quent ultrasonication were necessary to effect complete dissolution of diphenoxylate hydrochloride in chloroform prior to automated analysis. In addition to hastening tablet dispersion, the water retains atropine sulfate and water-soluble excipients, which are subsequently separated from the chloroform solution.

Bromphenol Blue Dye Solution—The pH of the dye solution prepared as specified is approximately 3.4. The effect of the pH of the dye solution on the color intensity of the resultant diphenoxylate–dye complex was investigated. The intensity was fairly constant between pH 2 and 4, with a maximum at pH 3–3.5. Above pH 4, the intensity decreased rapidly.

The concentration of the dye required in the solution was similarly determined by incrementing the weight ratio of dye to diphenoxylate hydrochloride until a constant color intensity was attained for the resultant complex. The amount of dye specified in the solution prepared for the assays is an excess of about twice that needed to react completely with the anticipated concentration of drug.

GLC—The collaborative study of the GLC method for atropine (11) cited references to reports of injection site decomposition. However, the authors of the collaborative study did not observe decomposition, which they attributed to the use of commercial silanized glass wool. GLC decomposition of the atropine was observed in the present study, even when using silanized glass wool, if an excessive amount of wool was placed in the injection site end of the column. The decomposition was evidenced by the appearance of one or more small peaks preceding the atropine and homatropine peaks. By using very little glass wool or none at all, the decomposition problem can be avoided.

Linearity Studies—The standard curve prepared for the GLC determination of atropine was linear over the range of standard concentrations specified.

Conformity to Beer's law was observed when concentrations of diphenoxylate hydrochloride introduced into the automated system were varied from 70 to 150% of the tablet dosage level. The manual dye complexation procedure also provided linear absorbance readings when the drug concentrations were varied from 50 to 150% of the level anticipated in solution sample extracts.

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Synthesis and Anticancer Activity of Novel Cyclic *N*-Hydroxyureas

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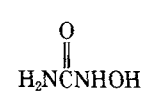
Abstract □ To overcome the disadvantages of hydroxyurea in anticancer therapy such as fast biotransformation and low potency, five cyclic *N*-hydroxyureas were synthesized. A new reaction was developed to prepare the desired products from the appropriate alkyl ω-haloalkylcarbamates with hydroxylamine. This reaction probably involves a two-step mechanism: nucleophilic substitution and intramolecular cyclization. The anticancer screening tests of these compounds were done both *in vitro* using tissue culture and *in vivo*. One compound, 1-hydroxy-1,3-diazacyclohexan-2-one, had anticancer activity comparable to hydroxyurea both *in vivo* and *in vitro*.

Keyphrases □ *N*-Hydroxyureas, cyclic—various derivatives synthesized, anticancer activity evaluated □ Anticancer activity—various cyclic *N*-hydroxyureas evaluated □ Structure-activity relationships—various cyclic *N*-hydroxyureas evaluated for anticancer activity

Hydroxyurea (I) was first synthesized in 1869 (1). Although hydroxyurea was reported not to be active against sarcoma 180 (2) and RC mouse mammary carcinoma (3) in mice, it demonstrated antitumor activities against cancers such as L-1210, Walker carcinoma, P-388 leukemia, and B16 melanoma in mice (4–6). Clinically, hydroxyurea is a simple organic compound currently used as a cancer chemotherapeutic agent in the treatment of chronic myeloid leukemia and in the management of malignant melanoma, head and neck cancers, and brain tumors concomitantly with X-ray therapy (7, 8).

Hydroxyurea immediately inhibits DNA synthesis (S phase) in various systems without any or with slight effect on the synthesis and metabolism of RNA and protein (9, 10). Hydroxyurea-induced inhibition of DNA synthesis is due primarily to interference with the biosynthetic reduction of ribonucleotides to deoxyribonucleotides by inhibition of the enzyme ribonucleotide reductase (11). This inhibition blocks the formation of deoxyribonucleotides required for *de novo* DNA synthesis.

Hydroxyurea has some advantages and disadvantages



I

Table I—Physical Properties of Cyclic *N*-Hydroxyureas

Compound	Formula	Melting Point[a] (Corrected)	Yield[b], %	Analysis, %[c] Calc.	Found
V*a*	$C_4H_8N_2O_2$	176.0–177.5°	18.5	C 41.37	41.98
				H 6.95	6.94
				N 24.02	24.12
V*b*	$C_5H_{10}N_2O_2$	115.0–117.0°	10.1	C 46.14	46.47
				H 7.75	7.52
				N 21.52	21.83
V*c*	$C_6H_{12}N_2O_2$	69.0–71.0°	<2	C 49.98	50.26
				H 8.39	8.67
				N 19.43	19.21
VI	$C_4H_7N_3O_3$	95.0–97.0° dec.	45.7	C 33.10	32.87
				H 5.53	4.86
				N 28.96	29.14
VII	$C_5H_9NO_2$	42–44°	16.5	C 52.16	50.71
				H 7.88	7.89
				N 12.17	11.91

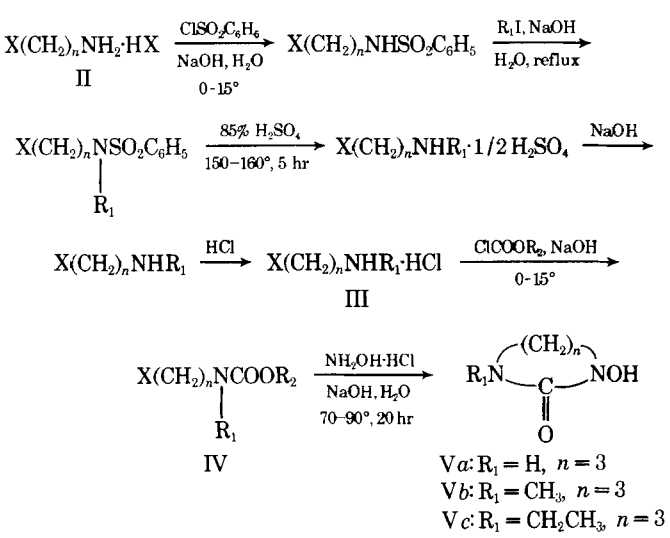
[a] Melting points were determined using a Thomas-Hoover capillary melting-point apparatus, Arthur H. Thomas Co., Philadelphia, Pa. [b] The percentage yield of the final step. [c] Performed by C. F. Geiger, Ontario, Calif.

as an anticancer drug. It has low toxicity (12), and no carcinogenicity or mutagenicity has been reported[1] (13–18). The disadvantages of hydroxyurea are that frequent relatively high doses are needed and rapid biotransformation occurs. The rapid elimination from the body and short half-life (19–22) are due to its high polarity (water solubility) and rapid hydrolysis. Some side effects such as GI disturbance and bone marrow depression were observed (4, 21).

Since medium size cyclic compounds (five- to seven-membered rings) are usually more resistant to hydrolysis than open-chain compounds because of the blockade of nucleophilic attack and since cyclic *N*-hydroxyureas should be more lipophilic than hydroxyurea itself, the cyclic *N*-hydroxyureas possessing the essential pharmacophore [C(=O)NOH] (23) may have higher intrinsic activity and a longer duration of action than hydroxyurea. This paper reports the results of such an investigation.

EXPERIMENTAL

Synthesis—The synthesis of six-membered cyclic *N*-hydroxyureas involved a multiple-step reaction (Scheme I). The primary amines (II, X = chloro or bromo) were obtained commercially, but the required secondary amines (III) were prepared from the corresponding primary amines (24, 25). The intermediate carbamates (IV, R_2 = methyl or ethyl) were prepared by the method of Hartman and Brethen (26).



Scheme I

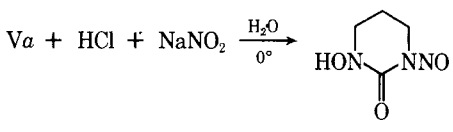
Six-membered cyclic *N'*-nitroso-*N*-hydroxyurea (VI) was synthesized by the nitrosation of the parent compound (V*a*) (27, 28) (Scheme II). The *N*-hydroxylactam, 1-hydroxy-5-methyl-2-pyrrolidinone (VII), was prepared by the reductive cyclization (29) from methyl 4-nitropentanoate (Scheme III). The identities of the desired products were unequivocally established by IR, NMR, and elemental analyses (Table I).

General Procedure for Carbamates (IV)—Fifty grams (0.38 mole) of 3-chloropropylamine hydrochloride was dissolved in 120 ml of distilled water in a 500-ml, three-necked, round-bottom flask equipped with a magnetic stirrer. The solution was cooled to 0° in an ice bath with stirring. Sodium hydroxide, 30.7 g (0.68 mole), was dissolved in 30 ml of distilled water and cooled to 0°. The base solution was added to the amine solution slowly, and the temperature was kept at around 5°. Then 41.6 g (0.38 mole) of ethyl chloroformate was added slowly to maintain the reaction mixture between 5 and 15° over 45 min.

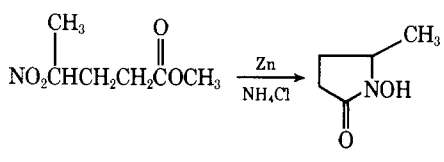
Stirring was continued for 2 hr at the ice-bath temperature and then for 2 hr at room temperature. Two layers separated upon standing. The organic layer (upper layer) was separated from the aqueous phase. The aqueous phase was extracted with 50 ml of chloroform three times. These chloroform extracts were combined and evaporated to dryness under reduced pressure, and the residue was combined with the organic phase. Ethyl *N*-3-chloropropylcarbamate was purified by fractional vacuum distillation.

Other intermediate carbamates also were prepared by the same procedure.

1-Hydroxy-1,3-diazacyclohexan-2-one (V*a*)—Fifty-seven grams (0.82 mole) of hydroxylamine hydrochloride was dissolved in 100 ml of distilled water in a 1-liter, three-necked, round-bottom flask equipped with a magnetic stirrer, and the solution was cooled to 0°. The sodium hydroxide solution was added to the hydroxylamine hydrochloride solution slowly to keep the temperature at around 5°. Then 83.4 g (0.55 mole) of methyl *N*-3-chloropropylcarbamate was added with vigorous stirring. The reaction mixture was kept at 0° for 2 hr, heated to 70°



Scheme II



Scheme III

[1] W. F. Benedict, Childrens Hospital, Los Angeles, Calif., personal communication.

Table II—Effect of V$_a$ against Lymphoid Leukemia L-1210 in BDF$_1$ Female Mice[a]

Treatment (Intraperitoneal)	Mean Survival (days), Treated/Control	Survival Range, days	Weight Change on Day 5, g	T/C
V*a*				
1000 mg/kg, qd (1–9)	13.17/8.40	13–14	−0.17	1.57
1500 mg/kg, qd (1–9)	13.50/8.40	13–15	−1.00	1.61
3000 mg/kg, qd (1–3)	14.50/9.67	14–15	−5.50	1.50[b]
I				
1000 mg/kg, qd (1–9)	15.17/8.40	15–16	−0.50	1.81
3000 mg/kg, qd (1–3)	14.00/9.67	14[c]	−5.50	1.45[b]

[a] Work performed at the Animal Tumor Research Facility of the LAC–USC Cancer Center. Groups of six animals were given 10^5 cells each intraperitoneally on Day 0. Drug treatment was started 24 hr after the transplants. Controls (10) received only isotonic sterile saline. [b] Two animals per treated group and six animals per control group were used because of the limited amount of V*a* available. [c] One animal died on Day 3 postinjection.

continuously for 18 hr over a water bath, and then cooled to 0°; the pH was adjusted to 4–5 by using concentrated hydrochloric acid.

For the removal of inorganic salts, the reaction mixture was poured into 1.5 liters of absolute ethanol at 60° and kept at this temperature for 15 min. This mixture was left in the refrigerator overnight and filtered. The filtrate was evaporated to dryness *in vacuo*, and a viscous liquid was obtained. Dry column chromatography (30) was used to isolate the desired product. The syrupy liquid was dissolved in a minimum amount of absolute ethanol and passed through a column packed with silica gel[2]. The gradient elution technique was used from pure chloroform to 40% ethanol in chloroform.

Silica gel thin-layer plates[3] were used for the detection of the content of each fraction. The desired product was obtained from a 10% ethanol in chloroform elution after the solvent was evaporated. Slightly pink-colored crystals were obtained and recrystallized from absolute ethanol.

1-Hydroxy-3-methyl-1,3-diazacyclohexan-2-one (V*b*)—This compound was prepared by the same procedure as V*a*, except that it was obtained from dry column chromatography using 5% ethanol–chloroform for elution. It was also recrystallized from absolute ethanol.

1-Hydroxy-3-ethyl-1,3-diazacyclohexan-2-one (V*c*)—This compound was prepared by the same procedure as V*a*, except that the desired product was obtained after passage through three columns. It eluted with pure chloroform from the first silica gel column. It also eluted with pure chloroform from the second alumina column. Finally, it eluted from the third silica gel column when 60% chloroform in hexane was used. It was analytically pure without further recrystallization.

1-Hydroxy-3-nitroso-1,3-diazacyclohexan-2-one (VI)—To a solution of 1-hydroxy-1,3-diazacyclohexan-2-one (7 g, 60 mmoles) in dilute hydrochloric acid at 0° was added a solution of sodium nitrite (4.4 g, 63 mmoles) slowly, with stirring, for 2 hr. The mixture was then left in a freezer for 2 hr. The solid residue was filtered and washed with cold water. The yellow residue was collected and dried *in vacuo* at 60° for 4 hr.

1-Hydroxy-5-methyl-2-pyrrolidinone (VII)—In a 150-ml round-bottom flask, 10.2 g (63.3 mmoles) of methyl 4-nitropentanoate and 3.4 g (63.3 mmoles) of ammonium chloride were dissolved in 80 ml of 50% ethanol. Zinc dust, 15.9 g (242.7 mmoles), was added slowly to this mixture over 20 min with stirring. The mixture was cooled in an ice bath for 30 min with continuous stirring and then heated over a water bath at 40° for 4 hr. It was then filtered, and the filtrate was evaporated to a viscous liquid under reduced pressure. This syrupy mixture was dissolved in 30 ml of 2 *N* HCl and then extracted with 50 ml of chloroform three times.

The extract was evaporated to dryness using a rotary evaporator. The residue was dissolved in a small amount of chloroform and passed through a silica gel column. Chloroform was used as an eluent. All ferric chloride-positive fractions were chromatographed on silica gel TLC plates. All TLC-identical fractions were combined and extracted with chloroform; the solvent was evaporated under reduced pressure. The residue was further purified by the sublimation technique.

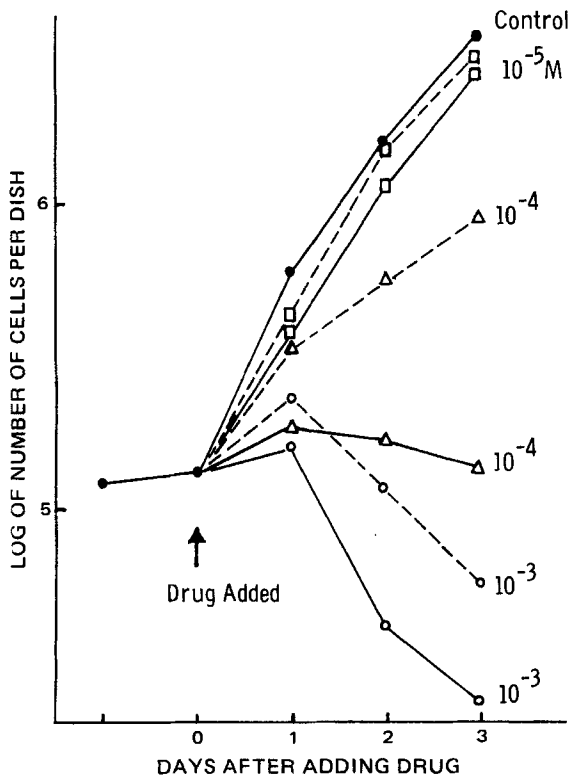


Figure 1—*Growth curve of 3T3 cells with and without drugs. The dead cells did not adhere to the plate and were washed out before counting. Key: ——, I; and - - -, V*a.

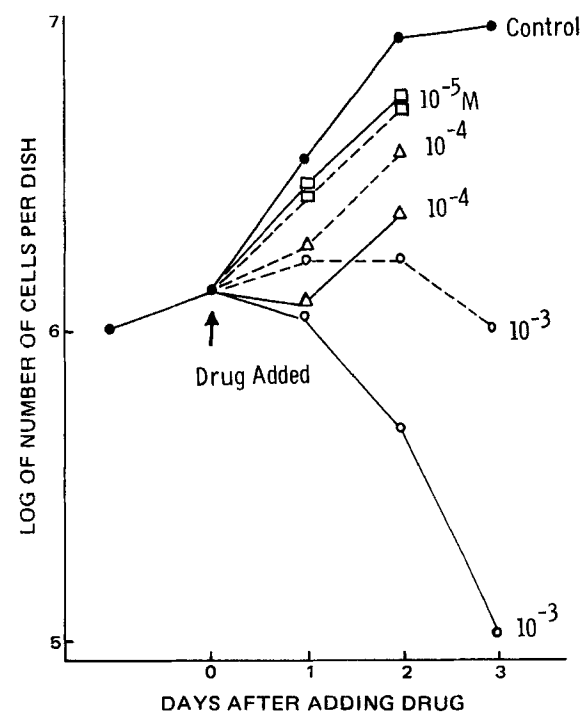


Figure 2—*Growth curve of H-35 cells with and without drugs. The dead cells were washed out and not counted. Key: ——, I; and - - -, V*a.

[2] Woelm dry-column chromatography, ICN.
[3] Eastman Kodak.

Table III—Effect of Cyclic *N*-Hydroxyureas against Lymphoid Leukemia L-1210 (Intraperitoneal) in CDF_1 Female Mice[a]

Treatment (Intraperitoneal)	Mean Survival (days), Treated/ Control	Weight Change on Day 5, g	T/C
V*a*			
400 mg/kg, q4d (1, 5, 9)	9.3/8.3	−1.4	1.12
200 mg/kg, q4d (1, 5, 9)	8.3/8.3	−0.4	1.00
100 mg/kg, q4d (1, 5, 9)	8.7/8.3	−0.2	1.04
V*b*			
600 mg/kg, qd (1–7)	9.0/9.0	−1.1	1.00
300 mg/kg, qd (1–7)	9.0/9.0	−0.5	1.00
150 mg/kg, qd (1–7)	8.5/9.0	−0.3	0.94
75 mg/kg, qd (1–7)	8.3/9.0	−0.2	0.92
37.5 mg/kg, qd (1–7)	8.3/9.0	−0.3	0.94

[a] National Cancer Institute supplied data.

***In Vitro* Anticancer Screening Tests**[4]—The inhibition of cell growth of one fibroblastic and two tumor cell lines in tissue culture was used for *in vitro* tests. The cell lines were BALB/c 3T3 mouse embryo fibroblasts clone 42, Reuber H-35 rat hepatoma, and human Wilms' tumor cells.

BALB/c 3T3 mouse fibroblasts were grown in Dulbecco's modified minimal essential medium (MEM) supplemented with 10% calf serum. The H-35 cells were grown in Eagle's minimal essential medium supplemented with sodium pyruvate, fourfold amino acids, vitamins, and 13% heat-inactivated fetal calf serum. Human Wilms' tumor cells (TuWi) were grown in medium 199 supplemented with 5% calf serum and 5% heat-inactivated fetal calf serum.

All cells were grown in 60- × 15-mm plastic dishes in a humidified incubator in an atmosphere of 95% air–5% CO_2 at 37°. Drug solutions were added to the cells at the beginning or in the middle of the logarithmic growth phase. Following a saline wash and mild trypsinization, the total cells per dish were counted with a hemocytometer under the microscope each day during the experimental period (Figs. 1–3).

Calculation of pI_{50}—The I_{50} is the molar concentration of the drug that inhibits 50% of normal cell growth. The I_{50}'s were calculated from the best-fit regression equations derived *via* the method of least squares using the probit-log of the concentration method (31). The value of pI_{50} is equal to $-\log I_{50}$. The larger the pI_{50} is, the more potent the drug is.

***In Vivo* Anticancer Screening Tests**—For *in vivo* studies, animals were obtained commercially[5]. The L-1210 leukemia[6] was maintained by serial transplants in DBA/2 mice. The *in vivo* anticancer screening tests were performed according to the National Cancer Institute screening protocol (32) (Tables II and III).

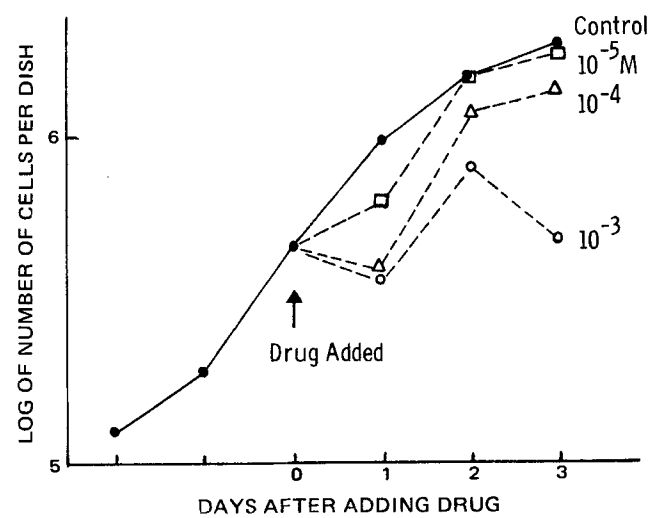


Figure 3—*Growth curve of Wilms' human tumor cells with (- - -) and without (—) the drug (Va).*

[4] All growth media were purchased from Grand Island Biological Co., Santa Clara, Calif.

[5] Simenson Laboratories, Gilroy, Calif.

[6] From Dr. G. R. Laster, Jr., Southern Research Institute, Birmingham, Ala.

Table IV—*In Vitro* Inhibition of BALB 3T3 Fibroblast Cell Growth by I and V*a*

Day	$pI_{50} \pm SE$ I	$pI_{50} \pm SE$ V*a*
1	4.61 ± 0.82	3.66 ± 0.85
2	4.70 ± 0.37	4.01 ± 0.29
3	4.72 ± 0.25	4.31 ± 0.29
ΔpI_{50} (Day 3 − Day 2)	0.02	0.30

RESULTS AND DISCUSSION

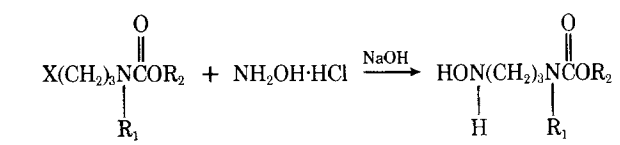
After many unsuccessful trials, the desired products were finally obtained by a new reaction. This reaction may involve a two-step mechanism (29, 33): (*a*) nucleophilic substitution (Scheme IV) and (*b*) intramolecular cyclization (Scheme V). The yield decreases as the alkyl group becomes bulkier, primarily due to the steric hindrance that decreases or prevents the cyclization (Table I).

Compound VII was synthesized by reductive cyclization, *i.e.*, reduction of NO_2 to NHOH, followed by intramolecular cyclization (Scheme VI).

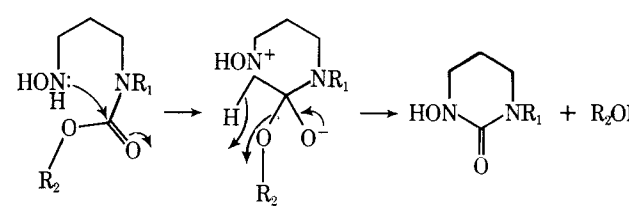
The growth curves of three cell lines with or without the drug are shown in Figs. 1–3. The pI_{50}'s for hydroxyurea and cyclic *N*-hydroxyureas are shown in Tables IV–VII. Morphologically, there is no difference between control cells and drug-treated cells in these three cell lines examined under the microscope.

These drugs were added to the logarithmic growth phase (Figs. 1–3) either at the beginning or the middle of this phase. However, if the cells in the plateau or other nondividing phase are used, the accuracy in assessment of antitumor activity becomes difficult (34). In the present experiments, the cell number declined 2 or 3 days after the drug solutions were added to the cells, indicating drug cytotoxicity. Hydroxyurea and the cyclic *N*-hydroxyureas were more cytotoxic toward fast growing cells such as H-35 and 3T3 cells than toward slower growing cells such as the Wilms' tumor line, as indicated by the lower pI_{50} against TuWi cells.

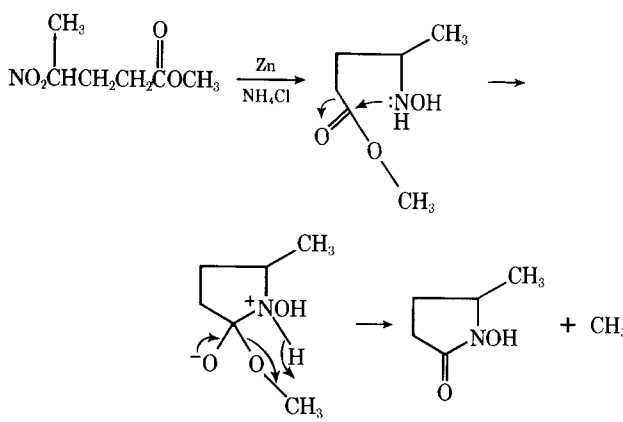
Compound V*a* was comparable to hydroxyurea in cytotoxicity on H-35 and 3T3 cells, as indicated by the close pI_{50} values. From Tables VI and VII, one can see a more favorable ΔpI_{50} value, reflecting greater stability or persistence of cytoxicity of the cyclic compounds.



Scheme IV



Scheme V



Scheme VI

Table V—*In Vitro* Inhibition of Rat H-35 Hepatoma Cell Growth by Hydroxyurea and Cyclic *N*-Hydroxyureas[a]

Compound	pI_{50} Day 1[b]	pI_{50} Day 2[b]	pI_{50} Day 3[b]
I	≃3	3.97 ± 0.32[c]	4.56 ± 0.32[c]
V*a*	≃3	3.33 ± 0.36	4.05 ± 0.35
V*b*	<3	3.29 ± 0.49	3.89 ± 0.39
V*c*	<3	2.96 ± 0.86	3.14 ± 0.63
VI	<3	3.35 ± 0.52	3.61 ± 0.43
VII	<3	2.43 ± 0.71	3.18 ± 0.58

[a] The pI_{50} values were obtained from the same experiment. [b] Days after adding the drugs. [c] Standard error.

Two cyclic compounds, V*a* and V*b*, tested by the National Cancer Institute, were not active against L-1210 at dose levels up to 400 and 600 mg/kg, respectively. Nevertheless, in this study V*a* at a dosage of 1000–3000 mg/kg (Table II) showed a highly significant activity against leukemia L-1210; this activity was comparable to hydroxyurea used as a positive control. Even though hydroxyurea itself was reported to be very active against L-1210 *in vivo* (5), it is known that the effectiveness of hydroxyurea is highly schedule dependent (35). Perhaps these new cyclic *N*-hydroxyureas (V*a* and V*b*) are also schedule dependent. The dose regimen used by the National Cancer Institute might not be frequent enough to show a definite anticancer activity.

Compound V*a* had comparable cytotoxicity *in vitro* and similar anticancer activity *in vivo* as compared to hydroxyurea. However, the negative results found by the National Cancer Institute appear to be due to the use of a suboptimal dose regimen. The other cyclic *N*-hydroxyureas apparently are less cytotoxic than either hydroxyurea or V*a*. Hydroxyurea always showed a lower ΔpI_{50} (Day 3 − Day 2) than the cyclic compounds (Tables IV, VI, and VII), reflecting the better persistence of the cyclic compounds under the testing conditions used.

A positive correlation between the log of molecular weight (mol. wt.) and the potency of several series of clinically used drugs, including anticancer drugs, was shown recently (35). The best-fit regression line for 15 different anticancer drugs was derived by using simple regression analysis. The equation is:

$$\log 1/C = 3.411 \log \text{mol. wt.} - 2.559$$

$$n = 15, r = 0.786, s = 0.837 \qquad \text{(Eq. 1)}$$

That is, the higher the molecular weight, the more potent the drug will be if the molecule fits into the receptor site and if the drug is active.

The optimal lipophilicity, as expressed by the logarithm of the 1-octanol–water partition coefficient (log *P*) of some anticancer drugs in an animal study, lies between 1 and −1 and the optimal log *P*'s for different series of anticancer drugs are different (36, 37). The parent compound (V*a*) of cyclic *N*-hydroxyurea is more cytotoxic than the *N'*-methylated derivative (V*b*), and V*b* is more cytotoxic than the *N'*-ethylated derivative (V*c*). These findings reflect a negative dependence on the lipophilicity and may indicate that hydrophilic character is necessary to have higher cytotoxicity for this series of compounds. In other words, the benefit derived from the greater stability of the cyclic structure may be offset by the undesired higher lipophilicity. Alternatively, there may be a very restricted bulk tolerance on the *N'*-substituent.

From this limited amount of information and previous findings (35), it appears that the *N*-hydroxyurea derivatives with substantially higher molecular weights and polar functional groups to maintain low lipophilicity (log *P*) may be worth pursuing.

Table VI—*In Vitro* Inhibition of Hepatoma H-35 Cell Growth by I and V*a*[a]

Day	pI_{50}[b] ± *SE* V*a*	pI_{50}[b] ± *SE* I
1	3.51 ± 0.59	3.80 ± 0.52
	2.87 ± 2.28	—
	3.23 ± 0.46	—
2	4.52 ± 0.68	4.63 ± 0.43
	3.43 ± 0.61	—
	3.51 ± 0.35	4.24 ± 0.35
3	—	—
	4.14 ± 0.52	—
	3.80 ± 0.34	4.06 ± 0.27
ΔpI_{50} (Day 3 − Day 2)[c]	+0.29	−0.18[c]

[a] Different experiments from Table V. [b] The pI_{50} values were obtained from three different experiments. [c] Calculated from only one experiment.

Table VII—*In Vitro* Inhibition of Wilms' Human Tumor Cell Growth by Hydroxyurea and Cyclic *N*-Hydroxyureas

Compound	pI_{50} Day 1[a]	pI_{50} Day 2[a]	pI_{50} Day 3[a]	ΔpI_{50} (Day 3 − Day 2)
I[b]	<3	4.29 ± 0.88	3.77 ± 0.38	−0.52
V*b*[b]	<3	2.48 ± 3.32	2.52 ± 1.12	0.04
VI[b]	<3	3.01 ± 1.13	3.70 ± 0.84	0.69
V*a*[c]	<3	<3	≃3.00	—

[a] Days after adding the drug. [b] The pI_{50} value of these compounds were obtained from the same experiment. [c] The pI_{50} value was obtained from a different experiment.

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In Vitro Photodecomposition of Uric Acid in Presence of Riboflavin II

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Abstract □ *In vitro* studies on the photodecomposition of uric acid in the presence of the monosodium salt of riboflavin 5′-phosphate in buffers at various pH values, in methanol, and in human plasma are reported. The decomposition rate increased with increasing pH and was independent of solvent or buffer species. The mechanism appears to be an energy transfer process involving triplet riboflavin and singlet oxygen. Riboflavin-enhanced photodecomposition of uric acid occurred *in vitro* in hyperuricemic human plasma.

Keyphrases □ Uric acid—*in vitro* photodecomposition in presence of riboflavin, effect of pH and solvent □ Riboflavin—effect on *in vitro* photodecomposition of uric acid, various pH's and solvents □ Photodecomposition—uric acid in presence of riboflavin, effect of pH and solvent □ Vitamins—riboflavin, effect on *in vitro* photodecomposition of uric acid, various pH's and solvents

The successful phototherapy of psoriasis was reported using methoxsalen and longwave UV light (1), and phototherapy alone was used to treat neonatal hyperbilirubinemia (2–4). Riboflavin was reported to enhance bilirubin photocatabolism in rats (5).

Recently, an *in vitro* photodecomposition of uric acid was observed in the presence of the monosodium salt of riboflavin 5′-phosphate (I) and visible light in aqueous solutions (6). Increasing the I concentration appeared to increase the disappearance rate of uric acid. The addition of potassium iodide (10^{-3} *M*) to the reaction mixture retarded photodecomposition.

Because of the potential clinical importance of this observation (treatment of hyperuricemia), experiments were undertaken to characterize the pH dependence of the reaction and to determine if the reaction would proceed in a nonaqueous solvent, if the reaction was oxygen dependent, and if photodecomposition would occur *in vitro* in human plasma.

EXPERIMENTAL

The irradiation light box used standardized test tubes distributed radially at equal distances (approximately 26 cm) measured from the center of the test tubes to the center of a vertically positioned, unfiltered 15-w fluorescent bulb[1] emitting visible light. The inside of the apparatus was painted flat black to reduce any internally reflected light, and the outside was draped with black cloth to eliminate external light. The light intensity was monitored using a light meter placed underneath a test tube and was held constant using a transformer[2].

The light intensity was approximately 40 foot-candles for all experiments, except the plasma study performed at approximately 50 foot-candles. Test tubes used for the irradiation[3] were standardized (±2%) by measuring[4] the absorbance of a 9.7×10^{-6} *M* aqueous I solution at 450 nm.

All solutions were prepared using deionized water, except for the methanol studies, and stock solutions of uric acid and riboflavin were prepared fresh daily. Irradiation, spectrophotometric analysis[5], and all sample preparation were performed in a darkened laboratory. Plasma samples, analyzed by high-pressure liquid chromatography (HPLC), were kept in light-resistant containers from which samples were withdrawn.

Reagents—Uric acid[6], lithium carbonate[6], and the monosodium salt of I[7] were used as received. All other solvents and reagents were reagent grade.

HPLC System and Conditions—HPLC analysis[8] was performed at a fixed wavelength of 280 nm, using a stainless steel column (0.61 m × 2 mm i.d.) packed with an anion-exchange material[9] (7). A Sorenson phosphate buffer, pH 5.9, was the eluting solution.

Buffers—Sorenson phosphate buffers of approximately pH 5, 6, 7, and 8 and a Delory and King carbonate buffer of approximately pH 9 were

[1] Sylvania F15T8-CW.
[2] Powerstat type 3PN11688, Superior Electric Co., Bristol, Conn.
[3] Pyrex No. 9800.
[4] Coleman Jr. II spectrophotometer.
[5] Beckman model 25 recording spectrophotometer.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Nutritional Biochemical Co., Cleveland, Ohio.
[8] Waters Associates liquid chromatograph model 6000 with Soltec recorder.
[9] VYDAK AM, Catalog No. 301, The Separations Group, Hesperia, Calif.