23287-26-5; *N*-(2-methoxyphenyl)benzimidoyl chloride, 59386-96-8; 2-(methoxycarbonyl)-6-methylphenyl *N*-(2-methoxyphenyl)-benzimidate, 88377-25-7; methyl *N*-benzoyl-*N*-(2-methoxyphenyl)-3-methylanthranilate, 88377-26-8; *N*-benzoyl-*N*-(2-methoxyphenyl)-3-methylanthranilic acid, 88377-27-9; 4,5-dimethoxy-9-acridanone, 88377-28-0; 2-nitro-3-methoxybenzoic acid, 4920-80-3; 2-amino-3-methoxybenzoic acid, 3177-80-8; 2-bromo-3-methoxybenzoic acid, 88377-29-1; *o*-anisidine, 90-04-0; *N*-(2-methoxyphenyl)-3-methoxyanthranilic acid, 88377-30-4; 4-methyl-9-oxoacridan-5-carboxylic acid, 24782-66-9; 3-methylanthranilic acid, potassium salt, 59425-36-4; 2-chlorobenzoic acid, 118-91-2; *N*-(2-carboxyphenyl)-3-methylanthranilic acid, 80841-45-8; 4-methoxy-9-oxoacridan-5-carboxylic acid, 88377-31-5; *N*-(2-carboxyphenyl)-3-methoxyanthranilic acid, 88377-32-6; 4-methoxy-9-chloroacridine-5-carbonyl chloride hydrochloride, 88377-33-7; *N*-methyl-4-methoxy-9-chloroacridine-5-carboxamide, 88377-34-8; *N*-(4-amino-3-methoxyphenyl)methanesulfonamide, 57165-06-7.

Potential Antitumor Agents. 41. Analogues of Amsacrine with Electron-Donor Substituents in the Anilino Ring

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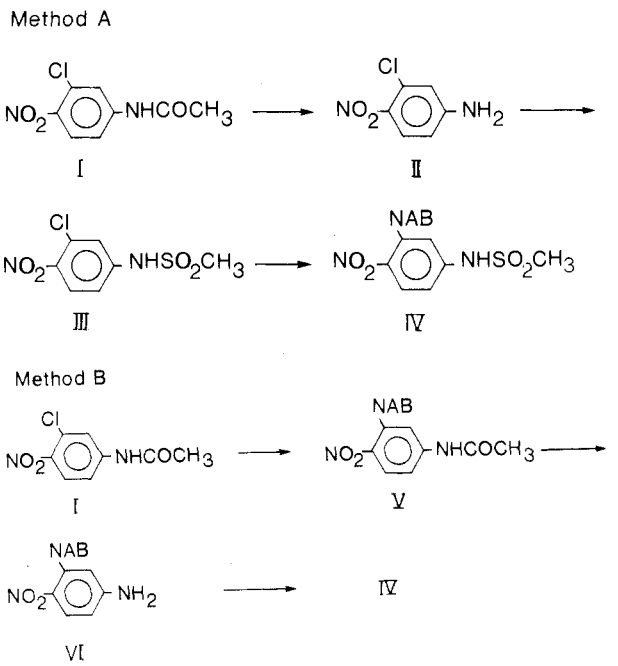
The preparation and antitumor activity of a series of 3′-alkylamino and 3′-dialkylamino analogues of amsacrine are reported. The results support previous work suggesting that the presence of electron-donating groups in the 3′-position of the anilino ring substantially enhance the antitumor activity of amsacrine analogues, possibly by the provision of high levels of electron density at the 6′-position. The alkylamino derivatives generally possess tighter DNA binding, higher levels of in vitro and in vivo antileukemic activity, and greater aqueous solubility than the corresponding amsacrine analogues.

During the early work that resulted in development of the clinical antileukemic agent amsacrine (*m*-AMSA, **6**)[1] from the general class of 9-anilinoacridine antitumor agents, the importance of electron-donor substituents on the anilino ring was noted.[2,3] For 1′-substituted derivatives of 9-anilinoacridine, there is an absolute requirement for electronically neutral or electron-donating groups at this position in order to preserve biological activity. The biochemical reasons for such a requirement are not entirely clear, for several important drug properties, such as acridine pK_a and drug–DNA binding constants, vary in a collinear fashion with the electronic properties of the 1′-substituent.[4] Similar structure–activity relationships were found for derivatives of the parent compound AMSA (**1**), where the effect of other aniline substituents on antitumor activity was examined.[3] For a series of 3′-substituents of varying electronic properties, those compounds (**2–4**) bearing electron-withdrawing groups proved inactive and nontoxic against the L1210 leukemia, both in vivo and in vitro, whereas those (e.g., **6**, **10**, and **11**) bearing small electron-donating groups at the 3′-position proved equally active to the parent compound. A hypothesis[3] that high electron density in the 6′-position of the anilino ring was required for antitumor activity was supported by the two AMSA analogues **12** and **13**. Both compounds have generally electron-deficient anilino rings, but the 2′-analogue **13**, where the electron-rich pyridyl nitrogen can act as a substitute for an electron-rich 6′-position, has appreciable biological activity.[3]

However, the substituent in the 3′-position has to be small enough to allow normal DNA binding of the drug to occur. An increase in the size of the 3′-substituent (e.g., **6–9**) results in steady decrease in biological activity and in the association constant (K) for the binding of the drugs to poly[d(A-T)][5,6] (Table I). This is compatible with current models of the drug–DNA complex, where the acridine chromophore binds by intercalation and the anilino ring makes further binding contacts in the minor groove.[7,8]

These results prompted the examination of other small 3′-substituents with powerful electron-donating properties,

Scheme I



and the 3′-OH and 3′-NH_2 compounds (**10** and **11**) have already been reported.[3] While possessing high activity, the dose potency of these compounds was no better than that

† Deceased 1981

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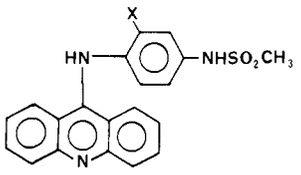
Table I. 3'-Substituted Derivatives of 4'-(9-Acridinylamino)methanesulfonanilide



no.	X	Rm[a]	pK_a[b]	σ_p	log K[c]	L1210 leukemia ID_{50}[d]	L1210 leukemia OD[e]	L1210 leukemia ILS[f]	ref
1	H	0.00	7.19	0.00	6.20	35	45	107	3
2	NO_2	0.06	4.86	0.78	5.61	9000	>500	*g*	3
3	Cl	0.23	6.34	0.23	5.45	1300	>500		3
4	F	0.08	6.62	0.06	5.65	800	>500		3
5	CH_3	0.15	7.36	−0.17	5.74	120	97	106	3
6[i]	OCH_3	0.18	7.43	−0.27	5.57	35	6.7	114	3
7	OCH_2CH_3	0.37	7.55	−0.24	5.68	600	250	29	5
8	OCH_2CH_2OH	0.03	7.53	−0.24	5.91	>1700	62.5	67	5
9	$OCH(CH_3)_2$	0.57	7.51	−0.45	5.17	>1700	>500		6
10	OH	0.09	7.56	−0.37	5.53	4000	25	88	3
11	NH_2	0.32	7.65	−0.66	6.28	1200	45	106	3
12	-N=	0.09	6.08		NA[h]	NA	>500		3
13	H, 2'-N=	0.13	6.20		5.57	140	110	118	3

[a] Rm values were determined as in ref 3, with AMSA (1) as a standard. [b] Determined by UV in 20% aqueous DMF, as detailed in ref 17. Aqueous pK_a values are approximately 0.6 unit higher. [c] Log K = binding constant to poly(dA-dT) determined by ethidium bromide displacement; see ref 4. [d] Nanomolar concentration of drug to inhibit growth of L1210 cells in culture by 50%, following a 48-h exposure; see ref 12. [e] OD (optimal dose) = dose of drug (in milligrams per kilograms per day) given ip on a qd 1-5 schedule that provides the highest ILS value in mice bearing 10^6 ip inoculated L1210 leukemia cells. [f] ILS = percentage increase in life span of drug-treated tumor-bearing controls; a value >20% is considered statistically significant. [g] Compound was inactive at the highest dose tested. [h] Data not available. [i] Amsacrine.

of the parent AMSA (1) and was reduced over that of amsacrine (6), possibly due to the observed oxidative instability of these *o*-aminophenol and *o*-phenylenediamine derivatives. The present paper details the synthesis and evaluation of amsacrine derivatives bearing various alkylamino and dialkylamino groups at the 3'-position. Alkylamino groups generally possess even greater electron-donating effects than amino or hydroxyl (e.g., σ_p −0.84 for $NHCH_3$, cf. −0.66 for NH_2), while providing compounds less sensitive to aerial oxidation. These compounds were found to possess exemplary antitumor activity in a number of in vitro and in vivo screening systems.

Chemistry. The compounds of Table II were prepared by acid-catalyzed coupling of 9-chloroacridine with the appropriate substituted aromatic amine under anhydrous conditions, as detailed previously.[9] These side chains in turn were prepared from the known[10] 3-chloro-4-nitroacetanilide (I) by one of the methods shown in Scheme I.

Most of the compounds were prepared by method A. Hydrolysis of the acetanilide (I) and treatment of the resulting amine (II) with methanesulfonyl chloride gave *N*-(3-chloro-4-nitrophenyl)methanesulfonamide (III). Treatment of this with a variety of amines (either neat or in aqueous solution) at elevated temperature provided the nitro precursors listed in Table IV. With the more volatile amines, confinement of the reaction in a pressure vessel gave improved yields.

While this method was satisfactory for most of the required compounds, some of the more hindered and strongly basic secondary amines failed to react or gave only negligible yields. Although the chlorine atom is labilized to nucleophilic replacement by the neighboring nitro group, the methanesulfonamide group under the basic conditions of the reaction is ionized to varying degrees, depending on the basicity of the amine component. Since the ionized form of the methanesulfonanilide is essentially inert to nucleophilic displacement of the chlorine, the reaction rate depends critically on the basicity of the amine reactant as well as on the steric hindrance around the amine nitrogen.

Thus, while the primary alkylamines (pK_a values of about 10.8) reacted readily, the more strongly basic pyrrolidine (pK_a = 11.3) required much longer reaction times. The more hindered strongly basic secondary amines diethylamine (pK_a = 10.5) and piperidine (pK_a = 11.1) reacted at negligibly slow rates, and an alternative route (method B, Scheme I) was employed for the preparation of these intermediates. The importance of the base strength of the amine as a variable governing the rate of the displacement reaction is demonstrated by the fact that morpholine, sterically equivalent to piperidine but with a pK_a almost 2 units lower (8.3), reacted readily under the same conditions. For amines unreactive under the above conditions, replacement of the chlorine of the nonionizable 3-chloro-4-nitroacetanilide itself proceeded more readily to give compounds IV. Hydrolysis of the acetanilide, followed by sulfonylation with methanesulfonyl chloride, gave the desired side chains for the preparation of the diethyl and piperidinyl compounds **34** and **36**. While the chloro group of the sulfonamide (III) could be replaced by aqueous methylamine under pressure to give IV (A = H; B = CH_3) directly, the requirement to prepare a number of different alkylsulfonamides of this structure made it more efficient to proceed via method B. Thus 3-(methylamino)-4-nitroaniline (VI, A = H; B = CH_3) was prepared, and selective sulfonylation with various alkylsulfonyl chlorides proceeded at low temperature in pyridine to give the side chains for the preparation of compounds **14–21** of Table II.

Reduction of the nitro group of the side-chain derivatives was carried out catalytically over Pd/C in MeOH, and the resulting unstable amines were used immediately for the preparation of the compounds listed in Table II. These products showed some instability as free bases and were normally handled, stored, and administered as salts.

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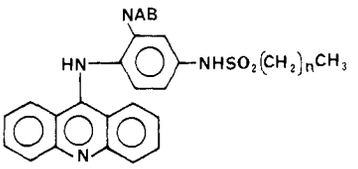
Table II. Physicochemical and Biological Data for 3′-Alkylamino Derivatives of Amsacrine



no.	*n*	A	B	Rm[a]	log *K*[b]	L1210 in vitro: ID_{50}[c]	P388 in vivo OD[d]	P388 in vivo ILS[e]
6 (amsacrine)				0.18	5.57	35	13.3	78
14	0	H	CH_3	0.17	6.42	71	13.3	152 (1)[f]
15	1	H	CH_3	0.37	6.62	43	13.3	99
16	2	H	CH_3	0.58	6.61	86	20	73
17	3	H	CH_3	0.71	6.66	102	45	67
18	4	H	CH_3	0.79	6.58	144	20	61
19	5	H	CH_3	0.83	6.41	443	100	36
20	6	H	CH_3	0.87	6.44	397	45	47
21	7	H	CH_3	0.92	6.51	217	30	42
22	0	H	CH_2CH_3	0.35	6.54	229	45	69
23	0	H	$(CH_2)_2CH_3$	0.54	6.57	286	66	*g*
24	0	H	$(CH_2)_3CH_3$	0.75	6.36	185	45	
25	0	H	$(CH_2)_4CH_3$	0.82	6.42	117	30	
26	0	H	$(CH_2)_5CH_3$	0.95	6.36	136	60	
27	0	H	$(CH_2)_6CH_3$	1.08	6.54	181	45	
28	0	H	CH_2CH_2OH	−0.07	6.49	86	45	89
29	0	H	$CH_2CH(OH)CH_2OH$	−0.30	6.71	219	45	84
30	0	H	$NHCOCH_3$	0.01	5.89	>1500		
31	0	H	cyclohexyl	0.80	6.30	470	45	
32	0	CH_3	CH_3	0.41	5.34	520	166	210 (2)
33	0	CH_3	CH_2CH_2OH	0.10	5.52	1400	100	100 (1)
34	0	CH_2CH_2	CH_2CH_3	0.49	5.35	4600	100	
35	0	$-(CH_2)_4-$		0.39	5.49	1300	45	
36	0	$-(CH_2)_5-$		0.60	5.69	>1600	100	
37	0	$-(CH_2)_2O(CH_2)_2-$		0.16	5.26	>1600	100	

[a] Footnote *a*, Table I. [b] Footnote *c*, Table I. [c] Footnote *d*, Table I. [d] OD = optimal drug dose (in milligrams per kilogram per day), administered intraperitoneally as a solution in 0.1 mL of 30%, v/v, ethanol/water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukaemia cells. [e] Footnote *f*, Table I. [f] Numbers in parentheses are the number of animals (out of a group fo six) that survived indefinitely. [g] Compound inactive (ILS <20%) at all doses.

Table III. Physical Data for the Compounds of Table II

no.	mp, °C	formula	anal.
14	261-263	$C_{21}H_{20}N_4O_2S\cdot HCl$	C, H, N, Cl
15	293-295	$C_{22}H_{22}N_4O_2S\cdot HCl$	C, H, N, Cl
16	294-296	$C_{23}H_{24}N_4O_2S\cdot HCl$	C, H, N, Cl
17	297-299	$C_{24}H_{26}N_4O_2S\cdot HCl$	C, H, N, Cl
18	284-286	$C_{25}H_{28}N_4O_2S\cdot HCl$	C, H, N, Cl
19	264-266	$C_{26}H_{30}N_4O_2S\cdot HCl$	C, H, N, Cl
20	249-251	$C_{27}H_{32}N_4O_2S\cdot HCl$	C, H, N, Cl
21	243-244	$C_{28}H_{34}N_4O_2S\cdot HCl\cdot H_2O$	C, H, N
22	268 dec	$C_{22}H_{22}N_4O_2S\cdot HCl$	C, H, N, Cl
23	285-286	$C_{23}H_{24}N_4O_2S\cdot HCl$	C, H, N, Cl
24	286-287	$C_{24}H_{26}N_4O_2S\cdot HCl$	C, H, N, Cl
25	260-261	$C_{25}H_{28}N_4O_2S\cdot HCl$	C, H, N, Cl
26	252-253	$C_{26}H_{30}N_4O_2S\cdot HCl$	C, H, N, Cl
27	242-243	$C_{27}H_{32}N_4O_2S\cdot HCl$	C, H, N, Cl
28	270-271	$C_{22}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl
29	254-256	$C_{23}H_{24}N_4O_4S\cdot HCl$	C, H, N, Cl
30	245-247	$C_{22}H_{20}N_4O_3S\cdot CH_3SO_3H$	C, H, N, S
31	276-277	$C_{26}H_{28}N_4O_2S\cdot HCl$	C, H, N, Cl
32	248-249	$C_{22}H_{22}N_4O_2S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
33	168-170	$C_{23}H_{24}N_4O_3S\cdot HCl\cdot 0.5H_2O$	C, H, N
34	205-207	$C_{24}H_{26}N_4O_2S\cdot HCl\cdot H_2O$	C, H, N
35	220-221	$C_{24}H_{24}N_4O_2S\cdot HCl$	C, H, N, Cl
36	252-253	$C_{25}H_{26}N_4O_2S\cdot HCl$	C, H, N, Cl
37	285-286	$C_{24}H_{24}N_4O_3S\cdot HCl$	C, H, N, Cl

Results and Discussion

Table II records pertinent physicochemical and biological properties for 3′-alkylamino derivatives of amsacrine (**14–37**). Binding of drugs to DNA was measured by the ability of the drugs to displace the fluorochrome ethidium bromide from DNA.[4,11] Displacement assays were conducted with the synthetic copolymers poly(dA-dT) and poly(dG-dC). However, as is the case with analogous compounds in the amsacrine series, little sequence selectivity was apparent, and DNA binding constants, corrected for quenching effects on ethidium fluorescence, are shown only for poly(dA-dT). The potency of the compounds against L1210 cells in culture was determined by the methods previously described,[12] and the in vivo P388 tests were conducted in this laboratory according to the NCI protocols.[9,13,14]

The 3′-$NHCH_3$ derivative (**14**) binds much more tightly to DNA than amsacrine (Table II) and, in fact, binds more strongly than any other 3′-substituted derivative listed in Table I. Extension of the alkyl chain off the 1′-sulfonamide group gave compounds **15–21**. As seen previously[12,15] for similar derivatives of amsacrine (**6**), levels of binding to poly(dA-dT) remained essentially constant, with the major variable changing being drug lipophilicity. Compound **14** proved to be considerably more active than amsacrine against the P388 leukemia in vivo (ILS of 152 compared

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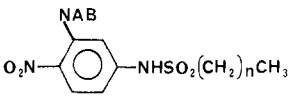
Table IV. Physical Data for Substituted Nitrophenyl Sulfonamides



n	A	B	mp, °C	formula	anal.
0	H	CH_3	191-193	$C_8H_{11}N_3O_4S$	C, H, N
1	H	CH_3	182-183	$C_9H_{13}N_3O_4S$	C, H, N
2	H	CH_3	208-209	$C_{10}H_{15}N_3O_4S$	C, H, N
3	H	CH_3	113-114	$C_{11}H_{17}N_3O_4S$	C, H, N
4	H	CH_3	115-116	$C_{12}H_{19}N_3O_4S$	C, H, N
5	H	CH_3	117-118	$C_{13}H_{21}N_3O_4S$	C, H, N
6	H	CH_3	95-96	$C_{14}H_{23}N_3O_4S$	C, H, N
7	H	CH_3	86-87	$C_{15}H_{25}N_3O_4S$	C, H, N
0	H	CH_2CH_3	154-155	$C_9H_{13}N_3O_4S$	C, H, N
0	H	$(CH_2)_2CH_3$	115-116	$C_{10}H_{15}N_3O_4S$	C, H, N
0	H	$(CH_2)_3CH_3$	161-163	$C_{11}H_{17}N_3O_4S$	C, H, N
0	H	$(CH_2)_4CH_3$	116-118	$C_{12}H_{19}N_3O_4S$	C, H, N
0	H	$(CH_2)_5CH_3$	89-90	$C_{13}H_{21}N_3O_4S$	C, H, N
0	H	$(CH_2)_6CH_3$	87-88	$C_{14}H_{22}N_3O_4S \cdot {}^1/_2C_6H_6$	C, H, N
0	H	CH_2CH_2OH	152-153	$C_9H_{13}N_3O_5S$	C, H, N
0	H	$CH_2CH(OH)CH_2OH$	153-155	$C_{10}H_{15}N_3O_6S$	C, H, N
0	H	cyclohexyl	193-194	$C_{13}H_{19}N_3O_4S$	C, H, N
0	CH_3	CH_3	159-160	$C_9H_{13}N_3O_4S$	C, H, N
0	$-(CH_2)_4-$		oil	$C_{11}H_{15}N_3O_4S$	C, H, N
0	$-(CH_2)_5-$		147-148	$C_{13}H_{17}N_3O_4S$	C, H, N
0	$-(CH_2)_2O(CH_2)_2-$		195-197	$C_{11}H_{15}N_3O_5S$	C, H, N

to 78), but activity steadily declined as the alkyl chain was lengthened to hexyl (compound **19**) and then remained essentially constant. The steady decline in in vivo activity as the lipophilicity is increased suggests that even the parent compound (**14**) has higher than optimal lipophilicity for in vivo P388 activity. The lipophilicity of **14**, as measured chromatographically, is identical with that of amsacrine (Rm of 0.17 compared to 0.18), which has independently been shown to be too lipophilic for optimal activity against the L1210 leukemia in vivo.[15]

However, in agreement with earlier QSAR studies for the 9-anilinoacridines,[6] drug lipophilicity is not a dominant determinant of activity in the 3′-methylamino series **14–21**, with even the octylsulfonamide (**21**) showing appreciable antitumor activity. One reason for the generally higher level of in vivo activity for the 3′-methylamino compounds (**14–21**) compared to their amsacrine analogues may be their greater solubility. Although appearing almost equally lipophilic in terms of chromatographic Rm values, they are in fact appreciably more soluble in aqueous solvents. Thus, compound **14** has a solubility in water of 0.49 mg/mL, compared to 0.12 mg/mL for amsacrine (**6**) (both compounds as hydrochloride salts at 20 °C).

Having established a base line for antitumor activity in this series with respect to lipophilicity, a second series of compounds (**22–27**) with alkyl side chains off the 3′-position were tested to determine the degree of bulk tolerance about this position. These compounds showed similar levels of DNA binding to the 1′-substituted series, and the binding did not fall off significantly as the chain was lengthened. The in vitro potency of the 3′-$NHCH_2CH_3$ derivative (**22**) was reduced threefold from that of the parent (**14**), but subsequent lengthening of the side chain had little further effect. The higher homologues (**23–27**) had ID_{50} values similar to or better than the corresponding 1′-homologues (**15–21**). However, the in vivo activity rapidly declined, with all compounds after the 3′-$NHCH_2CH_3$ derivative (**22**) proving inactive, although relatively toxic.

To further explore the degree of bulk tolerance at the 3′-position for in vivo activity, we prepared the hydroxylated derivatives **28** and **29** in order to provide more hydrophilic compounds. In the amsacrine series, the introduction of a hydroxyl group to give **8** from **7** resulted in increased DNA binding (Table I) and higher biological activity, although still below that of the parent, amsacrine (**6**). In the alkylamino series, hydroxylation of **22** to give **28** resulted in a similar binding increase but no improved biological activity. Dihydroxylation of the biologically inactive propyl derivative (**23**) gave a much more polar compound (**29**) with significant antitumor activity. However, none of the hydroxylated derivatives proved as potent or as active as the parent (**14**).

The amount of bulk tolerance around the 3′-position in the DNA binding site while able to accommodate quite large groups of small cross-section (ethylamino, dihydroxypropylamino), is not sufficiently large to fit N,N-disubstituted derivatives equally well. Thus, while the 3′-cyclohexylamine derivative (**31**) has DNA-binding properties not significantly different from the other 3′-alkylamino derivatives, the N,N-disubstituted compounds (**32–37**) have greatly reduced DNA affinity.

Previous work[6,7] has demonstrated the importance of the steric properties of 3′-substituents on the DNA binding and antitumor activity of 9-anilinoacridine derivatives. The present study sheds light also on the influence of the electronic properties of groups placed at this position. It can be seen from the combined results of Tables I and II that group electronic properties are an extremely important determinant of antitumor activity. Strongly electron-donating groups provide analogues with antitumor activity better than or comparable to amsacrine. The 3′-$NHCH_3$ group appears to be particularly effective in providing highly active, very potent compounds, and studies with a number of acridine-substituted analogues of **14** are in progress.

Experimental Section

Where analyses are indicated only by the symbols of the elements, results obtained were within ±0.4% of the theoretical values. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand, under the direction of Professor A. D. Campbell. Melting points were determined on an Electrothermal apparatus, using the supplied stem-corrected thermometer, and are as read. NMR spectra were

obtained on a Varian T-60 spectrometer (Me_4Si). Determination of Rm values for final products has been detailed previously.[16]

***N*-(3-Chloro-4-nitrophenyl)methanesulfonamide (III).** A stirred solution of 3-chloro-4-nitroaniline[10] (3.4 g, 20 mmol) in a mixture of dry pyridine (5 mL) and dimethoxyethane (10 mL) was treated dropwise at 0 °C with methanesulfonyl chloride (1.88 mL, 24 mmol). The mixture was kept at 20 °C for 1 h, heated at 100 °C for 30 min, and then concentrated under vacuum, and the residue shaken with excess dilute AcOH. The resulting solid was dissolved in 1 N NaOH, filtered, precipitated with 2 N HCl, and crystallized from MeOH as colorless needles (82% yield), mp 181–182 °C. Anal. ($C_7H_7ClN_2O_4S$) C, H, N.

***N*-[3-(Ethylamino)-4-nitrophenyl]methanesulfonamide (IV, A = H; B = CH_2CH_3).** A mixture of *N*-(3-chloro-4-nitrophenyl)methanesulfonamide (III; 2.5 g, 10 mmol) and 70% aqueous ethylamine (50 mL) was placed in a sealed pressure vessel and heated at 100 °C for 12 h. After cooling and diluting with water, the solution was clarified with charcoal/Celite and acidified with AcOH to give *N*-[3-(ethylamino)-4-nitrophenyl]methanesulfonamide (55%), which crystallized from aqueous MeOH, mp 154–155.5 °C (Table IV).

Similar procedures gave the side-chain precursors for compounds **23–25** and **32**. Reaction of less volatile amines proceeded at atmospheric pressure, as exampled below.

***N*-[3-[(2-Hydroxyethyl)amino]-4-nitrophenyl]methanesulfonamide (IV, A = H; B = CH_2CH_2OH).** *N*-(3-Chloro-4-nitrophenyl)methanesulfonamide (III; 3.0 g, 12 mmol) and ethanolamine (6 g, 100 mmol) were heated together at 120 °C until the starting material was consumed (TLC). The mixture was diluted with water (80 mL), acidified to pH 6 with 12 N HCl, and cooled well to give crude product, which was washed with cold water and crystallized from boiling water, followed by EtOAc, to give orange prisms (87% yield), mp 152–153 °C (Table IV).

***N*-[3-(Pentylamino)-4-nitrophenyl]methanesulfonamide [IV, A = H; B = $(CH_2)_4CH_3$].** *N*-(3-Chloro-4-nitrophenyl)-methanesulfonamide (III; 3.0 g, 12 mmol) and *n*-pentylamine (8 g, 92 mmol) were heated together under reflux for 8 h, cooled, and acidified. The resulting solid was extracted with 8 N HCl, and neutralization of the filtered solution with ammonia precipitated the crude product, which was crystallized from aqueous EtOH as orange prisms (70% yield), mp 116–118 °C (Table IV).

The hexylamino and heptylamino Derivatives (Table IV) were similarly prepared.

***N*-[3-[[(2-Hydroxyethyl)methyl]amino]-4-nitrophenyl]-methanesulfonamide (IV, A = CH_3; B = CH_2CH_2OH).** A solution of *N*-(3-chloro-4-nitrophenyl)methanesulfonamide (III; 5 g, 20 mmol) in 20 mL of *N*-methylethanolamine was heated at 100 °C for 12 h, and the excess solvent was removed under vacuum. The residue was treated with 10 mL of AcOH and extracted with EtOAc. After successive washings with minimum volumes of dilute aqueous AcOH and $NaHCO_3$, the EtOAc was dried ($MgSO_4$) and removed under vacuum to give the product sulfonamide as a bright red oil (97% yield): NMR (Me_2SO-d_6) δ 2.88 (s, 3 H, CH_3N), 3.07 (s, 3 H, CH_3S), 3.32 (t, 2 H, J = 5 Hz, CH_2N_2), 3.77 (t, 2 H, J = 5 Hz, CH_2O), 6.68 (dd, 1 H, J = 9 and 2 Hz, 6-H), 7.00 (d, 1 H, J = 2 Hz, 2-H), 7.68 (d, J = 9 Hz, 5-H).

***N*-[3-(Diethylamino)-4-nitrophenyl]methanesulfonamide (IV, A = B = CH_2CH_3).** A mixture of 3-chloro-4-nitroacetanilide (35 mmol) and diethylamine (50 mL of neat) was heated at 100 °C for 3 days in a pressure vessel. After the mixture was cooled, the excess diethylamine was removed under vacuum, and the oily residue was dissolved in 2 N ethanolic HCl and heated under reflux until all of the intermediate 3-(diethylamino)-4-nitroacetanilide had disappeared (about 1 h). The solution was evaporated to dryness, and the residue was basified with ammonia and extracted into EtOAc. Washing, drying, and removing the solvent gave 3-(diethylamino)-4-nitroaniline (93%) as a dark red oil, homogeneous on TLC: NMR ($CDCl_3$) δ 0.91 (t, 6 H, J = 7 Hz, CH_3), 2.64 (q, 4 H, J = 7 Hz, CH_2), 3.66 (br s, 2 H, NH_2), 5.13 (m, 2 H, 2-H and 6-H), 6.51 (d, 1 H, J = 8 Hz, 5-H).

The above nitroaniline was treated with methanesulfonyl chloride (1.1 equiv) in pyridine at 0 °C. After removal of the solvent, the residue was extracted into EtOAc, washed with 2 N AcOH, and extracted into 2 N NaOH. The aqueous layer was neutralized with 2 N AcOH and extracted with EtOAc to give *N*-[3-(diethylamino)-4-nitrophenyl]methanesulfonamide (88%) as a bright red oil: NMR ($CDCl_3$) δ 0.94 (t, 6 H, J = 7 Hz, CH_3), 2.58 (s, 3 H, SCH_3), 2.66 (q, 4 H, J = 7 Hz, CH_2), 5.52 (dd, 1 H, J = 2 and 9 Hz, 6-H), 5.78 (d, 1 H, J = 2 Hz, 2-H), 6.45 (d, 1 H, J = 9 Hz, 5-H).

***N*-[3-(1-Piperidinyl)-4-nitrophenyl]methanesulfonamide [IV, A, B = $-(CH_2)_5-$].** A mixture of 3-chloro-4-nitroacetanilide (3 g, 14 mmol) and piperidine (6 g, 70 mmol) was refluxed for 1 h, cooled, and neutralized with 2 N HCl. The precipitate was crystallized twice from aqueous MeOH to give **3-(1-piperidinyl)-4-nitroacetanilide** (76% yield), mp 123–124 °C. Anal. ($C_{13}H_{17}N_3O_3$) C, H, N. Acid hydrolysis of this product gave the amine as an orange oil, which was treated with methanesulfonyl chloride in pyridine as detailed above to provide the sulfonamide as orange prisms (87% yield from the acetanilide), mp 147–148 °C (Table IV).

***N*-[3-(Methylamino)-4-nitrophenyl]ethanesulfonamide.** A mixture of 3-chloro-4-nitroacetanilide (5 g, 24 mmol) and 40% aqueous methylamine solution (50 mL) was heated in a pressure vessel at 120 °C for 8 h. The cooled reaction mixture was concentrated under vacuum, and the residue was shaken with dilute AcOH. Crystallization of the solid gave **3-(methylamino)-4-nitroacetanilide** as orange needles (71% yield), mp 197–198 °C. Anal. ($C_9H_{11}N_3O_3$) C, H, N. Acid hydrolysis of this compound gave **3-(methylamino)-4-nitroaniline** as bronze needles from benzene–MeOH, mp 191–193 °C. Anal. ($C_7H_9N_3O_2$) C, H, N.

A stirred solution of the above nitroaniline (3.34 g, 20 mmol) in dry pyridine (15 mL) was treated dropwise at 0 °C with ethanesulfonyl chloride (2.0 mL, 21 mmol), kept at 20 °C for 1 h, and heated to 100 °C for 15 min. Excess pyridine was removed under vacuum, and the residue was triturated with dilute AcOH. The resulting solid was dissolved in 2 N NaOH, clarified, and reprecipitated with 2 N HCl to provide a TLC-homogeneous product, which was crystallized from aqueous EtOH as bronze needles (86% yield), mp 182–183 °C (Table IV).

Higher alkanesulfonamide homologous (Table IV) were prepared in a similar fashion and crystallized from aqueous EtOH or benzene in yields from 75 to 90%. The methanesulfonamide analogue (IV; A = H, B = CH_3) prepared by this method from methanesulfonyl chloride was identical in every respect with the product prepared from *N*-(3-chloro-4-nitrophenyl)methanesulfonamide (III) by method A.

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Dibenz[*b*,*e*]oxepinalkanoic Acids as Nonsteroidal Antiinflammatory Agents. 4. Synthesis and Evaluation of 4-(4,10-Dihydro-10-oxothieno[3,2-*c*][1]benzoxepin-8-yl)butanol and -butyric Acid and Related Derivatives

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4,10-Dihydro-10-oxothieno[3,2-*c*][1]benzoxepin-8-acetic acid (**6**) was previously reported as a potent antiinflammatory–analgesic agent characterized by an impressive therapeutic ratio in comparison with indomethacin. With the goal of finding compounds that might display even more favorable therapeutic ratios and/or enhanced antiinflammatory/analgesic properties in comparison to **6**, we synthesized 4-(4,10-dihydro-10-oxothieno[3,2-*c*][1]-benzoxepin-8-yl)butanol (**4b**) and -butyric acid (**5a**) and a series of related derivatives. All compounds were evaluated for potential analgesic activity in the phenylquinone-induced writhing (PQW) assay, for antiinflammatory activity in the carrageenan-induced paw edema (CPE) model and, where warranted, for gastric irritation (GI) liability. Of the compounds investigated, **4b** (HP 573) displays moderate analgesic-like activity in PQW, is approximately half as potent as indomethacin or **6** as an antiinflammatory agent in the CPE, and is characterized by an extremely low propensity to induce GI as reflected by comparison of the therapeutic ratios (GI ED_{50}/CPE ED_{50}: **4b** > 46, **6** = 9.9, indomethacin = 0.4). Compound **4b** was selected for clinical evaluation.

We previously reported the synthesis and biological evaluation of dihydro-10-oxofuro- and -thieno[3,2-*c*][1]-benzoxepin-8-acetic acids.[1-3] From this work, 4,10-dihydro-10-oxothieno[3,2-*c*][1]benzoxepin-8-acetic acid (**6**) emerged as a potent antiinflammatory–analgesic agent that is characterized by an impressive therapeutic ratio in comparison with indomethacin.[2] The fact that nonsteroidal antiinflammatory agents may exhibit gastric irritation (GI) on oral or parenteral administration is established, and the reduction of GI by molecular modification to minimize a high localization of the active drug in the gastrointestinal mucosa has been discussed by Stella.[4] With the goal of finding compounds that might display even more favorable therapeutic ratios and/or enhanced antiinflammatory/analgesic properties in comparison to **6**, we synthesized 4-(4,10-dihydro-10-oxothieno[3,2-*c*][1]benzoxepin-8-yl)butanol (**4b**) and -butyric acid (**5a**) and a series of related derivatives. On oral administration we hoped that these compounds would be absorbed without significant gastric irritation due to a local effect and then be converted to **6** as the, a priori, biologically active species. Support for **6** as possibly being the biologically active species is derived from analogy to the recently established biotransformation of the antiinflammatory agent fenbufen to 4-biphenylacetic acid, which was inferred to be the biologically active moiety based on in vitro and in vivo studies of fenbufen and its metabolities.[5] The synthesis of the acetoxyacetate derivative **7** was prompted by a report that enhanced antiinflammatory activity is associated with the acetoxyacetic acid analogue of indomethacin.[6] The oxazoline **11**, prepared for C-alkylation studies, also represents a masked form of **6**, and both **7** and **11** are reversible derivatives of **6**, at least in a chemical sense.

Chemistry. The synthesis of the various analogues is outlined in Schemes I and II, and the properties of the compounds are summarized in Table I. Condensation of methyl 3-(bromomethyl)-2-thiophenecarboxylate (**1**) with 4-(4-hydroxyphenyl)butanol (**2a**) or ethyl 4-(4-hydroxyphenyl)butyrate (**2b**) and hydrolysis afforded the intermediates **3a** and **3b**, respectively. Cyclization of **3a** with trifluoroacetic anhydride gave the trifluoroacetyl ester **4a** which after acid-catalyzed hydrolysis and esterification afforded the butanol analogue **4b** and the acetyl ester **4c**, respectively. Similar cyclization of **3b** gave the butyric acid derivative **5a** from which esters **5b–e** were prepared by standard methods.

Ester derivatives of **6** were previously reported.[7] The acetoxyacetate analogue **7** was prepared by condensation of **6** under mildly alkaline conditions with methyl bromoacetate. Selective borane–methyl sulfide reduction of **6**

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