N HCl, and the mixture was heated at reflux for 2 h, cooled, and filtered. The filtrate was evaporated under reduced pressure, and the residue was triturated with a mixture of Et_2O and EtOAc to yield a yellow solid. After two recrystallizations from aqueous MeOH, there was obtained 1.2 g (12% yield) of the HCl salt of **2** as a yellow powder, mp 222–224 °C dec. Anal. ($C_6H_{10}N_4O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

4-[(2-Methylhydrazino)methyl]benzaldehyde Thiosemicarbazone (3). To a solution of 8 g (0.04 mol) of 4-[(2-methylhydrazino)methyl]benzaldehyde[31] in 200 mL of absolute EtOH at 70 °C was added a solution of 3.7 g (0.04 mol) of thiosemicarbazide in 50 mL of EtOH. The resulting mixture was refluxed for 1 h, cooled, and diluted with 100 mL of H_2O. The brown solid which separated was collected by filtration, washed with aqueous EtOH, and dried to give 7 g (70% yield) of the HCl salt of **3**, mp 296–298 °C. Anal. ($C_9H_{13}N_5S \cdot HCl$) C, H, N.

4-(Bromomethyl)-*N*-(1-methylethyl)benzamide (8). A mixture of 5 g (0.023 mol) of α-bromo-*p*-toluic acid[38] and 50 mL of $SOCl_2$ was refluxed for 3 h. The resulting solution was evaporated under reduced pressure and the residual solid residue was dissolved in 100 mL of C_6H_6. To the solution cooled below 10 °C was added dropwise 2.7 g (0.046 mol) of 2-$PrNH_2$ in 50 mL of C_6H_6. After the reaction was complete, the reaction was stirred overnight at room temperature and filtered. The filtrate was concentrated in vacuo to yield a white solid, mp 135–137 °C. Two recrystallizations from aqueous MeOH gave 3 g (50% yield) of the amide **8**, mp 143–145 °C. It showed only one spot (R_f 0.55) on a $CHCl_3$–Al_2O_3 TLC plate. Anal. ($C_{11}H_{14}BrNO$) C, H, N.

Benzyl [[4-[[(1-Methylethyl)amino]carbonyl]phenyl]-methoxy]carbamate (9a). To a solution of 5 g (0.03 mol) of *N*-[(benzyloxy)carbonyl]hydroxylamine[32] in 100 mL of 70% EtOH was added 1.2 g (0.03 mol) of NaOH followed by 7.7 g (0.03 mol) of **8**. The mixture was heated under reflux for 2 h and cooled. On standing, the product precipitated as a white solid, mp 125–127 °C. One recrystallization from 40% EtOH gave 10 g (99% yield) of **9a**: mp 128–130 °C; MS *m/e* 342 (M^+). Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

4-[[(Methylamino)oxy]methyl]-*N*-(1-methylethyl)benzamide (4). To a stirred solution of 20 g (0.058 mol) of **9a** in 250 mL of Me_2CO was added 41.5 g (0.3 mol) of K_2CO_3 and 41.4 g

(0.3 mol) of CH_3I. The mixture was heated under reflux for 24 h. The reaction mixture was filtered and the filtrate concentrated in vacuo to yield the methylated intermediate **9b** as a yellow oil. This was treated with 30 mL of ethanolic HCl and the mixture stirred for 1 h at room temperature. It was then concentrated under reduced pressure and the residue triturated with Et_2O to afford a white solid, mp 182–186 °C dec. Two recrystallizations from EtOH–Et_2O gave 3.5 g (23% yield) of the HCl salt of **4**, mp 192–195 °C dec. Anal. ($C_{12}H_{18}N_2O_2$·HCl) C, H, N.

***N*-(1-Methylethyl)-4-[[(phenylmethylene)amino]-methyl]benzamide N^4-Oxide (10).** To a solution of 2 g (0.05 mol) of NaOH in 100 mL of absolute MeOH was added 6 g (0.05 mol) of *anti*-benzaldoxime[33] followed by 12.8 g (0.05 mol) of **8**. The mixture was heated for 4 h under reflux and cooled. After the mixture was left standing overnight, the product precipitated as white crystals, mp 180–183 °C. One recrystallization from $C_6H_5CH_3$ gave 2 g (13% yield) of **10**, mp 188–189 °C. Anal. ($C_{18}H_{20}N_2O_2$) C, H, N.

4-[(Methoxyamino)methyl]-*N*-(1-methylethyl)benzamide (5). To a refluxing solution of 7.5 g (0.025 mol) of **10** in 500 mL of $C_6H_5CH_3$ was added dropwise 33 g (0.26 mol) of $(CH_3)_2SO_4$. After the addition was complete, the mixture was heated for an additional hour and cooled, and the solvent was decanted. The gummy residue was dissolved in 100 mL of 15% H_2SO_4 and heated at 50 °C for 3 h. The resulting reaction mixture was stirred overnight and then extracted with $C_6H_5CH_3$ (3 × 30 mL). The aqueous phase was made alkaline with NH_4OH and extracted with $CHCl_3$ (3 × 30 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to yield a residue, which was dissolved in 100 mL of absolute EtOH, cooled to 5 °C, and stirred with 150 mL of ethanolic HCl overnight. Evaporation of the acidic mixture under reduced pressure and trituration of the residue with Et_2O gave a white solid. After recrystallization from EtOH–Et_2O, there was obtained 2 g (26% yield) of the HCl salt of **5**, mp 182–185 °C dec. Anal. ($C_{12}H_{18}N_2O_2$·HCl) C, H, N.

4-[[(Methylamino)carbonyl]amino]-*N*-(1-methylethyl)-benzamide (6a). To a solution of 4.8 g (0.027 mol) of 4-amino-*N*-(1-methylethyl)benzamide[35] in 150 mL of absolute EtOH was added 1.6 g (0.028 mol) of methyl isocyanate. The resulting solution was stirred overnight at room temperature. After cooling the solution in an ice bath, the product (2 g) separated from the solution as white crystals, mp 196–198 °C. Concentration of the filtrate produced another 3 g of solid with the identical melting point. Two recrystallizations from aqueous EtOH gave 3 g (50% yield) of **6a**, mp 209–210 °C. Anal. ($C_{12}H_{17}N_3O_2$·0.5H_2O) C, H, N.

4-[[(Methylamino)thioxomethyl]amino]-*N*-(1-methylethyl)benzamide (6b). To a solution of 8.9 g (0.05 mol) of 4-amino-*N*-(1-methylethyl)benzamide[35] in 200 mL of EtOH was added 3.65 g (0.05 mol) of methyl isocyanate at room temperature. After stirring for 18 h, the solution was evaporated in vacuo. The orange crystalline residue was recrystallized from aqueous EtOH to give a gray solid, mp 170–175 °C. Another recrystallization from the same solvent gave 3 g (25% yield) of analytically pure **6b**, mp 172–174 °C. Anal. ($C_{12}H_{17}N_3OS$) C, H, N.

4-[[[(Methylamino)carbonyl]amino]methyl]-*N*-(1-methylethyl)benzamide (7a). A mixture of 9 g (0.048 mol) of 4-cyano-*N*-(1-methylethyl)benzamide[36] in 150 mL of absolute EtOH and 25 mL of concentrated NH_4OH and 25 g of Raney nickel was hydrogenated at 4.2 kg/cm^2 of H_2 until 2 equiv of H_2 was absorbed. The mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in 250 mL of 95% EtOH. To this was added 2.7 g (0.48 mol) of methyl isocyanate at room temperature. After stirring the reaction mixture overnight, the product, which precipitated from the reaction mixture, was collected by filtration and recrystallized from aqueous EtOH to give 1.4 g (12% yield) of **7a**, mp 185–186 °C. Anal. ($C_{13}H_{19}N_3O_2$) C, H, N.

4-[[[(Methylamino)thioxomethyl]amino]methyl]-*N*-(1-methylethyl)benzamide (7b). 4-(Aminomethyl)-*N*-(1-methylethyl)benzamide, prepared from 6.6 g (0.035 mol) of the corresponding 4-cyano derivative[36] and Raney nickel as described in the preceding experiment, was dissolved in 150 mL of absolute EtOH and stirred overnight with 2.5 g (0.035 mol) of methyl isothiocyanate at room temperature. The product precipitated during the reaction period and was collected by filtration. It was recrystallized from EtOH to give 6 g (75% yield) of **7b**, mp 198–200 °C. Anal. ($C_{13}H_{19}N_3OS$) C, H, N.

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L-Chlorozotocin

Thomas P. Johnston,* George S. McCaleb,

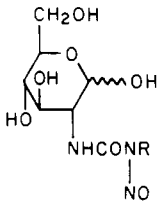
Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Tom Anderson, and Donald S. Murinson

Medicine Branch, National Cancer Institute, Bethesda, Maryland 20014. *Received October 10, 1978*

L-Chlorozotocin (2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-L-glucose) was synthesized in seven steps from L-arabinose for comparison with chlorozotocin, which is the D enantiomorph and an antineoplastic agent with clinical potential. Purification of the intermediate 2-amino-2-deoxy-L-glucose as the Schiff's base formed with 4-methoxybenzaldehyde ensured complete separation from the manno epimer. Comparative screening against leukemia L1210 with concurrent toxicity controls revealed no significant difference between D- and L-chlorozotocin in either activity or toxicity.

Current interest in chlorozotocin (**1**), an analogue of the

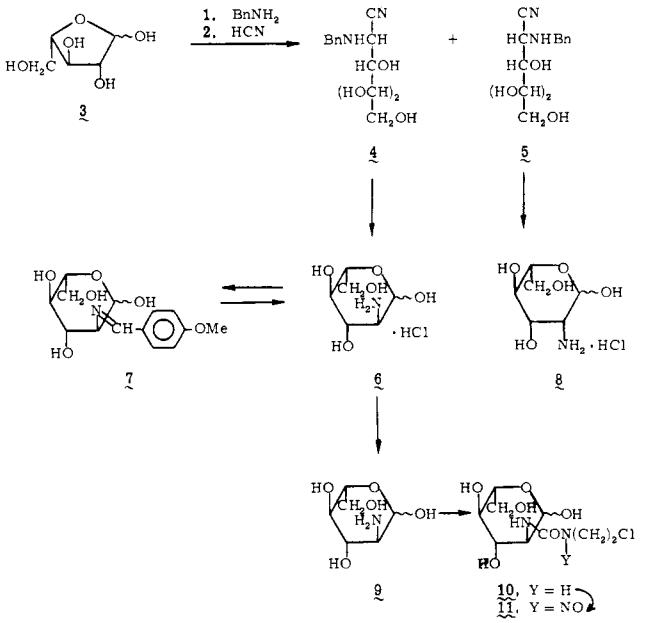


1, R = $(CH_2)_2Cl$
2, R = Me

antineoplastic antibiotic streptozotocin (**2**) and a prospective clinical drug, results from the observation of enhanced activity against leukemia L1210[1,2] and relatively low myelosuppression.[2] Derived from 2-amino-2-deoxy-D-glucose, **1** is in effect D-chlorozotocin. Since L-glucose is not actively transported in mammalian tissues,[3,4] the possible role of D-glucose-mediated transport of **1** was recently tested in a comparison of **1** and L-chlorozotocin (**11**) and found not to be evident: no significant difference in the antitumor and marrow-sparing effects of **1** and **11** was observed.[5] The synthesis of **11** (Scheme I) that enabled the above comparison is reported here along with prefatory screening against leukemia L1210.

The method used for the preparation of the intermediate 2-amino-2-deoxy-L-glucose hydrochloride (**6**) was an adaptation of the reported catalytic reduction of 2-deoxy-2-[(phenylmethyl)amino]-L-gluconononitrile (**4**) derived from L-arabinose (**3**).[6] Although the formation of **4** by the addition of hydrogen cyanide to *N*-(phenylmethyl)-L-arabinosylamine formed in situ is predominant,[7] this procedure prescribes a separation of **4** from its manno epimer **5** in order to avoid contamination of **6** with 2-amino-2-deoxy-L-mannose hydrochloride (**8**). We devised a convenient method that ensures a complete separation of **6** and **8**, which is independent of the stereochemical purity of **4**.

Scheme I



A basified aqueous solution of 2-amino-2-deoxy-D-glucose hydrochloride readily formed an insoluble Schiff's base with 4-methoxybenzaldehyde in near quantitative yield, whereas 2-amino-2-deoxy-D-mannose hydrochloride, whose free base is less basic than 2-amino-2-deoxy-D-glucose,[8-10] did not give evidence (TLC or precipitation) of reaction under the same conditions.[11] In a model experiment, a mixture of equal amounts of 2-amino-2-deoxy-D-glucose hydrochloride and 2-amino-2-deoxy-D-mannose hydrochloride gave only 2-deoxy-2-[[(4-methoxyphenyl)methylene]amino]-D-glucose under the above conditions. Thus, crude **6** containing **8** and ammonium chloride was purified as the Schiff's base **7**, which was

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