trend of $+\pi/+\sigma$ and away from the $-\pi/-\sigma^2$ relationship actually derived, and also to the presence of data points which are seriously aberrant even with respect to the $-\pi/-\sigma^2$ relationship. Although the failure of this Topliss tree provides a counterexample to the previous findings of consistent success in retrospective studies using the technique (ref 3), we note that no overall inefficiency in series development seems to have resulted from its use and that the resulting data were satisfactory for the more complex QSAR analyses described in this paper. Consequently, we tend to regard this study as somewhat supportive of use of the Topliss approach, provided that the Topliss models are supplemented by formal QSAR methods as more data are available.

10-(Alkylamino)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines. A Novel Class of Potential Neuroleptic Agents

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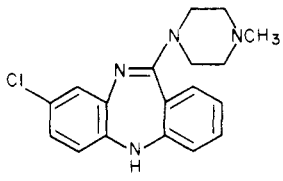
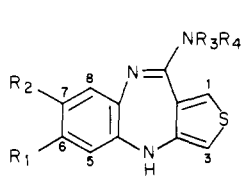
An intensive investigation of the structural requirements for CNS activity of the title compounds was undertaken. A synthesis of the precursor dihydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-ones was achieved and three routes for their conversion to the title compounds were developed. The compounds were tested for neuroleptic activity by means of the blockade of *d*-amphetamine lethality in aggregated mice and/or effects on locomotor activity in rats. Antidepressant activity was examined using inhibition of tetrabenazine-induced depression in mice. Most of the compounds were found to be potent neuroleptic agents with several exhibiting additional antidepressant activity.

As part of an intensive program of developing novel psychotropic agents, we became interested in potential neuroleptic agents which exhibit low extrapyramidal symptoms (EPS). Reports that clozapine (1) was the first



1



2

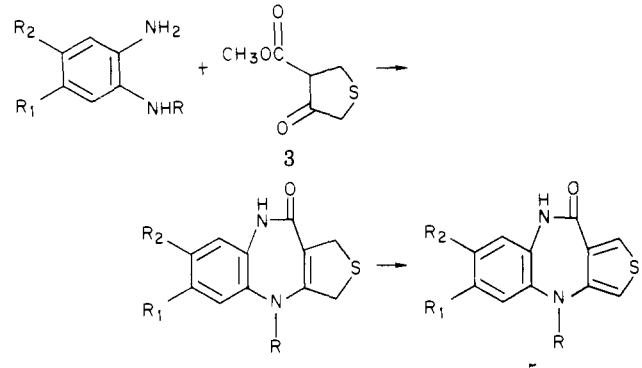
tricyclic antipsychotic drug that did not cause EPS[1] encouraged us to undertake a program of synthesis and investigation of a related tricyclic system, 4*H*-thieno[3,4-*b*][1,5]benzodiazepine[2] (2), as a source of agents with an improved therapeutic profile.

Chemistry. The thieno[3,4-*b*][1,5]benzodiazepin-10(9*H*)-one system (5)[3] was prepared as outlined in Chart I. The appropriately substituted *o*-phenylenediamine was condensed with methyl tetrahydro-4-oxo-3-thiophenecarboxylate[4] (3) in refluxing toluene or xylene to give a 1,3,4,9-tetrahydrothieno[3,4-*b*][1,5]benzodiazepin-10(9*H*)-one (4). Oxidation of 4 could be effected by fusion with elemental sulfur, treatment with sulfuryl chloride in refluxing chloroform, or, preferably, treatment with *N*-chlorosuccinimide in pyridine at 60 °C to give the fully aromatic thiophene derivative 5. When the above sequence was performed with unsymmetrical *o*-phenylenediamines ($R_1 \neq R_2$ or R = CH_3), separation of the isomeric products was most easily accomplished via fractional crystallization of the oxidized derivatives 5.

The desired title compounds 2 were prepared from the lactams 5 in one of three ways as summarized in Chart II. In method A, the principal method used for the preparation of the final compounds 2, lactam 5 was converted by the action of phosphorus pentasulfide in pyridine to a thiolactam 6. Alkylation of 6 with methyl iodide or dimethyl sulfate and base gave the methyl thioether 7, which reacted smoothly with a variety of amines to give the desired 2.

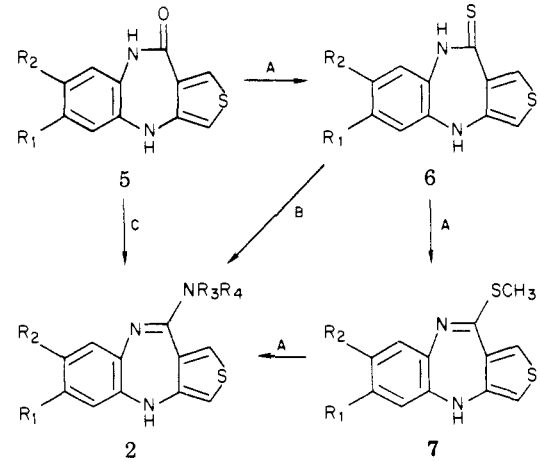
Alternatively, reaction of thiolactam 6 directly with the appropriate amine (e.g., *N*-methylpiperazine) gave 2 in moderate yield (method B). A more useful procedure is that reported by Fryer et al.[5] and is depicted as method

Chart I. Synthesis of Thieno[3,4-*b*][1,5]benzodiazepin-10(9*H*)-ones



R = H, CH_3

Chart II. Synthesis of 10-(Alkylamino)-4*H*-thieno-[3,4-*b*][1,5]benzodiazepines



method A, P_2S_5/pyr; $(CH_3)_2SO_4$/KOH; R_3R_4NH/heat
method B, R_3R_4NH/heat
method C, $R_3R_4NH/TiCl_4$

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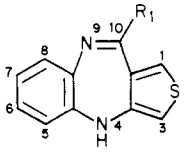
Table I. Biological Activity of 10-(Alkylamino)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines 2



compd	substituent	R_1	motor act.: MDD_{50},[a] mg/kg po	GAL: ED_{50},[b] mg/kg po
2a	H	-c-$N(CH_2CH_2)_2N$-CH_3	6.1	6.0
2b	6-Cl	-c-$N(CH_2CH_2)_2N$-CH_3	12.3	I[c]
2c	7-Cl	-c-$N(CH_2CH_2)_2N$-CH_3	13.9	ND[d]
2d	H	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	33.1	ND
2e	6,7-$(CH_3)_2$	-c-$N(CH_2CH_2)_2N$-CH_3	26.2	ND
2f	H	-$NHCH_2CH_2$-c-NC_5H_{10}	37	ND
2g	H	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	6.3	ND
2h	6,7-$(CH_3)_2$	-$NHCH_2CH_2$-c-NC_5H_{10}	~50	I
2i	6,7-$(CH_3)_2$	-c-NC_4H_6	~50	I
2j	H	-c-$N(CH_2CH_2)_2N$-$CH_2CH_3CH_3$	27	6.0
2k	6-CH_3O	-c-$N(CH_2CH_2)_2N$-CH_3	16.1	1.1
2l	H	-$N(CH_3)_2$	26.5	ND
2m	6-CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	9.0	5.8
clozapine			21	2
chlorpromazine			2	0.6

[a] Dose causing 50% reduction of motor activity in rats. [b] Protection vs. amphetamine lethality in grouped mice. [c] I = inactive at 20 mg/kg ip. [d] ND = not done.

C. In this method, titanium tetrachloride is used to catalyze the condensation of lactam **5** with amines to give **2**. The latter procedure could not be utilized with polyfunctional amines such as *N*-(β-hydroxyethyl)piperazine, probably as a result of undesired coordination and subsequent deactivation of the catalyst.

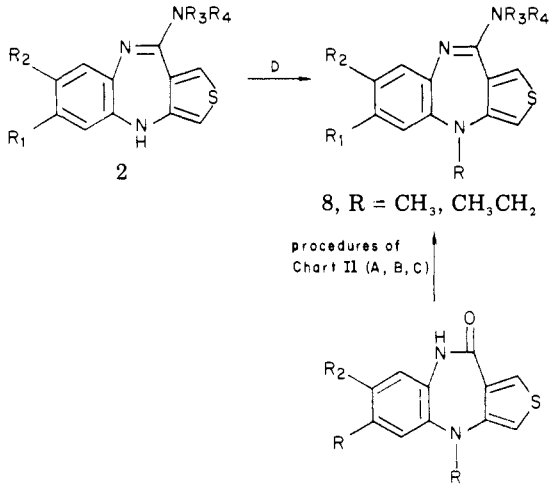
During the course of our investigation, we were interested in studying the effects of alkyl substitution at N-4 of the 10-(alkylamino)-4*H*-thienobenzodiazepines **2** on biological activity. Methods of preparation of these derivatives are outlined in Chart III. Preferably, thienobenzodiazepine **2** was reductively alkylated to give **8** using sodium borohydride in a modified procedure of Gribble.[6] Alternatively, lactam **5** (R = CH_3, prepared from an *N*-methyl-*o*-phenylenediamine according to the procedures in Chart I) was reacted in the manner outlined in Chart II, to form 4-*N*-alkylated thienobenzodiazepine **8**.

Pharmacology. The potential neuroleptic activity of the test compounds was assessed by their ability to antagonize *d*-amphetamine-induced lethality in grouped mice (GAL) and/or to inhibit motor activity in rats.[7] Activities of thienobenzodiazepines **2** and **8**, as well as the reference agents clozapine and chlorpromazine, are listed in Tables I and II.

General observations concerning structure–activity relationships may be made from these data. The nature of the basic substituent at C-10 of the thieno[3,4-*b*]-benzodiazepine is of key importance. *N*-Methyl- or *N*-ethylpiperazinyl substituents produce the most potent neuroleptic agents. Aromatic substitution at C-3 (**8o**), C-6 (**2b**, **2k**, 2m, and **8n**), and C-7 (**2c**, **8d**, **8e**, **8l**, and **8m**) usually caused attenuation of neuroleptic activity, with little apparent correlation between electron-withdrawing or -donating effects and biological activity. The best candidates appear to be the all-hydrogen (**2a**, **2d**, **2g**, **8a**-**8c**, and **8g**), 6-methoxy (**2k**), 6-methyl (**2m**), 7-chloro (**2c**, **8d**, **8e**, **8j**, and **8l**), 6,7-dichloro (**8i**), and 6,7-dimethyl (**2e**) derivatives.

Unfortunately, this promising neuroleptic activity was accompanied by a causation of catalepsy in rats (see Table III). Representative thienobenzodiazepines (**2a–c**, **2k**, **8a**, and **8d–e**) have ED_{50} values of 10–40 mg/kg po, while that

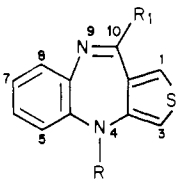
Chart III. 4-N-Alkyl Derivatives of 10-(Alkylamino)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines



of chlorpromazine is 7 and clozapine is 76 mg/kg. There is a positive correlation between the ability of known antipsychotic drugs to produce a cataleptic effect in laboratory animals and to cause parkinsonian effects in man.[8] Since this class of compounds exhibits a potential therapeutic effect (Tables I and II) in the same dose range which induces catalepsy (Table III), these thienobenzodiazepines appear to behave as classical neuroleptic agents (e.g., chlorpromazine) rather than as agents with improved therapeutic profiles (e.g., clozapine).

During the course of our regular screening program, it became apparent that 4-alkyl-substituted compounds (**8**) also exhibited potential antidepressant activity, as measured by antagonism of tetrabenazine (TBZ) induced depression of exploratory behavior in mice (Table II). The 4-methyl compounds **8a**,**b** and **8f–h** showed marked antidepressant activity, while the 4-ethyl compounds **8c**, **8p**, and **8g** were marginally active. Most aromatic substitution (**8d**,**e** and **8i–m**) caused inactivity, with the exception of

Table II. Biological Activity of 4-Alkyl-10-(alkylamino)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines 8



compd	substit	R	R_1	motor act.: MDD_{50},[a] mg/kg po	GAL: ED_{50},[b] mg/kg po	TBZ: MED[c] mg/kg po
8a	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	12	3.6	3.1
8b	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	8.7	4.8	3.1
8c	H	CH_3CH_2	-c-$N(CH_2CH_2)_2N$-CH_3	23	5.7	6.25
8d	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	17	1.4	I[d]
8e	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	20.4	2.0	I
8f	H	CH_3	-c-$N(CH_2CH_2)_2N$-H	I[e]	I[f]	3.1
8g	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	21.3	I	12.5
8h	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	ND[g]	ND	6.25
8i	6,7-Cl_2	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	35.9	8	I
8j	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	7.3	1.1	I
8k	6,7-$(CH_3)_2$	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	58	I	I
8l	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-$CH_2CH_2OC(=O)CH_3$	26.7	3.2	I
8m	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-$CH_2CH_2CH_3$	24	I	I
8n	6-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	ND	I	12.5
8o	3-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	I	I	3.1
8p	H	CH_3CH_2	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	~50	ND	I
8q	H	CH_3CH_2	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	28	ND	25
clozapine				21	2	I
chlorpromazine				2	0.6	I
imipramine				>50	>20	12

[a] Dose causing 50% reduction of motor activity in rats. [b] Protection vs. amphetamine lethality in grouped mice. [c] Minimum effective dose causing antagonism of tetrabenazine (TBZ) induced depression in mice. [d] I = inactive at 25 mg/kg po for TBZ. [e] I = inactive at 50 mg/kg ip for motor activity. [f] I = inactive at 20 mg/kg ip for GAL. [g] ND = not done.

Table III. Cataleptic Effects of Selected 10-(Alkylamino)-4*H*-thienobenzodiazepines 2 and 8

compd	catalepsy[a] induct.: ED_{50}, mg/kg po
2a	11
2b	16
2c	16
2k	15
8a	14
8d	40
8e	13
clozapine	76
chlorpromazine	7

[a] Induction of catalepsy in rats.

chlorine substitution at C-6 or -3 which resulted in retention of activity (**8n,o**).

While this class of compounds did not show the initially desired profile as neuroleptic agents with low EPS liability, the mixed action profile of **8a–q** as potential neuroleptic/antidepressant compounds is interesting. Recently, fluotracen was reported as a clinical agent with a combination of properties similar to those observed for **8**.[9] With current clinical interest in such compounds, thienobenzodiazepines such as **8a** may represent a unique class of mixed action CNS agents for further development.

Experimental Section

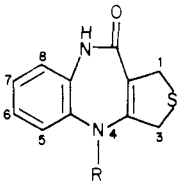
Melting points were determined on a Mel-Temp capillary block melting point apparatus and are uncorrected. Elemental analyses are within ±0.4% of theory, except where noted; exceptional compounds all exhibited proper spectral characteristics and were homogeneous by thin-layer chromatographic analysis. ^{1}H NMR measurements were obtained on a Varian Associates HA-100A spectrometer, and shift values are reported in δ downfield from tetramethylsilane as the internal standard. Mass spectral measurements and exact mass determinations were made on a AEI MS-9 mass spectrometer. The procedures reported herein represent general synthetic operations used for preparation of the compounds in Tables I–IX.

1,3,4,9-Tetrahydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-one (4a). A solution of methyl tetrahydro-4-oxo-3-thiophenecarboxylate[4] (40.0 g, 0.25 mol) and *o*-phenylenediamine (27.0 g, 0.25 mol) in toluene (1 L) was refluxed for 2.5 h using a Dean–Stark separator to remove water. The precipitate obtained upon cooling was collected by filtration and recrystallized from dimethylformamide/water to give the product (38.1 g, 70%) as a yellow crystalline solid: mp 216–218 °C, lit.[3] mp 225–230 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.60 (br s, 1 H, amine H), 8.31 (br s, 1 H, amide H), 6.80 (m, 4 H, aromatic H), 3.81 (s, 4 H, $-CH_2S-$).

4,9-Dihydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-one (5a). To a cooled slurry of **4a** (10.9 g, 0.05 mol) in anhydrous pyridine (100 mL) was added *N*-chlorosuccinimide (6.70 g, 0.05 mol) in portions such that the temperature of the reaction mixture remained between 10 and 15 °C. Upon the final addition, the mixture was warmed on a steam bath for 30 min, then cooled in an ice bath, and poured slowly into ice-cold water (1 L) with vigorous stirring. The precipitate was collected by filtration (10.6 g, 97%) and recrystallized from methanol/water to give the product as an off-white crystalline solid (6.50 g, 60%): mp 218–220 °C dec, lit.[3] mp 237–239 °C; ^{1}H NMR (Me_2SO-d_6) δ 9.67 (br s, 1 H, amide H), 8.08 (br s, 1 H, amine H), 8.03 (d, 1 H, thiophene H), 6.92 (m, 4 H, aromatic H), 6.54 (d, 1 H, thiophene H).

4,9-Dihydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-thione (6a). A mixture of **5a** (7.6 g, 0.035 mol) and phosphorus pentasulfide (10.5 g, 0.046 mol) was refluxed in anhydrous pyridine (100 mL) for 4 h. The reaction mixture was concentrated to dryness and then treated with 1 N sodium carbonate (to pH 7–7.2) and stirred vigorously overnight. The precipitate was collected and recrystallized from methanol to give the product as an orange crystalline solid (2.70 g, 34%): mp 210–212 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.16 (d, 1 H, thiophene H), 7.98 (br s, 1 H, amine H), 7.02 (m, 4 H, aromatic H), 6.52 (d, 1 H, thiophene H).

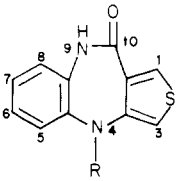
Table IV. Physical Properties of 1,3,4,9-Tetrahydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-ones **4**



no.	substit	% yield	mp, °C	formula[a]	recrystn solv
4a	4-H	70	216-218	$C_{11}H_{10}N_2OS$	DMF-H_2O
4b	4-CH_3	39	196-198	$C_{12}H_{12}N_2OS$	EtOAc
4c	4-H, 6,7-Cl_2	64	247-249	$C_{11}H_8Cl_2N_2OS$	DMF-EtOH
4d	4-H, 6,7-$(CH_3)_2$	73	234-236	$C_{13}H_{14}N_2OS$	DMF-H_2O
4e	4 H, mix of 6- & 7-Cl	76	233-235	$C_{11}H_9ClN_2OS$	DMF
4f	4-CH_2CH_3	26	195-197	$C_{13}H_{14}N_2OS$[b]	EtOAc
4g	4-CH_3, 6,7-Cl_2	36	281-283	$C_{12}H_{10}Cl_2N_2OS$	EtOAc
4h	4,6,7-$(CH_3)_3$	25	250-252	$C_{14}H_{16}N_2OS$	EtOAc
4i	4-H, 6-CH_3O	14	203-205	$C_{12}H_{12}N_2O_2S$[c]	MeCN
4j	4-H mix of 6- & 7-CH_3	45	202-204	$C_{12}H_{12}N_2OS$[d]	MeCN

[a] All compounds gave satisfactory (±0.4%) combustion analyses for C, H, N, S, and halogen where appropriate, except where noted. [b] Not purified; converted directly to 5h. [c] Not purified; converted directly to 5k. [d] Not purified; converted directly to 5l.

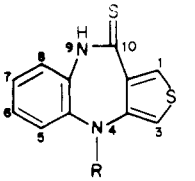
Table V. Physical Properties of 4,9-Dihydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-ones 5



no.	substit	% yield	mp, °C	formula[a]	recrystn solv
5a	4-H	60	218-220	$C_{11}H_8N_2OS$	MeOH-H_2O
5b	4-CH_3	33	224-225	$C_{12}H_{10}N_2OS$	MeOH-H_2O
5c	4-H, 6,7-Cl_2	80	286-288	$C_{11}H_6Cl_2N_2OS$	MeOH
5d	4-H, 6,7-$(CH_3)_2$	97	239-242	$C_{13}H_{12}N_2OS$[c]	MeOH-H_2O
5e	4-H, 6-Cl	*b*	279-281	$C_{11}H_7ClN_2OS$	MeOH
5f	4-H, 7-Cl	*b*	197-198	$C_{11}H_7ClN_2OS$	MeOH-H_2O
5g	4-CH_3, 7-Cl	47	244-246	$C_{12}H_9ClN_2OS$[d]	MeOH
5h	4-CH_2CH_3	59	201-202	$C_{13}H_{12}N_2OS$	MeOH-H_2O
5i	4-CH_3, 6,7-Cl_2	73	270-272	$C_{12}H_8Cl_2N_2OS$	MeOH-H_2O
5j	4,6,7-$(CH_3)_3$	97	239-242	$C_{13}H_{12}N_2OS$	MeOH-H_2O
5k	4-H, 6-CH_3O	47	125 dec	$C_{12}H_{10}N_2O_2S$[e]	
5l	4-H, 6-CH_3	35	184-188	$C_{12}H_{10}N_2OS$[f]	EtOH

[a] All compounds gave satisfactory (±0.4%) combustion analyses for C, H, N, S, and halogen when appropriate, except where noted. [b] 5e and 5f were prepared as the mixture from 4e in 83% yield and separated by fractional crystallization. [c] Not purified; converted directly to 6g. [d] Not purified; converted directly to 6e. [e] Not purified; converted directly to 2k. [f] Not purified; converted directly to 2m.

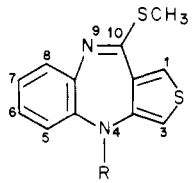
Table VI. Physical Properties of 4,9-Dihydro-10*H*-thieno[3,4-*b*][1,5]benzodiazepin-10-thiones 6



no.	substit	% yield	mp, °C	formula[a]	recrystn solv
6a	4-H	34	210-212	$C_{11}H_8N_2S_2$	MeOH
6b	4-CH_3	45	203-204	$C_{12}H_{10}N_2S_2$[b]	MeOH
6c	4-H, 7-Cl	36	197-198.5	$C_{11}H_7ClN_2S_2$[c]	MeOH-H_2O
6d	4-H, 6-Cl	27	235-237	$C_{11}H_7ClN_2S_2$	MeOH
6e	4-CH_3, 7-Cl	51	238-240	$C_{12}H_9ClN_2S_2$	MeOH
6f	4-CH_2CH_3	74	189-191	$C_{13}H_{12}N_2S_2$[d]	MeOH
6g	4-H, 6,7-$(CH_3)_2$	66	217-220	$C_{13}H_{12}N_2S_2$	MeOH-CH_2Cl_2

[a] All compounds gave satisfactory (±0.4%) combustion analyses for C, H, N, S, and halogen when appropriate, except where noted. Exceptional compounds were homogeneous by thin-layer chromatographic analysis. [b] C: calcd, 58.50; found, 58.01. [c] C: calcd, 49.52; found, 48.71. [d] S: calcd, 24.63; found, 24.12.

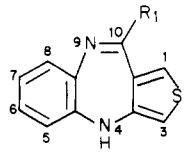
Table VII. Physical Properties of 10-(Methylthio)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines 7



no.	substit	% yield	mp, °C	formula[a]	recrystn solv
7a	4-H	65	128.5-130	$C_{12}H_{10}N_2S_2$[b]	MeOH-H_2O
7b	4-CH_3	80	113-115	$C_{13}H_{12}N_2S_2$	MeOH-H_2O
7c	4-H, 7-Cl	49	111-113	$C_{12}H_9ClN_2S_2$	MeOH-H_2O
7d	4-H, 6-Cl	74	152-154	$C_{12}H_9ClN_2S_2$[c]	MeOH-H_2O
7e	4-CH_3, 7-Cl	82	156-158	$C_{13}H_{11}ClN_2S_2$	MeOH-H_2O
7f	4-CH_2CH_3	95	138-140	$C_{14}H_{14}N_2S_2$	MeOH
7g	4-H, 6,7-$(CH_3)_2$	80	176-179	$C_{14}H_{14}N_2S_2$	EtOH

[a] All compounds gave satisfactory (±0.4%) combustion analyses for C, H, N, S, and halogen when appropriate, except where noted. Exceptional compounds were homogeneous by thin-layer chromatographic analysis. [b] C: calcd, 58.50; found, 58.98. [c] C: calcd, 51.33; found, 52.36.

Table VIII. Physical Properties of 10-(Alkylamino)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines 2



no.	substit	R_1	% yield (method)[a]	formula[b]	base salt[c]	mp, °C	recrystn solv
2a	H	-c-$N(CH_2CH_2)_2N$-CH_3	58 (A)	$C_{16}H_{18}N_4S$	FB	197.5-199	acetone-pet. ether
2b	6-Cl	-c-$N(CH_2CH_2)_2N$-CH_3	37 (A)	$C_{16}H_{17}ClN_4S$	FB	148-149[f]	acetone-pet. ether
2c	7-Cl	-c-$N(CH_2CH_2)_2N$-CH_3	57 (A)	$C_{16}H_{17}ClN_4S\cdot 2HCl\cdot H_2O$[d]	HCl	223-235[f]	
2d	H	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	72 (A)	$C_{17}H_{20}N_4OS\cdot 0.5C_4H_4O_4\cdot 0.25H_2O$[e]	F	207-208[f]	EtOH
2e	6,7-$(CH_3)_2$	-c-$N(CH_2CH_2)_2N$-CH_3	60 (A)	$C_{18}H_{22}N_4S$	FB	218-220	MeOH
2f	H	-$NHCH_2CH_2$-c-NC_5H_{10}	22 (A)	$C_{18}H_{22}N_4S$	FB	184-185[f]	MeOH
2g	H	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	32 (A)	$C_{17}H_{20}N_4S$	FB	193-196	EtOH
2h	6,7-$(CH_3)_2$	-$NHCH_2CH_2$-c-NC_5H_{10}	9 (A)	$C_{20}H_{26}N_4S$	FB	168-169	Et_2O
2i	6,7-$(CH_3)_2$	-c-NC_4H_6	57 (A)	$C_{17}H_{17}N_3S\cdot C_4H_4O_4\cdot 0.25H_2O$	F	239-240	EtOH
2j	H	-c-$N(CH_2CH_2)_2N$-$CH_2CH_2CH_3$	46 (A)	$C_{18}H_{22}N_4S$	FB	140-142	MeOH-H_2O
2k	6-CH_3O	-c-$N(CH_2CH_2)_2N$-CH_3	22 (B)	$C_{17}H_{20}N_4SO$	FB	208-211[f]	MeCN
2l	H	-$N(CH_3)_2$	89 (B)	$C_{13}H_{13}N_3S\cdot C_4H_4O_4$	F	185-189[f]	EtOH
2m	6-CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	69 (C)	$C_{17}H_{20}N_4S$	FB	212-217	MeCN

[a] Refer to Experimental Section for Methods. [b] All compounds gave satisfactory (±0.4%) combustion analyses for C, H, N, S, and halogen when appropriate, except where noted; exceptional compounds were homogeneous by thin-layer chromatographic analysis. [c] Free base = FB; hydrochloride = HCl; fumarate = F. [d] MS (for $C_{16}H_{17}ClN_4S$) calcd, 332.0862; found, 332.0842. [e] MS (for $C_{17}H_{20}N_4OS$) calcd, 328.1358; found, 328.1366. [f] Decomposition.

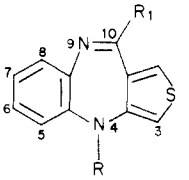
10-(Methylthio)-4*H*-thieno[3,4-*b*][1,5]benzodiazepine (7a). To a stirred solution of **6a** (1.34 g, 0.0058 mol) in dioxane (15 mL) was added, simultaneously, in four portions during the course of 1 h, a solution of potassium hydroxide (1.93 g, 0.035 mol) in methanol (10 mL) and dimethyl sulfate (2.21 g, 0.0175 mol) in methanol (4 mL). The mixture was stirred overnight, diluted with methanol, and filtered. The filtrate was concentrated to ca. 20 mL and precipitated with water. The precipitate was collected and recrystallized from methanol/water to give the product as a deep gold crystalline solid (0.92 g, 65%): mp 128.5–130 °C; ^1H NMR (Me_2SO-d_6) δ 7.70 (d, 1 H, thiophene H), 7.64 (br s, 1 H, amine H), 6.90 (m, 4 H, aromatic H), 6.43 (d, 1 H, thiophene H), 2.44 (s, 3 H, SCH_3).

10-(4-Methyl-1-piperazinyl)-4*H*-thieno[3,4-*b*][1,5]-benzodiazepine (2a). Method A. A solution of **7a** (0.98 g, 0.004 mol) in xylene (10 mL) was treated with *N*-methylpiperazine (1 mL, excess) and glacial acetic acid (2 drops) and refluxed until the starting material was consumed as evidenced by TLC analysis (ca. 4 h). The reaction mixture was concentrated, and the residue was dissolved in 2 N acetic acid (25 mL) and filtered through diatomaceous earth. The filtrate was then basified with concentrated ammonium hydroxide and the resulting precipitate was collected by filtration. Recrystallization of the solid from acetone/petroleum ether gave the product as a yellow solid (0.70 g, 58%): mp 197.5–199 °C; ^1H NMR (Me_2SO-d_6) δ 7.44 (d, 1 H, thiophene H), 7.36 (br s, 1 H, NH), 6.84 (m, 4 H, aromatic H), 6.57 (d, 1 H, thiophene H), 3.36, 2.46 (2 m, 4 H each, CH_2N), 2.24 (s, 3 H, NCH_3).

Method B. A mixture of thione **6a** (2.32 g, 0.01 mol), *N*-methylpiperazine (23 mL, 0.21 mol), and acetic acid (3 drops) was refluxed with stirring for 18 h. After cooling, the reaction mixture was poured into ice–water (1 L), and the resultant suspension was extracted with methylene chloride (three times). Filtration of the organic phase through magnesium silicate, concentration, and recrystallization from ethanol gave the product **2a** identical with that prepared above (1.0 g, 40%), mp 194–196 °C.

Method C. *N*-Methylpiperazine (32.9 mL, 0.297 mol) in dry toluene (17.7 mL) was added to a mechanically stirred mixture of toluene (148 mL), anisole (15.9 mL), and titanium tetrachloride (8.13 mL, 0.0744 mol). To this solution was added lactam **5a** (15.9 g, 0.0735 mol) and *N*-methylpiperazine (16.6 mL, 0.148 mol), and the mixture was refluxed for 6.5 h. The reaction mixture was

Table IX. Physical Properties of 4-Alkyl-10-(alkylamino)-4*H*-thieno[3,4-*b*][1,5]benzodiazepines 8



no.	substit	R	R_1	% yield (method)[a]	formula[b]	base/ salt[c]	mp, °C	recrystn solv
8a	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	65 (A)	$C_{17}H_{20}N_4S \cdot 0.75C_2H_5OH$	FB	65-69	MeOH
8b	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	82	$C_{17}H_{20}N_4S \cdot C_4H_4O_4$	F	185-186	EtOH
8c	H	CH_3CH_2	c-$N(CH_2CH_2)_2N$-CH_3	73 (A)	$C_{18}H_{22}N_4S \cdot 0.5C_4H_4O_4 \cdot 0.25H_2O$	F	195-198[g]	EtOH
8d	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	28 (A)	$C_{17}H_{19}ClN_4S \cdot 0.5C_4H_4O_4$	F	189.5-191[g]	EtOH
8e	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	50 (A)	$C_{18}H_{21}ClN_4OS \cdot 2HCl \cdot 0.5H_2O$	HCl	250[g]	MeOH-ether
8f	H	CH_3	-c-$N(CH_2CH_2)_2N$-H	40 (A)	$C_{16}H_{18}N_4S \cdot C_4H_4O_4 \cdot 0.5H_2O$	F	205-206[g]	MeOH
8g	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	50 (A)	$C_{18}H_{22}N_4OS \cdot 0.5C_4H_4O_4$	F	192-193[g]	EtOH
8h	H	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	30 (D)	$C_{18}H_{22}N_4S \cdot 2C_4H_4O_4$	F	216-219[g]	EtOH
8i	6,7-Cl_2	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	76 (D)	$C_{17}H_{18}Cl_2N_4S \cdot 0.5C_4H_4O_4$	F	225-226[g]	EtOH
8j	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	77 (A)	$C_{18}H_{21}ClN_4S \cdot C_4H_4O_4$	F	205-207	EtOH
8k	6,7-$(CH_3)_2$	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	100 (C)	$C_{19}H_{24}N_4S \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$	F	140-142	EtOH
8l	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_2CH_2O-C(=O)CH_3	94[d]	$C_{20}H_{23}ClN_4O_2S \cdot C_4H_4O_4$	F	156-158	EtOH
8m	7-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-$CH_2CH_2CH_3$	20 (A)	$C_{19}H_{23}ClN_4S \cdot C_4H_4O_4 \cdot 0.5H_2O$[e]	F	154-157	EtOH
8n	6-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	100 (D)	$C_{17}H_{19}ClN_4S$	FB	149-151	EtOH
8o	3-Cl	CH_3	-c-$N(CH_2CH_2)_2N$-CH_3	29	$C_{17}H_{19}ClN_4S \cdot C_4H_4O_4 \cdot C_2H_5OH$[f]	F	130-132[g]	EtOH
8p	H	CH_3CH_2	-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	69 (A)	$C_{19}H_{24}N_4OS \cdot 0.5C_4H_4O_4$	F	212-213	EtOH
8q	H	CH_3CH_2	-c-$N(CH_2CH_2)_2N$-CH_2CH_3	51 (D)	$C_{19}H_{24}N_4S \cdot 0.5C_4H_4O_4$	F	200-202	EtOH

[a] Refer to Experimental Section for Methods. [b] All compounds gave satisfactory (±0.4%) combustion analyses for C, H, N, S, and halogen when appropriate, except where noted. Exceptional compounds were homogeneous by thin-layer chromatographic analysis. [c] Free base = FB; hydrochloride = HCl; fumarate = F. [d] Acetic anhydride/pyridine acetylation of 8e. [e] MS (for $C_{19}H_{23}ClN_4S$) calcd, 374.1332; found, 374.1322. [f] MS (for $C_{17}H_{19}ClN_4S$) calcd, 346.1019; found, 346.1028. [g] Decomposition.

cooled to 60 °C and 2-propanol (23 mL) and diatomaceous earth (14 g) was added with concentrated ammonium hydroxide (21 mL). The solid was removed by filtration and thoroughly washed with toluene. The filtrate was washed with 3 N hydrochloric acid and the resulting aqueous phase was basified with concentrated ammonium hydroxide to cause the product to precipitate. The precipitate was collected by filtration and dissolved in methylene chloride, and the resultant solution was filtered through magnesium silicate and concentrated to dryness. The product was recrystallized from ethanol to give **2a** as yellow crystals (8.97 g, 41%), mp 197–200 °C.

4-Methyl-10-(4-methyl-1-piperazinyl)-4*H*-thieno[3,4-*b*][1,5]benzodiazepine (8a). Method D. Diazepine **2a** (11.9 g, 0.04 mol) in 97% formic acid (140 mL) was cooled with stirring in an ice bath while sodium borohydride pellets (13.5 g, 0.36 mol) were added, one pellet at a time. The mixture was stirred at room temperature until starting material was absent, as evidenced by TLC analysis (ca. 12 h). The reaction mixture was cooled, diluted with water, and made alkaline with concentrated ammonium hydroxide. The aqueous layer was extracted with chloroform (three times), and the combined organic layers were dried over magnesium sulfate and concentrated to dryness. The product was recrystallized from ethanol to give a yellow crystalline product (8.1 g, 65%), mp 84–86 °C. The product was found to contain ethanol of crystallization, which was not removed by prolonged drying in vacuo: ^{1}H NMR (Me_2SO-d_6) δ 7.49 (d, 1 H, thiophene H), 6.92 (m, 4 H, aromatic H), 6.80 (d, 1 H, thiophene H), 3.36 (m, 4 H, CH_2N), 3.10 (s, 3 H, CH_3N), 2.46 (m, 4 H, CH_2N), 2.25 (s, 3 H, CH_3N).

The product was reacted with fumaric acid (3.01 g, 0.026 mol) in ethanol (50 mL) to give the corresponding fumarate salt as a white crystalline solid, 9.1 g (82%), mp 185–186 °C.

3-Chloro-4-methyl-10-(4-methyl-1-piperazinyl)-4*H*-thieno[3,4-*b*][1,5]benzodiazepine Fumarate (8o). To a solution of the diazepine **8a** (2.34 g, 0.0075 mol) in chloroform (10 mL) was added sulfuryl chloride (1.4 mL, 0.017 mol) dropwise. The mixture was stirred for 0.5 h and triethylamine (0.7 mL, 0.008 mol) was added with stirring for an additional 1 h. Water was added, concentrated ammonium hydroxide was added to pH 10–12, and the layers were separated. The chloroform layer was filtered through magnesium silicate and concentrated to give a dark oil (1.9 g, 0.0055 mol, 73%). The oil was dissolved in ethanol (8 mL) and treated with fumaric acid (0.64 g, 0.0055 mol) in ethanol (18 mL). The product crystallized as a white solid (1.1 g, 29%) and was recrystallized from ethanol to give the analytical sample, mp 130–132 °C dec. The salt contains ethanol of crystallization which remains after prolonged drying in vacuo: ^{1}H NMR (Me_2SO-d_6) δ 7.36 (s, 1 H, thiophene H), 7.02 (br s, 4 H, aromatic H), 6.62 (s, 2 H, fumaric acid H), 3.93 (m, 4 H, CH_2N), 3.29 (m and s, 7 H, CH_3 and CH_2N), 2.94 (s, 3 H, CH_3).

Pharmacological Testing Methods. Antagonism of *d*-amphetamine lethality in grouped mice was determined using a previously reported procedure.[7] Groups of 10 mice were treated with the test compound at graded doses and placed in wire mesh cages (20 × 13 × 13.5 cm). After 30 min, *d*-amphetamine sulfate in saline was administered at a dose of 15 mg/kg, which causes 90–100% death in untreated grouped mice. Deaths were measured after 24 h. ED_{50} values were determined and defined as the dose of compound that prevented death in 50% of the test animals.

Effects of the compounds on locomotor activity in rats were determined as previously described[7] by oral treatment of groups of five rats with graded doses of the test compounds. Locomotor activity was determined for each individual rat as measured over a 5-min interval at the time of peak effect (previously measured using a selected dose of the compound) utilizing an Animex® activity counter. The MDD_{50} was measured from a linear regression analysis and is defined as the dose that produces 50% reduction in motor activity as compared to the control animals.

Inhibition of tetrabenazine-induced depression of exploratory behavior in mice was determined in the reported manner.[7] Groups of five mice were treated with a dose of the test compound orally, and after 1 h were treated with tetrabenazine hexamate (aqueous) at a dose of 30 mg/kg ip. Treated mice were placed on a horizontal disk (18-in. diameter) after 30 min and exploratory behavior was measured within 10 s according to an observational response rating scale. The MED (minimum effective dose) was established by dosing initially at 25 mg/kg orally and halving the dose until the test compound is found inactive in the above procedure.

Induction of catalepsy was determined using groups of six to eight rats which were treated orally with graded doses of the test compound.[10] Each rat was tested for catalepsy at various times from 30 min to 18 h after drug administration. Catalepsy is defined as the failure of rats to move from an unnatural posture caused by placing their paws upon four separate raised pedestals within 10 s. Untreated animals remove one or more paws from the pedestals during this test in less than 10 s (usually immediately). ED_{50} values were determined and defined as the dose of compound that caused catalepsy in 50% of the test animals.

Acknowledgment. The authors acknowledge their gratitude to the late Miss G. Wiegand for her initial discovery work and thank Dr. L. Crawley for the preparation of several compounds. In addition, we acknowledge Messrs. W. Fulmor and G. Morton and staff for the measurement and interpretation of spectral data and Mr. L. Brancone and staff for microanalytical data.

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Preparation and Purification of L-(±)-5-Formyl-5,6,7,8-tetrahydrofolic Acid

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Reinvestigation of the conversion of folic acid to leucovorin [L-(±)-5-CHO-THF] led to improved methods for the synthesis of this drug, which is suitable for clinical use. Also, methods were developed for the chromatographic and nonchromatographic purification of less pure samples of L-(±)-5-CHO-THF.

The active investigation of high-dose methotrexate with citrovorum factor [L-(−)-5-formyl-5,6,7,8-tetrahydrofolic acid, L-(−)-5-CHO-THF][1] rescue for the treatment of a number of solid tumors and hematologic malignancies requires large amounts of both methotrexate and leucovorin [L-(±)-5-CHO-THF].[2] Recently, our laboratory reported an improved method for the large-scale synthesis of methotrexate,[3] and in this paper we report improvements in the method for the large-scale preparation of L-(±)-5-CHO-THF.

The elegant work around 1950 by several research groups resulted in the synthesis of L-(±)-5-CHO-THF.[4,5] Although bioassay methods showed that mixtures of L-(±)-THF and reagents containing formate [e.g., HCO_2H, HCO_2Et, and $C_6H_5N(Me)CHO$] produced citrovorum factor activity, the method adopted for the isolation of the mixture of diastereomers involved the catalytic hydrogenation of 10-formylfolic acid (10-CHO-FA) in HCO_2H and treatment of the L-(±)-(5,10-CH-THF)$^+$ generated in situ with base at elevated temperatures to give crude L-(±)-5-CHO-THF, which was purified by column chromatography. Mechanisms involving the transformations of (5,10-CH-THF)$^+$ and 10-CHO-THF to 5-CHO-THF have been proposed.[6]

Initially, our efforts were directed toward the preparation and isolation of L-(±)-(5,10-CH-THF)$^+$ via FA, 10-CHO-FA, and L-(±)-10-CHO-THF. Treatment of FA·2H_2O with HCO_2H gave a diformylated derivative (^1H NMR), which was monodeformylated on recrystallization from H_2O to give 10-CHO-FA·H_2O. Chemical reduction of 10-CHO-FA to L-(±)-10-CHO-THF with $Na_2S_2O_4$, $NaBH_4$, or $NaBH_3CN$ (pH 6.7) under a variety of conditions was unsatisfactory, these reactions leading to mixtures of folates and decomposition products. Although the catalytic hydrogenation of 10-CHO-FA with Pt in HCO_2H was reported to occur readily with rapid stirring

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