

[EtOAc/petroleum ether (1:1)], 0.77 [CHCl<sub>3</sub>/CH<sub>3</sub>OH (9:1)], diacetate *R<sub>f</sub>* 0.34 [CHCl<sub>3</sub>/CH<sub>3</sub>OH/CH COOH (9:3:0.5)].

**Brain-Uptake Studies. Brain Penetration Index Measurements.** Each of the <sup>14</sup>C-labeled lipids was dissolved in 15% propyleneglycol in water (0.3–0.4 mL) and then injected subcutaneously (sc) into Balb-c mice (male) (20 ± 2 g). After 5 min the animals were sacrificed by cervical fracture, the brain and liver were removed, weighed, and homogenized in 8 and 10 mL, respectively, of brain protein solvent<sup>12</sup> (phosphate buffer containing sodium dodecyl sulfate and EDTA at pH 7.6), and aliquots were counted for <sup>14</sup>C in 10 mL of liquiscint (National Diagnostics) with a Beckman liquid scintillation counter. The <sup>14</sup>C counts were used to calculate the total quantity of the compound present in brain and liver per gram of tissue. The ratio of the amount in brain as a percent of that present in liver at 5 min was taken as the brain penetration index (BPI). BPI = ([brain]/[liver]) × 100.

**Brain Uptake Index Measurements.** Male Sprague-Dawley rats (200–300 g) were deeply anesthetized with Nembutal (Abbott Laboratories, Chicago), and the right carotid artery was dissected free. A 0.15-mL bolus of <sup>3</sup>H-labeled water and a <sup>14</sup>C-labeled test compound dissolved in 0.01 M HEPES (pH 7.4) was rapidly injected into the artery, and the animal was decapitated 5 s later.<sup>13</sup> The brain was quickly removed and the ipsilateral forebrain dissected out, rinsed, and homogenized in 0.5 mL of buffer consisting of 0.1% SDS, 6 M urea, 20 mM EDTA, and 10 mM sodium phosphate (pH 7.4). Duplicate aliquots of the homogenate were

added to 10 mL of Liquiscint (National Diagnostics), and the radioactivity was determined in a Beckman liquid scintillation counter. The results were corrected for quenching and spillover. A brain uptake index (BUI)<sup>9</sup> was calculated for each compound by the formula:

$$\text{BUI} = [^{14}\text{C cpm}/^3\text{H cpm}]_{\text{brain}} / [^{14}\text{C cpm}/^3\text{H cpm}]_{\text{injectate}}$$

**Pharmacology.** The lipid conjugate of GABA was used as the diacetate salt dissolved in 0.14 M saline. An injection volume of 0.1–0.2 cc for 20 ± 2 g Balb-c mice was used. Suppression of general motor activity following an intraperitoneal (ip) injection of each test compound in comparison to vehicle control was carried out on a Stoelting electronic activity monitor (EAM) apparatus.<sup>11</sup> Each data point represents the mean of the general motor activity of nine test mice, as compared to the results for nine control mice injected with the vehicle (0.14 M saline).

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**Registry No.** 1a, 123-94-4; 1b, 111-03-5; 1c, 2277-28-3; 2a, 108920-48-5; 2b, 108920-49-6; 2c, 108920-50-9; 3a, 108920-51-0; 3a-2HOAc, 108920-52-1; 3a-2TFA, 108920-58-7; [<sup>14</sup>C]-3a-2HOAc, 108920-61-2; 3b, 108920-53-2; 3b-2HOAc, 108920-54-3; [<sup>14</sup>C]-3b-2HOAc, 108920-63-4; 3c, 108920-55-4; 3c-2HOAc, 108920-56-5; 3c-2TFA, 108920-59-8; [<sup>14</sup>C]-3c-2HOAc, 108920-65-6; 3d, 93383-17-6; 3d-2HOAc, 108920-57-6; Box-GABA anhydride, 89231-63-0; [<sup>14</sup>C]-Boc-GABA anhydride, 89231-64-1.

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## Potential Antitumor Agents. 52. Carbamate Analogues of Amsacrine with in Vivo Activity against Multidrug-Resistant P388 Leukemia

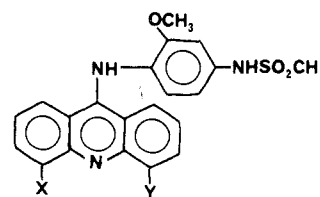
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Study of a series of aniline-substituted 9-anilinoacridines related to the antileukemic drug amsacrine showed that a 1'-carbamate group provided increased activity against the multidrug-resistant P388/ADR leukemia subline in vivo. Since activity against such resistant tumors is of great clinical significance, a series of acridine-substituted carbamate derivatives were evaluated against both wild-type and ADR/resistant P388 leukemia and the Lewis lung solid tumor in vivo. Structure-activity relationships for all three tumor lines were similar, with 3-halo-5-methyl and 3-halo-5-methoxy compounds proving the most active. This substitution pattern also provided the highest DNA binding. Such compounds (particularly the 3-chloro-5-methyl and 3-chloro-5-methoxy) have in vivo activity against wild-type P388 and Lewis lung comparable to that of the best amsacrine analogues previously developed (>50% cures), as well as P388/ADR activity. This work essentially completes the development of the amsacrine series of antitumor agents.

The acridine derivative amsacrine (1) is a useful clinical antitumor drug, albeit with a limited spectrum of action.<sup>1,2</sup> Work in our laboratory on analogues of amsacrine has concentrated on structural variants with a broader spectrum of action against experimental tumors, using particularly the mouse Lewis lung carcinoma.<sup>3</sup> This work has led to the 4-methyl-5-(methylcarbamoyl) analogue (2; CI-921, NSC 343 499), which is currently in clinical trial,<sup>4</sup> and other derivatives now under advanced evaluation.<sup>5-8</sup>



(1) X = Y = H

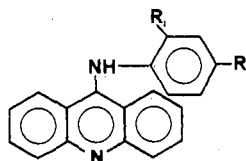
(2) X = CH<sub>3</sub>; Y = CONHCH<sub>3</sub>

One significant aspect of the clinical profile of amsacrine that has received little attention is the reports<sup>9,10</sup> of its

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Table I. In Vivo Activity of 9-Anilinoacridines against Adriamycin-Resistant Leukemia P388



no.	R <sub>1</sub>	R <sub>2</sub>	R <sub>m</sub> <sup>a</sup>	pK <sub>a</sub> <sup>b</sup>	log K <sup>c</sup> (AT)	E <sub>1/2</sub> <sup>d</sup>	in vivo activity			
							P388		P388/ADR	
							OD <sup>e</sup>	ILS <sub>max</sub> <sup>f</sup>	OD	ILS <sub>max</sub>
1	OCH <sub>3</sub>	NHSO <sub>2</sub> CH <sub>3</sub>	0.18	7.43	5.57	280	13.3	78	13.3	NA <sup>g</sup>
3	NHCH <sub>3</sub>	NHSO <sub>2</sub> CH <sub>3</sub>	0.17	7.47	6.42	145	13.3	152 (2) <sup>h</sup>	13.3	NA
4	N(CH <sub>3</sub> ) <sub>2</sub>	NHSO <sub>2</sub> CH <sub>3</sub>	0.41	7.25	5.34	195	166	210 (2)	100	41
5	OCH <sub>3</sub>	NHCOCH <sub>3</sub>	0.30	7.79	6.10	427	13.3	50	8.9	NA
6	NHCH <sub>3</sub>	NHCOCH <sub>3</sub>	0.30	7.80	6.79		65	96	65	NA
7	N(CH <sub>3</sub> ) <sub>2</sub>	NHCOCH <sub>3</sub>	0.57	7.59	5.58		65	NA	100	NA
8	OCH <sub>3</sub>	NHCOOCH <sub>3</sub>	0.50	7.93	6.24	378	30	86	45	33
9	NHCH <sub>3</sub>	NHCOOCH <sub>3</sub>	0.51	7.95	6.35	185	30	132	45	50
10	N(CH <sub>3</sub> ) <sub>2</sub>	NHCOOCH <sub>3</sub>	0.56	7.85	5.88	305	45	20	30	NA
11	OCH <sub>3</sub>	NHP(O)(OCH <sub>3</sub> ) <sub>2</sub>	0.37	8.21	6.18	290	8.9	67	8.9	NA
12	NHCH <sub>3</sub>	NHP(O)(OCH <sub>3</sub> ) <sub>2</sub>	0.32	8.09	6.82		13.3	118		NT <sup>i</sup>
13	Na(CH <sub>3</sub> ) <sub>2</sub>	NHP(O)(OCH <sub>3</sub> ) <sub>2</sub>	0.44	(8.17) <sup>j</sup>	6.43		100	97	100	NA

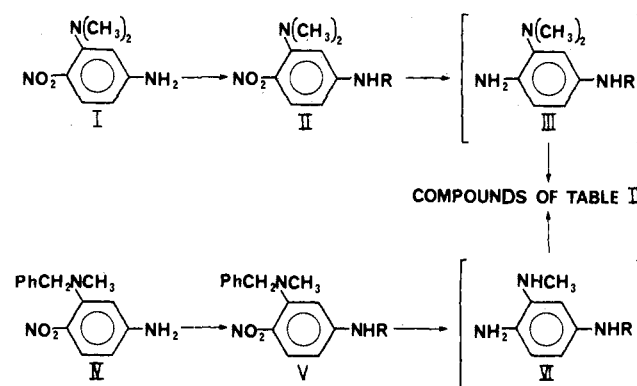
<sup>a</sup>R<sub>m</sub> values are a measure of drug lipophilicity and were determined as in ref 17, with 4'-(9-acridinylamino)methanesulfonamide as an internal reference. <sup>b</sup>pK<sub>a</sub> values for the acridine nitrogen were determined in 20% DMF as detailed in ref 17; add ca. 0.60 pK<sub>a</sub> units for the value in aqueous solution. <sup>c</sup>log K: binding constant (M<sup>-1</sup>) to poly[d(A-T)], determined by ethidium bromide displacement with correction for quenching. See ref 26. <sup>d</sup>E<sub>1/2</sub>: half-wave redox potential (mV) for the reversible two-electron oxidation to the quinone diimine, determined as detailed in ref 25. <sup>e</sup>OD: optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v EtOH/water on days 1, 5, and 9 after intraperitoneal inoculation of 10<sup>6</sup> P388 or ADR/P388 cells or on days 5, 9, and 13 after intravenous inoculation of 10<sup>6</sup> Lewis lung carcinoma cells. See ref 27. <sup>f</sup>ILS<sub>max</sub>: the percentage increase in lifespan of drug-treated tumor-bearing animals compared to nontreated tumor-bearing controls; values above 20% for P388 and ADR/P388 and above 40% for Lewis lung are considered statistically significant. <sup>g</sup>NA: compound not active at any dose level. <sup>h</sup>Numbers in parentheses indicate the number of animals in a group of 6 that were long-term survivors (50 days for P388 and P388/ADR and 60 days for Lewis lung); such animals are normally considered cured. <sup>i</sup>NT: compound not tested. <sup>j</sup>Approximate: compound too insoluble for accurate determination.

activity against various Adriamycin-resistant tumors in the clinic. Since Adriamycin is a very widely used drug, and since resistance to it is usually pleiotropic (with collateral resistance to many other drugs of widely differing structures and mechanism of action), this aspect of amsacrine activity is of interest. Analogues with enhanced activity against Adriamycin-resistant tumor lines would be potentially as useful as those with a broadened spectrum of activity.

In this paper we evaluate a series of 9-anilinoacridine derivatives against both the P388 leukemia and an Adriamycin-resistant subline. The basis of drug resistance in cell lines such as P388/ADR (developed by repeated passage in the presence of increasing concentrations of Adriamycin<sup>11</sup>) is currently under intense debate.<sup>12</sup> Anthracycline resistance in these cell lines is associated with an increased, energy-dependent drug efflux process<sup>13</sup> and with the amplification of genes coding for a plasma membrane glycoprotein (P-glycoprotein).<sup>14</sup> The mechanism of this process is not clear, but it recognizes a number of drugs of widely differing structure apart from the anthracyclines, including amsacrine.<sup>15</sup>

Thus amsacrine has an IC<sub>50</sub> of 49 nM against the wild-type P388 cell line, but a value of 460 nM against P388/ADR,<sup>16</sup> giving an IC<sub>50</sub> ratio of 9.4. Studies of the IC<sub>50</sub> ratios of the number of amsacrine analogues showed that

## Scheme I



a: R=COCH<sub>3</sub>  
b: R=COOCH<sub>3</sub>  
c: R=P(O)(OCH<sub>3</sub>)<sub>2</sub>

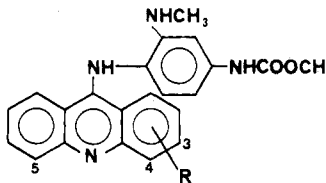
this ratio could be significantly lowered by certain substituents on the aniline ring.<sup>16</sup> Since IC<sub>50</sub> ratios of carcinoma cell lines vs. leukemia lines have previously been shown to predict activity against such tumors in vivo, it was decided to evaluate a selection of acridine-substituted amsacrine derivatives against the P388/ADR leukemia in vivo. Once a clear choice of aniline substitution pattern to give the best in vivo activity emerged, a series of acridine-substituted analogues of this parent compound would be prepared and evaluated against both the P388/ADR leukemia and the advanced Lewis lung carcinoma<sup>3</sup> in vivo, to determine structure-activity relationships against each tumor line and compare these with previous studies.

## Chemistry

The compounds of Tables I and II were prepared by acid-catalyzed coupling of the appropriate 9-chloro-

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Table II. Physicochemical and Biological Data for Carbamate Derivatives



no.	R	Rm <sup>a</sup>	log K <sup>b</sup>	in vitro IC <sub>50</sub> <sup>c</sup>		in vivo data					
				L1210	HCT-8	P388		P388/ADR		LL	
						OD <sup>d</sup>	ILS <sub>max</sub> <sup>e</sup>	OD	ILS <sub>max</sub>	OD	ILS <sub>max</sub>
9	H	0.51	6.35	284	290	30	132	45	50	30	112 (1) <sup>f</sup>
14	2-CH <sub>3</sub>	0.70	5.62	436	850	45	69	45	NA	13.3	NA <sup>g</sup>
15	3-F	0.63	5.77	200	420	65	110	100	21	100	83
16	3-Cl	0.73	6.13	174	550	65	104	65	35	45	92
17	3-Br	0.70	6.87	341	800	45	101	45	29	45	86
18	3-I	0.65	6.12	110	350	45	92	100	NA	65	68
19	3-CH <sub>3</sub>	0.78	6.72	152	270	8.9	105	13.3	NA	8.9	58
20	3-OCH <sub>3</sub>	0.45	6.74	170	330	45	108	65	89	45	85 (1)
21	3-NO <sub>2</sub>	0.40	6.58	5060		30	48	20	NA	65	49
22	4-F	0.37	5.82	1400	2800	65	103	100	43	65	77 (2)
23	4-Cl	0.42	6.30	906	4500	30	30	45	22	65	NA
24	4-CH <sub>3</sub>	0.56	6.74	101	190	13.3	114	20	43	20	122 (2)
25	4-OCH <sub>3</sub>	0.41	6.57	540	630	30	98	30	35	30	167 (3)
26	4-CONH <sub>2</sub>	0.06	6.00	1600	3600	100	32	100	NA	65	NA
27	4-CONHCH <sub>3</sub>	0.43	6.48	840	2750	65	60	65	NA	65	58
28	3-F, 5-CH <sub>3</sub>	0.52	7.37	520	610	65	43	65	47	45	126
29	3-Cl, 5-CH <sub>3</sub>	0.67	7.52	185	330	45	173	45	79	45	143 (4)
30	3-Br, 5-CH <sub>3</sub>	0.69	7.52	180	230	45	286 (1)	45	42	30	82 (1)
31	3,5-(CH <sub>3</sub> ) <sub>2</sub>	0.57	7.69	52	62	8.9	189	20	76	13.3	61
32	3-CH <sub>3</sub> , 5-CH <sub>3</sub>	0.51	6.95	44	80	20	181	13.3	33	20	171 (3)
33	3-F, 5-OCH <sub>3</sub>	0.45	6.27	940	1600	100	125	45	NA	45	52
34	3-Cl, 5-OCH <sub>3</sub>	0.54	6.93	660	1100	100	177	100	73	65	133 (3)
35	3-Br, 5-OCH <sub>3</sub>	0.55	6.43	360		65	98	65	20	100	31 (1)
36	3-CH <sub>3</sub> , 5-OCH <sub>3</sub>	0.61	7.34	11		45	128	45	57	65	107 (3)
37	4,5-(CH <sub>3</sub> ) <sub>2</sub>	0.46	7.34	490		45	154 (3)	45	43	100	161 (1)
38	4,5-(OCH <sub>3</sub> ) <sub>2</sub>	0.41	7.37	865	1180	65	120 (1)	45	NA	65	45
39	4-CH <sub>3</sub> , 5-CONHCH <sub>3</sub>	0.50	7.12	1200	1850	20	99	30	35	30	84
40	4-OCH <sub>3</sub> , 5-CONHCH <sub>3</sub>	0.45	6.95	1750		65	92	45	31	65	47

<sup>a</sup> See footnote a, Table I. <sup>b</sup> See footnote b, Table I. <sup>c</sup> IC<sub>50</sub>: concentration of drug in nM to inhibit growth of leukemia L1210 or human colon tumor (HCT-8) cells in culture by 50% after a 40 h exposure; see ref 28. <sup>d</sup> See footnote e, Table I. <sup>e</sup> See footnote f, Table I. <sup>f</sup> See footnote h, Table I. <sup>g</sup> See footnote g, Table I.

acridines and substituted anilines. The 3-substituted 4-aminomethanesulfonanilides for the preparation of compounds 1, 3, and 4 and the 3-methoxy derivatives for compounds 5, 8, and 11 have been described.<sup>17-19</sup> Side chains for compounds 7, 10, and 13 were elaborated from 3-(dimethylamino)-4-nitroaniline (I),<sup>8</sup> as shown in Scheme I. The 4-amino derivatives III were very unstable and were used without isolation.

Side chains for compounds 6, 9, and 12 were similarly prepared from 3-(benzylmethylamino)-4-nitroaniline (IV).<sup>5</sup> Reaction with the appropriate reagent followed by hydrogenation over Pd/C reduced the nitro group and simultaneously effected debenzoylation to give the unstable amines VI. However, the prolonged reaction times needed for debenzoylation were inconvenient in the case of Vb, used for preparation of the many compounds of Table II. Fortunately the stability of the carbamate group in this case meant that hydrolytic debenzoylation could be employed, and the resulting methyl *N*-[3-(methylamino)-4-nitrophenyl]carbamate was used as the precursor.

The end products of Table II were often difficult to crystallize, and purification by the method given in the

Experimental Section was usually necessary before crystalline samples could be obtained.

## Results and Discussion

Table I gives in vivo results for amsacrine (1) and 11 anilino-substituted derivatives against both the parent (wild-type) P388 leukemia and the P388/ADR subline. Amsacrine itself shows no activity against P388/ADR, and the 3'-NHCH<sub>3</sub> derivative 3 is also inactive, despite greatly enhanced activity against the parent line. The 3'-N(CH<sub>3</sub>)<sub>2</sub> derivative 4 shows some activity, but at very high doses. While the corresponding 1'-NHCOCH<sub>3</sub> analogs 5-7 showed higher levels of DNA binding and were much more resistant to oxidation (and thus possibly to oxidative metabolism), they had lower in vivo activity against wild-type P388 and were inactive against P388/ADR. The dimethyl phosphoramidate compounds 11-13 showed the highest levels of DNA binding, but were also inactive against P388/ADR. Overall, the highest activity against the resistant leukemia line was shown by the carbamate derivatives 8-10, which were also the most lipophilic compounds. The most interesting was the 3'-(methylamino) 1'-carbamate 9, which showed high activity in the wild-type P388, the highest activity of any of the derivatives against the P388/ADR, and in addition produced long-term survivors in the Lewis lung (LL) carcinoma model (Table II).

Structure-activity studies with acridine-substituted derivatives of several series of 9-anilinoacridines, including analogues of 1,<sup>21,22</sup> 3,<sup>5</sup> 4,<sup>8</sup> and 11,<sup>23</sup> have invariably dem-

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onstrated the significant influence of acridine substitution on biological activity, and in each case, compounds of superior activity have resulted from these SAR studies. We thus decided to similarly study the SAR for acridine substitution of the carbamate **9**, aiming to maximize activity against the ADR/P388 leukemia, and to compare for the set of compounds SAR against this tumor, the wild-type P388, and the LL.

Physicochemical data and the results of testing against all three tumor systems are shown in Table II for the parent carbamate **9**, 14 monosubstituted derivatives (**14–27**), and 13 disubstituted compounds (**28–40**). As noted above, replacement of a 1'-NHSO<sub>2</sub>CH<sub>3</sub> group ( $\pi$  value -1.18) by 1'-NHCOOCH<sub>3</sub> ( $\pi$  value -0.37) gives an expected increase in lipophilicity as measured by R<sub>m</sub> values. The difference between the parent compounds (**3** and **9**) is 0.33 units, and this is reflected throughout the series, with the carbamates having R<sub>m</sub> values on average 0.35 units higher (data from Table II and ref. 5). This is equivalent to 0.70 log *P* units, which is very close to the difference of 0.81 in  $\pi$  values between the 1'-substituents.

The monosubstituted compounds show DNA binding constants broadly similar to those of the corresponding 1'-NHSO<sub>2</sub>CH<sub>3</sub> derivatives,<sup>5</sup> suggesting that the 1' group has little effect on binding. While the 2-methyl compound has depressed binding compared to the parent in both series, both 3- and 4-substituents generally enhance binding. As observed previously,<sup>5</sup> the 3,5-disubstitution pattern appears to enhance binding by more than that expected from simple addition of the effects of the two individual substituents. However, the 1'-carbamate does have a significant effect on in vitro cytotoxicity (as noted earlier in a comparison of the parent compounds **3** and **9**). The nearly 9-fold decrease in cytotoxicity against the L1210 shown by the carbamate is also reflected by the acridine-substituted compounds, which are on average 7-fold-less cytotoxic than their 1'-NHSO<sub>2</sub>CH<sub>3</sub> counterparts (data from Table II and ref. 5). However, this difference between the two series is not as marked for cytotoxicity against the HCT-8 human colon tumor line, with the result that the carbamate compounds in general show a better IC<sub>50</sub> ratio (ratio of IC<sub>50</sub> values for L1210 and HCT-8). Thus amsacrine (**1**) has a ratio of 3.6 in favor of the leukemia, and the 3'-NHCH<sub>3</sub> compound **3** has a ratio of 2.5, but the carbamate **9** has a ratio of 1.0, indicating improved selectivity for the solid tumor line. For acridine-substituted derivatives of amsacrine itself and for a series of 3'-NHCH<sub>3</sub> derivatives,<sup>5</sup> low values of this ratio have been shown to correlate with in vivo activity against the LL solid tumor, and the low ratio shown by **9** was a further encouragement for development of the series.

The compounds of Table II were tested in vivo against all three tumor lines over the full dose range from inactive to toxic. The optimal dose (OD) values approximate to the LD<sub>10</sub> and can be used as a comparative measure of acute toxicity. The ILS<sub>max</sub> values are the percentage increase in lifespan of treated over nontreated groups at the OD and are a rough measure of tumor cell selectivity. Dosage protocols in all cases were qd4 × 3 (on days 1, 5, and 9 after inoculation of the P388 and P388/ADR and

days 5, 9, and 13 after inoculation of the LL; see Table I footnotes). Against the wild-type P388, the monosubstituted carbamates **14–27** showed similar potency but slightly lower activity than their 1'-NHSO<sub>2</sub>CH<sub>3</sub> counterparts (Table II and ref. 5). In each series the 3- and 4-methyl groups markedly improved dose potency. Among the disubstituted compounds, the same general trends are seen in both series; the 3-halo-5-methyl and 3-halo-5-methoxy substitution patterns provide the most active compounds, but the carbamate series overall shows slightly lower activity.

Against the LL carcinoma, the two series again show roughly comparable activity. Given the significantly lower IC<sub>50</sub> ratios shown by the carbamates compared to their 1'-NHSO<sub>2</sub>CH<sub>3</sub> counterparts (see above), one might have hoped for significantly improved activity against the LL, but the carbamate series overall shows only a slight superiority (Table II and ref. 5). In both series the favored substitution patterns are again 3-halo-5-methyl and 3-halo-5-methoxy, although the 4-methyl and 4-methoxy carbamates also show good activity, being capable of effecting about 50% of cures.

However, the most interesting set of data was the activity of the carbamate compounds against the P388/ADR tumor line. As noted above, among various 9-anilinoacridine derivatives, the 1'-carbamate group did seem to confer a definite advantage for P388/ADR activity (Table I), and the major question was whether there were also clear-cut SAR for acridine substitution, different to that for wild-type P388 and LL activity. The answer from the data of Table II is, however, clearly negative. Among the (admittedly limited) set of compounds studied, in vivo activity against the P388/ADR is depressed compared to that against the wild type, but clearly follows the same broad SAR. Thus monosubstitution at either the 3- or 4-position has little effect, and the most effective substitution patterns are again 3-halo-5-methyl and 3-halo-5-methoxy (or rather, more narrowly, 3-chloro-5-methyl and 3-chloro-5-methoxy). These two compounds (**29** and **34**), together with the 3,5-dimethyl compound (**31**) are the only ones to show ILS<sub>max</sub> values above 70% and are thus clearly separated from the rest. While the higher potency of **31** might suggest it as a better candidate, in fact the superior LL activity of the two halogen derivatives **29** and **34** proclaim these as the two best compounds of the series, and they are under advanced evaluation.

## Conclusions

Since the introduction of amsacrine (**1**) into clinical practice as an antileukemic agent,<sup>1,2</sup> a great many analogues have been prepared and evaluated in our laboratory, in a search primarily for compounds with a broader spectrum of activity against solid tumors. This search has been successful, resulting in the agent (**2**) (4-methyl-5-(methylcarbamoyl)amsacrine) now in clinical trial and several 3'-(alkylamino) derivatives under advanced evaluation. In the present work we have systematically examined a number of 9-anilinoacridines with varying 3'- and 1'-substituents for improved activity against the multi-drug-resistant P388/ADR leukemia subline, since compounds with improved activity against Adriamycin-resistant tumors would be of great significance.<sup>9,10</sup> The 1'-carbamate group did give compounds with sufficient activity against both the P388/ADR and the LL to warrant further study of SAR for acridine substitution. For these compounds, SAR for activity against all three tumor lines studied (wild-type and ADR-resistant P388 and LL) proved to be identical and to be similar to those already observed for the related 3'-NHCH<sub>3</sub>, 1'-NHSO<sub>2</sub>CH<sub>3</sub> series,

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with 3-halo-5-methoxy compounds being the most active. This is the same substitution pattern that maximizes DNA binding, and the results for all three tumor lines may only reflect this. However, the best of the carbamate compounds (29 and 34) show sufficiently high activity to be added to the small number of amsacrine analogues now under advanced evaluation.

This work essentially completes the development of the amsacrine series of compounds, which was begun<sup>20</sup> in 1972. Systematic substitution of both the aniline and the acridine rings of 9-anilinoacridine has provided a group of compounds of sufficiently high activity (at least against the wild-type P388 and the LL) that these tumors can no longer be used to rank them, since they all provide at least 50% of long-term survivors (Table II and ref 5 and 8). Results from the clinical evaluation of 2 are now awaited before a decision is made whether a third compound from the series might go forward for human trials.

### Experimental Section

Where analyses are indicated by symbols of the elements, analytical results obtained were within  $\pm 0.4\%$  of the theoretical. Analyses were performed by the Microchemical Laboratory, University of Otago, New Zealand. Melting points were determined on an Electrothermal apparatus with the maker's stem-corrected thermometer and are as read. The progress of reactions and the purity of products were monitored by TLC on  $\text{SiO}_2$ . Cyclic voltammetry was carried out on a Princeton Applied Research M173 instrument, using the methods given in ref 25. NMR spectra were measured on a Bruker WP-60.

**Methyl N-[4-(Butyramido)-3-methoxyphenyl]carbamate.** A stirred solution of *N*-(4-amino-2-methoxyphenyl)butanamide<sup>24</sup> (5 g, 25.5 mmol) in pyridine (20 mL) was treated at 3 °C with  $\text{CH}_3\text{COCl}$  (2.9 g, 1.2 equiv). The mixture was kept at 20 °C for 10 min, and most of the solvent was removed in vacuo. The residue was triturated with water, and the resulting solid was collected, washed well with water, and crystallized from aqueous EtOH (6.1 g, 91%), mp 141–142 °C. Anal. ( $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_4$ ) C, H, N.

**Compound 8 of Table I.** A solution of the above carbamate (2.7 g, 10 mmol) in 2 N ethanolic HCl was heated under reflux for 1 h. The solution was evaporated to dryness under reduced pressure, and the residue was azeotroped twice with EtOH to remove water and ethyl butyrate. The resulting hydrochloride and 9-chloroacridine (2.0 g, 0.95 equiv) were dissolved in MeOH and heated under reflux for 10 min. The volume was then reduced by boiling, and EtOAc was added until crystallization began. The solution was then kept at 20 °C for 4 h, and the hydrochloride salt of 8 was collected (2.5 g, 65%), mp 214–216 °C. Anal. (Table III).

**Methyl N-[3-(Dimethylamino)-4-nitrophenyl]carbamate (IIb).** A solution of 3-(dimethylamino)-4-nitroaniline (**I**)<sup>8</sup> (4 g, 22 mmol) in pyridine (20 mL) was treated with  $\text{CH}_3\text{COCl}$  (2.5 g, 1.2 equiv) at 0 °C. The mixture was allowed to warm to 20 °C, water was added, and the solvents were removed in vacuo. Trituration with 1 N HCl gave a solid, which was crystallized from EtOH as needles (4.4 g, 83%), mp 122–123 °C. Anal. ( $\text{C}_{10}\text{H}_{13}\text{N}_3\text{O}_4$ ) C, H, N.

**Methyl N-[3-(Methylamino)-4-nitrophenyl]carbamate.** Similar treatment of 3-(benzylmethylamino)-4-nitroaniline (**IV**)<sup>8</sup> with  $\text{CH}_3\text{COCl}$  gave crude **Vb**, which was hydrolyzed by heating under reflux for 2 h in EtOH/water/concentrated HCl (20:7:3). The EtOH was then allowed to boil off until the product separated as orange crystals (75% yield). A sample was recrystallized from

Table III. Analytical Data for the New Compounds of Tables I and II

no.	mp, °C	formula	analyses
5	224–226	$\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
6	292–294	$\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}\cdot\text{HCl}$	C, H, N, Cl
7	270–271	$\text{C}_{25}\text{H}_{22}\text{N}_4\text{O}\cdot\text{HCl}$	C, <sup>a</sup> H, N, Cl
8	214–216	$\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_3\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
9	268 dec	$\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
10	260–262	$\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_2\cdot\text{HCl}$	C, H, N, Cl
12	230 dec	$\text{C}_{22}\text{H}_{23}\text{N}_4\text{O}_3\text{P}\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
13	245 dec	$\text{C}_{23}\text{H}_{25}\text{N}_4\text{O}_3\text{P}\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N
14	267 dec	$\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_2\cdot\text{HCl}$	C, H, N, Cl
15	246–250 dec	$\text{C}_{22}\text{H}_{19}\text{FN}_4\text{O}_2\cdot\text{HCl}$	C, H, N, Cl
16	250 dec	$\text{C}_{22}\text{H}_{19}\text{ClN}_4\text{O}_2\cdot\text{HCl}$	C, H, N, Cl
17	270 dec	$\text{C}_{22}\text{H}_{19}\text{BrN}_4\text{O}_2\cdot\text{HCl}$	C, H, N, Cl
18	240 dec	$\text{C}_{22}\text{H}_{19}\text{IN}_4\text{O}_2\cdot 2\text{HCl}$	C, <sup>b</sup> H, N
19	272–274	$\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_2\cdot\text{HCl}$	C, H, N, Cl
20	>360	$\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_3\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
21	>360	$\text{C}_{22}\text{H}_{18}\text{N}_5\text{O}_4\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N
22	249–252	$\text{C}_{22}\text{H}_{19}\text{FN}_2\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
23	209 dec	$\text{C}_{22}\text{H}_{19}\text{ClN}_4\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
24	283–285	$\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_2\cdot 2\text{HCl}$	C, H, N, Cl
25	238–240	$\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_3\cdot 2\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
26	270–275	$\text{C}_{23}\text{H}_{21}\text{N}_6\text{O}_3\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
27	260 dec	$\text{C}_{24}\text{H}_{23}\text{N}_5\text{O}_3\cdot 2\text{HCl}$	C, H, N, Cl
28	225 dec	$\text{C}_{23}\text{H}_{21}\text{FN}_4\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
29	>310	$\text{C}_{23}\text{H}_{21}\text{ClN}_4\text{O}_2\cdot 2\text{HCl}$	C, <sup>b</sup> H, N, Cl
30	>360	$\text{C}_{23}\text{H}_{21}\text{BrN}_4\text{O}_2$	C, H, N, Cl
31	295–298	$\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
32	289–292	$\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_3\cdot 2\text{HCl}$	C, H, N, Cl
33	>350	$\text{C}_{23}\text{H}_{21}\text{FN}_4\text{O}_3\cdot 2\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
34	>300	$\text{C}_{23}\text{H}_{21}\text{ClN}_4\text{O}_3\cdot 2\text{HCl}$	C, H, N
35	240 dec	$\text{C}_{23}\text{H}_{21}\text{BrN}_4\text{O}_3\cdot 2\text{HCl}$	C, H, N
36	239 dec	$\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_3\cdot 2\text{HCl}\cdot\text{H}_2\text{O}$	C, H, N, Cl
37	265–267	$\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_2\cdot\text{HCl}$	C, <sup>b</sup> H, N, Cl
38	220 dec	$\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_4\cdot\text{HCl}$	C, H, N, Cl
39	>360	$\text{C}_{25}\text{H}_{25}\text{N}_5\text{O}_3\cdot\text{HCl}$	C, H, N, Cl
40	283–285	$\text{C}_{25}\text{H}_{25}\text{N}_5\text{O}_4\cdot\text{HCl}$	C, <sup>a</sup> H, N, Cl

<sup>a</sup>C out by 0.5%. <sup>b</sup>C out by 0.6%.

aqueous EtOH, mp 195 °C. Anal. ( $\text{C}_9\text{H}_{11}\text{N}_3\text{O}_4$ ) C, H, N.

**Dimethyl N-[3-(Dimethylamino)-4-nitrophenyl]phosphoramidate (IIc).** A suspension of 3-(dimethylamino)-4-nitroaniline (**I**)<sup>8</sup> (10 g, 55 mmol) in  $\text{POCl}_3$  (200 mL) was heated under reflux for 2 h, when all of the solid had dissolved. Excess  $\text{POCl}_3$  was removed under reduced pressure, and the oily residue was dissolved in a solution of excess NaOMe in MeOH at  $-78$  °C. The basic solution was poured into 2 L of water containing  $\text{NaH}_2\text{PO}_4$  buffer, and the product was extracted EtOAc. Removal of the solvent and chromatography on  $\text{SiO}_2$  (eluting with EtOAc) gave dimethyl *N*-[3-(dimethylamino)-4-nitrophenyl]phosphoramidate (5.91 g, 37%) as a dark red oil: NMR ( $\text{CDCl}_3$ )  $\delta$  2.93 (s, 6 H,  $\text{CH}_3\text{N}$ ), 3.90 (d,  $J = 12$  Hz, 6 H,  $\text{CH}_3\text{O}$ ), 4.83 (m, 1H), 6.66 (m, 1 H, aromatic 2-H), 7.90 (m, 2 H, aromatic 5-H and 6-H).

**Dimethyl N-[3-(Benzylmethylamino)-4-nitrophenyl]phosphoramidate (Vc).** Treatment of 3-(benzylmethylamino)-4-nitroaniline (**IV**)<sup>5</sup> with dimethyl phosphorobromidate gave a yellow oil, which, after decolorization with charcoal, was crystallized from MeOH (46% yield), mp 128–128.5 °C. Anal. ( $\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}_5\text{P}$ ) C, H, N.

**General Preparation of the Compounds of Table II: Compound 9 of Table II.** A suspension of methyl *N*-[3-(methylamino)-4-nitrophenyl]carbamate (2.0 g, 8.9 mmol) in MeOH (100 mL) was hydrogenated over Pd/C until  $\text{H}_2$  uptake ceased (30 min). The solution was filtered to remove catalyst and immediately added to solid 9-chloroacridine (0.95 equiv). A trace of HCl was added, and the mixture was heated under reflux until homogenous and for a further 10 min. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and 2 N  $\text{NH}_4\text{OH}$ . The organic layer was washed with water and extracted with 1 N aqueous  $\text{CH}_3\text{SO}_3\text{H}$  (100 mL). This aqueous layer was then basified with 2 N  $\text{NH}_4\text{OH}$  and the free base extracted into EtOAc. The organic layer was washed with water and saturated NaCl, dried, and evaporated to give the free base as a red solid. This was dissolved in MeOH (20 mL), and concentrated HCl (2–3 equiv) was added. The mixture was heated to boiling, and EtOAc was added dropwise until crystallization

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of the hydrochloride salt began. Recrystallization from MeOH/EtOAc gave the pure monohydrochloride of compound 9 (3.08 g, 85%), mp 268 °C dec. Anal. (Table III).

The other compounds were prepared similarly. In cases where the monohydrochloride salt was not crystalline, recrystallization from MeOH/EtOAc/HCl gave the crystalline dihydrochloride salts (see Table II). Many of the compounds were hygroscopic and crystallized as hydrates.

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**Registry No.** 1, 51264-14-3; 3, 88412-78-6; 4, 88412-94-6; 5, 108835-44-5; 5-HCl, 108835-45-6; 6, 108835-46-7; 6-HCl, 108835-47-8; 7, 108835-48-9; 7-HCl, 108835-49-0; 8, 90125-87-4; 8-HCl, 108835-50-3; 9, 88914-50-5; 9-HCl, 88913-92-2; 10, 88914-59-4; 10-HCl, 88914-01-6; 11, 82720-41-0; 12, 88914-47-0; 12-HCl, 88913-89-7; 13, 108835-51-4; 13-HCl, 108835-52-5; 14, 108835-53-6; 14-HCl, 108835-54-7; 15, 88914-53-8; 15-HCl, 88913-95-5; 16, 88914-54-9; 16-HCl, 88913-96-6; 17, 88914-55-0; 17-HCl, 88913-97-7; 18, 108868-10-6; 18-2HCl, 108835-55-8; 19, 88914-52-7; 19-HCl, 88913-94-4; 20, 108835-56-9; 20-HCl, 108835-57-0; 21, 108835-58-1; 21-HCl, 108835-59-2; 22, 108835-60-5; 22-HCl, 108835-61-6; 23, 108835-62-7; 23-HCl, 108835-63-8; 24, 88914-58-3; 24-2HCl, 88914-00-5; 25, 108835-64-9; 25-2HCl, 108835-65-0; 26, 108835-66-1; 26-HCl, 108835-67-2; 27, 108835-68-3; 27-2HCl, 108835-69-4; 28, 108835-70-7; 28-HCl, 108835-71-8; 29, 88914-56-1; 29-2HCl, 88913-98-8; 30, 88914-57-2; 31, 108835-72-9; 31-HCl, 108835-73-0; 32, 108835-74-1; 32-2HCl, 108835-75-2; 33, 108835-76-3; 33-2HCl, 108835-77-4; 34, 108835-78-5; 34-2HCl, 108835-79-6; 35, 108835-

80-9; 35-2HCl, 108835-81-0; 36, 108835-82-1; 36-2HCl, 108835-83-2; 37, 108835-84-3; 37-HCl, 108835-85-4; 38, 108835-86-5; 38-HCl, 108835-87-6; 39, 108835-88-7; 39-HCl, 108835-89-8; 40, 108835-90-1; 40-HCl, 108835-91-2; I, 55851-38-2; IIb, 108835-92-3; IIc, 108835-93-4; IV, 88914-75-4; Vb, 88914-83-4; Vc, 88914-78-7; 2-MeO-4-NH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHCOPr, 59988-64-6; 3-MeO-4NHCOPrC<sub>6</sub>H<sub>3</sub>NHCO<sub>2</sub>Me, 88149-78-4; 3-NHMe-4-NO<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHCO<sub>2</sub>Me, 108835-94-5; 2-OMe-4-NHAcC<sub>6</sub>H<sub>3</sub>NH<sub>2</sub>, 93973-25-2; 2-NHMe-4-NHAcC<sub>6</sub>H<sub>3</sub>NH<sub>2</sub>, 108835-95-6; 2-NMe<sub>2</sub>-4-NHAcC<sub>6</sub>H<sub>3</sub>NH<sub>2</sub>, 108835-96-7; 3-OMe-4-NH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHP(O)(OMe)<sub>2</sub>, 86187-37-3; MeOP(O)BrOMe, 24167-74-6; 3-Me<sub>2</sub>N-4-NH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHCO<sub>2</sub>Me, 88915-02-0; 3-N(Me)CH<sub>2</sub>Ph-4-NH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHP(O)(OMe)<sub>2</sub>, 108835-97-8; 3-NMe<sub>2</sub>-4-NH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHP(O)(OMe)<sub>2</sub>, 108835-98-9; 3-NHMe-4-NH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHCO<sub>2</sub>Me, 88914-84-5; 9-chloroacridine, 1207-69-8; 9-chloro-2-methylacridine, 16492-09-4; 9-chloro-3-fluoroacridine, 2377-16-4; 3,9-dichloroacridine, 35547-70-7; 3-bromo-9-chloroacridine, 35547-72-9; 9-chloro-3-iodoacridine, 88914-90-3; 9-chloro-3-methylacridine, 16492-10-7; 9-chloro-3-methoxyacridine, 16492-14-1; 9-chloro-3-nitroacridine, 1744-91-8; 9-chloro-4-fluoroacridine, 3829-32-1; 4,9-dichloroacridine, 10166-44-6; 9-chloro-4-methylacridine, 16492-11-8; 9-chloro-4-methoxyacridine, 16492-15-2; 9-chloro-4-aminocarbonylacridine, 63178-96-1; 9-chloro-4-[(methylamino)carbonyl]acridine, 63178-97-2; 9-chloro-3-fluoro-5-methylacridine, 88914-95-8; 3,9-dichloro-5-methylacridine, 88914-96-9; 3-bromo-9-chloro-5-methylacridine, 88914-98-1; 9-chloro-3,5-dimethylacridine, 88914-93-6; 9-chloro-3-methoxy-5-methylacridine, 88914-94-7; 9-chloro-3-fluoro-5-methoxyacridine, 102940-93-2; 3,9-dichloro-5-methoxyacridine, 88914-97-0; 3-bromo-9-chloro-5-methoxyacridine, 6534-56-1; 9-chloro-3-methyl-5-methoxyacridine, 88914-99-2; 9-chloro-4,5-dimethylacridine, 63345-58-4; 9-chloro-4,5-dimethoxyacridine, 89784-84-9; 9-chloro-4-methyl-5-[(methylamino)carbonyl]acridine, 88915-00-8; 9-chloro-4-methoxy-5-[(methylamino)carbonyl]acridine, 88377-34-8.

## 17-Heteroaryl Esters of Corticosteroids. 2. 11 $\beta$ -Hydroxy Series

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The preparation and topical antiinflammatory potencies of a series of 17-furoyl and -thenoyl esters of 9 $\alpha$ -fluoro-11 $\beta$ -hydroxy-16-methyl and 9 $\alpha$ -chloro-11 $\beta$ -hydroxy-16-methyl corticosteroids are described. The 17 $\alpha$ -esters were introduced to the 9 $\alpha$ -fluoro 11-ketones or to the appropriate  $\Delta^{9(11)}$  compounds by direct acylation with the appropriate heteroaryl carbonyl chloride in the presence of 4-(dimethylamino)pyridine. Functionalization of the C ring was completed by standard methods. The most extensively studied heterocyclic acyl group was 2-furoyl, but 3-furoyl and 2- and 3-thenoyl derivatives were also investigated. Antiinflammatory potencies were measured in mice by a 5-day modification of the Tonelli croton oil ear assay. The most potent topical antiinflammatory agents were 1e, dexamethasone 17-(2'-furoate) 21-propionate, and 2c, the 21-chloro 17-(2'-furoate) in the 9 $\alpha$ -chloro series, both being 6 times as potent as betamethasone 17-valerate. Several other 9 $\alpha$ -chloro-11 $\beta$ -hydroxy-17-heteroaryl carboxylates (2a, 2b, 2d, and 2g) were at least 4 times as potent as betamethasone 17-valerate. Evaluation of 2c in the clinic confirmed that the compound is a potent topical antiinflammatory agent in humans.

In this paper we describe a new class of topical corticosteroids bearing 17-heteroaryl ester groups.<sup>1</sup> The preceding publication focused on 9 $\alpha$ ,11 $\beta$ -dichloro corticosteroids;<sup>2</sup> herein described are their 11 $\beta$ -oxygenated counterparts. The 17-position has been functionalized with furoyl and thenoyl esters. As introduction of the 17-heteroaromatic ester groups resulted in high topical an-

tiinflammatory potencies in the 9,11-dichloro series,<sup>2</sup> we expected high topical antiinflammatory potencies in the 11 $\beta$ -hydroxy series. The potency in this class of 17-esters was generally high, and some of the compounds exceeded the potency of the most potent topical corticosteroids tested in our laboratories. The 9 $\alpha$ -fluorinated compounds are delineated in Table I, while the 9 $\alpha$ -chloro and 9-unsubstituted compounds are in Table II, with appropriate substitution at positions 6, 16, and 21.

### Chemistry

In the preceding paper we reported a process utilizing direct esterification of the 17-hydroxy group with the ap-

(1) Shapiro, E. L. U.S. Patent 4472393, Sept 18, 1984; *Chem. Abstr.* 1985, 102, 95905k.

(2) Shapiro, E. L.; Gentles, M. J.; Tiberi, R. L.; Popper, T. L.; Berkenkopf, J.; Lutsky, B.; Watnick, A. S. *J. Med. Chem.* 1987, 30, 1068.