MeOH. Anal. ($C_{16}H_{21}N_3O_3S$) C, H, N, S.

6-Methyl-8β-[(methylthio)methyl]-2-azaergoline Methanesulfonic Acid Salt (7c) from 7b. A solution of 7.5 g (0.15 mol) CH_3SH in 250 mL of DMF was cooled in ice. NaH, 7.2 g (0.15 mol; 50% in mineral oil), was added in portions with stirring. To the resulting mixture was then added a solution of 4.2 g (12 mmol) of **7b** in 75 mL of DMF. The cooling bath was removed, and the mixture was stirred at 25 °C for 2 h. Water was added, and the product was extracted with EtOAc. The extract was washed with H_2O and brine, dried, and concentrated. The crude product, 3.16 g (90%), was purified by chromatography on Florisil using $CHCl_3$ with 1–5% MeOH as eluant: yield 2.55 g; mp 223–226 °C. The pure base was suspended in 60 mL of boiling MeOH. To the suspension was added a solution of 0.6 mL of CH_3SO_3H in 5 mL of MeOH. The hot solution was filtered and cooled: yield 3.07 g (67%); mp 290 °C dec. Anal. ($C_{17}H_{25}N_3O_3S$) C, H, N, S.

[4aR-(2β,4aβ,10bα)]-7-Amino-1,3,4,4a,5,10b-hexahydro-2-(methoxymethyl)-4-methylbenzo[f]quinolin-6-(2H)-one (9a) from 8a. A solution of 730 mg (1.93 mmol) of **8a** in 60 mL of MeOH and 60 mL of 10% NaOH was heated under reflux under N_2 for 1 h. It was diluted with H_2O, and the product was extracted with $CHCl_3$–*i*-PrOH. The extract was washed with brine, dried, and concentrated. The crude product was purified by chromatography on 30 g of Florisil using $CHCl_3$–2% MeOH as eluant: yield 340 mg (51%); mp 140–141 °C from Et_2O. Anal. ($C_{16}H_{22}N_2O_2$) C, H, N. These conditions brought about both hydrolysis of the formyl group and displacement of the mesylate ester. When the reaction was conducted at 25 °C (1.75 h), only the amide was hydrolyzed to give **9b** in 87% yield.

8β-(Methoxymethyl)-6-methyl-2-azaergoline Methanesulfonic Acid Salt (7a) from 7b. A solution of 2.4 mmol of **7b** in 100 mL of MeOH and 5 mL of 40% trimethylbenzylammonium methoxide in MeOH was heated under reflux for 48.5 h. The solution was then cooled and diluted with H_2O, and the product was extracted with $CHCl_3$. The extract was washed with brine, dried (Na_2SO_4), and concentated. The crude product was purified by chromatography on 300 g of Florisil using $CHCl_3$–2% MeOH as eluant: yield 550 mg; mp 195–196 °C. The mesylate salt was prepared in MeOH–Et_2O: yield 670 mg (63%); mp 226–230 °C. Anal. ($C_{17}H_{25}N_3O_4S$) C, H, N, S.

[4aR-(2β,4aβ,10bα)]-N-[1,2,3,4,4a,5,6,10b-Octahydro-2-(methoxymethyl)-6-oxo-4-propylbenzo[f]quinolin-7-yl]-formamide Maleate Salt (8d). A solution of 1.9 g (5 mmol) of 8β-(methoxymethyl)-6-propylergoline methanesulfonic acid salt[9] in 50 mL of H_2O and 50 mL of MeOH was added to a solution of 2.14 g (10 mmol) of $NaIO_4$ in 200 mL of H_2O. The resulting mixture was stirred for 2.25 h. Excess aqueous $NaHCO_3$ was then added, and the product was extracted with $CHCl_3$. The extract was washed with brine and dried (Na_2SO_4), and the solvent was distilled. The product was purified by chromatography on 35 g of Florisil using $CHCl_3$/1–2% MeOH as eluant. The maleate salt was prepared in MeOH–Et_2O: yield 1.1 g (51%); mp 172–173 °C. Anal. ($C_{23}H_{30}N_2O_7$) C, H, N.

Isolated Smooth Muscle Testing of 7a. All tissues were removed from animals after killing them by a blow on the head. Tissues were placed in physiological salt solution (Krebs, pH 7.4) at room temperature and then dissected free of fat and connective tissue. Whole tissues, or strips prepared from them, were suspended in 10-mL organ baths containing Krebs solution maintained at 37.5 °C and aerated with a 5% carbon dioxide–95% oxygen mixture. The tissues were attached to Grass FT-03 isometric transducers connected to a Grass Model 7 polygraph recorder and allowed to equilibrate for 1–2 h before drug addition. Compound **7a** was dissolved in H_2O and added to the 10-mL tissue bath in a volume of 0.1 mL.

Acknowledgment. The authors thank G. M. Maciak and associates for the microanalyses. We are indebted also to Dr. D. T. Wong, F. B. Bymaster, Dr. R. R. Ruffolo, J. E. Waddell, and R. W. Kattau for pharmacological studies.

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Tricyclics with Analgesic and Antidepressant Activity. 1. [[(Alkylamino)ethyl]thio]dibenz[b,f]oxepins and 10,11-Dihydro Derivatives

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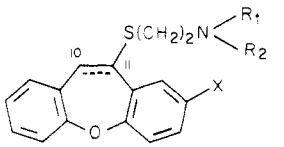
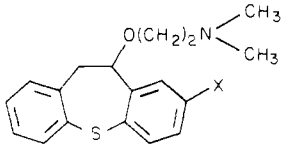
Received October 9, 1979

A series of [[(alkylamino)ethyl]thio]dibenz[b,f]oxepins (I) and their 10,11-dihydro derivatives (II) was synthesized and subjected to broad analgesic/CNS screening. Several analogues of both types, carrying small N-substituents and frequently a nuclear fluorine function, have been found to possess potent analgesic activity in the phenylquinone writhing assay (PQW) and the tail-flick test in mice. Many of these compounds also exhibited significant activity in antagonizing tetrabenazine-induced ptosis, as exemplified by **10b**, **16b**, and **18b**. Results from the mouse jumping test indicated low physical dependence potential for these compounds, and further evidence for a nonnarcotic profile was provided by the absence of significant naloxone interactions with the tail-flick response. Compound **10b** did not produce tolerance in mice following chronic administration in the PQW screen.

A previous publication[3] from this laboratory has described the synthesis and pharmacology of a series of spiro[dibenz[b,f]oxepinpiperidine] derivatives, some of which displayed potent oral analgesic activity by inhibiting phenylquinone-induced writhing in mice. As part of a continued program aimed at discovering tricyclic analgesics with a nonclassic profile, or pain-relieving agents of multiple clinical utility, we have synthesized a series of [[(alkylamino)ethyl]thio]dibenz[b,f]oxepins (I) and their



I, 10,11-dehydro
II, 10,11-dihydro



III, X = H
IV, X = Cl

10,11-dihydro derivatives (II) for broad analgesic/CNS

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(3) H. H. Ong, J. A. Profitt, T. C. Spaulding, and J. C. Wilker, *J. Med. Chem.*, **22**, 834 (1979).

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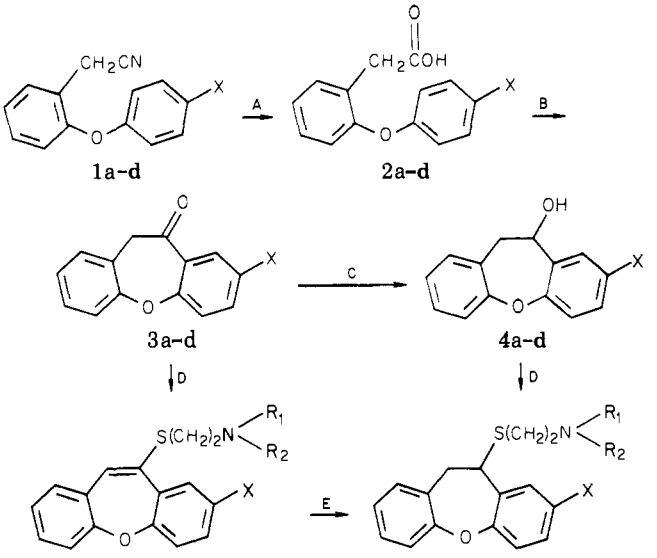
Scheme I



5a-d, $R_1 = R_2 = CH_3$
6a,d, $R_1 = R_2 = C_2H_5$
7a, $R_1 = R_2 = i\text{-}C_3H_7$
8a, $R_1 = H$; $R_2 = (CH_2)_2C_6H_5$

16a-d, $R_1 = R_2 = CH_3$
17a,c,d, $R_1 = R_2 = C_2H_5$
22a, $R_1 = H$; $R_2 = (CH_2)_2C_6H_5$

a, X = H; b, X = F; c, X = Cl; d, X = SCH_3

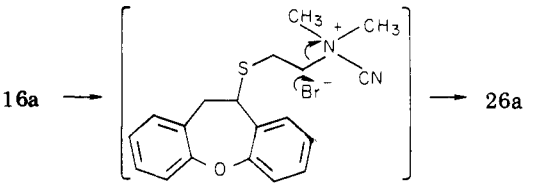
screening. Compounds of this type appeared of interest to us because there had only been a few reported examples of tricyclics incorporating a sulfur moiety in the side chain.[4,5] It is interesting to note that two clinically investigated antipsychotic agents, amethothepine (III) and amethoclothepine (IV),[6] bear topographical resemblance to some of the proposed target compounds, **6a** and **6c**. Although the overall lipophilicity would not be substantially different, transposition of oxygen and sulfur of this type, however, can envoke significant changes in the steric and electronic properties of the molecule, factors which may be translated into differences in chemical stability, biological profile, and potency, as well as fate of metabolism.

Chemistry. Synthetic sequences leading to the initial target compounds, i.e., **5a-d** and **16a-d**, are depicted in Scheme I. Phenoxyphenylacetonitriles **1a-d** were hydrolyzed with potassium hydroxide (method A) to give **2a-d** in up to 90% yield. Cyclization of **2a-d** to ketones **3a-d** was best carried out by the intramolecular Friedel-Crafts reaction: conversion to the corresponding acid chlorides, followed by ring closure with aluminum chloride (method B). Ketones **3a-d** so prepared were reduced smoothly with ethanolic sodium borohydride (method C) to give alcohols **4a-d** in nearly quantitative yields. With the exception of **2b**, **3b**, and **4b** of the fluorine-substituted series, all other intermediates shown in Scheme I had been previously reported in the literature.

Several different approaches were used in attempts to synthesize **5a-d** by dehydrative coupling of **3a-d** with β-(dimethylamino)ethanethiol. The most satisfactory one involved the use of boron trifluoride in glacial acetic acid (method D) and, as shown in Table I, this procedure could be applied with equal success to a number of aminoethanethiols with bulkier N-substituents (**6a**, **6d**, and **7a**) or an ethanethiol bearing a secondary amino function (**8a**). In the latter case, the desired condensation occured exclusively, with no evidence of an enamine formation. It is also worth noting that, despite the wide application of boron trifluoride etherate in the conversion of ketones to 1,3-dithianes, very little, if any, had been previously reported on its potential use in the synthesis of vinyl sulfides.[7]

By a similar procedure, alcohols **4a-d** were converted to **16a-d** in yields ranging from 24 to 85%. The low recovery of **16b** and **16c**, in contrast to **16a** and **16d**, was probably due to the presence of a highly competing elimination reaction, as evidenced by the isolation of large quantities of 2-fluoro- and 2-chlorodibenz[*b*,*f*]oxepin, respectively, in each instance. A new route to **16a-d** was thus developed; it involved the direct saturation of Δ^{10} double bonds in **5a-d**, and the reagent of choice for this purpose was found to be magnesium in methanol (method E) as recently described by Watt, Profitt, and Corey.[8] As shown in Table I, compounds **5a-d** could be converted by this procedure to **16a-d** in uniformly good yields. In addition to its effectiveness, economy, and convenience, the magnesium in methanol reduction appeared to leave nuclear halogens essentially intact; it also tolerated well the presence of heteroatoms such as bivalent sulfur and basic nitrogen.[9]

Further structural modifications of **5a-d** and **16a-d** by varying the N-substituents are depicted in Scheme II. In an initial attempt to prepare **18a-d** by N-demethylation of **6a-d**, the latter were treated with cyanogen bromide in the presence of potassium carbonate, according to the modified von Braun procedure.[10] Somewhat surprisingly, the products isolated were not the expected *N*-cyanamides, rather some nitrogen-free compounds characterized as the bromoethylthio derivatives **26a-d** (method F). The



"abnormal" cleavage of the C-N linkages (as shown) was obviously facilitated by the presence of the β-thio moiety, and a similar activating effect was observed when **26a-d** were found to be quite reactive in nucleophilic displacements by an amine (method G). Thus, we have discovered an efficient method of derivatization of the amino function in the 10,11-dihydrodibenz[*b*,*f*]oxepin series, which not only permitted easy access to **18a-d** but also rendered possible the synthesis of the more complex derivatives such as **23a**, **24a**, and **25a**.

When **5a** was treated with cyanogen bromide in a similar manner, the corresponding bromoethylthio compound **27a** was obtained only in 42% yield, along with 50% of the "normal" *N*-cyanamide **14a**. A preferred method for demethylation of **5a-d** was via carbamate (**9a-d**) formation (method H) and the subsequent hydrolysis with potassium hydroxide in ethylene glycol (method I). The secondary

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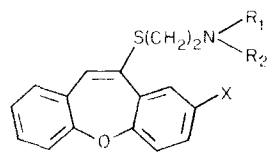
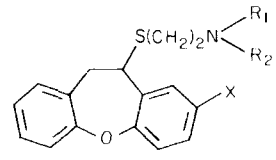
(5) V. Bartl, J. Holubek, J. Nemec, M. Bartosova, and M. Protiva, *Collect. Czech. Chem. Commun.*, **43**, 2427 (1978).
(6) A. Burger Ed., "Medicinal Chemistry", Part II, 3rd ed, Wiley-Interscience, New York, 1970, p 1443.
(7) A recent report described the use of titanium tetrachloride in the synthesis of vinyl sulfides from ketones and mercaptans; attempts to apply this method to our system, however, have so far been unsuccessful. T. MuKaiyama and K. Saigo, *Chem. Lett.*, 479 (1973).
(8) J. A. Profitt, D. W. Watt, and E. J. Corey, *J. Org. Chem.*, **40**, 127 (1975).
(9) J. A. Profitt and H. H. Ong, *J. Org. Chem.*, **44**, 3972 (1979).
(10) H. Rapoport, C. H. Lovell, H. R. Reist, and M. E. Warren, Jr., *J. Am. Chem. Soc.*, **89**, 1942 (1967).

Table I. 11-[(β-Aminoethyl)thio]dibenz[*b*,*f*]oxepins and Dihydro Derivatives[a]



5-15 (type I)



16-25 (type II)

no.	X	R_1	R_2	starting material	method	mp, °C	yield,[b] %	recrystn solvent[c]	formula	anal.
5a	H	CH_3	CH_3	3a	D	147-148	80	E-G	$C_{18}H_{19}NOS \cdot C_2H_2O_4$[f]	C, H, N, S
5b	F	CH_3	CH_3	3b	D	196-198	68	A	$C_{18}H_{18}FNOS \cdot HBr$	C, H, Br, N
5c	Cl	CH_3	CH_3	3c	D	183-184	41	E-G	$C_{18}H_{18}ClNOS \cdot C_2H_2O_4$[f]	C, H
5d	SCH_3	CH_3	CH_3	3d	D	148-150	83	A-E-G	$C_{19}H_{21}NOS_2 \cdot C_2H_2O_4$[f]	C, H
6a	H	C_2H_5	C_2H_5	3a	D	138-140	67	A-E	$C_{20}H_{23}NOS \cdot C_2H_2O_4$[f]	C, H, N
6d	SCH_3	C_2H_5	C_2H_5	3d	D	184-186	69	A	$C_{21}H_{25}NO_2S \cdot HBr$	C, H, N
7a	H	i-C_3H_7	i-C_3H_7	3a	D	65-66	54	H	$C_{22}H_{27}NOS$	C, H, N
8a	H	H	$(CH_2)_2C_6H_5$	3a	D	164-165	57	A-E-G	$C_{24}H_{23}NOS \cdot C_4H_4O_4$[g]	C, H, N
9a	H	CH_3	$C(=O)OC_6H_5$	5a	H	103-104	65	E-H	$C_{24}H_{21}NO_3S$	C, H, N
9b	F	CH_3	$C(=O)OC_6H_5$	5b	H	*e*	72	*d*	$C_{24}H_{20}FNO_3S$	C, H, F, N
9c	Cl	CH_3	$C(=O)OC_6H_5$	5c	H	*e*	65	*d*	$C_{24}H_{20}ClNO_3S$	*h*
9d	SCH_3	CH_3	$C(=O)OC_6H_5$	5d	H	*e*	72	*d*	$C_{25}H_{23}NO_3S_2$	C, H, N
10a	H	CH_3	H	9a	I	207-208	82	E-G	$C_{17}H_{17}NOS \cdot C_2H_2O_4$[f]	C, H, N
10b	F	CH_3	H	9b	I	135-136	69	A-E-G	$C_{17}H_{16}FNOS \cdot C_4H_4O_4$[g]	C, H, F, N
10c	Cl	CH_3	H	9c	I	153-154	63	E-G	$C_{17}H_{16}ClNOS \cdot C_4H_4O_4$[g]	C, H, Cl, N
10d	SCH_3	CH_3	H	9d	I	160-162	97	A	$C_{18}H_{19}NO_2S_2 \cdot C_4H_4O_4$[g]	C, H, N
11a	H	CH_3	C_2H_5	10a	J	126-128	47	A-E	$C_{19}H_{21}NOS \cdot C_2H_2O_4$[f]	H, N, C[i]
11d	SCH_3	CH_3	C_2H_5	10d	J	112-114	46	E-F	$C_{20}H_{23}NOS_2 \cdot C_4H_4O_4$[g]	C, H, N
12a	H	CH_3	$CH_2C{\equiv}CH$	10a	J	140-142	26	A-E-G	$C_{20}H_{19}NOS \cdot C_2H_2O_4$[f]	C, H, N
13a	H	CH_3	CH_2-c-C_3H_5	10a	J	137-139	54	F	$C_{21}H_{23}NOS \cdot C_2H_2O_4$[f]	C, H, N
14a	H	CH_3	CN	5a	F	54-56	51	G-H	$C_{18}H_{16}N_2OS$	C, H
15a	H			27a	G	181-183	52	E-G	$C_{20}H_{21}NO_2S \cdot C_2H_2O_4$[f]	C, H, N
16a	H	CH_3	CH_3	5a 4a	E D	168-169	81 85	A-G	$C_{18}H_{21}NOS \cdot C_2H_2O_4$[f]	C, H, N, S
16b	F	CH_3	CH_3	5b 4b	E D	169-171	90 24	A	$C_{18}H_{20}FNOS \cdot C_2H_2O_4$[f]	C, H, F, N
16c	Cl	CH_3	CH_3	5c 4c	E D	139-141	88 30	A-G	$C_{18}H_{20}ClNOS \cdot C_2H_2O_4$[f]	C, H, N
16d	SCH_3	CH_3	CH_3	5d 4d	E D	100-102	72 44	A-E	$C_{19}H_{23}NOS_2 \cdot C_4H_4O_4$[g]	C, H, N
17a	H	C_2H_5	C_2H_5	4a	D	109-111	40	A	$C_{20}H_{25}NOS \cdot C_2H_2O_4$[f]	C, H, N
17c	Cl	C_2H_5	C_2H_5	4c	D	127-128	21	A	$C_{20}H_{24}ClNOS \cdot C_2H_2O_4$[f]	C, H, N
17d	SCH_3	C_2H_5	C_2H_5	4d	D	118-120	46	A	$C_{21}H_{27}NOS_2 \cdot HBr$	C, H, N
18a	H	CH_3	H	26a	G	102-104	56	A-E	$C_{17}H_{19}NOS \cdot C_4H_4O_4$[g]	C, H, N, S
18b	F	CH_3	H	26b	G	118-120	57	A-G	$C_{17}H_{18}FNOS \cdot C_4H_4O_4$[g]	C, H, F, N
18c	Cl	CH_3	H	26c	G	138-140	62	A-G	$C_{17}H_{18}ClNOS \cdot C_4H_4O_4$[g]	C, H, Cl, N

18d	SCH_3	CH_3	H	26d	G	138–140	75	E-G	$C_{18}H_{21}NOS_2 \cdot C_4H_4O_4$[g]	C, H, N
19a	H	C_2H_5	H	16a	G	205–207	86	F	$C_{18}H_{21}NOS \cdot C_2H_2O_4$[f]	C, H, N
20b	F	$CH_2CH{=}CH_2$	H	16b	G	100–102	61	A–E	$C_{19}H_{20}FNOS \cdot C_4H_4O_4$[g]	C, H, F, N
21b	F	CH_2-c-C_3H_5	H	16b	G	124–125	27	A–E	$C_{20}H_{22}FNOS \cdot C_4H_4O_4$[g]	C, H, F, N
22a	H	$(CH_2)_2C_6H_5$	H	8a	E	125–126	87	A–E	$C_{24}H_{25}NOS \cdot C_4H_4O_4$[g]	C, H, N
22b	F	$(CH_2)_2C_6H_5$	H	26b	G	132–134	66	A–E	$C_{24}H_{24}FNOS \cdot C_4H_4O_4$[g]	C, H, N
23a	H			26a	G	196–198	73	A–E–G	$C_{20}H_{23}NO_2S \cdot C_2H_2O_4$[f]	H, N, C[j]
24a	H			26a	G	180–182	68	A–G	$C_{21}H_{26}N_2OS \cdot 2HBr$	C, H, N
25a	H			26a	G	176–178	53	E–F	$C_{28}H_{28}NO_2S \cdot HCl$	C, H, F, N

[a] All compounds exhibited IR, ^1H NMR, and MS spectra consistent with the structures. [b] Isolated yield; no efforts were made to optimize these yields. [c] A = acetone; B = benzene; C = cyclohexane; D = water; E = ether; F = ethanol; G = methanol; H = hexane; I = pentane. [d] Purified by column chromatography over silica gel, dichloromethane as eluant. [e] Isolated as a heavy oil. [f] Acid oxalate salt. [g] Acid maleate salt. [h] Mass spectrum: *m/e* 437, molecular ion of the free base. [i] C: calcd, 61.23; found, 60.77. [j] C: calcd, 62.82; found, 62.39.

amines **10a–d** so prepared could be readily alkylated in DMF with an appropriate halide to **11a–d**, **12a**, and **13a** (method J).

Pharmacology and Discussion of Results

The title compounds were evaluated in a battery of five pharmacological tests to assess potential analgesic/CNS activity; results from these studies are given in Table III. Additional biological data for selected compounds showing marked activity in the primary screens will also be presented in conjunction with SAR discussions.

Analgesic activity was determined by measuring the inhibition of phenyl-*p*-quinone-induced writhing (PQW) and the delay in response to noxious heat stimuli in mice (D'Amour–Smith tail-flick method). The PQW test is used to detect both weak and strong analgesic, while the tail-flick assay is sensitive to the opiate-like, strong analgesics.

In the PQW assay, optimum activity of the pentazocine range was found in **10a,b** of type I and **16b** and **18b** of type II, all of which carry N-substituents no greater than a methyl radical. Increasing the size of one or both N-substituents in the unsubstituted series (X = H) clearly resulted in a reduction of activity, at least among the type I congeners, as exemplified by **6a**, **7a**, and **11a**. Similar correlations, however, are less evident among the type II compounds, although the most active analogues from this group are all free of steric bulk at the nitrogen terminals (**16b** and **18a,b**). It is interesting to note that the presence of a *N*-phenethyl group, a typical agonist pharmacophore for the benzomorphans and morphinans, exerts, surprisingly, a detrimental effect on the PQW activity of the present series. Investigation of the effect of nuclear substitution has led to the observation that while a fluoro substituent enhanced activity among some type II compounds (**16b** and **18b** vs. **16a** and **18a**), either no effect or a negative one was observed in the type I series (**10b** and **5b** vs. **10a** and **5a**), and replacement of the hydrogen at C_2 with chlorine generally caused a reduction in activity (**5c**, **10c**, **17c**, and **18c**). The effect of a methylthio group in this test has remained somewhat ambiguous.

In general, the tail-flick potency exhibited by the title compounds in Table III ranged from weakly active[11] to approximately one-half or three-fourths as active as morphine (**16b** and **18b**, respectively), including some which were on the same order of pentazocine (**5d**, **10a**, **10b**, and **18b**). Analogous to the PQW findings, optimum activity in the tail-flick assay also resides in analogues with small N-substituents (R_1, $R_2 \leq$ methyl) and none of the compounds showing marked or moderate activity in this test carries a chlorine substituent.

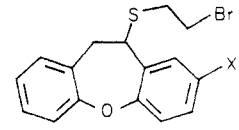
In addition to analgesic efficacy, compounds **10b**, **16b**, and **18b** were shown to be independent of a narcotic receptor interaction: no significant effect on their tail-flick potency was observed following subcutaneous administration of naloxone.[12] Furthermore, none of these compounds exhibited a high degree of dependence-producing potential in the naloxone-precipitated jumping test as described by Way et al.[13] At doses which totaled 40–60

(11) With the exception of 13a and 18d, all compounds tested caused a delayed response to the noxious heat stimulus; ED_{50} values were not determined if the response was less than 50%.

(12) The ED_{50} values (95% confidence limits) before and after naloxone (10 mg/kg sc) for **10b** were 11.3 (6.7–22.3) and 8.3 (4.4–21.3) mg/kg sc; for **18b**, the ED_{50} values were 5.1 (1.9–9.1) and 7.2 (2.6–13.4) mg/kg sc, respectively. Administration of **16b** at 25 mg/kg sc resulted in 100% analgesia in mice, regardless of the presence or absence of naloxone (10 mg/kg sc).

(13) E. L. Way, H. H. Loh, and F. Shen, *J. Pharmacol. Exp. Ther.*, **167**, 1 (1969).

Table II. 11-[(β-Bromoethyl)thio]-10,11-dihydrodibenz[b,f]oxepins[a]



compd	X	starting material	method	mp, °C	yield,[b] %	recrystn solvent[c]	formula	anal.
26a	H	16a	F	77-78	85	H	$C_{16}H_{15}BrOS$	C, H, Br
26b	F	16b	F	46-48	84	H	$C_{16}H_{14}BrFOS$	C, H, F, Br
26c	Cl	16c	F	*e*	67	*d*	$C_{16}H_{14}BrClOS$	C, H, S
26d	SCH_3	16d	F	64-66	31	H	$C_{17}H_{17}BrOS_2$	C, H, Br

[a-e] See corresponding footnotes to Table I.

Table III. Pharmacology of 11-[(β-Aminoethyl)thio]dibenz[b,f]oxepins and Dihydro Derivatives

compd[a]	PQW writhing ED_{50}, mg/kg sc	tail flick ED_{50}, mg/kg sc	TBZ ptosis ED_{50}, mg/kg ip	MTZ lethality ED_{50}, mg/kg po	amphetamine lethality ED_{50}, mg/kg po
5a	5.8 (5.1-6.5)	>25	8.4 (6.9-11.0)	~50[d]	>20
5b	10.3 (9.3-11.3)	23.9 (5.6-24.0)	4.3 (3.9-4.8) 5.6 (4.8-6.8)[b]	>50	>20
5c	>25	>25	>20	>50	>20
5d	5.3 (4.2-6.4)	14.1 (5.2-27.3)	9.4 (7.8-11.8)	>50	>20
6a	~25[d]	>25	>20	>50	>20
6d	>25	>25	>20	>50	>20
7a	>25	>25	~25[d]	>50	c
8a	13.6 (13.3-13.9)	⩾25[d]	>20	>50	>20
10a	2.3 (1.6-3.0)	11.6 (6.4-22.0)	3.4 (3.1-3.8)	~50[d]	>20[e]
10b	2.3 (1.9-2.8) 24.7 (21.9-27.4)[b]	11.3 (6.7-22.3)	0.32 (0.26-0.38) 2.30 (2.1-2.5)[b]	>50	>50
10c	~16.0[d]	⩾25[d]	5.9 (5.2-6.8)	>50	⩾20[d]
10d	⩽25[d]	>25	6.1 (5.4-6.8)	>50	>20
11a	~25[d]	>25	20[d]	>50	c
11d	~25[d]	>25	9.0 (7.5-11.3)[b]	>50	>20
12a	~25[d]	>25	12.2 (10.4-14.9)	>50	c
13a	6.0 (5.9-6.2)	>25	>20	>50	c
15a	>25	>25	c	>50	c
16a	>25	>25	7.0 (5.8-8.9)	~50[d]	>20
16b	1.9 (1.5-2.3)	7.9 (6.4-9.5)	2.0 (1.9-2.1) 7.6 (6.4-9.5)[b]	52.3 (20.5-108.6)[h]	>20
16c	<25[f]	>25	3.5 (3.1-3.9) 11.7 (8.3-20.3)[b]	30.0 (23.3-47.5)[h]	c
16d	10.4 (10.2-10.6)	>25	16.0 (12.1-23.3)	>50	>20
17a	6.9 (6.1-7.7)	⩾25[d]	>20	>50	>20
17c	14.8 (11.2-21.3)	>25	>20	>50	c
17d	~25[d]	>25	>20	>50	>20
18a	4.8 (4.4-5.2)	11.6 (6.5-16.2)	0.9 (0.8-1.3) 3.4 (2.8-4.7)[b]	~50[d]	>20
18b	1.4 (1.1-1.7) 13.0 (12.6-13.3)[b]	5.1 (2.0-9.1)	0.8 (0.7-0.8) 3.0 (2.4-3.5)[b]	31.6 (8.3-40.5)[h]	>20
18c	5.3 (4.8-5.8) 11.3 (10.9-11.8)[b]	22.4 (6.4-27.5)	1.5 (1.3-1.7) 6.1 (4.5-9.3)[b]	<50[f]	>20
18d	6.4 (6.1-6.8)	>25	~12.7[d]	~50[d]	>20
19a	6.6 (6.2-7.1)	>25	~20[d]	>50	c
20b	10.6 (10.3-11.1)	>25	3.5 (2.9-4.2)	>50	>20
21b	10.7 (10.2-11.2)	>25	>20	>50	>20
22a	9.3 (9.2-9.5)	>25	>20	>50	23.0 (18.9-30.1)
22b	>10[g]	>25	c	>50	>20
23a	⩽25[d]	>25	>20	>50	>20
24a	6.3 (6.2-6.3)	>25	>20	>50	c
25a	10.9 (10.1-11.9) 9.5 (9.0-9.9)[b]	>25	>20	>50	>20
pentazocine	2.4 (2.1-2.9)	14.6 (7.4-24.4)		>25	>20
morphine	0.36 (0.33-0.40)	3.8 (1.7-9.5)	8.1 (6.8-9.7)	>25	>20
imipramine	~11[e]	>25	1.3 (0.9-1.7)	~50[d]	>20
diazepam				1.4 (0.9-2.4)	>20
chloropromazine				>50	2.8 (1.9-4.3)

[a] The vehicle control used in all five biological tests consists of distilled water and a few drops of Tween 80. [b] Determined by oral administration. [c] Not determined. [d] Estimated graphically without statistical analysis. [e] Determined by intraperitoneal administration. [f] ED_{50} value was not determined due to poor dose-response relationships. [g] Overt effects required a lower initial screening dose. At 10 mg/kg sc, 34% inhibition was observed.

times the ED_{50} values, there was only a 7-40% incidence of jumping in mice. Using the phenylquinone-induced writhing test, there was no apparent tolerance following a 4-day administration of **10b** at a total dose of 128 mg/

kg.[14]

Antidepressant activity of the title compounds was determined by measuring their ability to prevent tetrabenazine-induced ptosis (TBZ) in mice. As can be seen from Table III, a preliminary SAR pattern deduced from this assay bears some similarity to that obtained from analgesic testing: compounds displaying significant activity in this screen were, again, those bearing short chain N-substituents (**10b**, **16b**, and **18a–c**). The potentiating effect of a fluorine substituent was most dramatically demonstrated in **10b**, which was 10 times more active than its unsubstituted congener (**10a**) and 4 times more active than imipramine. With a methylthio group at C_2, activity diminished drastically, whereas chloride exerted a negative effect only on the type I targets. It is noteworthy that the anti-TBZ activity was markedly reduced with the introduction of an additional aromatic ring (**8a**, **22a**, and **25a**).

Anticonvulsant activity of the title compounds was assessed by measuring their ability to prevent pentylenetetrazol (MTZ) induced lethality in mice. Some activity was shown by a number of compounds of both structural types, among them, **5a**, **10a**, **16a–c**, and **18a–c**. To round out the CNS profile, compounds **10b**, **16b**, and **18b** were tested in the Geller conflict procedure[15a,b] for potential anxiolytic activity, all of which were found inactive at 16 mg/kg ip.

Antipsychotic activity was assessed in the amphetamine aggregation toxicity model (AAT) for the majority of target compounds listed in Table III and in the Sidman avoidance paradigm[16] for a selected few (**10b**, **16b**, and **18b**). Only **10c** and **22a** exhibited weak activity in the AAT, and at 10 mg/kg, ip, none of the three compounds tested was active in blocking the conditioned avoidance-response in rats.

In summary, the synthesis and biological evaluation of a series of [(β-aminoethyl)thio]dibenz[b,f]oxepins and their dihydro derivatives have led to the identification of **10b**, **16b**, and **18b** as potentially nonnarcotic analgetic agents with an unique profile. Of special appeal to us is the combination of analgesic and antidepressant activity shown by **10b**, as well as by several of its congeners, in view of the growing number of reports contending the therapeutic advantages of antidepressants in the management of pain.[17a-c]

Experimental Section

The structures of all compounds are supported by their IR (Perkin-Elmer 457) and ^1H NMR (JEOLCO C6OHL; tetramethylsilane) spectra. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectral data were determined with a Finnigan Model 4000 GS–MS equipped with a INCOS data system. Where analyses were indicated only by symbols of the elements, the analytical results obtained for those elements (performed by Micro-Tech Laboratories, Skokie, Ill.) were within 0.4% of theoretical values.

2-(4-Fluorophenoxy)phenylacetic Acid (2b). Method A. A mixture of 2-(4-fluorophenoxy)phenylacetonitrile (**1b**;[3] 4.85 g, 21.3 mmol) and 3.4 g of potassium hydroxide in 32 mL of 80% aqueous ethanol was stirred at reflux for 3 h. The cooled solution was concentrated in vacuo to a solid residue, and the residue was redissolved in 50 mL of water. Extraction with ether removed a small amount of neutral material, and the aqueous solution was carefully acidified to pH 1 with concentrated hydrochloric acid. The precipitated solid was filtered off, washed, and air-dried to give 4.73 g (90%) of white prisms, mp 97–98.5 °C. Recrystallization from cyclohexane did not change the melting range. Anal. ($C_{14}H_{11}FO_3$) C, H, F.

2-Phenoxyphenylacetic acid (2a) was obtained from **1a** in 88% yield by method A as colorless crystals, mp 89–91 °C (lit.[18] 90–91 °C).

2-(4-Chlorophenoxy)phenylacetic acid (2c) was obtained from **1c** in 76% yield by method A as off-white crystals, mp 120–122 °C (lit.[18] 119–121.5 °C).

2-[4-(Methylthio)phenoxy]phenylacetic acid (2d) was obtained from **1d** in 82% yield by method A as colorless crystals, mp 98–100 °C (lit.[18] 96–99 °C).

2-Fluoro-10,11-dihydro-11-oxodibenz[b,f]oxepin (3b). Method B. To 3.3 g (13.3 mmol) of **2b** was added 5.6 mL of freshly distilled thionyl chloride, and the mixture was heated on a steam bath for 10 min. The excess reagent was removed under reduced pressure, and the residue was dissolved in 30 mL of 1,2-dichloroethane to form a clear solution, which was then added dropwise to a mixture of 1.94 g of aluminum chloride in 5 mL of 1,2-dichloroethane. The mixture was stirred at reflux for 2 h, cooled, and poured onto 150 g of ice–water. The organic material was extracted into ether, washed with brine, and dried over $MgSO_4$. Removal of solvent in vacuo left a reddish solid, which was decolorized with Darco and recrystallized from cyclohexane to give 2.43 g (80%) of off-white crystals, mp 85.5–87.5 °C. Anal. ($C_{14}H_9FO_2$) C, H, F.

10,11-Dihydro-10-oxodibenz[b,f]oxepin (3a) was obtained from **2a** in 77% yield by method B to give prisms, mp 57–58 °C (lit.[18] 56 °C).

2-Chloro-10,11-dihydro-11-oxodibenz[b,f]oxepin (3c) was obtained from **2c** in 71% yield by method B to give off-white crystals, mp 83–85 ° C (lit.[18] 83–84 °C).

10,11-Dihydro-2-(methylthio)-11-oxodibenz[b,f]oxepin (3d) was obtained from **2d** in 69% yield by method B to give white prisms, mp 62–64 °C (lit.[18] 64–65 °C).

2-Fluoro-10,11-dihydrodibenz[b,f]oxepin-11-ol (4b). Method C. To a solution of **3b** (1.0 g, 4.4 mmol) in 10 mL of absolute ethanol was added finely pulverized sodium borohydride (0.58 g) in portions. The solution was stirred at room temperature for 1 h and poured onto 50 mL of ice–water. Ethanol was removed by distillation at reduced pressure until the volume was reduced to 20 mL. To this solution was then added 35 mL of 10% sodium hydroxide and the organic material was extracted thrice with ether (100-mL portions). The dried ($MgSO_4$) ethereal solution was concentrated in vacuo to a brownish oil, which was purified by column chromatography over silica gel; elution with chloroform gave 1.0 g (99%) of a pale yellowish oil, **4b**, which was analytically pure. Anal. ($C_{14}H_{11}FO_2$) C, H.

10,11-Dihydrodibenz[b,f]oxepin-10-ol (4a) was obtained from **3a** in 89% yield by method C to give a heavy oil, bp 150–154 °C (1.5 mmHg) [lit.[18] 184–186 °C (3.5 mmHg)].

2-Chloro-10,11-dihydrodibenz[b,f]oxepin-11-ol (4c) was obtained from **3c** in 91% yield by method C as rhombic crystals, mp 65–67 °C (lit.[18] 63–65 °C).

10,11-Dihydro-2-(methylthio)dibenz[b,f]oxepin-11-ol (4d) was obtained from **3d** in 88% yield by method C as colorless needles: mp 76–77 °C (lit.[18] 75–77 °C).

10-[[β-(Dimethylamino)ethyl]thio]dibenz[b,f]oxepin Oxalate (5a). Method D. A mixture of **3a** (1.2 g, 5.7 mmol) and 2.4 g of β-(dimethylamino)ethylthiol hydrochloride in 10 mL of glacial acetic acid containing 2 mL of boron trifluoride was stirred at room temperature for 16 h, followed by heating on a steam bath for 30 min. The cooled solution was poured onto 200 g of crushed ice and, without delay, was basified with a large excess of 40% sodium hydroxide. The basic material was extracted into ether, washed, dried and concentrated to a reddish oil. The crude amino sulfide was purified by column chromatography over silica gel; elution with 15% methanol–CH_2Cl_2 (v/v) afforded a colorless oil

(14) The ED_{50} for vehicle was 2.3 (1.9–2.8) mg/kg sc and for **10b** was 2.0 (1.9–2.1) mg/kg sc.

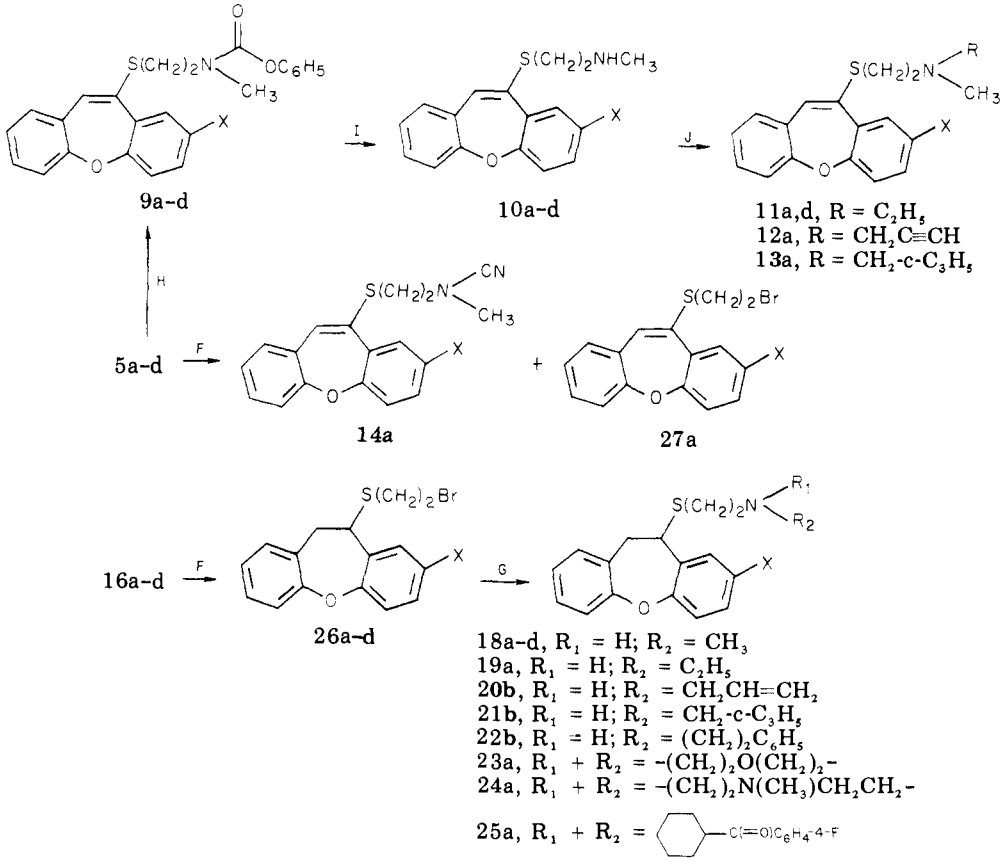
(15) (a) I. Geller, J. T. Kulak, and J. Seifter, *Psychopharmacologia*, **3**, 374 (1962); (b) I. Geller and J. Seifter, *ibid.*, 1, 482 (1960).

(16) C. I. E. Niemegeers, F. J. Verbruggen, and P. A. J. Janssen, *Psychopharmacologia*, **16**, 161 (1969).

(17) (a) *J. Am. Med. Assoc., Med. News*, **240**, 1225 (1977); (b) R. Lee and P. S. J. Spencer, *J. Int. Med. Res.*, **5** (Supplement 1), 146 (1977); G. Beaumont, *ibid.*, **4**, (Supplement 2), 56 (1976).

(18) V. Seidlova, K. Pelz, E. Allerova, I. Jirkovsky, J. Metysova, and M. Protiva, *Collect. Czech. Chem. Commun.*, 34, 2258 (1969).

Scheme II



a, X = H; b, X = F; c, X = Cl; d, X = SCH_3

which was converted to a crystalline oxalate (**5a**) in ether. Properties of **5a**, and of **5b–d**, **6a–d**, **7a**, and **8a** prepared in a similar manner, are included in Table I.

10-[[β-(Dimethylamino)ethyl]thio]-10,11-dihydrodibenz[*b*,*f*]oxepin Oxalate (16a). Method E. To 3.0 g (10.0 mmol) of the free base from **5a** in 50 mL of anhydrous methanol (Mallinckrodt, Karl Fischer) was added, at 10 °C, 1.2 g (50 mmol) of magnesium shavings. The reaction mixture was stirred at 10–20 °C for 30 min and at room temperature for 1 h. The methanolic solution was then decanted from the unreacted magnesium and acidified cautiously with a large excess of 3 N hydrochloric acid. The acidic mixture was extracted thrice with 50-mL portions of $CHCl_3$, and the combined organic solution was washed with water, dried ($MgSO_4$), and concentrated to a brownish oil. The crude product, which was homogeneous by TLC (silica gel, 25% methanol–CH_2Cl_2), was converted to 3.3 g (85%) of a crystalline oxalate (**16a**). Properties of **16a**, and of **16b–d** and **22a** prepared in a similar manner, are included in Table I.

Method D. Alternatively, **16a** was prepared by method D from 1.6 g (7.5 mmol) of **4a**, 3.2 g of β-(dimethylamino)ethylthiol hydrochloride, and 2.6 mL of boron trifluoride etherate in 13 mL of glacial acetic acid as previously described for **5a**. After working up, 2.4 g (81%) of **16a** was obtained, which was identical with an authentic sample prepared by method E. Similarly, **16b–d** and **17a,c,d** could be prepared by method D from **4b–d** with varying degrees of success; yields from these reactions are also included in Table I.

10-[(β-Bromoethyl)thio]-10,11-dihydrodibenz[*b*,*f*]oxepin (26a). Method F. A solution of 5.5 g (18 mmol) of **16a** in 100 mL of anhydrous $CHCl_3$ was added dropwise to 2.8 g of cyanogen bromide in 50 mL of $CHCl_3$ in the presence of 6.0 g of potassium carbonate. After stirring at room temperature for 10 min, the mixture was filtered and the filtrate was concentrated to a thick oil which crystallized on standing. The crude product was recrystallized from hexane to give 4.2 g (65%) of **26a**. Properties of **26a**, and of **26b–d** prepared in a similar manner, are included in Table II.

10,11-Dihydro-10-[[β-(methylamino)ethyl]thio]dibenz[*b*,*f*]oxepin Maleate (18a). Method G. Anhydrous methylamine was bubbled through a solution of **26a** (2.6 g, 7.7 mmol) in 30 mL of DMF until it became saturated. The solution was stoppered and allowed to stand at room temperature for 16 h before 150 g of ice–water was added. The organic materials were extracted with three 100-mL portions of ether, and the combined ether solution was washed four times with water to remove the bulk of DMF and excess of methylamine. After drying over $MgSO_4$, the ether solution was treated with an ethereal solution of maleic acid to give 1.75 g (56%) of **18a**. Properties of **18a**, and of **18b–d** prepared in a similar manner, are included in Table I.

2-Fluoro-10,11-dihydro-11-[[β-(phenethylamino)ethyl]thio]dibenz[*b*,*f*]oxepin Maleate (22b). Method G. A mixture of 3.3 g (9.3 mmol) of **26b** and 1.5 g of sodium bicarbonate in 24 mL of anhydrous Me_2SO containing a few crystals of potassium iodide was stirred at room temperature for 5 h. The mixture was diluted with water and extracted thrice with 150-mL portions of ether, and the combined ether solution was dried over $MgSO_4$. Removal of solvent in vacuo left a brownish oil, which was chromatographed over a column of silica gel packed in CH_2Cl_2. Elution with 5% methanol–CH_2Cl_2 (v/v) yielded, as the main component, a colorless oil which was converted to 3.12 g (66%) of a crystalline maleate (**22b**) with ethereal maleic acid. Properties of **22b**, and of **19a**, **20b**, **21b**, and **23a–25a** prepared in a similar manner, are included in Table I.

Reaction of Cyanogen Bromide with 10-[[β-(Dimethylamino)ethyl]thio]dibenz[*b*,*f*]oxepin (5a). Method F. A solution of the free base (1.0 g, 3.4 mmol) from **5a** in 20 mL of $CHCl_3$ was added dropwise to a stirred mixture of 0.36 g of cyanogen bromide and 2.3 g of potassium carbonate in 10 mL of $CHCl_3$. The mixture was refluxed for 2 h, cooled, and filtered. Upon concentration in vacuo at 60 °C, a colorless oil remained, which was found to be a 1:1 mixture by TLC (silica gel F-254, CH_2Cl_2). The crude products were thus separated by column chromatography over silica gel packed in CH_2Cl_2. Elution with a large excess of CH_2Cl_2 gave, first, 0.47 g (42%, R_f 0.88) of **27a**: mp 106–107 °C; needles from hexane. Anal. ($C_{16}H_{13}BrOS$) C, H, Br, S. Further elution with CH_2Cl_2 gave 0.53 g (50%, R_f 0.51)

of 14a. Properties of 14a are included in Table I.

10-[[β-[N-Methyl-N-(phenoxycarbonyl)amino]ethyl]thio]dibenz[b,f]oxepin (9a). Method H. A solution of 9.8 g (32.8 mmol) of the free base from **5a** in 50 mL of CH_2Cl_2 was stirred at room temperature with 10.0 g of potassium carbonate while 5.65 g (36.1 mmol) of phenyl chloroformate in 50 mL of CH_2Cl_2 was added dropwise over 60 min. The mixture was stirred at room temperature for an additional 24 h, filtered, and diluted with 200 mL of ether. The organic solution was washed with 10% sodium hydroxide and water and dried over $MgSO_4$ overnight. Removal of the solvents under reduced pressure left a reddish oil, which was purified by passing through a short column of silica gel packed in ether; elution with ether afforded 10.6 g (80%) of **9a**, which was analytically pure. Properties of **9a**, and of **9b–d** prepared in a similar manner, are included in Table I.

10-[[β-(Methylamino)ethyl]thio]dibenz[b,f]oxepin Oxalate (10a). Method I. A mixture of **9a** (1.6 g, 4 mmol) and 3.4 g of potassium hydroxide in 40 mL of ethylene glycol was heated at 150–160 °C for 60 min, during which a clear solution was formed. The cooled solution was diluted with 100 g of ice–water and extracted thrice with ether (100-mL portions), and the combined ether solution was dried over K_2CO_3. Removal of the solvent under reduced pressure left a viscous oil which resisted all attempts to crystallization. The crude product was thus converted to 1.25 g (82%) of a crystalline oxalate (**10a**) with ethereal oxalic acid. Properties of **10a**, and of **10b–d** prepared in a similar manner, are included in Table I.

10-[[β-(N-Ethyl-N-methylamino)ethyl]thio]dibenz[b,f]-oxepin Oxalate (11a). Method J. A mixture of 1.5 g (5.3 mmol) of the free base from **10a**, 1.1 g of ethyl iodide, 1.48 g of sodium bicarbonate, and 1.46 g of potassium iodide in 20 mL of DMF was stirred at 80–85 °C for 16 h. The cooled reaction mixture was diluted with 75 mL of water and extracted thrice with 75-mL portions of ether, and the combined solution was dried over $MgSO_4$. Removal of the solvent under reduced pressure yielded a brownish oil, which was chromatographed over adsorption alumina (Fisher) packed in ether. Elution with ether gave the analytically pure tertiary amine which was converted to 1.0 g (47%) of **11a** with ethereal oxalic acid. Properties of **11a**, and of **11d**, **12a**, and **13a** prepared in a similar manner, are included in Table I.

Phenylquinone-Induced Writhing in Mice (PQW). The procedure employed was a modification of the method of Siegmund et al.[19] Groups of five male CD-1 Charles River mice weighing 18–24 g were administered the test drug (sc or po) 15, 30, 60, and 90 min prior to the injection of a phenyl-*p*-benzoquinone solution (0.125% in a 5% aqueous ethanol solution) at the initial screening dose of 25 mg/kg. Control mice were treated with an equal volume of vehicle. After phenylquinone injection, the mice were placed individually in 1000-mL beakers and 5 min later the number of writhes was recorded for a 10-min period. The peak time of test drug activity was thereby determined. A dose–response study was performed in a similar manner, except that ten animals were used at the peak time of activity. Animals were dosed and tested in a randomized manner using four drug doses and one control group. Drug activity is expressed as the percent inhibition of the number of writhes, and an estimated ED_{50} is calculated by a computerized linear-regression analysis.

Tolerance to the antiwrithing activity of **10b** was determined after chronic administration (four times per day at 2-h intervals for 4 days). Doses were increased as a multiple of its ED_{50} value on each successive day. Testing was carried out in the same manner as described above, 16–18 h after the last dose.

Modified D'Amour–Smith Analgesia (Tail-Flick) in Mice. The procedure used was a modification of the test developed D'Amour and Smith[20] and quantified according to Hayashi and Takemori.[21] Groups of ten male Charles River (CD-1) mice were individually placed in a restraining Plexiglas compartment and, subsequently, a noxious stimulus was produced by an intense light beam. The subjects quickly responded by flicking their tails. This reaction time, the intervals between stimulus onset and response, was measured and recorded. Prior to drug administration, two control readings of reaction time were taken. Subjects were discarded if their reaction times varied by more than 1 s or if their inclusion would cause the spread of reaction times to exceed 3 s. For both sets of control readings, a cutoff (co) time was thus determined. Test compounds were administered subcutaneously and control mice received an equal volume (10 mL/kg) of vehicle. For a time response, the animals were tested 15, 30, 45, and 60 min after dosing. Animals which responded after the determined co values were called positive, indicative of analgesia. For a dose range, the animals were tested at the peak time with a minimum of three doses in addition to vehicle control. Percent analgesia was calculated for each dose, and estimated ED_{50} values were determined using a linear-regression analysis.

Antagonism of Tetrabenazine-Induced Ptosis in Mice.[22] The test compound was administered by intraperitoneal injection to male mice (Charles River CD-1) weighing 20 to 30 g in groups of five. Tetrabenazine methanesulfonate (40 mg/kg, ip) was administered 30 min later, and after another 30 min the mice were placed in individual containers. Ptosis was then evaluated on a three-point scale: eyes closed = 2; eyes half-opened = 1; eyes open = 0. A linear-regression analysis of the ptosis scores was used to compute ED_{50} values and 95% confidence intervals.

Antagonism of Metrazol (Pentamethylenetetrazol) Lethality in Mice. Groups in six male (Charles River CD-1) mice weighing 18–30 g were administered the test drug per os at 30, 60, 90, and 120 min prior to the injection of a Metrazol solution (K & K, 150 mg/kg, ip) at the initial screening dose of 40 mg/kg. Control mice were treated with an equal volume of vehicle (distilled water and a drop of Tween 80). Those animals surviving 15 min after metrazol injection were considered protected. The time period with the greatest percent protected was used for the dose-range study, which was performed in a similar manner except that 50 animals (10 per group) were tested. Animals were tested in a randomized manner using four drug doses and one control group. ED_{50} values and 95% confidence limits are calculated by means of a linear-regression analysis.

Antagonism of Amphetamine Aggregate Toxicity in Mice.[23] Groups of ten male (Charles River CD-1) mice weighing 18–28 g were administered the test compound (po or ip) and placed in separate plastic carriers (10.5 × 8 × 6 in.) to maintain group integrety; control subjects received the distilled water–Tween 80 solution, and all administrations were in volume proportionate to mL/100 g. Sixty minutes postdrug the mice were dosed with *d*-amphetamine sulfate by subcutaneous injection (21 mg/kg), and immediately afterwards the mice of each treatment group were aggregated in "stick cages" (10 × 10 × 10 cm wire mesh with 0.25-in. holes) in groups of five. These cages were subsequently placed in close-fitting containers which were maintained at an elevated temperature of 80–84 °F. At the end of the 1st h postamphetamine, the unprotected mice began to expire and, to maintain a semblance of the aggregated condition, the expired mice were removed and replaced by marked, untreated mice. This was done at 15-min intervals for the following 4 h, which were also convenient intervals for examination of chamber temperature. At the end of 5-h postamphetamine, the number dead in each treatment group was counted. All control subjects (solvent + amphetamine) should have expired. Drug activity is expressed as the percent protection against lethality, and ED_{50} values are calculated by a linear-regression analysis with 95% confidence limits.

Acknowledgment. The authors express their appreciation to Marc Agnew and Peter Kranack for spectral data and to Minh G. Ma, Karin Theurer, Adrienne Minet, and Mark Szewczak for performing pharmacological assays. We also gratefully acknowledge Eve Memoli for assistance in preparation of this manuscript.

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