3.6 Hz, H-1), 4.7 (q, 1, H-2), 4.24 (m, 3, H-4, H-5a, H-5b), 2.46 (s, 3, tosyl CH_3), 2.12 (q, 2, H-3a, H-3b), 1.37, 1.27 (both s, 6, *gem*-dimethyl). Anal. ($C_{15}H_{20}O_6S$) C, H, S.

3,5-Dideoxy-5-iodo-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (24). A solution of **23** (2.5 g), 2-butanone (50 mL), and NaI (5 g) was boiled under reflux for 24 h. The same workup as previously described afforded a clear oil that crystallized from cold ethyl acetate–*n*-hexane as needles but melted at room temperature: $[\alpha]^{25}_D$ -14.1° (*c* 2.3, $CHCl_3$); NMR ($CDCl_3$) δ 5.86 (d, 1, $J_{1,2}$ = 3.6 Hz, H-1), 4.76 (q, 1, H-2), 4.4 (m, 1, H-4), 3.53, 3.40 (both s, 2, H-5a, H-5b), 2.3 (m, 2, H-3a, H-3b), 1.56, 1.33 (both s, 6, *gem*-dimethyl). Anal. Calcd for $C_8H_{13}IO_3$: C, 33.82; H, 4.61. Found: C, 33.35; H, 4.50.

Polarimetric Studies. The procedure used for the periodate oxidation and borohydride reduction has been reported previously.[12,23] 9-(5-Deoxy-β-D-*erythro*-pent-4-enofuranosyl)adenine (**8**) was the reference and was oxidized for 18 h. Because of trans hydroxyl groups, nucleoside **6** was oxidized for 5 days. The final solution of dialcohol **7** derived from **6** had $[\alpha]_D$ +59°, whereas the solution derived from **8** had $[\alpha]_D$ +55°.

Acknowledgment. This work was supported by Grant CA13802 from the National Cancer Institute, National Institutes of Health.

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Antileukemic Activity of Substituted Ureidothiazoles, Ureidothiadiazoles, and Related Compounds

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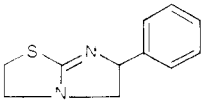
A number of ureidothiazole and ureidothiadiazole derivatives related to ethyl 4-[[(2-thiazolylamino)carbonyl]-amino]benzoate were prepared and evaluated against the leukemia P-388 tumor system in mice. Preliminary structure activity relationship study revealed that, among other considerations, active compounds of this series contain either an "isothioureido" [>N–C(S–)=N–] or an "isothiosemicarbazono" [>N–C(S–)=N–N=] structural unit.

A comparison of the structures of a number of nitrogen- and sulfur-containing heterocyclic compounds possessing antineoplastic activity reveals a definite structural arrangement of certain key atoms. Levamisole[1] (**1**) was



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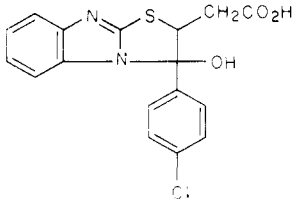

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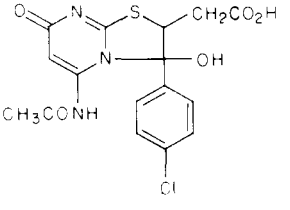

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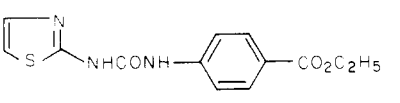
reported to have antitumor activity against Lewis lung carcinoma,[2] rhabdomyosarcoma,[3] other experimental systems,[4] and man.[5] This drug acts as a nonspecific stimulant of the immune system.[6] Levamisole and its analogues are inhibitors of alkaline phosphatase.[6] A fused benzimidazolylthiazoleacetic acid derivative **2** and a pyrimidylthiazoleacetic acid derivative **3** were also known to be active against Lewis lung tumor.[7]

Recently, a ureidothiazole derivative **4** (Carbolabs, Inc.)



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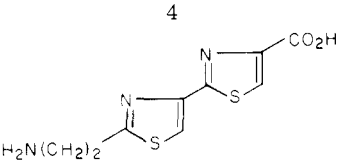

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was found to possess interesting biological activity.

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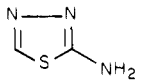
Whereas this compound had only borderline activity against leukemia L1210 (T/C 125 at 200 mg/kg), its activity against P-388 was higher (T/C 162 at the same dosage). Of particular interest is its activity against B-16 melanoma (T/C 167 at 100 mg/kg). However, this compound is not active against Lewis lung tumor.

In connection with a synthetic study of the antitumor antibiotic bleomycin, a bithiazolecarboxylic acid[8] **5** and some related derivatives were synthesized in this laboratory. None of these compounds exhibited antineoplastic activity in experimental animal test systems. An examination of these active and inactive thiazole derivatives revealed that the presence of a nitrogen atom at position 2 of a thiazole or related ring systems is important to the antineoplastic action. The significance of this "isothioureido" structural unit is further substantiated by the fact that 2-amino-1,3,4-thiadiazole (**6**) and 2,2′-(methylene-



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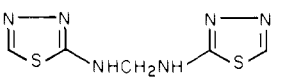

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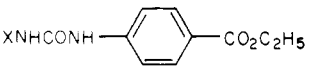
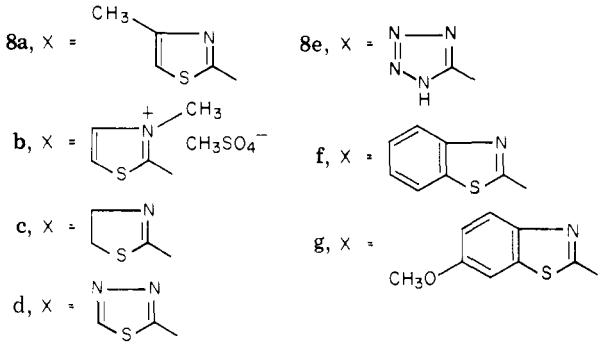
diamino)bis(1,3,4-thiadiazole) (**7**) were also reported to possess antitumor activity.[9-14]

Studies of the mechanism of action of **6** and **7** suggested that these compounds interfere with guanosine monophosphate (GMP) biosynthesis by inhibiting inosine monophosphate (IMP) dehydrogenation, thus preventing the conversion of IMP to xanthosine 5-phosphate.[13,14] Consequently, preparation of properly designed compounds incorporating the isothioureido structural feature may furnish agents with both antileukemic and antitumor activity. Our initial approach was to modify various parts of structure **4** in order to gain additional information on the relationship between the structure and biological activity.

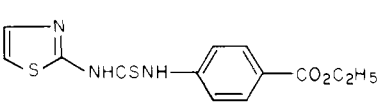
Modification of the Thiazole Portion of 4. This includes preparation of methylated derivatives **8a**, a di-





hydrothiazole derivative **8c**, a thiadiazole analogue **8d**, a tetrazole analogue **8e**, and two benzothiazole derivatives **8f,g** to study the relationship between the nature of the heterocyclic ring to antineoplastic activity.

Modification of the Ureido Linkage of 4. Only one compound, the thioureido analogue **9**, was prepared for the present study.



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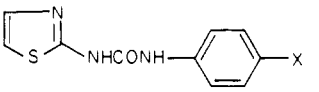
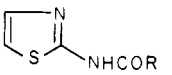
Modification of the Terminal Ester Group of 4. This includes replacement of the ester by other atoms or functional groups, including a carboxylic acid (**10a–g**).

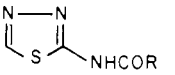


10a, X = H 10e, X = CN
b, X = CH_3 **f**, X = NO_2
c, X = Cl **g**, X = CO_2H
d, X = F

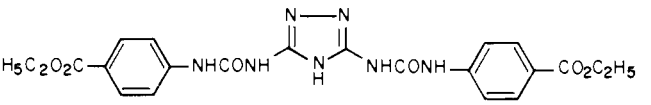
Miscellaneous Modifications. Compounds **11a–f**, **12a–f**, and **13** with a variety of single and combined



11a, R = OC_2H_5
b, R = CH_2Cl
c, R = CF_3
d, R = $NHCH_3$
e, R = NH-C_6H_4-2-CF_3
f, R = $NHSO_2$-C_6H_4-4-CH_3



12a, R = OC_2H_5
b, R = CH_2Cl
c, R = CF_3
d, R = NH-C_6H_5
e, R = NH-C_6H_4-4-CN
f, R = NH-C_6H_4-4-NO_2



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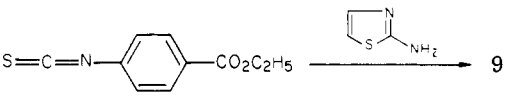
modifications of **4** were prepared to study the lipophilic, steric, and electronic effects of different substituents on biological activity.

Chemistry. The N,N′-disubstituted ureas were prepared from substituted amines and appropriate isocyanates. To avoid the formation of diarylureas, which are often formed as major contaminants and are difficult to separate from the desired products, the reagents used had to be azeotropically dried prior to use, and benzene was chosen as the general reaction solvent. Most compounds prepared by this method were obtained in 85% to quantitative yields.

Attempted preparation of ethyl *p*-(isothiocyanato)-benzoate (**14**) by treatment of ethyl *p*-aminobenzoate with thiophosgene in benzene or other organic solvents, according to the procedure of Potts et al.,[15] failed to yield the expected product. It was later found that compound **14** could be readily obtained by conducting the reaction in aqueous HCl. Analogous to the preparation of the N,N′-disubstituted ureas, condensation of **14** with 2-aminothiazole in benzene gave the desired thioureido compound **9**.



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Compounds **11a,b** were prepared by refluxing 2-aminothiazole with ethyl chloroformate and with chloroacetyl chloride, respectively, in benzene without an acid scavenger until no more HCl was liberated from the boiling reaction mixture. Compounds **12a,b** were prepared in a similar manner except that 2-amino-1,3,4-thiadiazole was used in place of 2-aminothiazole for the condensation. Compounds **11c** and **12c** were obtained by treatment of the appropriate amino heterocyclic compound with $(CF_3CO)_2O$ at room temperature. All compounds in this group were found to be very soluble in many organic solvents, including ether and hexane.

Biological Activity and Discussion. Preliminary screening results of the ureidothiazoles, ureidothiadiazoles, and related compounds are shown in Table I. Compounds

Table I. Antileukemic Activity of Substituted Ureidothiazoles, Ureidothiadiazoles, and Related Compounds against Leukemia P-388[a]

compd	formula (analyses)	mp, °C	yield, %	λ_{max}, nm (log ϵ)	dose, mg/kg	survival	wt diff	T/C, %
4[b]	$C_{13}H_{13}N_3O_3S$ (C, H, N)	258-260	87	290 (4.45)	200	5/6	-2.6	162
					100	6/6	-1.8	143
					50	6/6	-2.2	149
					25	6/6	0.7	145
8a	$C_{14}H_{15}N_3O_3S \cdot 0.5H_2O$ (C, H, N)	235-237	98	290 (4.61)	200	6/6	-5.8	138
					100	6/6	-2.6	127
					50	6/6	-2.2	109
8b	$C_{16}H_{17}N_3O_3S_2 \cdot 0.25H_2O$ (C, H, N)	218-220	95	225 (4.19), 267 (4.00), 320 (4.78)	200	4/6	-2.2	90
					100	6/6	-1.3	100
8c	$C_{15}H_{15}N_3O_3S$ (C, H, N)	200-201	94	242 (3.92), 294 (4.64)	400	6/6	-2.7	92
					200	6/6	2.3	87
8d[c]	$C_{12}H_{12}N_4O_3S$ (C, H, N)	266-268	100	277 (4.55), 310 (3.75)	100	6/6	4.9	134
					50	12/12	2.7	138
					25	12/12	2.1	132
					12.5	6/6	1.2	119
8e	$C_{11}H_{12}N_6O_3$ (C, H, N)	268-270	65	276 (4.49)	400	6/6	3.1	108
					200	6/6	1.5	100
8f	$C_{17}H_{15}N_3O_3S$ (C, H, N)	290-292	97	240 (4.09), 290 (4.66), 298 (4.95), 320 (3.86)	400	6/6	4.6	90
					200	6/6	3.2	90
8g	$C_{15}H_{17}N_3O_4S \cdot 0.5H_2O$ (C, H, N)	285-287	100	243 (4.13), 298 (4.60)	400	6/6	3.3	89
					200	6/6	2.6	89
9	$C_{13}H_{13}N_3O_2S_2$ (C, H, N)	180-182	61	317 (4.44)	400	5/6	3.6	92
					200	5/6	4.3	92
					100	6/6	-2.7	95
10a	$C_{10}H_9N_3OS$ (C, H, N)	260	100	234 (4.02), 267 (4.43)	200	6/6	2.0	93
					100	6/6	0.1	96
10b	$C_{11}H_{11}N_3OS$ (C, H, N)	258-260	100	238 (3.98), 268 (4.41)	200	6/6	3.1	105
					100	6/6	-1.9	99
10c	$C_{10}H_8ClN_3OS$ (C, H, N)	262-264	95	243 (4.02), 268 (4.43)	100	6/6	-2.9	118
					50	6/6	2.6	111
10d	$C_{10}H_8FN_3OS$ (C, H, N)	238-240	100	233 (3.93), 266 (4.38)				
10e	$C_{11}H_8N_4OS$ (C, H, N)	244-246	93	255 (4.00), 283 (4.59), 314 (3.39)	100	16/18	4.4	160
					50	18/18	3.0	132
					25	18/18	2.0	124
10f	$C_{10}H_8N_2O_3S$ (C, H, N)	272-274	100	245 (4.07), 324 (4.38)	50	6/6	2.5	142
					25	6/6	1.8	127
					12.5	6/6	1.6	112
10g	$C_{11}H_9N_3O_3S \cdot H_2O$ (C, H, N)	188-190	100	285 (4.62)	200	6/6	2.8	163
					100	6/6	1.5	150
					50	6/6	2.0	143
					25	6/6	1.1	125
11a	$C_5H_5N_3O_2S$ (C, H, N)	153-155	100	256 (4.05)	200	6/6	0.3	108
					100	6/6	0.7	102
11b	$C_5H_4ClN_3OS$ (C, H, N)	160-162	100	276 (4.00)	100	6/6	1.4	116
					50	6/6	0.2	103
11c	$C_5H_4FN_3OS$ (C, H, N)	173-175	94	298 (3.98)	400	6/6	4.3	108
					200	4/6	4.2	99
					100	6/6	2.4	104
11d	$C_5H_5N_3OS$ (C, H, N)	215-216	95	257 (4.09)	200	6/6	2.0	97
					100	5/6	0.6	96
					50	6/6	0.9	105
11e	$C_{11}H_8F_3N_3OS$ (C, H, N)	318-320	95	237 (3.89), 266 (4.24)				
11f	$C_{11}H_{11}N_3O_3S \cdot 0.5H_2O$ (C, H, N)	218-220	98	264 (4.23)	200	6/6	1.5	99
					100	6/6	1.7	106
12a	$C_5H_5N_3O_2S$ (C, H, N)	202-204	94	242 (3.93)	200	6/6	2.8	151
					100	6/6	2.2	142
					50	6/6	0.7	132
					25	6/6	0.4	108
12b	$C_4H_4ClN_3OS$ (C, H, N)	210-212	88	251 (3.95)	200	6/6	2.5	100
					100	6/6	0.5	104
12c	$C_5H_4F_3N_3OS$ (C, H, N)	162-164	80	282 (3.97)	50	12/12	2.9	161
					25	11/12	2.4	130
					12.5	12/12	1.3	110
12d	$C_5H_5N_4OS$ (C, H, N)	275-276	100	257 (4.41)	25	6/6	1.5	104
					12.5	6/6	1.1	104
12e	$C_{11}H_8N_4OS$ (C, H, N)	280-282	100	272 (4.53)	200	4/6	4.3	146
					100	6/6	3.1	140
					50	12/12	2.1	131
					25	12/12	1.7	122

Table I (*Continued*)

compd	formula (analyses)	mp, °C	yield, %	λ_{max}, nm (log ϵ)	dose, mg/kg	survival	wt diff	T/C, %
12f	$C_9H_7N_5O_3S \cdot 0.5H_2O$ (C, H, N)	281-283	100	318 (4.32)	400	5/6	-6.2	142
					200	5/6	-4.2	132
					100	6/6	-3.0	124
13	$C_{22}H_{23}N_5O_6$ (C, H, N)	>360	96	282 (4.75)	100	6/6	-2.4	88
					50	6/6	-0.4	89

[a] Ascitic fluid implanted in BDF_1 mice. Treatment started 24 h after implant. Treatment schedule: qd 1-9. For the general screening procedure and data interpretation, cf. R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, 1 (1972); Instruction Booklet 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., 1977. [b] Against B-16 melanoma: T/C 153, 167, 166, 147, and 134 at 400, 200, 100, 50, and 25 mg/kg, respectively. Treatment schedule: qd 1-9. [c] Nontoxic and inactive against B-16 melanoma at doses of 6.25-50 mg/kg.

4, **10e,g** and **12a,c** possess inhibitory activity against leukemia P-388 with T/C values of 150 or higher; moderate activity (T/C 130–149) was observed with compounds **8a,d**, **10f**, and **12e,f**. The remaining compounds were inactive against the P-388 system. Some structure–activity relationship observations of compounds of this type can be summarized as follows.

(1) Both the thiazolylureidobenzoate ester **4** and its free acid **10g** are equally active against leukemia P-388.

(2) Activity is retained, albeit at a somewhat lower level, with very slight structural modification of the thiazole ring of **4** (compare compound **4** with compounds **8a** and **8d**). Excessive structural modifications resulted in inactive compounds (cf. compounds **8b,c,e–g**).

(3) Electron-withdrawing functions, such as a nitro or a cyano group, substituted on the phenyl ring para to the ureido linkage (compounds **10e,f** and **12e,f**) in both the thiazole and thiadiazole series retained antileukemic activity. Unsubstituted or other substituents on the phenyl ring resulted with inactive compounds.

(4) Modification of the ureido linkage of **4** with either a thioureido (**9**) or a ureidosulfonyl (**11f**) linkage does not afford active compounds.

(5) Both the urethane (**12a**) and the trifluoroacetamide (**12c**) derivatives of 1,3,4-thiadiazole possess good antileukemic activity. This could well be explained by their facile in vivo conversion to the parent 2-amino-1,3,4-thiadiazole (**6**), which was reported to possess antitumor activity.[13,14] It is of interest to note that the two corresponding derivatives in the thiazole series (compounds **11a,c**) are inactive. The preceding information leads us to postulate that the thiazole and the thiadiazole derivatives may not necessarily have the same mode of action. The latter compounds possessing an "isothiosemicarbazono" structural unit—a necessary but not sufficient factor for activity—provide an interesting guideline for future structural modification work.

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 ±0.4% of the theoretical values.

Preparation of compounds **10a**,[16] **11a**,[17] **11b**,[18] **11c**,[19] **11d**,[20] **12b**,[21] and **12d**[22] has been reported.

Ethyl 4-[[(2-Thiazolylamino)carbonyl]amino]benzoate (4). A stirred suspension of 2.6 g (0.026 mol) of 2-aminothiazole in 250 mL of C_6H_6 was azeotropically dried for 1 h. To the cooled mixture was added 5 g (0.0026 mol) of dry ethyl *p*-isocyanatobenzoate in 50 mL of anhydrous C_6H_6, and the resulting mixture was refluxed for 2 h. After standing overnight, the solid product was collected by filtration, washed with C_6H_6 (2 × 20 mL) and Et_2O (3 × 30 mL), and dried to give 6.6 g of **4**, mp 198–200 °C (resolidified and remelted at 255 °C). An analytical sample was prepared by recrystallization from a mixture of EtOH and petroleum ether: mp 201–203 °C (resolidified and remelted at 258–260 °C).

Other substituted ureas were prepared from appropriate isocyanates and amines in a similar manner.

2-[[[[4-(Ethoxycarbonyl)phenyl]amino]carbonyl]amino]-3-methylthiazolium Methyl Sulfate (8b). A mixture of 1.5 g (0.005 mol) of **4**, 45 mL of $C_6H_5NO_2$, and 10 mL of xylene was heated at 100 °C in an oil bath until solution was achieved. To this was added 3 mL of $(CH_3)_2SO_4$ in 1 min and the resulting solution was heated at 110–120 °C for 15 min, with stirring. Immediately, a solid started to precipitate from the hot reaction solution. The mixture was cooled and diluted with 200 mL of Et_2O. The product was collected by filtration, washed with Et_2O (2 × 50 mL), and dried to give 2.1 g of **8b**, mp 212–214 °C. An analytical sample was obtained as long needles by recrystallization of the product from MeOH: mp 218–220 °C.

Ethyl 4-[[(2-Thiazolylamino)thioxomethyl]amino]benzoate (9). To a 0 °C solution of 10 g (0.06 mol) of ethyl *p*-aminobenzoate in 24 mL of concentrated HCl and 100 mL of HCl was rapidly added 9 g (0.08 mol) of $CSCl_2$. The mixture was stirred in an ice bath for 2 h and then at room temperature overnight. The resulting solid was collected by filtration and washed with H_2O. It was then dissolved in 150 mL of Me_2CO. To the solution was added 100 mL of H_2O and the mixture was allowed to stand overnight. The purified solid intermediate, ethyl *p*-(isothiocyanato)benzoate, was collected by filtration and dried to yield 13 g, mp 53–55 °C (lit.[15] mp 51–53 °C).

To a solution of 3 g (0.03 mol) of 2-aminothiazole in 150 mL of dry C_6H_6 was added a solution of 6 g (0.03 mol) of ethyl *p*-(isothiocyanato)benzoate in 200 mL of dry C_6H_6. The mixture was heated under reflux for 5 h. After standing overnight, the mixture was evaporated to dryness under reduced pressure. The residual solid was triturated with 50 mL of petroleum ether and filtered. The solid was washed with petroleum ether (2 × 20 mL) to give 8.3 g of crude **9**, mp 157–200 °C. Recrystallization from C_6H_6 gave 5.5 g of pure **9**, mp 180–182 °C.

4-[[(2-Thiazolylamino)carbonyl]amino]benzoic Acid (10g). A suspension of 0.4 g of **4**, 50 mL of 5% aqueous Na_2CO_3, and 10 mL of EtOH was stirred overnight. It was then heated on a steam bath for 1 h whereupon a clear solution was obtained. A small amount of solid impurity was removed by filtration and the filtrate acidified to pH 5 with AcOH. The resulting solid product was collected by filtration and washed with H_2O (2 × 10 mL) to give a quantitative yield of the acid, **10g**, mp 188–190 °C.

Ethyl 1,3,4-Thiadiazol-2-ylcarbamate (12a). To a cooled suspension of 7.6 g (0.075 mol) of 2-amino-1,3,4-thiadiazole in 150 mL of C_6H_6 was added slowly 9.8 g (0.09 mol) of ethyl chloroformate. After the addition was complete, the mixture was refluxed under N_2 for 7 h and evaporated to dryness under reduced pressure. The residue was triturated with 20 mL of petroleum ether, filtered, and washed with petroleum ether to give 12.6 g of the urethane, **12a**, mp 192–195 °C. Recrystallization from EtOH gave 7.4 g of purified **12a**, mp 202–204 °C.

2-Chloro-*N*-(1,3,4-thiadiazol-2-yl)acetamide (12b) was prepared in a similar manner from chloroacetyl chloride and 2-amino-1,3,4-thiadiazole: mp 210–212 °C (lit.[21] mp 178–180 °C).

2,2,2-Trifluoro-*N*-(1,3,4-thiadiazol-2-yl)acetamide (12c). To a stirred suspension of 8 g (0.08 mol) of 2-amino-1,3,4-thiadiazole in 150 mL of dry C_6H_6 was added, with ice cooling and

exclusion from moisture, 26 g (0.12 mol) of $(CF_3CO)_2O$. The resulting solution was stirred continuously in an ice bath for 1 h and then at room temperature overnight. The solvent and excess reagent were evaporated under reduced pressure. The residue was dissolved in 200 mL of Et_2O. A small amount of solid was removed by filtration and the filtrate was added to 500 mL of petroleum ether. The resulting solid was collected by filtration, washed with petroleum ether (2 × 20 mL), and dried to give 12.5 g of **12c**, mp 161–163 °C. An analytical sample was prepared by an additional reprecipitation from Et_2O and petroleum ether: mp 162–164 °C.

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Ergot Alkaloids. Synthesis of Nitrosourea Derivatives of Ergolines as Potential Anticancer Agents

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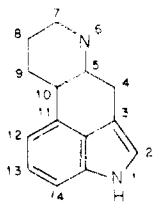
Nitrosourea derivatives of ergolines have been synthesized for the purpose of obtaining agents with both prolactin- and tumor-inhibitory activity. Two derivatives of 8-amino-6-methylergoline (3), 8-[3-(2-chloroethyl)-3-nitrosoureido]-1-nitroso-6-methylergoline (**5c**) and 8-[3-(2-chloroethyl)-3-nitrosoureido]-6-methylergoline (**5a**), have been prepared. In addition, nitroso (7) and chloroethylcarbamyl (8) derivatives of elymoclavine (6) are reported. Compounds **5a** and **5c** have activity against L1210 leukemia in mice but only moderate prolactin-inhibiting activity. The chloroethylcarbamyl derivative 8 of elymoclavine is a potent prolactin inhibitor.

A number of reports have shown that compounds containing the ergoline nucleus (1) are effective inhibitors



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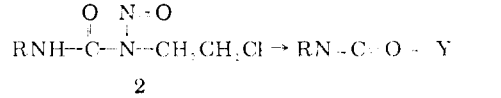
of prolactin release.[1] Previously, we reported an attempt to prepare potential irreversible prolactin inhibitors by attaching alkylating groups at the 8 position of the ergoline skeleton.[2] As an extension of this work, an alkylating nitrosourea group has been incorporated into the ergoline system in an attempt to prepare compounds which are distributed in such a way that both prolactin and tumor inhibitory activity can be achieved with the same molecule.[3]

Ergolines appear to inhibit prolactin release from the anterior pituitary gland by interacting with the prolactin-inhibiting factor (PIF) receptor.[4] As an example, an ergolinylnitrosourea could possibly target a tumor located in the pituitary gland. Such a compound could be potentially useful in the treatment of Forbes-Albright Syndrome, a condition which is the result of a pituitary tumor in which excessive amounts of prolactin are produced leading to persistent lactation.

The *N*-(2-chloroethyl)-*N*-nitrosoureas **2** decompose (eq



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1) to yield an isocyanate and a variety of other reactive species (Y).[5] The interaction of one or more of these

