Table IV—Antidiabetic Activity of Substituted Cyclic Sulfonylthiourea Derivatives

Compound	Reduction in Plasma Glucose Level Compared with Control, %	Statistical Significance Value of p
Phenformin	10	<0.01[a]
IIc	1.5	0.05
IIIc	4.5	<0.05[a]
VI*d*	3.0	<0.01[a]
VIIc	2.0	0.05
VIIIc	8.0	<0.01[a]
IXc	4.0	<0.01[a]
X*b*	2.0	0.05
XI*a*	4.0	<0.01[a]

[a] Statistically significant.

(IV or V, 0.01 mole) and ethyl bromoacetate (0.011 mole) in absolute ethanol (50 ml) was refluxed with stirring for 4–6 hr, concentrated, and allowed to crystallize. The products obtained were recrystallized from ethanol (Table II).

Synthesis of VIII and IX—A mixture of IV or V (0.01 mole) and ethyl α-bromopropionate (0.011 mole) in absolute ethanol (50 ml) was refluxed with stirring for 6–8 hr, concentrated, and allowed to crystallize. The products obtained were recrystallized from ethanol as colorless needles (Table II).

Synthesis of X and XI—A mixture of IV or V (0.01 mole) and ethyl β-bromopropionate (0.011 mole) in absolute ethanol (50 ml) was refluxed with stirring for 6–8 hr, concentrated, and allowed to crystallize. The products were recrystallized from 95% ethanol as colorless needles (Table III).

In addition to the aromatic protons at δ 7.0–8.1, the PMR spectra of VIc showed a multiplet at δ 0.8–1.7 for the $(CH_2)_3$ protons of the butyl group together with the methylene group of the cyclic ring, a singlet at δ 2.4 characteristic of the methyl group at C-3 of the pyrazole ring, a singlet at δ 1.3 for the CH_3 of the butyl group, and a singlet at δ 6.4 for the proton at C-4 of the pyrazole ring.

In addition to the aromatic protons at δ 6.8–7.9, the PMR spectra of XIc showed a singlet at δ 2.3 for the two methyl groups together with the two methylene groups of the cyclic ring.

DISCUSSION

Biological Testing—Compounds IIc, IIIc, VI*d*, VIIc, VIIIc, IX*c*, X*b*, and XI*a* were tested for hypoglycemic activity using alloxanized female albino mice weighing 20–25 g. Alloxan, 100 mg/kg of body weight, was injected into the tail vein in saline solution (10 mg/ml). Three days later, the mice were given the test compounds orally in suspension in 1% carboxymethylcellulose sodium at the rate of 0.2 mmole/kg of body weight.

Each day, four mice were used as a control group and one group of four mice was given the standard drug phenformin in a dose of 100 mg (0.4 mmole)/kg of body weight. Up to six groups of four mice received test compounds. Blood samples were taken at 0, 1, and 3 hr.

Blood was collected into 0.04% NaF solution. Glucose was determined by the microcolorimetric copper reduction technique of Haslewood and Strookman (4). Results are expressed as a percentage reduction of plasma glucose level compared to the control value.

Statistical Significance—Statistical significance was assessed using the Student t test. Statistical significance was accepted where the calculated t value exceeded the tabulated t value at the 0.05 level.

From the data presented in Table IV, it is obvious that VIII possesses marked hypoglycemic activity. While IIIc, IX*c*, and XI*a* possess only moderate hypoglycemic activity, the potency of these compounds is more than their corresponding thiourea analogs.

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Synthesis of New Polyamine Derivatives for Cancer Chemotherapeutic Studies

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Received July 28, 1980, from the **Midwest Research Institute, Kansas City, MO 64110,* the ‡*University of Missouri–Kansas City, Kansas City, MO 64110,* and the §*Mid-America Cancer Center, University of Kansas Medical Center, Kansas City, KS 66103.* Accepted for publication January 15, 1981.

Abstract □ Selected homologs, analogs, and acylated derivatives of spermine and spermidine, together with several heterocyclic and aromatic compounds containing a novoldiamine side chain, were prepared and evaluated biologically. Several compounds possessed activity against B-16 melanoma and human epidermoid carcinoma of the nasopharynx.

Keyphrases □ Polyamines—synthesis of derivatives, cancer chemotherapy □ Chemotherapy—synthesis of polyamine derivatives for cancer chemotherapy □ Derivatives—polyamines, synthesis, cancer chemotherapy

The importance of many naturally occurring polyamines, such as putrescine, spermidine, and spermine, to the growth of living cells is well established (1–4). It was reported recently that increased amounts of certain polyamines were found in rapidly proliferating biological systems (*e.g.*, chick embryo cells, rat regenerating liver cells, and wound-healing tissues) (5–10), as well as in neoplastic cells of animals and humans (11–16). The higher polyamine levels are believed to be contributed by ornithine decarboxylase activity in these tissues (17). Elevated polyamine levels also were detected in the urine of cancer patients (18–20). The high excretion level of polyamines declined when the patients were in remission, and chemotherapy treatment with methotrexate, cytosine arabinoside, 5-azacitidine, or fluorouracil resulted in polyamine depletion in tumor cells (21–23).

Since cellular protein synthesis is affected by polyamines at the transcription and translation level (24) and at least one role of polyamines is to organize the structure and activity of tRNA (25), it was postulated that properly designed analogs of polyamines could be useful in oncology studies. This concept was substantiated by previous reports (26–29) that some spermine and spermidine analogs of both synthetic and plant origin demonstrated inter-

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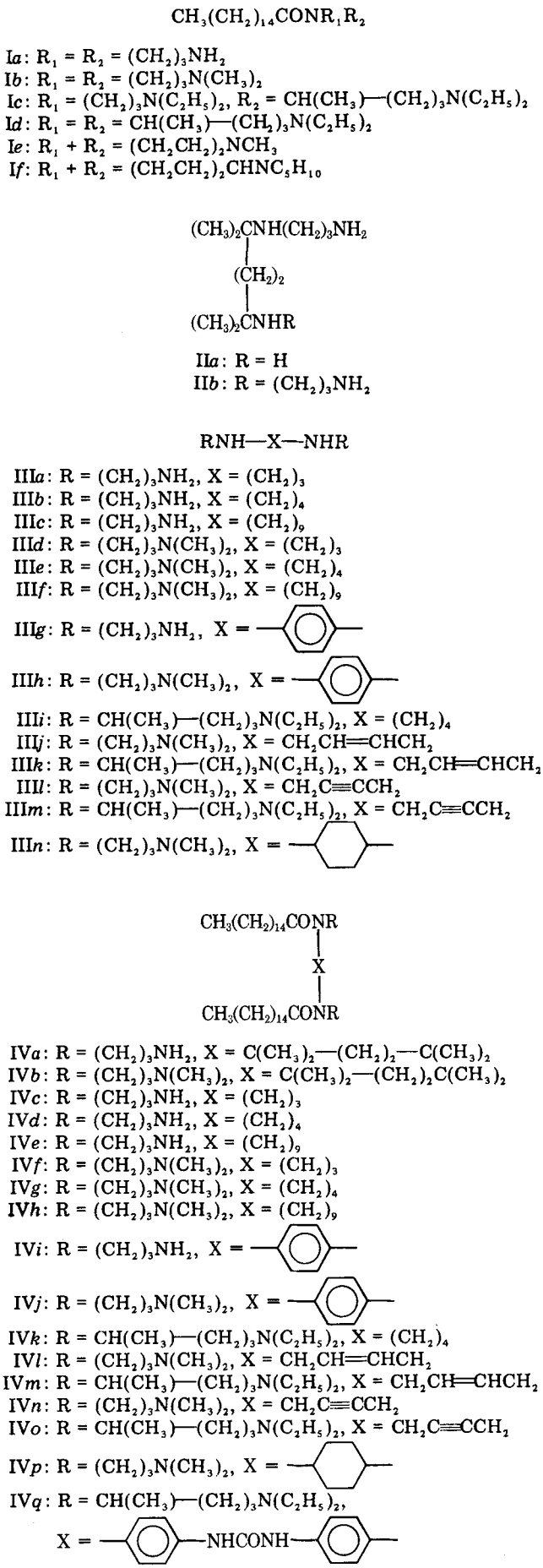
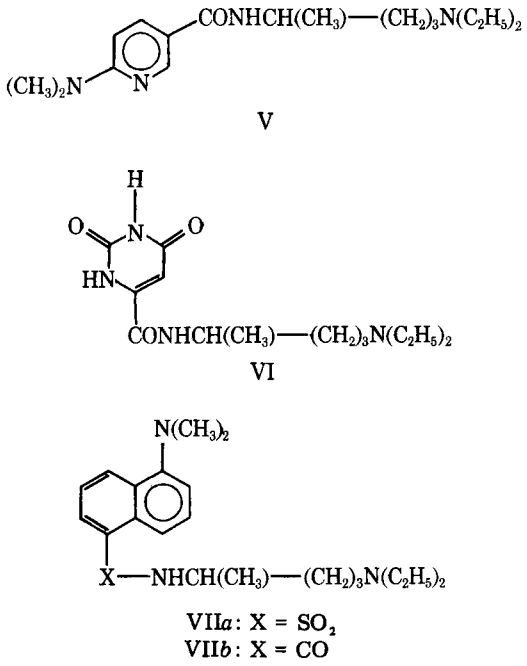




esting *in vitro* and *in vivo* antineoplastic activity. Based on the structure–activity relationship information provided by these investigators and the general biochemical and biological aspects of polyamines, selective analogs, homologs, and acylated derivatives of spermine and spermidine (I–IV), as well as several heterocyclic and aromatic compounds containing a novoldiamine side chain (V–VIII), were prepared and their biological activity was evaluated at the National Cancer Institute.

EXPERIMENTAL

Chemistry—*N*,*N*-Bis(2-cyanoethyl)amine (VIII*a*), prepared by cyanoethylation of ammonia with acrylonitrile (30), was treated with palmitoyl chloride to yield the hexadecaneamide, VIII*b*. Catalytic reduction of VIII*b* with Raney nickel in ethanolic ammonia (31) gave I*a*, isolated as a dihydrochloride salt, mp 240–243° dec. The corresponding dimethyl homolog I*b* was obtained from I*a* and a mixture of formic acid and formamide, according to the method of Clarke *et al.* (32). Compounds Ic and I*d* were prepared by reductive alkylation of 5-diethylamino-2-pentanone (IX) with 3-diethylaminopropylamine (X*a*) and novoldiamine (XI), respectively, followed by acylation. Treatment of 1-methylpiperazine (XII) and 1,4′-bipiperidine (XIII) with palmitoyl chloride in tetrahydrofuran in the presence of triethylamine gave I*e* (isolated as a monohydrochloride salt, mp 150–152°) and I*f* (isolated as a free base, mp 68–69°), respectively.

The tetramethyl homologs of spermidine and spermine, II*a* (isolated as a diphosphate salt, mp 269–271°) and II*b* (disphosphate, mp 239–241°), were prepared by mono- and dicyanoethylation of 2,5-diamino-

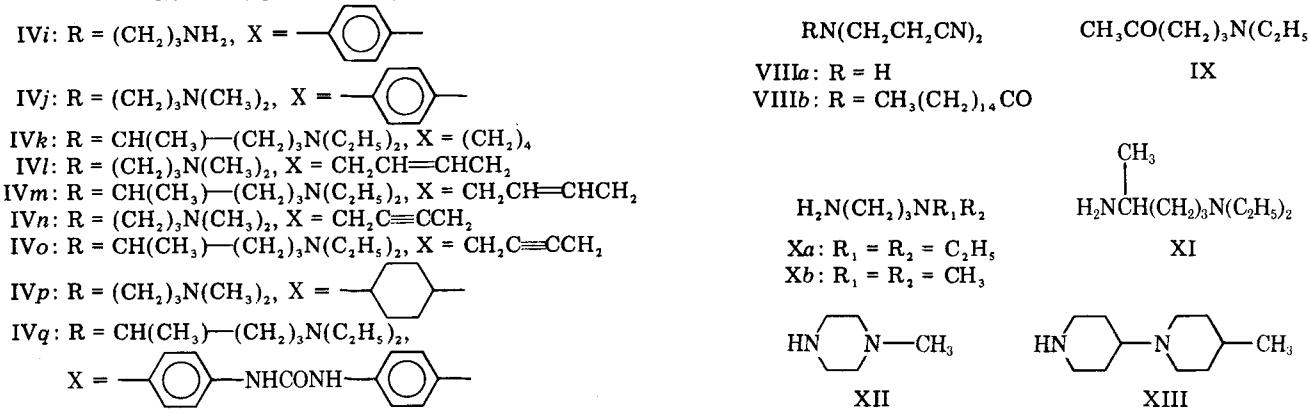
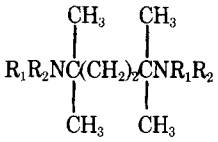
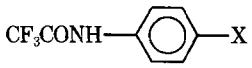


Table I—Antineoplastic Activity Screening Results of Acylated and Some Unacylated Polyamines

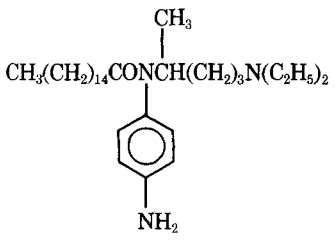
Compound	P-388 Leukemia Dose, mg/kg	P-388 Leukemia T/C	B-16 Melanoma Dose, mg/kg	B-16 Melanoma T/C	KB Cell Culture ED_{50}, μg/ml
I*a*	12.5	109	—	—	0.96
I*b*	6.25	101	—	—	0.024
I*c*	1.5	98	3.13	104	0.29
I*d*	6.25	99	3.13	91	0.026
I*e*	25.0	115	—	—	1.7
I*f*	300.0	100	—	—	100.0
II*a*	200.0	106	—	—	—
II*b*	3.13	115	—	—	—
III*e*	100.0	110	—	—	29.0
III*i*	25.0	92	50.0	Toxic	100.0
			25.0	224	
			12.5	125	
			6.5	133	
			3.13	125	
III*k*	3.13	100	6.25	105	100.0
III*l*	100.0	105	—	—	100.0
III*m*	3.13	108	1.56	105	2.2
IV*a*	6.25	114	12.5	111	16.0
IV*b*	25.0	98	—	—	2.3
IV*c*	25.0	109	—	—	2.0
IV*d*	400.0	111	—	—	—
IV*e*	50.0	97	—	—	100.0
IV*f*	1.56	106	1.56	106	2.6
IV*g*	12.5	90	—	—	2.3
IV*h*	3.13	108	6.25	118	12.0
VI*i*	100.0	98	—	—	—
IV*j*	3.0	101	—	—	8.9
IV*k*	25.0	93	3.13	104	26.0
IV*l*	6.25	113	50.0	155	2.2
			25.0	163	
			12.5	177	
IV*m*	12.5	102	3.13	98	2.4
IV*n*	25.0	95	—	—	2.5
IV*o*	50.0	101	12.5	106	0.63
IV*p*	3.13	90	—	—	18.0
IV*q*	100.0	93	—	—	—
V	25.0	123	—	—	—
VI	50.0	106	—	—	—
VII*a*	6.25	96	6.25	104	—
XIV*b*	100.0	128	—	—	—
XVIII*a*	12.5	117	—	—	—
XVIII*b*	25.0	98	—	—	—



XIV*a*: $R_1 = R_2 = H$
XIV*b*: $R_1 = H$, $R_2 = (CH_2)_2CN$
XIV*c*: $R_1 = CH_3(CH_2)_{14}CO$, $R_2 = (CH_2)CN$



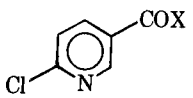
XV*a*: X = NO_2
XV*b*: X = NH_2
XV*c*: X = $NHCH(CH_3)—(CH_2)_3N(C_2H_5)_2$



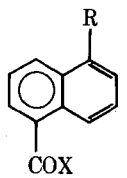
XVI

2,5-dimethylhexane (XIV*a*) with acrylonitrile, followed by catalytic hydrogenation. Acylation of the intermediate dinitrile XIV*b* with palmitoyl chloride and subsequent reduction yielded IV*a*, mp 70–73°. Reductive methylation (32) of IV*a* afforded IV*b*. Compounds IV*c* and IV*d* were prepared by similar sequential steps through intermediates III*a*–III*i* from the appropriate α,ω-diamines.

The unsaturated spermine analogs were prepared according to a reported method (33). Treatment of *trans*-1,4-dichloro-2-butene with 3-dimethylaminopropylamine (X*b*), followed by acylation of the intermediate diamine III*j*, yielded IV*l*, mp 58–60°. The novoldiamine analog IV*m* was prepared from III*k* in an analogous manner. Similarly, the corresponding butyne derivatives IV*n* (mp 40–41°) and IV*o* were prepared from 1,4-dichloro-2-butyne through the intermediates III*l* and III*m*, respectively. For the preparation of the cyclohexyl derivative IV*p*, the intermediate tetramine III*n* was obtained by reductive alkylation



XVII*a*: X = OH
XVII*b*: X = $NHCH(CH_3)—(CH_2)_3N(C_2H_5)_2$



XVIII*a*: R = H, X = OH
XVIII*b*: R = NO_2, X = OH
XVIII*c*: R = NO_2, X = $NHCH(CH_3)—(CH_2)_3N(C_2H_5)_2$
XVIII*d*: R = NH_2, X = $NHCH(CH_3)—(CH_2)_3N(C_2H_5)_2$

of 1,4-cyclohexanedione and X*b*. Acylation of III*n* readily yielded IV*p*, mp 110–115°.

The urea derivative IV*q* was prepared as follows. *p*-Nitrotrifluoroacetanilide (XV*a*), prepared from *p*-nitroaniline and trifluoroacetic anhydride, was reduced to the corresponding amino derivative XV*b*. This reaction was followed by reductive alkylation with IX to give XV*c*. Acylation of XV*c* with palmitoyl chloride and subsequent hydrolysis with potassium carbonate gave the *p*-phenylenediamine derivative XVI. Treatment of XVI with diethylcarbamoyl chloride in pyridine, according to a reported method (34), yielded IV*q*, mp 313–315°.

Condensation of 6-chloronicotinic acid (XVII*a*) with novoldiamine in the presence of dicyclohexylcarbodiimide yielded the chloronicotinamide XVII*b*. Amination of XVII with aqueous dimethylamine gave V (monophosphate, mp 105–107° dec.). The orotamide derivative VI (mp 196–198°) was obtained by refluxing a mixture of novoldiamine and the methyl ester of orotic acid (35) in 2-methoxyethanol[1]. The naphthalenesulfonamide VII*a* (dihydrochloride, mp 77–80°) was prepared by treatment of dansyl chloride with novoldiamine. For the preparation of the naphthalenecarboxamide VII*b* (monophosphate, mp 211–214° dec.), the starting material 1-naphthoic acid (XVIII*a*) was nitrated according to previously reported methods (36, 37). Condensation of the resulting nitroacid XVIII*b* and XI, followed by hydrogenation and reductive alkylation, yielded VII*b*.

Biological Activity—Test results (Table I) were obtained from the National Cancer Institute. Biological screening was conducted in mice with leukemia P-388 and B-16 melanoma and an *in vitro* system with human epidermoid carcinoma of the nasopharynx (KB cell culture). (For the general procedure and data interpretation, see Refs. 38 and 39.)

None of the polyamines showed good activity against leukemia P-388. On the other hand, *N,N'*-bis(3-dimethylaminopropyl)-*N,N'*-bis(palmitoyl)-*trans*-1,4-diamino-2-butene (IV*l*) and *N,N'*-bis(5-diethylamino-2-pentyl)-1,4-butanediamine (III*i*) exhibited inhibitory activity against B-16 melanoma. Compound IV*l* gave test/control (T/C) values of 155, 163, and 177 at doses of 50, 25, and 12.5 mg/kg, respectively. Compound III*i* had a T/C value of 224 at 25 mg/kg but was toxic at 50 mg/kg.

For the KB cell culture, inhibitory activity was demonstrated when the ED_{50} value was <6 μg/ml. Based on this criterion, several acylated and unacylated polyamines (I*a*–I*e*, III*m*, IV*b*, IV*c*, IV*f*, IV*g*, and IV*l*–IV*o*) demonstrated inhibitory activity. Three of the most active compounds (I*b*–I*d*) are all monoacylated polyamines that showed ED_{50} values at concentrations of 0.024, 0.29, and 0.026 μg/ml, respectively.

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[1] Methyl Cellosolve.