with monochromatic Cu Kα radiation using about 24 h for data collection in the ω scan mode.

The structure was found by classical Patterson and Fourier refinement methods, followed by full-matrix least-squares refinement computed on a Burroughs B6700. When the conventional R factor was 8.5%, examination of a difference map showed that the largest errors in the model were due to anisotropic vibration of the chloride ion and to the hydrogen atoms whose positions were obvious in this map. Full-matrix refinement with anisotropic temperature factor for the chloride ion only, keeping the H atoms B's constant, converged with an R factor of 4.8%. This refinement also included anomalous dispersion corrections to the atomic scattering factors for all non-H atoms. To determine the absolute configuration, the positions were changed to $\bar{x}$, $\bar{y}$, and $\bar{z}$ and refined in the same way as before. This refinement on the mirror form dropped the R factor to 3.1%, showing clearly that this second trial structure has the correct absolute configuration. Intense low-angle reflections were clearly affected by secondary extinction so that in these final refinements the 29 reflections having sine θ/λ less than 0.15 Å^{-1} were left out. The resulting structure is pictured in Figure 2 and the atomic positions are listed in Table II. The bond angles and distances along with standard deviations are given in Tables II and IV (see paragraph at the end of paper concerning supplementary material).

Opiate Receptor Binding. Guinea pig brain minus cerebellum homogenate was prepared as previously described.[17,18] The homogenate was prepared for the binding assay as 2% wet homogenate pellet weight in 100 mM Tris-HCl buffer without Na^+ present. [^{3}H]Etorphine (33 Ci/mmol, Amersham) was employed as the ^{3}H-labeled opiate ligand. The homogenate (250 μL), [^{3}H]etorphine (final concentration 2.5×10^{-9} M), varying concentrations of (+)-, (−)-, or (±)-**1b**, and Tris-HCl (to a final volume of 500 μL) were used in each determination. The homogenate was allowed 15 min equilibration time with the test compounds at 23 °C before the addition of the ^{3}H-labeled ligand and then incubated at 37 °C for 30 min prior to filtration (Whatman GF/B filters). The filters were placed in counting vials, and the [^{3}H]etorphine was solubilized from the membrane fragments by vortexing in 1 mL of absolute EtOH and counted in 10 mL of dioxane-based (Bray's solution) scintillation cocktail. Each concentration was run in quadruplicate samples, and the mean of the nondisplaced minus the mean of the displaced with 200X excess of levorphanol was plotted as probit values vs. log concentration. The IC_{50} values thus represent the ability of (−)-, (+)- and (±)-**4b** to displace 50% of the [^{3}H]etorphine bound at 2.5 nM concentration.

Acknowledgment. The authors are indebted to S. K. Guthrie for technical assistance, E. L. May of NIH, NIAMDD, for determination of analgetic activities, and to B. M. Cos of the Addiction Research Foundation, Palo Alto, CA, for help in setting up the opiate receptor binding assay. The research was supported in part by NIH Grant NS 05192 and a Scholarly Activity Grant made by the University of the Pacific to D.S.F.

Supplementary Material Available: Tables II–IV containing bond angles and distances along with standard deviations (2 pages). Ordering information is given on any current masthead page.

Isoxicam and Related 4-Hydroxy-*N*-isoxazolyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxides. Potent Nonsteroidal Antiinflammatory Agents

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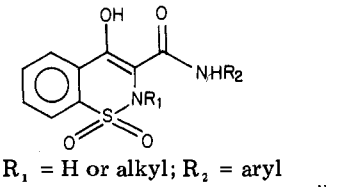
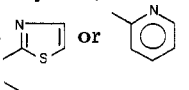
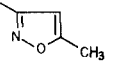
A novel series of antiinflammatory agents, *N*-isoxazolyl-3-carboxamides of 4-hydroxy-2*H*-1,2-benzothiazine 1,1-dioxide, was synthesized and evaluated as antiinflammatory agents in the carrageenin-induced rat paw edema (CIRPE) assay and adjuvant-induced polyarthritis (AIP) assay. Several analogues were found to be equipotent or more potent than aspirin and phenylbutazone. Structure–activity relationships are discussed. One of the compounds, 4-hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine 3-carboxamide 1,1-dioxide (**3a**; isoxicam), was found to be 3 times as potent as phenylbutazone in the CIRPE and in the therapeutic AIP assays. Isoxicam (**3a**) is presently undergoing phase III clinical trial as an antiarthritic drug.

Independent studies in our laboratories,[1] as well as studies by Lombardino et al.,[2] have shown that certain enolizable amides (**1**) have useful antiinflammatory properties. More recently, Lombardino et al.[3] have reported that replacement of the aryl group (R_2) on the amide with

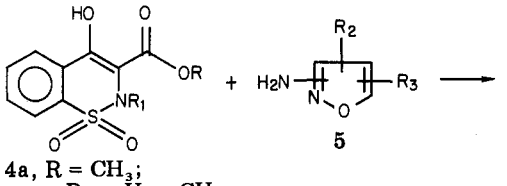


1, R_1 = H or alkyl; R_2 = aryl

2, R_1 = CH_3; R_2 =  or

3a, R_1 = CH_3; R_2 = 

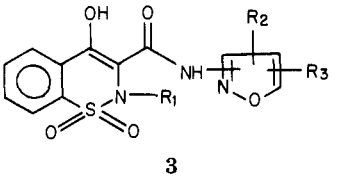
Scheme I



4a, R = CH_3; R_1 = H or CH_3

b, R = Et; R_1 = H or CH_3

5



3

(1) H. Zinnes, N. A. Lindo, J. C. Sircar, M. L. Schwartz, and J. Shavel, Jr., *J. Med. Chem.*, 16, 44 (1973).
(2) J. G. Lombardino, E. H. Wiseman, and W. M. McLamore, *J. Med. Chem.*, 14, 1171 (1971).

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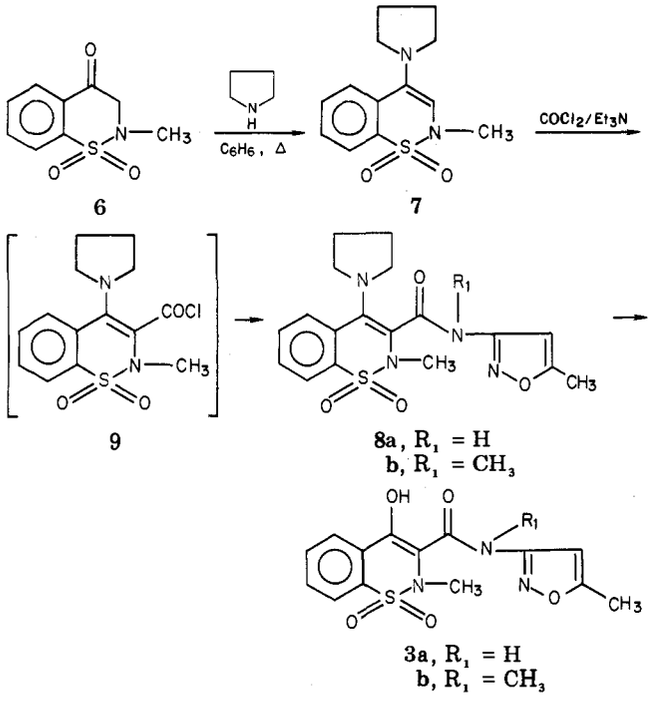
Scheme II



2-thiazole (sudoxicam) or 2-pyridyl (piroxicam) results in clinically[4,5] efficacious compounds, **2**, having increased potency and biological half-life. We describe here a series of related compounds of structure **3** in which the amide nitrogen is substituted with an isoxazole group. One of these, isoxicam (**3a**), is presently undergoing clinical evaluation.

Chemistry. Isoxicam and related amides (**3**) were prepared by heating the ester[2,6] (**4**) with the appropriate 3- or 5-isoxazolamines (**5**) in refluxing xylene or toluene using molecular sieves to remove the alcohol that was formed (Scheme I).

Starting materials (**5**) are commercially available, except for 3-isoxazolamine which was prepared in accordance with the reported procedure.[7] Acetylation of **3a** gave the acetyl derivative (**3c**), which is probably a prodrug of isoxicam.

Isoxicam (**3a**) was also synthesized by the sequence outlined in Scheme II. Pyrrolidine enamine (**7**), prepared from the ketone (**6**),[8] was converted to the acid chloride (**9**) by reaction with phosgene in the presence of an equivalent of triethylamine. This intermediate was reacted without further purification with 2 equiv of 5-methyl-3-isoxazolamine to give the enamine amide, **8a**, in 62% overall yield. Hydrolysis to isoxicam (**3a**) was effected in 92% yield by heating **8a** with a mixture of acetic acid and hydrochloric acid on a steam bath.

The same method was used to synthesize *N*-methylisoxicam (**3d**), which could not be prepared by Scheme I.

A third method for preparing isoxicam (**3a**) is illustrated in Scheme III. The amide **10** obtained by chloroacetylation of 5-methyl-3-isoxazolamine was reacted with sodium saccharin in *N*,*N*-dimethylformamide (DMF) to give the N-substituted saccharin amide **12**. Treatment of **12** with sodium methoxide in DMF resulted in ring enlargement to the desired 1,2-benzothiazine system, but these reaction conditions also caused opening of the isoxazole and reclosure to the oxadiazole **13**. The structural assignment was supported by the spectral data.

This transformation could also be carried out stepwise. Keeping the temperature at 25–30 °C resulted in the isolation of the ring-opened compound **11a**, which could be converted to **13** by utilizing the original reaction conditions (sodium methoxide in DMF at 60–70 °C). This suggests that the rearrangement takes place as depicted through the intermediate **3b**. Further support for this mechanism was given by conversion of **3b** to **13** by treatment with sodium methoxide in DMF at 60 °C. This type of isoxazole rearrangement has been discussed by Van Der Plas.[9]

Compound **13** was readily methylated with methyl sulfate to give **14**. Generation of the desired isoxicam (**3a**) was achieved by heating **14** with triethylamine in xylene at 115–120 °C. The latter reaction could be reversed by heating **3a** with sodium methoxide in DMF at 60 °C. The overall yield (from **12**) was 54%. Isoxicam (**3a**) was also obtained by methylation of **3b**.

In addition, two open-chained analogues were synthesized for comparison of biological activity. Treatment of **12** with aqueous alkali gave the acid **11b**.

The related open-chained compound **20** was prepared by a series of reactions starting with *N*-methylsaccharin (**15**) (Scheme IV). Treatment of the latter with sodium methoxide in methanol in the presence of excess dimethyl sulfate resulted in ring opening and methylation to give **16**. This was reacted with dimethyl sulfoxide anion, and the resulting crude β-ketosulfoxide **18** was reduced with aluminum amalgam to give the ketone **17**. Reactions of **17** with sodium hydride and dimethyl carbonate gave rise to the ester **19**, which was not purified. It was aminolyzed with 3-isoxazolamine to give the target compound **20**.

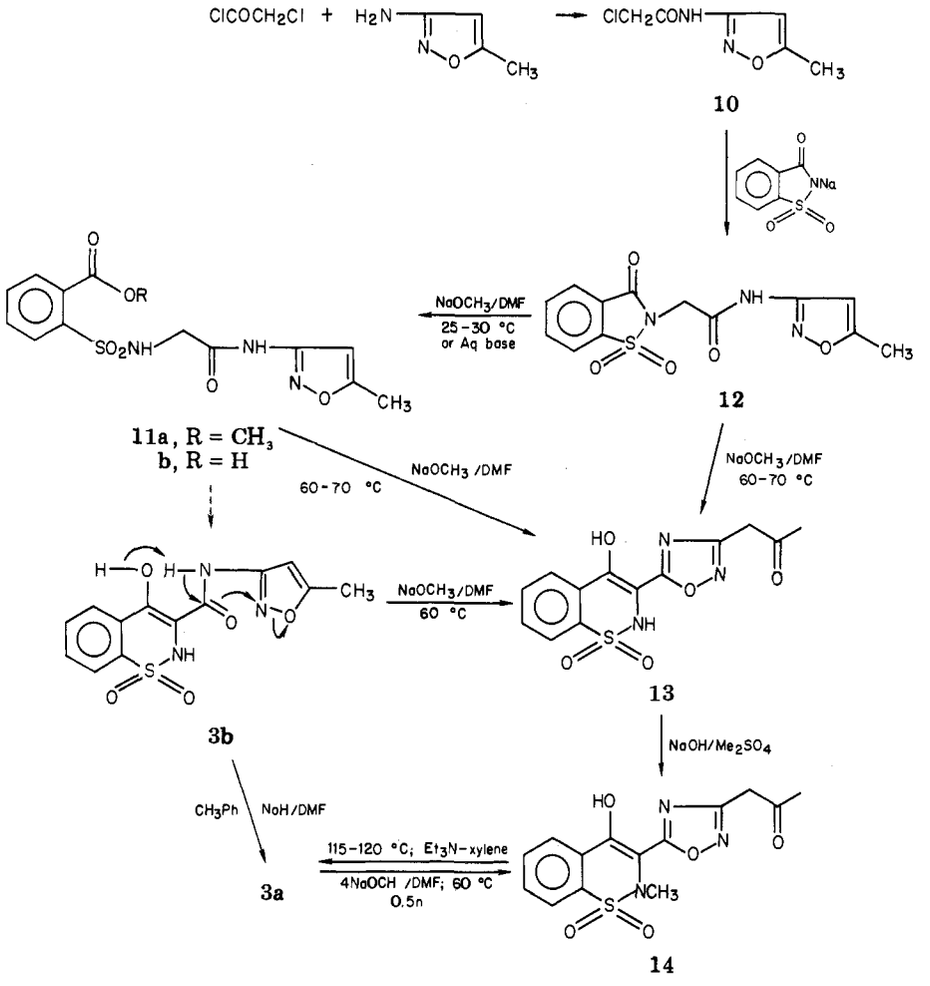
Biological Results and Discussion

These enolic 1,2-benzothiazine-3-carboxamides were screened for antiinflammatory activity by means of the carrageenin-induced rat paw edema (CIRPE) test. Those compounds which met our criteria for activity at doses of 100 mg/kg or less were evaluated further in both prophylactic and therapeutic adjuvant-induced polyarthritis (AIP) procedures.[10] The results are presented in Table I.

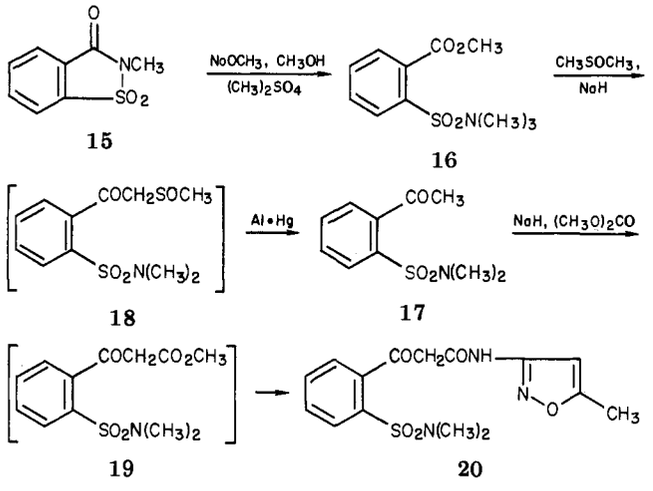
The most active compounds of the series were the 3-aminoisoxazole derivatives, **3a** (isoxicam) and **3e**, whose structures are characterized by the presence of a free amide hydrogen (R_3 = H), a 4-hydroxyl (X = OH), and methyl substitution on the sulfonamide nitrogen (R_1 = CH_3). The

(3) (a) J. G. Lombardino and E. H. Wiseman, *J. Med. Chem.*, **15**, 848 (1972). (b) J. G. Lombardino, E. H. Wiseman, and J. Chiaini, *ibid.*, **16**, 493 (1973). (c) J. G. Lombardino, *ibid.*, **24**, 39 (1981).
(4) (a) J. Chiaini, E. H. Wiseman, and J. G. Lombardino, *J. Med. Chem.*, 14, 1175 (1971). (b) E. H. Wiseman, Y. H. Chang, and J. G. Lombardino, *Arzneim-Forsch.*, **26**, 1300 (1976).
(5) (a) F. Ginsberg, T. Appelboom, and J. P. Famaey, *Curr. Ther. Res.*, **28**, 570 (1980) and the references cited therein. (b) M. Weintraub, R. F. Jacox, D. Angevine, and E. C. Atwater, *J. Rheumatol.*, **4**, 393 (1977).
(6) C. R. Rasmussen, U.S. Patent 3 501 466 (1970); J. D. Genzer and F. C. Fontsere described a modified process for the synthesis of the methyl ester **4** in U.S. Patent 3 960 856 (1976).
(7) I. Iwani and N. Nakamura, *Chem. Pharm. Bull.*, **14**, 1277 (1966).
(8) H. Zinnes, R. Comes, F. Zuleski, A. Caro, and J. Shavel, Jr., *J. Org. Chem.*, **30**, 2241 (1965).
(9) H. C. Van Der Plas, "Ring Transformations of Heterocycles", Vol. 1, Academic Press, London, and New York, 1973, Chapter 3.
(10) (a) G. DiPasquale, C. L. Rassaert, R. S. Richter, and L. V. Tripp, *Arch. Int. Pharmacodyn. Ther.*, **203**, 92 (1973). (b) G. DiPasquale, C. Rassaert, R. Richter, P. Welaj, J. Gingold, and R. Singer, *Agents Actions*, **5**, 256 (1975).
(11) C. A. Winter, E. A. Risky, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1963).

Scheme III



Scheme IV



o-acetyl derivative, **3c**, which showed comparable activity, is probably a prodrug of **3a** (isoxicam). The two related open-chain compounds, **11b** and **20**, were inactive at the screening dose.

The 5-aminoisoxazole derivatives (**3f**, **3g**, and **3h**) were less active. Likewise, absence of substitution on the sulfonamide nitrogen (**3b**), methylation on the amide nitrogen (**3d**), or different 4-substitution (**8a**) all resulted in considerably diminished activity.

An in-depth comparison of adjuvant-induced polyarthritis testing of isoxicam (**3a**) and reference compounds, phenylbutazone and indomethacin, has been reported elsewhere.[10b] These studies clearly showed isoxicam (**3a**) to be more potent than phenylbutazone.[12] It was less potent than indomethacin, but the difference in potency in the chronic type of test was much less than that observed in an acute model, such as the CIRPE. The same publication[10b] also reported the results of ulcerogenicity testing, as well as classical toxicity data, which demonstrated isoxicam (**3a**) to be considerably less toxic than these and other reference antiinflammatory agents.

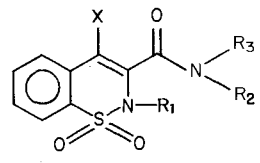
Compound **3a** (isoxicam) thus represents a novel class of nonsteroidal antiinflammatory agents which is different from the classical carboxylic acids series. Presently, **3a** is undergoing phase III clinical trials.

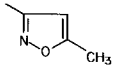
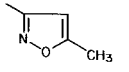
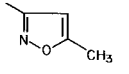
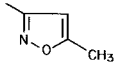
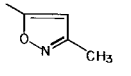
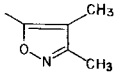
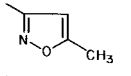
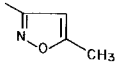
Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus that was calibrated against known standards. Each analytical sample was homogeneous by TLC and had IR, UV, and ^{1}H NMR spectra compatible with its structure. Spectral data are given only for novel compounds. The ultraviolet and infrared spectra were obtained, respectively, with a Beckman DKI spectrophotometer and a Baird Model 455 double-beam

(12) For purposes of initial screening and the derivation of structure–activity relationships within the series, the results of AIP testing were reported, as tabulated, in terms of the minimum dose required for statistically significant reduction of edema in the injected paw. Thus, the tabulated data would suggest that isoxicam (**3a**) and phenylbutazone are equipotent. However, a valid comparison requires evaluation of all of the major parameters measured in the AIP procedure. These include the dose required to inhibit edema in both injected and noninjected paw, the extent of edema inhibition at comparable doses, and measurement of body weight and organ weights. These data are given in ref 10b.

Table I. Antiinflammatory Activity of *N*-Isoxazolyl-3-carboxamides of 4-Hydroxy-2*H*-1,2-benzothiazine 1,1-Dioxide



no.	R_1	R_2	R_3	X	synth scheme	yield, %	mp, °C	recrystn solvent	formula	anal.	CIRPE: MED,[b] mg/kg po	AIP: MED,[b] mg/kg po prophylactic	AIP: MED,[b] mg/kg po therapeutic
3a	CH_3		H	OH	I	49	258–259 dec	DMF	$C_{14}H_{13}N_3O_5S$	C, H, N, S	12.5	1.56	1.56
3b	H		H	OH	I	58	254–257 dec	dioxane	$C_{13}H_{11}N_3O_5S$	C, H, N, S	100	<25.0	25.0
3c	CH_3		H	OAc	*c*	64	193–194 dec	EtOAc	$C_{16}H_{15}N_3O_6S$	C, H, N, S	6.25	5.0	1.56
3d	CH_3		CH_3	OH	*c*	52	145–148	ether–CH_2Cl_2	$C_{15}H_{15}N_3O_5S$	C, H, N, S	100		
3e	CH_3		H	OH	I	32	237–240 dec	EtOAc	$C_{13}H_{11}N_3O_5S$	C, H, N, S	25	6.25	6.25
3f	CH_3		H	OH	I	35	234–239 dec	EtOAc	$C_{13}H_{11}N_3O_5S$	C, H, N, S	100		
3g	CH_3		H	OH	I	43	249–254 dec	dioxane	$C_{14}H_{13}N_3O_5S$	C, H, N, S	100	25	
3h	CH_3		H	OH	I	55	190–192 dec	MeOH	$C_{15}H_{15}N_3O_5S$	C, H, N, S	100		
8a	CH_3		H		II	62	197–200 dec	THF	$C_{18}H_{20}N_4O_4S$	C, H, N, S	100	25.0	
8b	CH_3		CH_3		II	52	148–151	THF–ether	$C_{19}H_{22}N_4O_4S$	C, H, N, S	100		
11b					*c*	24	214–216 dec	MeOH	$C_{13}H_{13}N_3O_6S$	C, H, N, S	In[d]		
13					*c*	71	187–189	MeOH	$C_{13}H_{11}N_3O_5S$	C, H, N, S	In[d]		
14					*c*	85	143–145	*e*	$C_{14}H_{13}N_3O_5S$	C, H, N, S	In[a]		
20					*c*	47	134–135.5	MeOH	$C_{15}H_{17}N_3O_5S$	C, H, N, S	In[d]		
		Phenylbutazone									37.5	1.56	1.56
		Indomethacinin									0.37	0.39	0.39
		Aspirin									100		

[a] Analyses for C, H, N, S were within ±0.4% for all compounds except as indicated. [b] MED = minimum effective dose; CIRPE = carrageenin-induced rat paw edema; AIP = adjuvant-induced polyarthritis; confidence limit, $p < 0.05$. [c] See Experimental Section. [d] Inactive at 100 mg/kg. [e] Not recrystallized.

instrument. The former were determined as solutions in 95% ethanol and the latter as Nujol mulls. The ^1H NMR spectra were determined with a Varian A-60 spectrometer using Me_4Si as an internal standard. The abbreviations br, s, d, t, and m refer to broad, singlet, doublet, triplet, and multiplet, respectively, and the number in parentheses refer to the number of protons represented by the given signal. All compounds analyzed within ±0.4% of theoretical values for C, H, N, and S. The reported yields for the products obtained were not maximized. The procedure for the preparation of **3a** may be considered as a general method of aminolysis (Scheme I).

Biological Test Procedures. Carrageenin-Induced Rat Paw Edema (CIRPE). The antiinflammatory activity of these compounds was determined by means of the *carrageenin-induced rat paw edema assay* described by Winter et al.[11] Male rats (Charles-River strain) were arranged in groups of 8–10. Test compounds were administered orally as aqueous suspensions, and the phlogistic agent (0.05 mL of 1% carrageenin in 0.9% sterile sodium chloride solution) was injected 1 h later into the plantar area of the left hind paw of each animal. The size of the injected and uninjected hind paws of the unanesthetized rat was determined 3 h later by insertion into mercury to a previously positioned mark on the skin over the lateral malleolus. Differences in mercury displacement produced by the injected and noninjected paws were recorded, and a statistical analysis of measurements with control and treated groups was obtained. The activities recorded in Table I refer to the oral screening dose.

Adjuvant-Induced Polyarthritis (AIP). Male albino rats (Charles-River-Lewis strain) weighing 150 ± 10 g were arranged in groups of 10. On day 1, heat-killed dry *Mycobacterium butyricum* (Difco) was suspended in light mineral oil (250 μg/0.05 mL) and injected into the left hind paw of each rat.[10] The test agents were administered prophylactically (days 1–20) or therapeutically (days 14–27). In the therapeutic assay, all the animals were evaluated on day 14 and only the "responders" were used (polyarthritic lesions in both hind paws evident). The responders were randomly arranged into groups of 10 and treated on days 14–27. The animals were sacrificed on day 21 (prophylactic assay) or day 28 (therapeutic assay), and the hind paw weights were recorded. The hind paw weight changes represent differences between day 1 and day 21 or day 14 and day 28.

The test agents were obtained from Merck Sharp & Dohme (indomethacin) and Ciba-Geigy (phenylbutazone). All the agents were administered as aqueous suspensions at a constant volume of 0.2 mL/rat.

4-Hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (3a; Scheme I). A mixture of 40.5 g (0.15 mol) of ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**4b**),[6] 20.6 g (0.21 mol) of 5-methyl-3-isoxazolamine, and 2500 mL of xylene was heated at reflux for 24 h in a Soxhlet apparatus, the thimble of which contained 60 g of Linde type 4A molecular sieves. The mixture was cooled to 25 °C, and the resulting crystalline precipitate was collected and washed with ether to give 44 g of crude product. Recrystallization from 1600 mL of 1,4-dioxane gave 34.7 g (49%) of **3a**: UV (EtOH) λ_{max} 320 nm (ε 16 210), 238 (11 350); IR (Nujol) ν_{max} 3292, 1625, 1590, 1177 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.48 (s, 3, CH_3), 2.95 (s, 3, NCH_3), 6.81 (s, 1, $C_{4'}$ H), 7.7–8.3 (m, 4, aromatic), 9.42 (s, 1, NH), 12.2–13.2 (br, 1, OH).

4-(Acetyloxy)-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (3c). A slurry of 10.05 g (0.03 mol) of 4-hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide (**3a**) in 100 mL of tetrahydrofuran was cooled to 0 °C, and 1.29 g (0.03 mol of NaH) of a 57% mineral oil dispersion of sodium hydride was added in small portions. The mixture was allowed to warm slowly to 25 °C and stirred at this temperature until hydrogen evolution had ceased. It was then cooled to 0 °C, a solution of 2.37 g (0.03 mol) of acetyl chloride in 50 mL of tetrahydrofuran was added, and the resulting mixture was stirred at 25 °C for 17 h. The solvent was removed and the residue was stirred with 600 mL of water. Filtration gave 13 g of crude product, mp 180–182 °C dec. Recrystallization from ethyl acetate gave 7.2 g (64%) of crystalline material, **3c**: IR (Nujol) ν_{max} 3280, 1762, 1683, 1605, 1160, 1140 cm^{-1}; ^1H NMR (Me_2SO-d_6) 2.35 (s, 3, CH_3), 2.40 (s, 3, $OCOCH_3$), 3.10 (s, 3, NCH_3), 6.67 (s, 1, $C_{4'}$ H), 7.85 (m, 4, aromatic), 11.40 (br, 1, NH, D_2O exchangeable).

2-Methyl-4-(1-pyrrolidinyl)-2*H*-1,2-benzothiazine 1,1-Dioxide (7). A mixture of 129 g (0.611 mol) of **6**,[8] 93 mL (1.12 mol) of pyrrolidine, and 1700 mL of benzene was placed in a flask equipped with a Dean–Stark water separator and was heated at reflux for 72 h under nitrogen atmosphere. Evaporation of benzene under reduced pressure gave 153 g of enamine (**7**), mp 137–140 °C; an analytical sample of **7** was prepared by recrystallization from an ether–benzene mixture and had mp 143–145 °C; IR (Nujol) ν_{max} 1590, 1175 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0 (m, 4, CH_2CH_2), 2.90 (m, 4, CH_2NCH_2), 3.15 (s, 3, NCH_3), 5.83 (s, 1, vinyl), 7.4–8.0 (m, 4, aromatic). Anal. ($C_{13}H_{16}N_2O_2S$) C, H, N, S.

2-Methyl-*N*-(5-methyl-3-isoxazolyl)-4-(1-pyrrolidinyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (8a; Scheme II). A solution of 2.5 g (0.025 mol) of carbonic chloride in 23 mL of benzene was diluted with 50 mL of dry tetrahydrofuran. This was cooled to −40 °C, and a solution of 6.6 g (0.025 mol) of 2-methyl-4-(1-pyrrolidinyl)-2*H*-1,2-benzothiazine 1,1-dioxide (**7**) and 2.5 g (0.025 mol) of triethylamine in 200 mL of tetrahydrofuran was added slowly with stirring over a period of 30 min while the temperature was maintained at −40 to −55 °C. The reaction mixture was then stirred at room temperature for 4 h and cooled to −70 °C, and a solution of 5.5 g (0.055 mol) of 5-methyl-3-isoxazolamine in 100 mL of tetrahydrofuran was slowly added. The mixture was allowed to warm to room temperature, stirred for 64 h, treated with ice–water, and extracted with dichloromethane. The organic layer was washed twice with water, dried (Na_2SO_4), and evaporated. Trituration of the residue with ether gave 6.0 g (62%) of crystalline product, mp 184–188 °C dec, which was sufficiently pure for use in the next step. Recrystallization from tetrahydrofuran gave 4.8 g of analytically pure **8a**: UV (EtOH) λ_{max} 364 nm (ε 13 400), 236 (14 200); IR (Nujol) ν_{max} 3200, 1627, 1612, 1180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0 (m, 4, CH_2CH_2), 2.5 (s, 3, CH_3), 2.75 (s, 3, NCH_3), 3.6 (m, 4, CH_2NCH_2), 6.9 (s, 1, $C_{4'}$ H), 7.85 (m, 4, aromatic), 9.1 (br, 1, NH, D_2O exchangeable).

***N*,2-Dimethyl-*N*-(5-methyl-3-isoxazolyl)-4-(1-pyrrolidinyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (8b).** A solution of 26.4 g (0.1 mol) of **7** and 16.4 mL (0.12 mol) of triethylamine in 400 mL of tetrahydrofuran was added to a cooled (−20 °C) solution of 110 mL of 12.5% carbonic dichloride in benzene and 100 mL of tetrahydrofuran during 1.5 h. After the addition was over, the reaction mixture was stirred for 3.0 h at room temperature and then cooled again to −40 °C. A solution of 12.4 g (0.1 mol) of *N*,5-dimethyl-3-isoxazolamine and 16.4 mL (0.12 mol) of triethylamine in 110 mL of tetrahydrofuran was added slowly to the reaction mixture during 30 min at −40 °C, stirred at room temperature for 2.0 h, and then heated at reflux for 20 h. Excess solvent was removed by distillation, and the reaction mixture was decomposed with ice–water and extracted with CH_2Cl_2. The crude product was dissolved in 500 mL of dry ether, and the solution was filtered to remove insoluble impurities. The filtrate on standing gave 30.8 (76%) of **8b**. This was recrystallized twice from tetrahydrofuran–ether to give 21.0 g (52%) of analytically pure product (**8b**): UV (EtOH) λ_{max} 367 nm (ε 9600), 241 (14 800); IR (Nujol) ν_{max} 1638, 1585, 1165, 1183 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.87 (m, 4, CH_2CH_2), 2.2 (s, 3, CH_3), 2.7 (s, 3, NCH_3), 3.18 (m, 4, CH_2NCH_2), 3.58 (s, 3, $CONCH_3$), 6.2 (s, 1, $C_{4'}$ H), 7.6 (m, 4, aromatic).

4-Hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (3a from 8a). A solution of 1.2 g of unrecrystallized 2-methyl-*N*-(5-methyl-3-isoxazolyl)-4-(1-pyrrolidinyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide (**8a**) in 30 mL of glacial acetic acid was heated on a steam bath, and 30 mL of 1 N hydrochloric acid was added. Heating was continued for 45 min, during which time some of the product separated from solution. The reaction mixture was diluted with ice–water to a volume of 300 mL and filtered to give 1.0 g (97%) of material, mp 255–260 °C dec. This was shown by mixture melting point and comparison of infrared spectra to be identical with **3a** prepared by the aminolysis method (Scheme I).

4-Hydroxy-*N*,2-dimethyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (3d). A mixture of 2.0 g (0.005 mol) of *N*,2-dimethyl-*N*-(5-methyl-3-isoxazolyl)-4-(1-pyrrolidinyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide

(8b), 25 mL of acetic acid, and 25 mL of 1 N HCl was heated on a steam bath for 10 min to give a clear solution. The clear reaction mixture was allowed to stand at room temperature for 1.5 h and then poured onto ice. The product, which crystallized, was filtered, washed, and dried. The crude product (1.5 g) was recrystallized from an ether–CH_2Cl_2 mixture to give 0.9 g (52%) of 3d: UV (EtOH) λ_{max} 322 nm (ϵ 10 400), 235 (12 800); IR (Nujol) ν_{max} 1628, 1597, 1550, 1182 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.3 (s, 3, CH_3), 2.65 (s, 3, NCH_3), 3.4 (s, 3, $CONCH_3$), 5.95 (s, 1, $C_{4'}$ H), 7.70 (m, 4, aromatic), 13.3 (s, 1, OH, D_2O exchangeable).

2-Chloro-*N*-(5-methyl-3-isoxazolyl)acetamide (10). To 800 mL of chloroform was added 118 g (1.12 mol) of 5-methyl-3-isoxazolamine, followed by 118 g (1.5 mol) of pyridine. To this solution was added 147 g (1.3 mol) of chloroacetyl chloride, the addition temperature being maintained at 0–10 °C. The reaction was then stirred at room temperature for 1 h, and the product 10 was collected by filtration and dried in a vacuum desiccator to give 116 g (56%) of 2-chloro-*N*-(5-methyl-3-isoxazolyl)acetamide, mp 192–195 °C. **Caution:** Since this material is irritating to the skin, caution should be exercised in handling. Anal. ($C_6H_7ClN_2O_2$) C, H, N, Cl.

***N*-(5-Methyl-3-isoxazolyl)-3-oxo-1,2-benzisothiazole-2-(3*H*)-acetamide 1,1-Dioxide (12).** To 2.31 L of DMF was added 479 g (2.75 mol) of 2-chloro-*N*-(5-methyl-3-isoxazolyl)acetamide (10), followed by 699 g (2.9 mol) of the sodium salt of 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide dihydrate. The mixture was heated to 100 °C, and this temperature was maintained for 2 h. The cooled reaction mixture was poured into 8.0 L of water and filtered, and the wet cake was recrystallized from 15.0 L of ethanol to give 658 g (74.8%) of *N*-(5-methyl-3-isoxazolyl)-3-oxo-1,2-benzisothiazole-2(3*H*)acetamide 1,1-dioxide: mp 218–220 °C. Anal. ($C_{13}H_{11}N_3O_5S$) C, H, N, S.

1-[5-(4-Hydroxy-2*H*-1,2-benzothiazin-3-yl)-1,2,4-oxadiazol-3-yl]-2-propanone *S*,*S*-Dioxide (13). To 300 mL of DMF was added 67.5 g (1.25 mol) of sodium methoxide. This was heated to 55 °C, and a solution of 100 g (0.31 mol) of *N*-(5-methyl-3-isoxazolyl)-3-oxo-1,2-benzisothiazole-2(3*H*)-acetamide 1,1-dioxide (12) in 350 mL of DMF was added. The temperature was maintained at 60–70 °C for 30 min and poured into aqueous acid. This afforded 71 g (71%) of crude product (13).

Purification was achieved by washing with hot aqueous methanol and this afforded 13: UV (dioxane) λ_{max} 327 nm (ϵ 16 400); IR (Nujol) ν_{max} 3180, 1720, 1600, 1140 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.3 (s, 3, CH_3), 4.2 (s, 2, CH_2), 7.9 (m, 4, aromatic), 9.0–11.0 (br, 2, OH and NH; D_2O exchangeable).

1-[5-(4-Hydroxy-2-methyl-2*H*-1,2-benzothiazin-3-yl)-1,2,4-oxadiazol-3-yl]-2-propanone *S*,*S*-Dioxide (14). To 160 mL of 56% aqueous proprietary solvent no. 3 (SoLOX) was added 3.21 g (0.01 mol) of 13. The mixture was cooled to 5 °C, and 20 mL of 1 N NaOH was added dropwise with the temperature being maintained below 10 °C. To this was added 1.8 g (0.015 mol) of dimethyl sulfate, and the reaction mixture was stirred for 3 h, at a maximum temperature of 25 °C. Acidification with 6 N HCl afforded 2.8 g (85%) of analytical 1-[5-(4-hydroxy-2-methyl-2*H*-1,2-benzothiazin-3-yl)-1,2,4-oxadiazol-3-yl]-2-propanone *S*,*S*-dioxide (14). This was also made from 3a by reacting with 4 equiv of sodium methoxide in DMF at 60 °C for 30 min: UV (dioxane) λ_{max} 327 nm (ϵ 16 450); IR (Nujol) ν_{max} 3305, 1730, 1600, 1140 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.3 (s, 3, CH_3), 3.0 (s, 3, NCH_3), 4.2 (s, 2, CH_2), 8.0 (m, 4, aromatic), 9.2 (br, 1, OH; D_2O exchangeable).

4-Hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (3a). To 80 mL of xylene was added 4.0 g (0.012 mol) of 14, followed by 0.4 g (0.0046 mol) of triethylamine, and the temperature was maintained to 115–120 °C for 30 min. The reaction mixture was cooled and filtered to afford 3.6 g (90%) of 3a, mp 252–254 °C. This was identical in all respects with 3a prepared in the first procedure.

4-Hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide (3a) via Methylation of 3b. To a mixture of 0.01 mol of NaH (as a 57% dispersion in mineral oil) and 3.21 g (0.01 mol) of 3b was added 20 mL of dry DMF. The mixture was stirred for 1 h, during which time hydrogen evolved and the dark brown anion was formed. A solution of 1.42 g (0.01 mol) of methyl iodide in DMF was added, and the reaction mixture was stirred at 60 °C for 4 h and then at 25 °C for 16 h. It was diluted with 20 mL of water and stirred for 1 h. The resulting precipitate was collected, washed with two 10-mL portions of DMF and one 20-mL portion of methanol, and air-dried: yield 2.7 g; mp 259–262 °C dec. TLC, IR, ^1H NMR, and mmp confirmed it as 3a.

1-[5-(4-Hydroxy-2*H*-1,2-benzothiazin-3-yl)-1,2,4-oxadiazol-3-yl]-2-propanone *S*,*S*-Dioxide (13) from 3b. To a suspension of 1.35 g of sodium methoxide in 7 mL of DMF was added over 5 min at a temperature below 30 °C a solution of 2.0 g (0.0062 mol) of 3b in 6 mL of DMF. A deep red solution was formed. The reaction mixture was heated at 65–70 °C for 30 min, cooled to 30 °C, and then poured over a mixture of 2.5 mL of concentrated HCl and 40 mL of water at 0–5 °C to give a light-yellow precipitate. It was filtered, washed with water, and dried. Recrystallization from methanol gave 1.32 g of 13, mp 186–187 °C, identical with 13 made from 12 in one step.

Methyl 2-[[[2-[(5-Methyl-3-isoxazolyl)amino]-2-oxoethyl]amino]sulfonyl]benzoate (11a). To 60 mL of DMF was added 13.5 g (0.25 mol) of sodium methoxide. To this slurry at 15 °C was added a solution of 20 g (0.062 mol) of *N*-(5-methyl-3-isoxazolyl)-3-oxo-1,2-benzisothiazole-2(3*H*)-acetamide 1,1-dioxide (12) dissolved in 70 mL of DMF. The reaction mixture was stirred at 25–30 °C for a 0.5 h and was acidified and extracted with chloroform. These extracts were washed with sodium bicarbonate and water, dried (Na_2SO_4), and concentrated to an oil, which was triturated with petroleum ether to give 16.3 g (75%) of 11a: mp 153–156 °C; ^1H NMR (Me_2SO-d_6) δ 2.35 (s, 3, CH_3), 3.80 (s, 2, CH_2), 3.85 (s, 3, OCH_3), 6.48 (s, 1, $C_{4'}$ H), 7.30–8.10 (m, ~6, aromatic and NH), 10.87 (br, 1, SO_2NH). Anal. ($C_{14}H_{15}N_3O_6S$) C, H, N, S.

2-[[[2-[(5-Methyl-3-isoxazolyl)amino]-2-oxoethyl]amino]sulfonyl]benzoic Acid (11b). The crude product 12 obtained from 3.0 g of sodium 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide and 1.5 g of 10 was dissolved in excess 1 N sodium hydroxide solution at room temperature and filtered. Acidification of the filtrate with concentrated HCl precipitated 11b as a white solid (1.3 g), mp 212–214 °C dec. Recrystallization from methanol gave 700 mg of an analytical sample (11b): IR (Nujol) ν_{max} 3260, 1710, 1690, 1620 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.35 (s, 3, CH_3), 3.85 (br, 2, CH_2), 6.48 (s, 1, $C_{4'}$ H), 7.20–8.10 (m, ~6, aromatic and NH), 10.85 (br, 1, SO_2NH).

1-[5-(4-Hydroxy-2*H*-1,2-benzothazin-3-yl)-1,2,4-oxadiazol-3-yl]-2-propanone *S*,*S*-Dioxide (13). To 24 mL of DMF was added 4.89 g (0.09 mol) of sodium methoxide. This was heated to 55 °C, and a solution of 8.0 g (0.023 mol) of 11a dissolved in 28 mL of DMF was added. A temperature of 60–65 °C was maintained for 30 min, and the reaction mixture was cooled and acidified. This afforded 4.8 g (65%) of crude product (13). Purification ($MeOH/H_2O$) afforded 13, mp 186–187 °C, which was identical in all respects with the compound 13 made in one step from 12.

Methyl 2-[(Dimethylamino)sulfonyl]benzoate (16). To a slurry of 103 g (0.05 mol) of sodium 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide in 650 mL of DMF was added 45 mL (0.05 mol) of dimethyl sulfate. The ensuing exotherm raised the temperature to 40 °C. The mixture was stirred for 1 h and treated portionwise with 81 g (0.15 mol) of sodium methoxide, the temperature being maintained at 40 °C by cooling. Stirring was continued for 10 min, and 140 mL (0.15 mol) of dimethyl sulfate was added dropwise at such a rate and with sufficient cooling to moderate the vigorous reaction (dimethyl ether evolved). In cases where the reaction mixture solidified, it was diluted with additional DMF. The mixture was stirred at room temperature for 2 h and poured into 3 L of ice–water, and the resulting precipitate was collected and dissolved in ether. The solution was washed successively with 1 N sodium hydroxide and water, dried over magnesium sulfate and evaporated. Recrystallization of the residue from isopropyl ether gave 58.4 g of 16: mp 61–64 °C; IR (Nujol) ν_{max} 1700 cm^{-1}. This was used in the next step without further purification.

2-Acetyl-*N*,*N*-dimethylbenzenesulfonamide (17). The procedure employed was based on that of Corey and Chaykovsky.[13] A mixture of 22.3 g (0.53 mol of NaH) of a 57% mineral oil

(13) E. J. Corey, and M. Chaykovsky, *J. Am. Chem. Soc.*, 87, 1345 (1965).

dispersion of sodium hydride and 300 mL of dry dimethyl sulfoxide was stirred at 50–70 °C, under nitrogen until gas was no longer evolved. The resulting grayish-green solution was diluted with 150 mL of dry tetrahydrofuran and cooled in an ice bath, and a solution of 58.4 g of methyl 2-[(dimethylamino)sulfonyl]-benzoate (16) in 100 mL of tetrahydrofuran was added over a period of 10 min. The mixture was stirred at room temperature for 45 min, poured into 2.0 L of ice–water which had previously been acidified to pH 2–3 by the addition of HCl, and *quickly* extracted with chloroform. Washing the chloroform solution three times with cold water (to remove all traces of acid), drying over $MgSO_4$, and evaporation of the solvent gave 57.9 g of crude ketosulfoxide as a pale brown syrup which was found to crystallize on long standing. The crude sulfoxide was immediately reduced as follows: it was dissolved in 2500 mL of 10% aqueous tetrahydrofuran and treated with aluminum amalgam prepared from 65 g of aluminum as described by Corey and Chaykovsky;[13] the reaction temperature was maintained at 10–20 °C, and the reaction time was 20 min. The reaction mixture was filtered, the filtrate was evaporated to remove the tetrahydrofuran, and the residue was dissolved in ether. The ether solution was washed successively with cold 1 N sodium hydroxide and water, dried, and evaporated. Recrystallization of the residue from diisopropyl ether gave 23.1 g of 17, mp 92.5–93.5 °C. Anal. ($C_{10}H_{13}NO_3S$) C, H, N, S.

3-[2-[(Dimethylamino)sulfonyl]phenyl]-*N*-(5-methyl-3-isoxazolyl)-3-oxopropanamide (20). A mixture of 6.8 g (0.03 mol) of 2-acetyl-*N*,*N*-dimethylbenzenesulfonamide (17), 0.09 mol of sodium hydride, and 50 mL of dimethyl carbonate was heated at reflux for 1.5 h. Methanol was added to destroy the excess sodium hydride, and the mixture was poured into ice–water and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated to a syrup. This crude β-keto ester (19) was dissolved in 300 mL of xylene and was heated at reflux with 3.3 g (0.033 mol) of 5-methyl-3-isoxazolamine for 18 h using a Soxhlet apparatus, the thimble of which contained Linde 4A molecular sieves. The reaction mixture was extracted with cold 1 N sodium hydroxide, and the aqueous layer was acidified and extracted with dichloromethane. The dichloromethane solution was evaporated to a residue, which was crystallized from methanol to give 7.1 g of 20, mp 133–136 °C. Recrystallization from ethyl acetate gave 5.0 g (47.5%) of an analytical sample: IR (Nujol) ν_{max} 3300, 1712, 1673, 1618, 1163 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.35 (s, 3, CH_3), 2.65 [s, 6, $N(CH_3)_2$], 4.05 (s, 2, CH_2, D_2O exchangeable), 6.60 (s, 1, $C_{4'}$ H), 7.70 (m, 4, aromatic), 10.85 (br, 1, NH, D_2O exchangeable).

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Enhanced Antitumor Properties of 3′-(4-Morpholinyl) and 3′-(4-Methoxy-1-piperidinyl) Derivatives of 3′-Deaminodaunorubicin

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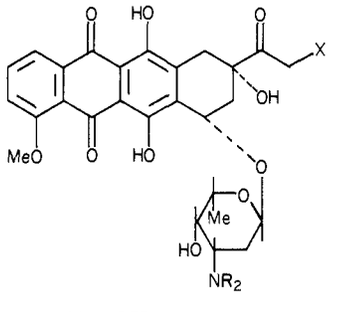
Reductive N,N-dialkylation of daunorubicin with 2,2′-oxydiacetaldehyde and $NaBH_3CN$ occurred in two steps without interruption and with cyclization to form 3′-(4-morpholinyl)-3′-deaminodaunorubicin. This derivative retained the antitumor efficacy of doxorubicin against mouse leukemia P388 but at one-fortieth the dose; hence, it is the most potent anthracycline analogue synthesized so far. The 4-methoxy-1-piperidinyl derivative, similarly prepared with 3-methoxyglutaraldehyde, showed improved efficacy against P388, though at normal doses. Results with a series of analogues indicate that incorporation of the N in the new ring and the presence of an ether O at the 4-position are critical for enhanced activity.

New analogues of doxorubicin (adriamycin, 1) and



1, X = OH; R = H
2, X = H; R = H
3, X = H; R = $CH_2C_6H_5$

daunorubicin (2) that might be more active or less toxic as cancer drugs are of considerable importance.[1] Doxorubicin appears to be the most active single agent against cancer. It is a principal agent in the treatment of an unusually wide number of solid tumors, and along with daunorubicin it is used in the treatment of leukemias. Despite this, many patients with these presumably treatable tumors fail to respond, and there are other types of tumors (colon cancer, melanoma) where essentially no patients respond. All patients treated with these drugs, however, encounter some risk of cardiotoxicity. Over 500 derivatives and analogues have been screened for antitumor properties just by the National Cancer Institute.[2] Many were active but could not be extensively evaluated. We have been exploring reductive alkylation[3] of the amino function with aldehydes and ketones in the presence of $NaBH_3CN$, as a useful one-step method for the semisynthesis of active analogues with altered patterns of biological effects.[4,5] *N*,*N*-Dibenzyldaunorubicin (3) is one example with markedly superior activity against mouse leukemia P388, even though it required higher doses and did not show the expected interactions with DNA. Because of superior activity also against murine colon and mammary tumors, 3 is currently under preclinical development at the NCI.

The series of active *N*-alkyl derivatives included the *N*,*N*-pentamethylene derivative of 1, with the amino N

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