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Irreversible Enzyme Inhibitors. 197.†,‡ Water-Soluble Reversible Inhibitors of Dihydrofolate Reductase with Potent Antitumor Activity Derived from 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine

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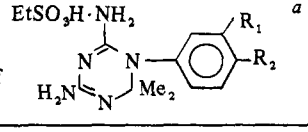
A series of 39 derivatives of the title compound was prepared with the object of optimizing reversible inhibition of dihydrofolate reductase and cell membrane transport while achieving sufficient water solubility for effective intravenous administration. Four compounds which met all the criteria were selected for antitumor testing *in vivo*. Of these, three showed excellent activity against Walker 256 ascites, intramuscular Walker 256, and Dunning leukemia ascites in the rat. Overall, the most active compound *in vivo* was 1-[3-chloro-4-(*m*-dimethylcarbamoylbenzyloxy)]phenyl-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine ethanesulfonate (**36**).

An earlier report from this laboratory demonstrated that several potent reversible as well as irreversible inhibitors of dihydrofolate reductase are highly effective in promoting cures# of the Walker carcinosarcoma 256 and Dunning leukemia tumor systems in the rat when given by intraperitoneal injection.[2] From a clinical standpoint it would be desirable to administer such a compound by intravenous infusion, so that administration could be discontinued at once should toxicity develop. Unfortunately, the inhibitors which have shown the greatest effectiveness *in vivo*[2] lack sufficient aqueous solubility. Solubilities of representative compounds **1**–**4** are given in Table I. Even the most soluble of these (**3**), a potent agent against Walker 256 ascites when administered intraperitoneally, was not soluble enough to achieve a toxic dose intravenously.[3] It was proposed that insufficient solubility in body fluids hindered the distribution of **3**, thus accounting for its substantially lower activity against intramuscular Walker 256 when given intraperitoneally.[3]

Consequently, a study was undertaken to prepare a series of reversible inhibitors of dihydrofolate reductase with the object of achieving a solubility in water of at least 25 mg/ml (50 mg/ml being preferred), while at the same time maintaining sufficient binding to the enzyme and cell membrane transport. The combination of the last two factors is reflected in the ED_{50} against L1210 leukemic cell culture.[4,5] The solubilization study was limited exclusively to reversible inhibitors on the basis of (a) their demonstrated equal or superior effectiveness *in vivo* compared to the corresponding irreversible inhibitors (sulfonyl fluorides),[2] (b) the apparent metabolic degradation of the sulfonyl fluoride group,[6,7] and (c) the desolubilizing effect of the fluorosulfonyl moiety, as seen by comparing **3** and **4** (Table I).

Because inhibitors **2** and **3**, which contain an amide bridge, were considerably more soluble than **1**, the investigation of other amide-containing side chains appeared promising. Certain obvious structural modifications expected to increase water solubility, such as the introduction of

Table I. Solubility of  [a]

No.	R_1	R_2	Solubility, mg/ml (H_2O)
1	Cl	$O(CH_2)_3OC_6H_5$	<0.5
2	Cl	$OCH_2CONHC_6H_5$	5
3	H	$(CH_2)_2CONHC_6H_4$-*m*-CH_3	10[b]
4	H	$(CH_2)_2CONHC_6H_3$-3-CH_3-4-SO_2F	1.1[c]

[a] See ref 2 for biological data for these compounds. [b] Data from ref 3. [c] F. R. White, Drug Research and Development, NCI, unpublished data.

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‡For the previous paper in this series see Baker and Ashton.[1]

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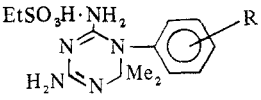
#The term "cure" is used here throughout to mean an *apparent* cure; all surviving treated animals were sacrificed at the end of the fixed evaluation period.

Table II. Inhibition of Dihydrofolate Reductase and L1210 Cell Culture by 

No.	R[a]	I_{50},[b] μM	ED_{50},[c] μM	ED_{50}/I_{50}	Solubility,[d] mg/ml (H_2O)
5	3-Cl-4-$OCH_2CON(Me)C_6H_5$	0.013	94	7000	60
6	3-Cl-4-OCH_2CONMe_2	0.069	20	300	⩾100
7	3-Cl-4-OCH_2CONEt_2	0.023	0.023	1	25
8	3-Cl-4-OCH_2CO-*c*-$N(CH_2CH_2)_2O$	0.014	1.1	100	90
9	3-Cl-4-OCH_2CO-*c*-$N(CH_2)_4$	0.051	1.2	25	⩾100
10	3-Cl-4-OCH_2CO-*c*-$N(CH_2)_5$	0.034	0.09	3	85
11	4-$OCH_2CONHC_6H_5$	0.013	8.6	700	15
12	4-$OCH_2CON(Me)C_6H_5$	0.69	>100	>100	⩾100
13	4-OCH_2CONMe_2	0.55	>100	>200	⩾100
14	4-OCH_2CONEt_2	0.19	>100	>500	⩾100
15	4-OCH_2CO-*c*-$N(CH_2CH_2)_2O$	0.051	0.8	20	⩾100
16	4-OCH_2CO-*c*-$N(CH_2)_4$	0.22	18	80	50
17	4-OCH_2CO-*c*-$N(CH_2)_5$	0.075	4	50	⩾100
18	3-$OCH_2CONHC_6H_5$	0.14	88	600	1
19	3-$OCH_2CON(Me)C_6H_5$	0.21	90	400	60
20	3-OCH_2CONMe_2	3.6	180	50	30
21	3-OCH_2CO-*c*-$N(CH_2CH_2)_2O$	14	27	2	90
22	4-$CH_2CON(Me)C_6H_5$	0.10	4	40	⩾100
23	4-CH_2CONMe_2	0.23	9	40	⩾100
24	4-CH_2CONEt_2	0.17	15	100	⩾100
25	4-CH_2CO-*c*-$N(CH_2CH_2)_2O$	0.075	40	500	⩾100
26	4-$CH_2CON(Me)CH_2C_6H_5$	0.050	10	200	35
27	4-CH_2CN	0.12	7	60	⩾100
28	4-$(CH_2)_2CON(Me)C_6H_5$	0.027	65	3000	⩾100
29	4-$(CH_2)_2CONMe_2$	0.089	100	1000	⩾100
30	4-$(CH_2)_2CONEt_2$	0.052	11	200	70
31	4-$(CH_2)_2CONPr_2$	0.045	11	200	2.5
32	4-$(CH_2)_2CO$-*c*-$(CH_2CH_2)_2O$	0.048	67	1000	⩾100
33	4-$(CH_2)_2CON(Me)CH_2C_6H_5$	0.049	0.5	10	10
34	3-Cl-4-OCH_2-C_6H_4-*m*-$CONHC_6H_5$	0.010	0.01	1	15
35	3-Cl-4-OCH_2-C_6H_4-*m*-$CON(Me)C_6H_5$	0.0075	0.007	1	<1
36[e]	3-Cl-4-OCH_2-C_6H_4-*m*-$CONMe_2$	0.019	0.9	50	80
37	3-Cl-4-OCH_2-C_6H_4-*m*-$CONEt_2$	0.0072	0.0006	0.1	30
38	3-Cl-4-OCH_2-C_6H_4-*m*-CO-*c*-$N(CH_2CH_2)_2O$	0.014	0.004	0.3	45
39	3-Cl-4-OCH_2-C_6H_4-*m*-CO-*c*-$N(CH_2)_4$	0.014	0.003	0.2	5
40	3-Cl-4-OCH_2-C_6H_4-*m*-CO-*c*-$N(CH_2)_5$	0.0095	0.004	0.4	10
41	3-Cl-4-$OCH_2C_6H_5$	0.030	0.03	1	<0.5
42	3-Cl-4-OCH_2-C_6H_4-*p*-SO_2NMe_2	0.033	0.013	0.4	<0.5
43	3-Cl-4-OCH_2-C_6H_4-*m*-CN	0.031	6×10^{-6}	2×10^{-4}	0.5

[a] Numbered from triazinyl junction = 1: *c*-$N(CH_2CH_2)_2O$, morpholino; c-$N(CH_2)_4$, pyrrolidino; c-$N(CH_2)_5$, piperidino. [b] I_{50} = concentration for 50% inhibition of Walker 256 rat tumor dihydrofolate reductase when assayed with 6 μM dihydrofolate, 30 μM NADPH, and 0.15 *M* KCl in 0.05 *M* Tris buffer (pH 7.4) as previously described.[4] [c] ED_{50} = concentration for 50% inhibition of L1210 cell culture. [d] Approximate values. [e] Data taken in part from ref 3.

charged substituents, were avoided because sufficient lipophilicity must be maintained for membrane transport. It was theorized that introduction of semipolar groupings could increase solubility without necessary loss in transport. Another factor to consider is that transport of inhibitors of this type is very sensitive to steric and conformational effects; compounds of comparable polarity can differ dramatically in their relative abilities to penetrate the cell membrane.

Among the structural modifications tested were variation of the acyl and amino moieties of the amide side chain, presence or absence of the 3-chloro substituent, and placement of the amide moiety meta to the triazine ring. The biological results of this study are discussed below.

In Vitro **Evaluation.** Thirty-nine candidate reversible inhibitors of dihydrofolate reductase were prepared as ethanesulfonate salts and evaluated for inhibition of the Walker 256 enzyme, inhibition of growth of L1210 mouse leukemia cell culture, and solubility in H_2O (Table II).

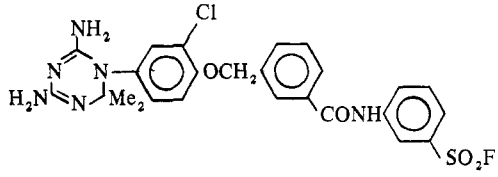
Compounds of the first series **5–10** were analogs of **2** in which substituents on the amide nitrogen were varied. All of these compounds were good reversible inhibitors. The N-methylated analog **5** of **2** had good solubility but showed very poor activity against the L1210 cell culture. Of the dialkylamides tested, **7** and **10** had ED_{50} values substantially better than that of **2** (0.6 μM);[2] **8** and **9** were comparable to **2**. The diethylamide **7**, which had the lowest ED_{50} in this series, was also the least soluble.

The next series **11–17** was similar to the first, except that the 3-chloro substituent was absent. This modification led to increased solubility but also to poorer cell culture potency and, in most cases, somewhat poorer enzyme inhibition. The highly soluble morpholino derivative **15** was surprising, in that its ED_{50} was comparable to that of **2** and the corresponding 3-chloro analog **8**.

Inhibitors **18–21** had the oxyacetamide side chain meta to the triazine ring. Compounds **18** and **19** were only fair reversible inhibitors; **20** and **21** were exceptionally poor. Each of these compounds demonstrated only weak activity against the L1210 cell culture. Of some interest is the extraordinarily low solubility of **18**.

Compounds **22–26**, containing a 4-acetamide grouping, were fair to good reversible inhibitors and in general quite soluble. However, none of these inhibitors had a sufficiently low ED_{50}. The related acetonitrile derivative **27** had properties very similar to those of the dimethylcarboxamide **23**.

Several triazines containing a propionamide side chain (**28–33**) were also prepared. These showed good reversible binding, and several had excellent solubility. With the exception of **33**, these compounds had poor ED_{50}'s. The ineffective transport of **28** could be attributed to the rigid, planar bulk of the *N*-methylanilide moiety. It was hypothesized that the *N*-benzyl-*N*-methylamide **33** might show better transport as a result of its greater conformational flexibility. This was borne out by the fact that the ED_{50}/I_{50} ratio of **33** was 300-fold better than that of **28**. (Surprisingly, a similar modification in the acetamide series led to no improvement; *cf.* **26** and **22**.) Unfortunately, the solubility of **33** was too low to be useful.



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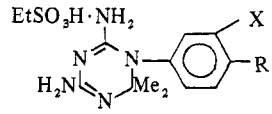
The exceptionally potent *in vitro* activity of **44** (ED_{50} = 0.0007 μM, ED_{50}/I_{50} = 0.01)[8] raised the question of whether a reversible analog of this compound could be sufficiently soluble while retaining good transport. A series of related amides **34–40** was thus prepared. The unsubstituted anilide **34**, besides being an excellent reversible inhibitor, was found to be quite potent against the L1210 cell culture. Although its solubility of 15 mg/ml was not high enough, the prospects for developing more soluble analogs were encouraging. N-Methylation, which in several other instances increased solubility, in this case (**35**) resulted in a dramatic decrease in solubility; transport was unchanged. The dimethylamide **36**, as hoped, was very soluble, and although transport was impaired, the ED_{50} was still comparable to that of **2**. The diethylamide **37** was near the minimum acceptable solubility, but it was 1000-fold more inhibitory against the L1210 cell culture than **36**. The morpholine amide **38** also demonstrated excellent cell culture potency and good solubility. Whereas the pyrrolidine and piperidine amides **39** and **40** were comparable in activity to **38**, they were poorly soluble.

The unsubstituted 3-chloro-4-benzyloxy compound **41** was evaluated for comparison purposes. As expected, it was very insoluble and was actually less effective against the L1210 cell culture than several of the amide derivatives. It is of interest that the corresponding 4-benzyloxy compound without the 3-chloro substituent is reported to have antimalarial, anthelmintic, antibacterial, and antiviral activity.[9] Substitution of a dimethylsulfonamide group in the para position of **41** to give **42** had little effect. The excellent

Table III. Activity of Selected Inhibitors against Walker Carcinosarcoma 256



8, X = Cl; R = OCH_2CO-c-$N(CH_2CH_2)_2O$ (NSC-140380)
15, X = H; R = OCH_2CO-c-$N(CH_2CH_2)_2O$ (NSC-140020)
36, X = Cl; R = OCH_2-C_6H_4-*m*-$CONMe_2$ (NSC-139105)
37, X = Cl; R = OCH_2-C_6H_4-*m*-$CONEt_2$ (NSC-143010)

Compd	Control group	Initial cell level[a]	MST[b] of controls	Day of evaluation	Antitumor activity: Dose, mg/kg/day 0.1	0.2	0.4	0.8	1.56	3.13	6.25	12.5	25	50	100	200
A. Walker 256 Ascites, Intraperitoneal Administration[c,d]																
8	A	10^6	9.0	40								4C	(111)	(122)	(100)	5T
	B	10^6	7.0	40				6C	6C	6C	6C	6C				
	C	10^5	7.0	45	(128)	1C	1C	3C								
15	A	10^6	9.0	40								5C	6C	4C	2C,1T	6T
	B	10^6	7.0	40				(128)	(128)	(128)	2C	4C				
36[e]	D	10^5	8.0	30								9C/10	1T/10	2T/10	10T/10	
	B	10^6	7.0	40				6C	6C	6C	5C					
	C	10^5	7.0	45	6C	6C	4C	5C								
37	E	10^5	9.0	45						8C/8	6C/8	2C/8	(88)	4T/8	8T/8	
	F	10^5	9.5	45		4C	4C	6C	6C	6C	6C	3C	2C	1T		
	G	10^5	9.0	45	3C	4C	5C	5C	6C	5C						
B. Walker 256 Ascites, Intravenous Administration[c,f]																
8	H	10^5	7.0	45						(150)	2C	(157)	(200)	(178)		
	I	10^5	9.5	45			(94)	(94)	(94)	(126)[g]						
36	H	10^5	7.0	45						(235)	3T	6T	6T			
	J	10^5	8.0	45						3C	2C	2T	5T	6T		
	I	10^5	9.5	45			(105)	(126)	4C	(163)						
37	K	10^5	9.0	45			(116)	(127)	(200)	(200)	3C	6T	6T			
C. Intramuscular Walker 256, Intraperitoneal Administration[h,i]																
8	L	10^6		7							27	21	4	4		
36	L	10^6		7						11	0	1	0			
	M	10^6		7			103	67	29	6	0					
37	N	10^6		7							5	1	1	2	(6T)	
	M	10^6		7			109	70	25	8	0	0				

[a] Approximate number of cells in tumor inoculum. [b] Median survival time in days. [c] The antitumor activity is the number of "cures" (C), *i.e.*, survivors at day of evaluation, or number of toxic deaths (T) out of a test group of six animals, unless otherwise indicated. Where no cures or toxic fatalities occurred, the ratio of the MST[b] of treated animals to that of controls, expressed as per cent, is given in parentheses. [d] On days 1–9, single ip doses were administered. [e] Data taken in part from ref 3. [f] On days 1–9, single iv doses were administered. [g] One possible toxic death also reported. [h] The antitumor activity is the ratio of mean tumor weight in treated animals to that of controls, expressed as per cent. The number of toxic deaths (T), if any, out of a test group of six animals is given in parentheses. [i] On days 3–6, single ip doses were administered.

Table IV. Physical Constants of O_2N-C$_6$H$_4$-R

No.	R[a]	Method	Yield,[b] %	Mp, °C	Formula[c]
45	3-Cl-4-$OCH_2CON(Me)C_6H_5$	A	64[d]	149–150	$C_{15}H_{13}ClN_2O_4$
46	3-Cl-4-OCH_2CONMe_2	B	58[e]	113–114	$C_{10}H_{11}ClN_2O_4$
47	3-Cl-4-OCH_2CONEt_2	B	52[f]	79–81	$C_{12}H_{15}ClN_2O_4$
48	3-Cl-4-OCH_2CO-c-$N(CH_2CH_2)_2O$	B	80[d]	179–181	$C_{12}H_{13}ClN_2O_5$
49	3-Cl-4-OCH_2CO-c-$N(CH_2)_4$	B	65[e]	153–154	$C_{12}H_{13}ClN_2O_4$
50	3-Cl-4-OCH_2CO-c-$N(CH_2)_5$	B	67[e]	98–99	$C_{13}H_{15}ClN_2O_4$
51	4-$OCH_2CONHC_6H_5$	A	79[d]	172–173[g]	
52	4-$OCH_2CON(Me)C_6H_5$	A	68[h]	118	$C_{15}H_{14}N_2O_4$
53	4-OCH_2CONMe_2	B	64[d]	126–127	$C_{10}H_{12}N_2O_4$
54	4-OCH_2CONEt_2	B	80[e]	64–65	$C_{12}H_{16}N_2O_4$
55	4-OCH_2CO-c-$N(CH_2CH_2)_2O$	B	81[d]	138–139	$C_{12}H_{14}N_2O_5$
56	4-OCH_2CO-c-$N(CH_2)_4$	B	64[e]	136–137	$C_{12}H_{14}N_2O_4$
57	4-OCH_2CO-c-$N(CH_2)_5$	B	77[e]	71–72	$C_{13}H_{16}N_2O_4$
58	3-$OCH_2CONHC_6H_5$	A	61[i]	127–128[j]	
59	3-$OCH_2CON(Me)C_6H_5$	A	64[d]	149–150	$C_{15}H_{14}N_2O_4$
60	3-OCH_2CONMe_2	B	74[e]	100–101	$C_{10}H_{12}N_2O_4$
61	3-OCH_2CO-c-$N(CH_2CH_2)_2O$	B	75[i]	106–107	$C_{12}H_{14}N_2O_5$
62	4-$CH_2CON(Me)C_6H_5$	A	46[k]	92–93	$C_{15}H_{14}N_2O_3$
63	4-CH_2CONMe_2	B	39[e]	90–91[l]	
64	4-CH_2CONEt_2	B	87[m]	Oil	
65	4-CH_2CO-c-$N(CH_2CH_2)_2O$	B	56[e]	120–121	$C_{12}H_{14}N_2O_4$
66	4-$CH_2CON(Me)CH_2C_6H_5$	B	61[e]	105–106	$C_{16}H_{16}N_2O_3$
67	4-CH=$CHCON(Me)C_6H_5$	A	68[d]	179–180	$C_{16}H_{14}N_2O_3$
68	4-CH=$CHCONMe_2$	B	62[e]	183–184	$C_{11}H_{12}N_2O_3$
69	4-CH=$CHCONEt_2$	B	37[d,n]	154–155	$C_{13}H_{16}N_2O_3$
70	4-CH=$CHCONPr_2$	B	41[e]	78	$C_{15}H_{20}N_2O_3$
71	4-CH=CHCO-c-$N(CH_2CH_2)_2O$	B	79[d]	198–200	$C_{13}H_{14}N_2O_4$
72	4-CH=$CHCON(Me)CH_2C_6H_5$	B	66[e]	133–135	$C_{17}H_{16}N_2O_3$
73	3-Cl-4-OCH_2-C_6H_4-*m*-$CONHC_6H_5$	A	49[i]	161–162[o]	$C_{20}H_{15}ClN_2O_4$
74	3-Cl-4-OCH_2-C_6H_4-*m*-$CON(Me)C_6H_5$	A	47[e]	125–126	$C_{21}H_{17}ClN_2O_4$
75	3-Cl-4-OCH_2-C_6H_4-*m*-$CONMe_2$	B[p]	97[q]	117–118[r]	$C_{16}H_{15}ClN_2O_4$
76	3-Cl-4-OCH_2-C_6H_4-*m*-$CONEt_2$	B	78[e]	135–137	$C_{18}H_{19}ClN_2O_4$
77	3-Cl-4-OCH_2-C_6H_4-*m*-CO-c-$N(CH_2CH_2)_2O$	B	63[e]	139–141	$C_{18}H_{17}ClN_2O_5$
78	3-Cl-4-OCH_2-C_6H_4-*m*-CO-c-$N(CH_2)_4$	B	57[e]	113–114	$C_{18}H_{17}ClN_2O_4$
79	3-Cl-4-OCH_2-C_6H_4-*m*-CO-c-$N(CH_2)_5$	B	79[e]	98–99	$C_{19}H_{19}ClN_2O_4$
80	3-Cl-4-$OCH_2C_6H_5$	C[s]	72[d]	121–122	$C_{13}H_{10}ClNO_3$
81	3-Cl-4-OCH_2-C_6H_4-*p*-SO_2NMe_2	C	16[d,e,t]	167–169	$C_{15}H_{15}ClN_2O_5S$
82	3-Cl-4-OCH_2-C_6H_4-*m*-CN	C[u]	71[d]	159	$C_{14}H_9ClN_2O_3$

[a] Numbered from NO_2 = 1. [b] Yield of analytically pure material except where indicated. [c] *Anal.* C, H, N. [d] Recrystallized from 2-methoxyethanol-H_2O. [e] Recrystallized from EtOAc–petroleum ether (bp 65–110°). [f] Recrystallized from MeOH-H_2O. [g] Lit. [T. H. Minton and H. Stephen, *J. Chem. Soc.*, **121**, 1591 (1922)] mp 170°. [h] Recrystallized from EtOH-H_2O. [i] Recrystallized from EtOH. [j] Lit.[g] mp 125°. [k] Recrystallized from C_6H_6–petroleum ether (bp 65–110°). [l] Lit. [K. Kindler, *Justus Liebigs Ann. Chem.*, **431**, 187 (1923)] mp 91°. [m] Yield of crude product. [n] Recrystallized from MeOH. [o] Analytical sample had mp 162–163°. [p] In formation of acid chloride, MeCN rather than C_6H_6 used as cosolvent. [q] Yield of unrecrystallized product, homogenous on tlc. [r] Analytical sample, recrystallized from EtOH-H_2O, had mp 119° [s] Prepared from commercial benzyl bromide. [t] Overall yield for bromination and alkylation. [u] Prepared from commercial α-bromo-*m*-tolunitrile.

solubility of **27** suggested that the introduction of cyano groups as solubilizing substituents should be investigated further; with this in mind, the *m*-cyano derivative **43** of **41** was synthesized. Although the solubility of **43** was disappointing, its potency against the L1210 cell culture ($ED_{50} = 6 \times 10^{-6}$ *μM*, confirmed) is noteworthy.

Arbitrary criteria were set in order to evaluate the inhibitors. According to these guidelines, to be worthy of *in vivo* testing the compound should have $I_{50} < 0.1$ *μM*, $ED_{50} < 2$ *μM*, and solubility ≥50 mg/ml of H_2O. Of the 39 triazines studied, 28 met the I_{50} requirement, 17 met the ED_{50} requirement, and 23 met the solubility criterion. Five inhibitors (**8–10**, **15**, **36**) met all three conditions. Four representative compounds were ultimately chosen for *in vivo* investigation. Inhibitors **8** and **15** provided a comparison for assessing the effect of the 3-chloro substituent. The other compounds submitted for *in vivo* testing were **36** and its analog **37**. Although the solubility of **37** was somewhat less than desired, its selection was warranted on the basis of its outstanding *in vitro* activity.

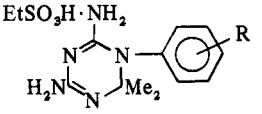
In Vivo **Evaluation.** The four selected triazines (**8**, **15**, **36**, **37**) were administered intraperitoneally on days 1-9 to rats bearing the Walker 256 ascites tumor (Table IIIA). All of the compounds were effective in promoting cures over a range of dose levels, the order of activity being **36** ≥ **37** > **8** ≫ **15**. A comparison of **8** and **15** indicates that whereas these two inhibitors were virtually indistinguishable *in vitro*, the 3-chloro substituent of **8** is markedly activity-enhancing *in vivo*. It is also of interest that **36** and **37** are active over approximately the same range of dosages, even though **37** was 1000-fold more potent than **36** in the L1210 cell culture assay.

In delayed treatment studies on Walker 256 ascites, compounds **36** and **37** given intraperitoneally on days 5–13 produced, respectively, 4/6–6/6 and 3/6–6/6 45-day survivors over a range of 0.8–12.5 mg/kg/day.**

As could be predicted, the inhibitors were less effective when administered intravenously against Walker 256 ascites than when given by the localized intraperitoneal route (Table IIIB). Still, compounds **8**, **36**, and **37** were capable of producing some cures under these conditions, although the curative dose range was narrow and, at least in the case of **36** and **37**, bordered on being toxic.

The ability of the compounds to reach a tumor target in

**Median survival time of untreated rats was 9.0 days.

Table V. Physical Constants of 

No.	R[a]	Method	Yield,[b] %	Mp, °C dec	Formula[c]
5	3-Cl-4-$OCH_2CON(Me)C_6H_5$	D[d]	27[e]	191–194	$C_{22}H_{29}ClN_6O_5S$
6	3-Cl-4-OCH_2CONMe_2	D[f]	30[e]	204–205	$C_{17}H_{27}ClN_6O_5S$
7	3-Cl-4-OCH_2CONEt_2	D[f]	38[e]	216–217	$C_{19}H_{31}ClN_6O_5S$
8	3-Cl-4-OCH_2CO-c-$N(CH_2CH_2)_2O$	D[d,g,h]	40[e]	199–200	$C_{19}H_{29}ClN_6O_6S$
9	3-Cl-4-OCH_2CO-c-$N(CH_2)_4$	D[d,g]	61[e]	167–168	$C_{19}H_{29}ClN_6O_5S \cdot 0.5C_3H_6O$
10	3-Cl-4-OCH_2CO-c-$N(CH_2)_5$	D[d]	45[i]	201–202	$C_{20}H_{31}ClN_6O_5S$
11	4-$OCH_2CONHC_6H_5$	D[d]	59[e]	222–223	$C_{21}H_{28}N_6O_5S$
12	4-$OCH_2CON(Me)C_6H_5$	D[d]	52[j]	198–199	$C_{22}H_{30}N_6O_5S$
13	4-OCH_2CONMe_2	D[d]	40[e]	205–206	$C_{17}H_{28}N_6O_5S$
14	4-OCH_2CONEt_2	D[f]	45[j]	199–200	$C_{19}H_{32}N_6O_5S$
15	4-OCH_2CO-c-$N(CH_2CH_2)_2O$	D[d]	50[e]	219–220	$C_{19}H_{30}N_6O_6S$
16	4-OCH_2CO-c-$N(CH_2)_4$	D[d,g]	69[e]	219–221	$C_{19}H_{30}N_6O_5S$
17	4-OCH_2CO-c-$N(CH_2)_5$	D[f]	57[e]	205–206	$C_{20}H_{32}N_6O_5S$
18	3-$OCH_2CONHC_6H_5$	E[d]	46[e]	212–213	$C_{21}H_{28}N_6O_5S$
19	3-$OCH_2CON(Me)C_6H_5$	D[d]	32[e]	216–217	$C_{22}H_{30}N_6O_5S$
20	3-OCH_2CONMe_2	D[d]	22[e]	205–206	$C_{17}H_{28}N_6O_5S \cdot 0.5H_2O$
21	3-OCH_2CO-c-$N(CH_2CH_2)_2O$	D[d]	18[e]	193–194	$C_{19}H_{30}N_6O_6S \cdot H_2O$
22	4-$CH_2CON(Me)C_6H_5$	D[d]	44[j]	199–200	$C_{22}H_{30}N_6O_4S \cdot 0.5H_2O$
23	4-CH_2CONMe_2	D[d]	57[j]	200–201	$C_{17}H_{28}N_6O_4S$
24	4-CH_2CONEt_2	D[f,h]	54[i]	201–202	$C_{19}H_{32}N_6O_4S$
25	4-CH_2CO-c-$N(CH_2CH_2)_2O$	D[d]	64[e]	206–207	$C_{19}H_{30}N_6O_5S \cdot H_2O$
26	4-$CH_2CON(Me)CH_2C_6H_5$	D[d]	69[e]	203–205	$C_{23}H_{32}N_6O_4S$
27	4-CH_2CN	F[f,k]	63[e]	207	$C_{15}H_{22}N_6O_3S$
28	4-$(CH_2)_2CON(Me)C_6H_5$	D[d,l]	34[i]	198	$C_{23}H_{32}N_6O_4S$
29	4-$(CH_2)_2CONMe_2$	D[d,l]	44[i]	199–200	$C_{18}H_{30}N_6O_4S$
30	4-$(CH_2)_2CONEt_2$	D[d,h,l]	28[i]	187–188	$C_{20}H_{34}N_6O_4S$
31	4-$(CH_2)_2CONPr_2$	D[h,l]	45[i]	200–201	$C_{22}H_{38}N_6O_4S$
32	4-$(CH_2)_2CO$-c-$N(CH_2CH_2)_2O$	D[d,l]	39[j]	187–189	$C_{20}H_{32}N_6O_5S \cdot H_2O$
33	4-$(CH_2)_2CON(Me)CH_2C_6H_5$	D[l]	50[j]	206–207	$C_{24}H_{34}N_6O_4S$
34	3-Cl-4-OCH_2-C_6H_4-*m*-$CONHC_6H_5$	D[d,m]	9[e]	>150	$C_{27}H_{31}ClN_6O_5S \cdot H_2O$
35	3-Cl-4-OCH_2-C_6H_4-*m*-$CON(Me)C_6H_5$	D[n]	30[e]	219–221	$C_{28}H_{33}ClN_6O_5S$
36	3-Cl-4-OCH_2-C_6H_4-*m*-$CONMe_2$	D[d]	71[e]	215–216[o]	$C_{23}H_{31}ClN_6O_5S$
37	3-Cl-4-OCH_2-C_6H_4-*m*-$CONEt_2$	D[d]	63[e]	199–200[p]	$C_{25}H_{35}ClN_6O_5S$
38	3-Cl-4-OCH_2-C_6H_4-*m*-CO-c-$N(CH_2CH_2)_2O$	D[n]	72[e]	201–202	$C_{25}H_{33}ClN_6O_6S$
39	3-Cl-4-OCH_2-C_6H_4-*m*-CO-c-$N(CH_2)_4$	D[n]	46[e]	211–212	$C_{25}H_{33}ClN_6O_5S$
40	3-Cl-4-OCH_2-C_6H_4-*m*-CO-c-$N(CH_2)_5$	D[n]	53[e]	217–219	$C_{26}H_{35}ClN_6O_5S$
41	3-Cl-4-$OCH_2C_6H_5$	D[d,g]	39[e]	219–220	$C_{20}H_{26}ClN_6O_4S$
42	3-Cl-4-OCH_2-C_6H_4-*p*-SO_2NMe_2	D[d]	61[e]	213–214	$C_{22}H_{31}ClN_6O_6S_2$
43	3-Cl-4-OCH_2-C_6H_4-*m*-CN	D[n]	28[e]	206–207	$C_{21}H_{25}ClN_6O_4S$

[a] Numbered from triazinyl junction = 1. [b] Yield of analytically pure material except where indicated. [c] *Anal.* C, H, N. [d] 2-Methoxyethanol used as hydrogenation solvent. [e] Recrystallized from *i*-PrOH–H_2O. [f] MeOH used as hydrogenation solvent. [g] EtOH added to condensation reaction mixture as cosolvent. [h] Et_2O added after completion of reaction to aid in precipitation of product. [i] Recrystallized from *i*-PrOH–petroleum ether (bp 65–110°). [j] Recrystallized from *i*-PrOH. [k] MeOH added to condensation reaction mixture as cosolvent. [l] *p*-Nitrocinnamamides reduced in presence of $EtSO_3H$. [m] DMF was added to the condensation reaction mixture as cosolvent and removed by evaporation *in vacuo* when reaction was complete; after addition of fresh Me_2CO, the mixture was stirred at reflux until product precipitated. [n] THF used as hydrogenation solvent. [o] Analytical sample had mp 214–215° dec. [p] Analytical sample had mp 199–201° dec.

sufficient concentration *via* the blood stream was also an important factor when tested by the intraperitoneal route against intramuscular Walker 256 in the leg of the rat (Table IIIC). Inhibitors **8**, **36**, and **37** were effective in producing complete or nearly complete inhibition of tumor growth, with **36** and **37** showing the greatest activity.

The pattern of activity against Dunning leukemia ascites in the rat closely paralleled that against Walker 256 ascites when the inhibitors were administered intraperitoneally. The order of activity was **36** > **37** > **8** ⩾ **15**, with **36** producing a substantial fraction of 40- or 45-day survivors at doses of 0.2–6.25 mg/kg/day.††

In contrast to the high order of activity shown against the Walker 256 and Dunning leukemia tumor systems, the inhibitors were inactive against L1210 mouse leukemia. Compound **36** also proved ineffective when screened against a variety of other mouse tumors, including P388 leukemia, Lewis lung carcinoma, and B16 melanocarcinoma. The reasons for the lack of activity in these mouse tumor systems are not understood.

It is presumed that "nonclassical" antifolates such as **36** enter cells by passive diffusion rather than active transport. The exceptional activity of **36** and related compounds against Walker carcinosarcoma 256 gives support to this assumption. Walker 256 apparently has a poor active transport system for folic acid and is thus resistant to the "classical" folate antagonist, amethopterin, which is dependent on active transport for cellular uptake.[10,11] The poor response to amethopterin of certain leukemias in mice[12,13] and in man[14,15] has also been correlated, at least in part, with impaired transport of the drug. The potential therapeutic value of a potent nonclassical antifolate in such cases has been discussed elsewhere.[3]

††Administered on days 1–9; median survival time of controls was 6.0 days.

Experimental Section

All analytical samples gave combustion values for C, H, and N within 0.4% of theoretical values. These microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Each compound

had ir and uv spectra in agreement with its assigned structure; purity was confirmed by tlc using Brinkmann silica gel GF. Melting points are uncorrected and were taken in capillary tubes on a Mel-Temp block.

α-(2-Chloro-4-nitrophenoxy)-*m*-toluic acid, a precursor for the synthesis of inhibitors **34–40,** has already been described.[8] The base-catalyzed ester hydrolysis was modified by using aqueous DMSO in place of aqueous MeOH. In this medium the reaction was essentially complete in 1 hr at 80–85°, with yields of up to 79% being obtained.

***N*-Arylamides (Method A).** Conversion of the nitro-substituted acid to the acid chloride and condensation with the arylamine in toluene at reflux was performed as previously described.[8] Compounds prepared in this manner are listed in Table IV.

***N,N*-Dialkylamides (Method B). *N,N*-Diethyl-*p*-nitrophenoxyacetamide (54).** A mixture of 983 mg (5.0 mmol) of *p*-nitrophenoxyacetic acid, 4 ml of $SOCl_2$, and 8 ml of C_6H_6 was stirred under reflux for 4.5 hr, then cooled, and spin evaporated *in vacuo*. The residual oil was dissolved in 8 ml of CH_2Cl_2 and cooled in an ice bath. The cold solution of acid chloride was added slowly with stirring to a chilled solution of 802 mg (11 mmol) of Et_2NH in 10 ml of CH_2Cl_2. Stirring was continued under protection from moisture for 10 min, while maintaining the temperature below 10°. Next, the mixture was diluted with 35 ml of CH_2Cl_2 and shaken with 50 ml of H_2O. The organic layer was washed with 1% HCl (1 × 50 ml) and 5% Na_2CO_3 (3 × 50 ml), then dried ($MgSO_4$), and evaporated to dryness. Upon trituration with petroleum ether (bp 30–60°) and scratching, the residual oil solidified. Recrystallization from EtOAc–petroleum ether (bp 65–110°) afforded 1.01 g (80%) of very pale yellow crystals, mp 64–65° (tlc in 1:1 EtOAc–petroleum ether). *Anal.* ($C_{12}H_{16}N_2O_4$) C, H, N. For additional compounds prepared by this method, see Table IV.

2-Chloro-4-nitrophenyl Benzyl Ethers (80–82) (Method C). The α-bromination of substituted toluenes and subsequent reaction with 2-chloro-4-nitrophenol were carried out in the usual manner.[8] Physical properties of the ethers are given in Table IV.

The reaction of *N,N*-dimethyl-*p*-toluenesulfonamide with NBS was complicated by side reactions, resulting in a low overall yield of **81.** A possible alternative route to compound **76,** involving α-bromination of *N,N*-diethyl-*m*-toluamide followed by reaction with 2-chloro-4-nitrophenol, was unsuccessful on account of the mixture of products formed when the diethyltoluamide was treated with NBS.

4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine Ethanesulfonates (5–43) (Methods D–F). The nitro intermediates **45–82** were hydrogenated over PtO_2 (Method D), Raney Ni (Method E), or 5% Pd/C (Method F); the resulting crude amines were condensed with cyanoguanidine and Me_2CO[16] in the presence of $EtSO_3H$ as previously described.[8] Compounds obtained by this procedure are shown in Table V.

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Studies on Psychotropic Drugs. 18.[1] Synthesis and Structure–Activity Relationships of 5-Phenyl-1,3-dihydro-2*H*-thieno[2,3-*e*][1,4]diazepin-2-ones

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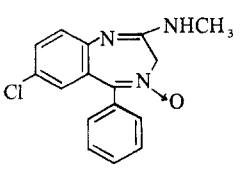
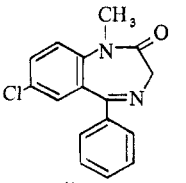
A series of 5-phenyl-1,3-dihydro-2*H*-thieno[2,3-*e*][1,4]diazepin-2-ones was synthesized and evaluated for CNS depressant activity. Structure–activity relationships were discussed.

It is well known that a number of 1,4-benzodiazepine derivatives show potent antianxiety activity.[2] From the standpoint of bioisosterism, we synthesized a series of thienodiazepine derivatives, which have a thieno moiety in place of the benzo moiety of the benzodiazepine ring system, starting from readily obtainable 2-amino-3-benzoylthiophenes according to Gewald, *et al.*[3] 5-Phenyl-1,3-dihydro-2*H*-thieno[2,3-*e*][1,4]diazepin-2-ones thus synthesized were pharmacologically screened and, as was expected, several compounds in this series **(84, 109,** and **110)** were found to show higher CNS depressant activity than CDP and not less than DZP. These compounds were observed to have similar low toxicity to CDP and DZP.



chlordiazepoxide (CDP)



diazepam (DZP)

Chemistry. The synthetic route to thienodiazepine derivatives is shown in Scheme I.

Starting materials, 2-amino-3-benzoylthiophenes (I), were prepared by the reaction of ω-cyanoacetophenone with aldehyde or ketone and sulfur in the presence of catalytic amine (Table I). Compounds of type I were converted into corresponding aminoacetamide derivatives IV *via* methods A and B, respectively.

Method A. Benzyloxycarbonylaminoacetamide intermediates II were synthesized by condensation of I with benzyloxycarbonylaminoacetyl chloride in $CHCl_3$ at low