101773-16-4; (*Z*)-**12**, 101760-48-9; (*E*)-**12**, 101760-57-0; (*Z*)-**13**, 101760-49-0; (*E*)-**13**, 101760-58-1; **14**, 101760-50-3; (*Z*)-**15**, 101760-51-4; (*E*)-**15**, 101760-59-2; **16**, 101760-52-5; **17**, 101760-53-6; **18**, 101760-54-7; **19**, 101760-55-8; **20**, 101760-56-9; ethyl *p*-aminobenzoate, 94-09-7; hydroxylamine hydrochloride, 5470-11-1; 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine, 1007-99-4; ethyl *p*-[*N*-propargyl-*N*-[3-[(2,5-diamino-3,4-dihydro-4-oxopyrimidin-6-yl)amino]acetonyl]amino]benzoate, 101760-60-5; diethyl glutamate hydrochloride, 1118-89-4; 7,8-dihydro-10-propargylfolic acid, 101760-61-6; 5,6,7,8-tetrahydro-10-propargylfolic acid, 101760-62-7; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2.

Adriamycin Analogues. Preparation and Biological Evaluation of Some Thio Ester Analogues of Adriamycin and *N*-(Trifluoroacetyl)adriamycin 14-Valerate[1]

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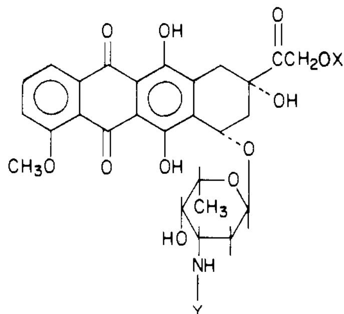
On the consideration that the highly active DNA-nonbinding adriamycin analogues *N*-(trifluoroacetyl)adriamycin 14-valerate and *N*-(trifluoroacetyl)adriamycin 14-*O*-hemiadipate undergo initial metabolic conversion to *N*-(trifluoroacetyl)adriamycin by the action of nonspecific serum and tissue esterases, a number of *N*-(trifluoroacetyl)-adriamycin 14-thio esters have been prepared and studied for in vitro growth inhibition, vs. human-derived CCRF-CEM leukemic lymphocytes, and in vivo antitumor activity, vs. murine P388 leukemia, relative to the rate of thio ester deacylation induced by esterases present in mouse serum. Products were obtained by reaction of *N*-(trifluoroacetyl)-14-bromodaunorubicin with thioacetic, thiopropionic, thiobutyric, thiovaleric, and thiobenzoic acids in ethanol, in the presence of potassium carbonate. Because little is known about similar thio ester derivatives of adriamycin itself, the corresponding adriamycin 14-thio esters were also prepared and evaluated for antitumor activity; with these products, determination of their extent of interaction with calf thymus DNA was also performed. For the adriamycin thio ester products, significant in vivo anti-P388 activity was seen with the thioacetate, thiovalerate, and thiobenzoate derivatives, although no compound matched the curative effects of *N*-(trifluoroacetyl)adriamycin 14-valerate in this system. With respect to the *N*-(trifluoroacetyl)adriamycin 14-thio ester products, although the corresponding oxo ester analogues are all significantly biologically active, none of the thio ester derivatives showed activity in vitro or in vivo.

For some time these laboratories have been engaged in the search for improved analogues of the antitumor antibiotic adriamycin (doxorubicin, **1**). Since its clinial introduction in the early 1970s, **1** has become the most widely used chemotherapeutic agent in cancer medicine. While most text and reference sources continue to suggest that the antitumor and cytotoxic properties of this agent are due mechanistically to direct drug–DNA binding, we questioned the validity of this hypothesis and the reported structural requirement for a basic 3′-aminoglycoside function as a necessary condition for the expression of biological activity in the anthracycline system.[2] Accordingly, we set about investigating a broad range of semisynthetic structural variants of **1** with altered DNA-binding properties. This approach led to the design, synthesis, preclinical development, and clinical introduction of the novel DNA-nonbinding analogue *N*-(trifluoroacetyl)-adriamycin 14-valerate (AD 32, **2**).[3,4] In animal model



systems, **2** shows the double advantage, compared to **1**, of being therapeutically superior and very much less toxic.[3,5–8] Extensive clinical trials with **2** have demonstrated significant activity against human disease, with little of the toxicities normally seen with **1**.[9–11] No patient receiving **2** has experienced cardiac toxicity, regardless of the total accumulated dose received. Because of solubility problems with **2**; *N*-(trifluoroacetyl)adriamycin 14-*O*-hemiadipate, a second-generation DNA-nonbinding analogue of **2** with improved water solubility, has been prepared[12,13] and is expected to enter clinical trials shortly. Various pharmacology studies with **2** and its newer hemiadipate analogue in vitro and in vivo have established that these agents have broad-range intrinsic biological activity, without the

(1) Work started at Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

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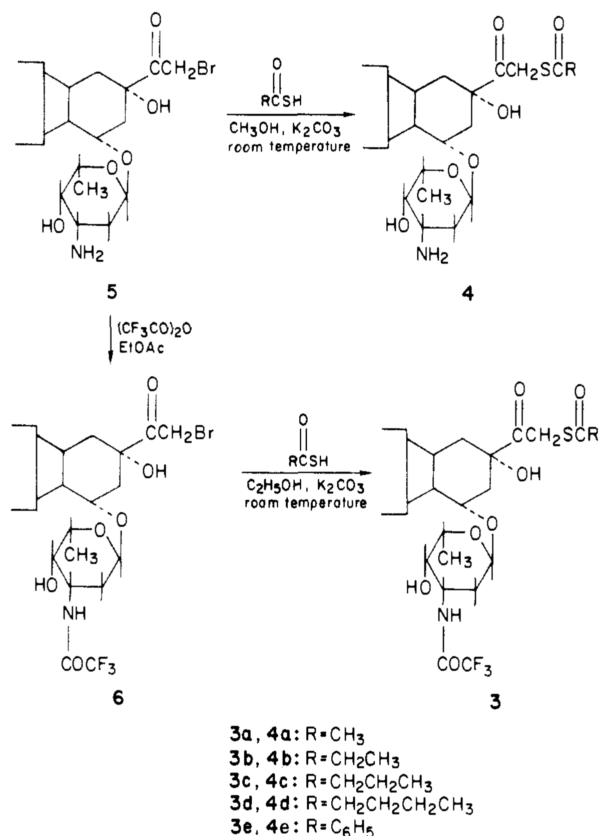
Scheme I



need for conversion into **1** or other DNA-binding species for the expression of their effects.

The work described in this paper was undertaken in connection with structure–activity studies relating to the novel analogue **2**. The initial and major metabolic event involving **2**, in humans and laboratory animals, is known to be loss of the valerate ester, with consequent conversion of **2** into *N*-(trifluoroacetyl)adriamycin.[10,14-17] This biotransformation is brought about by ubiquitously occurring nonspecific serum and tissue esterases. On the consideration that thio esters (**3**) might serve as less adequate substrates than oxo esters for these enzymes, a number of thio ester analogues of **2** have been prepared and evaluated for in vitro growth inhibition and in vivo antitumor activity, relative to the rate of esterase-mediated deacylation. Review of the literature revealed little information about analogous adriamycin 14-thio esters (**4**), other than that adriamycin 14-thioacetate (**4a**) and 14-thiobenzoate (**4e**) were included in a patent specification[18] and claimed to be clearly less effective than **1** in the treatment of P388 tumor-bearing mice.[18,19] For completeness of study, we accordingly prepared the series of **4**, including **4a** and **4e**, corresponding to the *N*-(trifluoroacetyl)adriamycin 14-thio esters mentioned above, and compared all products in internally consistent bioassay experiments.

The synthetic route used to obtain the thioester products **3** and **4** is shown in Scheme I. *N*-(Trifluoroacetyl)-14-iododaunorubicin, a common intermediate in these laboratories and the compound normally used to prepare the oxo analogues of **3**, could not be used in this work. We have recently shown that this iodo compound undergoes a rapid redox reaction with thio alcohols, with consequent formation of *N*-(trifluoroacetyl)daunorubicin and alkyl disulfides.[20,21] A pilot study with the 14-iodo intermediate and thioacetic acid showed similar reduction of the iodomethyl ketone side-chain function to acetyl. Instead, 14-bromodaunorubicin (**5**), prepared according to details in the patent literature,[22] was converted into *N*-(trifluoroacetyl)-14-bromodaunorubicin (**6**), as previously reported.[21] Treatment of **6** with thio acids in absolute ethanol, in the presence of potassium carbonate, afforded smooth reactions and high yields of **3** after chromatographic purification. Compound **5**, on treatment with thio acids under similar conditions (methanol instead of ethanol solvent), gave good yields of **4**, which were characterized as their hydrochloride salts. Commercially available thioacetic acid, thiopropionic acid, and thiobenzoic acid were used for the corresponding preparations; thiobutyric and thiovaleric acids were made from the corresponding oxo acids by refluxing with phosphorus pentasulfide. All products were purified by open-column chromatography on Biosil A silicic acid, checked for homogeneity by both thin-layer and high-performance liquid chromatography, and characterized by infrared and nuclear magnetic resonance spectral data and microchemical analyses.

Target compounds were evaluated in vitro for their ability to inhibit the growth of human leukemic cells and for the relative rate at which esterases in mouse serum effected side-chain deacylation. Compounds **4a–e** were also assayed for their extent of interaction with DNA. Similar assays with **3a–e** were considered unnecessary, as these compounds, like **2**, should be incapable of binding with nucleic acids; however, to validate this point, compounds **3b** and **3e** were tested in a similar fashion to **4a–e**

Table I. In Vitro Data for Adriamycin 14-Thio Esters and *N*-(Trifluoroacetyl)adriamycin 14-Thio Esters

compd	ID_{50},[a] μM	$T_{1/2}$,[b] min	A/A_0[c] (λ 480 nm)
adriamycin (1)	0.06		0.60
AD 32 (2)	0.24	10	0.96
3a	>5.0	18	*d*
3b	2.8	80	0.99
3c	>5.0	42	*d*
3d	>5.0	22	*d*
3e	3.8	*e*	0.98
4a	0.90	6	0.61
4b	0.72	5	0.61
4c	0.56	10	0.63
4d	0.45	8	0.65
4e	2.3	8	0.65

[a] Versus CCRF-CEM cells in culture; 48-h continuous drug exposure. [b] Time for esterases in unfractionated mouse serum (1% in pH 7.0 carbonate buffer) to reduce the concentration of test compound by 50% due to thio ester deacylation. [c] Extent of DNA binding; calf thymus DNA; pH 7.0 Tris buffer; drug/DNA molar ratio = 0.1. [d] Not determined because of expected lack of DNA interaction (see text). [e] 10% hydrolysis at 60 min.

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(22) Societa Farmaceutici Italia, Brit. Patent 1217133, 1970.

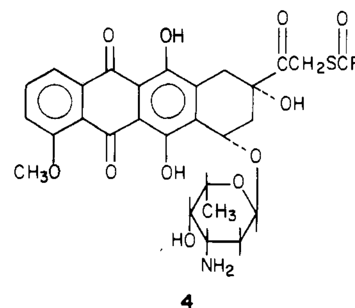
and were indeed found not to bind to calf thymus DNA. The results of these in vitro studies, together with reference values for **1** and **2**, are provided in Table I.

Growth-inhibition assays utilized CCRF-CEM cells, a lymphoblastic leukemic line of human origin maintained in continuous suspension culture. Details of the assay procedure have been described elsewhere.[23] The ID_{50} values shown in Table I indicate the concentration of drug required to bring about a 50% reduction in the growth of treated cells, as compared to untreated control cultures; values of $<1.0\ \mu M$ represent significant activity. Thus, with the exception of the aromatic derivative **4e**, all of the adriamycin 14-thio esters **4** showed high growth-inhibitory activity against this cell line; although differences were not great, activity was seen to increase with increasing chain length of the thioacyl function. While **2** and most other 14-oxo esters of *N*-(trifluoroacetyl)adriamycin have ID_{50} values in this assay in the range of 0.2–0.8 μM, none of the corresponding trifluoroacetamide thio esters **3** showed activity.

To determine the rate of enzyme-mediated deacylation, unfractionated mouse serum was used under defined experimental conditions. Drug was incubated at 37 °C with pH 7.0 carbonate buffer containing 1% mouse serum pooled from 10 animals and maintained frozen at −70 °C, except as needed. The $T_{1/2}$ values in Table I represent the time required for the initial concentration of test compound to be reduced by 50%, as monitored by high-performance liquid chromatography. With **2** and related oxo esters in this system, the disappearance of starting material is marked by the increasing appearance of the hydrolysis product, *N*-(trifluoroacetyl)adriamycin. With the thio esters, the assay is somewhat more difficult, as the disappearance of starting material is accompanied by the appearance of multiple signals, indicative of the instability of the resulting 14-thio alcohol products. Despite this, the rate of decay of the test compound could still be monitored directly. The $T_{1/2}$ values shown for the trifluoracetamide derivatives **3** clearly indicate that the presence of the sulfur function in these thio ester products significantly retards deacylation as compared to **2** and related oxo esters. This finding, however, makes the unexpected more rapid rate of deacylation observed with the adriamycin thio esters **4** all the more surprising.

The ability of **4a–e** to bind with double-helical calf thymus DNA was determined spectrophotometrically at 480 nm by the spectral shift method.[24,25] In this assay, the absorption of light energy by the drug alone (A_0), and on admixture with DNA (A), is determined and expressed as the ratio A/A_0; a value of unity indicates no drug–DNA interaction, while values significantly less than 1.0 represent considerable drug–DNA complexation. Thus, the A/A_0 values shown for **4** indicate these compounds to interact with calf thymus DNA to almost the same extent as parent **1**. The use of this assay system as a first approximation of the extent of anthracycline–DNA binding has been validated by the demonstrated lack of DNA interaction of **2**, first shown by us by this procedure[26] and now established by various other techniques.[27-31]

Table II. In Vivo Antitumor Data for Adriamycin 14-Thio Esters vs. Murine P388 Lymphocytic Leukemia[a]



compd	R	optimal dose,[b] mg/kg qd 1–4	% ILS[c]
adriamycin (1)		3.0	+157
AD 32 (2)		50.0	curative
4a	CH_3	25.0	+220
4b	CH_2CH_3	20.0	+80
4c	$CH_2CH_2CH_3$	10.0	+90
4d	$CH_2CH_2CH_2CH_3$	10.0	+130
4e	C_6H_5	10.0	+140

[a] B6D2F1 male mice; 10^6 tumor cell inoculum ip day 0; treatment ip. [b] Highest nontoxic dose. [c] Percent increase in life span relative to untreated controls. Experiment terminated on day 60; all tumor-bearing treated animals died, except for those in the AD 32 treatment groups.

In vivo antitumor evaluation of target compounds utilized the murine P388 leukemia system (10^6 tumor cell inoculum ip, treatment ip once daily on days 1–4), essentially according to the standard protocols of the National Cancer Institute.[32] All the adriamycin 14-thio ester products **4** showed activity in this system; antitumor data for compounds **4a–e** are presented in Table II. In contradistinction to the earlier report,[17,18] both the thioacetate and the thiobenzoate exhibited significant antitumor activity: the thiobenzoate **4e** was about equiactive with **1**, and the thioacetate **4a** was superior, in increasing the life span of tumor-bearing mice. Compound **4a** is, however, less potent than **1**, requiring about 8 times as much drug to bring about its optimal therapeutic effect. Among the 4 homologous members of the adriamycin alkyl thio ester series, although **4a** was the most effective, the next higher homologue was the least active; activity then began to increase with increasing thioacyl chain length (and increasing lipophilicity). All of the trifluoracetamide thio esters **3** were inactive at all doses tested up to toxicity. Data shown in the table for **1** and **2** were derived from the P388 antitumor assays for the thio ester analogues, in which experiments **1** and **2** were included as positive controls; the values for these compounds are fully consistent with historical data for **1** and **2** obtained over many years in these laboratories.

The present report shows that adriamycin thio ester analogues **4**, which bind strongly with DNA, exhibit in vivo antitumor activity. Beyond this, however, analysis of the bioassay data indicates relatively little relationship among the various biological properties studied. Compound **4a**, the most active thio ester analogue of **1** in vivo, clearly was

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not the most growth inhibitory of the compounds against cultured leukemic cells. The thiopropionate **4b** interacted equally as well as **4a** with isolated calf thymus DNA but was inferior to **4a** with respect to in vivo antitumor activity. While only one-fifth as active in in vitro growth inhibition, **4e** was equal to or perhaps somewhat better than **4d** in in vivo antitumor activity. Neither in vitro growth inhibition nor in vivo antitumor activity correlated closely with the rate of deacylation of the thio ester side chain. Disappointing was the finding that, although all of the corresponding 14-oxo esters of *N*-(trifluoroacetyl)adriamycin are active in vivo in the P388 system (with the least active compound, the acetate, being at least as effective as **1**),[4] none of the thio ester analogues of **2** showed activity. Thus, while in the adriamycin series the thioacyl function results in retention of antitumor activity but with a loss in potency, in the adriamycin–trifluoroacetamide series, the presence of the 14-thioacyl function is clearly associated with the loss of all biological activity.

Experimental Section

TLC on silica gel G (Analtech), with either $CHCl_3$–MeOH, 9:1 (solvent system A), or $CHCl_3$–MeOH, 4:1 (solvent system B), as eluant, was used for identification and evaluation of homogeneity. Column chromatography was performed on Biosil A silicic acid (100–200 mesh; Bio-Rad Laboratories). HPLC analyses were done with a Waters Associates instrument fitted with a μ-Bondapak/phenyl reverse-phase column, with acetonitrile–pH 4.0 ammonium formate buffer as the mobile phase (gradient elution from 32% to 65% over 6 min; flow rate 3.5 mL/min; flow fluorescence detection, excitation at 482 nm). Infrared spectra were recorded on a Perkin-Elmer Model 1320 spectrophotometer as KCl pellets; all compounds exhibited characteristic bands for the anthracycline nucleus at 3500 (OH), 1730 (C=O), 1620, and 1580 (quinone) cm^{-1}. Proton NMR spectra were recorded on a Varian T60-A spectrometer in $CDCl_3$ with tetramethylsilane as internal standard.

***N*-(Trifluoroacetyl)adriamycin 14-Thioacetate (3a).** To a stirred mixture of *N*-(trifluoroacetyl)-14-bromodaunorubicin (**6**; 250 mg, 0.33 mmol) and anhydrous K_2CO_3 (100 mg) in absolute ethanol (50 mL) was added thioacetic acid (25 μL, 0.35 mmol) at room temperature. After 10 min the reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with pH 7 buffer (230 mL). The organic extract was dried (Na_2SO_4) and concentrated to dryness, and the residue was chromatographed on Biosil A (8 g). $CHCl_3$–MeOH (1%) eluted pure **3a** (210 mg, 85%), homogeneous on TLC (A, R_f 0.68) and HPLC (t_r = 6.71 min); NMR δ 1.33 (d, J = 6 Hz, 3 H, 5′-CH_3), 2.40 (s, 3 H, $SCOCH_3$), 4.00 (s, 3 H, Ar OCH_3), 5.18 (br s, 1 H, 7-H), 5.47 (br s, 1 H, 1′-H), 6.75 (d, J = 9 Hz, 1 H, $NHCOCF_3$), 7.23–8.00 (m, 3 H, aromatic), 13.12 (s, 1 H, phenolic OH), 13.92 (s, 1 H, phenolic OH). Anal. ($C_{31}H_{30}F_3NO_{12}S$) C, H, N, S; F: calcd, 8.17; found, 7.68.

***N*-(Trifluoroacetyl)adriamycin 14-Thiopropionate (3b).** Thiopropionic acid (30 μL, 0.36 mmol) was added to a mixture of the bromo compound **6** (250 mg, 0.33 mmol) and anhydrous K_2CO_3 (100 mg) in absolute ethanol (50 mL) at room temperature with stirring. After 10 min the product was worked up as before. Chromatography on Biosil A (8 g) with $CHCl_3$–MeOH (1%) yielded pure **3b** (202 mg, 87%), homogeneous on TLC (A, R_f 0.68) and HPLC (t_r = 7.26 min); NMR δ 1.22 (t, J = 7 Hz, 3 H, $SCOCH_2CH_3$), 1.33 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.03 (s, 3 H, Ar OCH_3), 5.20 (br s, 1 H, 7-H), 5.50 (br s, 1 H, 1′-H), 6.77 (d, J = 9 Hz, 1 H, $NHCOCF_3$), 7.20–8.00 (m, 3 H, aromatic), 13.07 (s, 1 H, phenolic OH), 14.00 (s, 1 H, phenolic OH). Anal. ($C_{32}H_{32}F_3NO_{12}S$) C, H, F, N, S.

***N*-(Trifluoroacetyl)adriamycin 14-Thiobutyrate (3c).** Thiobutyric acid was prepared by heating butyric acid (10 g) with excess phosphorus pentasulfide (10 g) in toluene (100 mL) overnight and distilling the product the next day. The fraction boiling at 134–138 °C was used for the reaction.

To a stirred solution of **6** (250 mg, 0.33 mmol) and anhydrous K_2CO_3 (100 mg) in absolute ethanol (50 mL) at room temperature was added thiobutyric acid (50 μL). After 10 min, the reaction product was worked up as before and chromatographed on Biosil A (8 g). $CHCl_3$–MeOH (0.5%) eluted pure **3c** (205 mg, 79%), homogeneous on TLC (A, R_f 0.69) and HPLC (t_r = 7.70 min); NMR δ 1.03 (t, J = 6 Hz, 3 H, $SCO(CH_2)_2CH_3$), 1.30 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.03 (s, 3 H, Ar OCH_3), 5.19 (br s, 1 H, 7′-H), 5.47 (br s, 1 H, 1′-H), 6.76 (m, J = 9 Hz, 1 H, $NHCOCF_3$), 7.23–8.01 (m, 3 H, aromatic), 13.25 (s, 1 H, phenolic OH), 13.96 (s, 1 H, phenolic OH). Anal. ($C_{33}H_{34}F_3NO_{12}S$) C, H, F, N, S.

***N*-(Trifluoroacetyl)adriamycin 14-Thiovalerate (3d).** Thiovaleric acid (bp 145 °C) was prepared from valeric acid in like manner to the preparation of thiobutyric acid from butyric acid.

To a stirred solution of **6** (250 mg, 0.33 mmol) and anhydrous K_2CO_3 (100 mg) in absolute ethanol (50 mL) at room temperature was added thiovaleric acid (65 μL). The reaction was stopped after 20 min and the product was worked up as before. Chromatography of the residue on Biosil A (8 g) with $CHCl_3$ yielded pure **3d** (216 mg, 82%), homogeneous on TLC (A, R_f 0.71) and HPLC (t_r = 8.07 min); NMR δ 0.95 (t, J = 6 Hz, 3 H, $SCO(CH_2)_3CH_3$), 1.28 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.03 (s, 3 H, Ar OCH_3), 5.15 (m, 1 H, 7-H), 5.50 (m, 1 H, 1′-H), 6.75 (d, J = 6 Hz, 1 H, $NHCOCF_3$), 7.24–8.02 (m, 3 H, aromatic), 13.13 (s, 1 H, phenolic OH), 14.00 (s, 1 H, phenolic OH). Anal. ($C_{34}H_{36}F_3NO_{12}S$) H, F, N, S; C: calcd, 55.35; found, 54.93.

***N*-(Trifluoroacetyl)adriamycin 14-Thiobenzoate (3e).** Thiobenzoic acid (45 μL, 0.38 mmol) was added to a stirred mixture of **6** (250 mg, 0.33 mmol) and anhydrous K_2CO_3 (100 mg) in absolute ethanol (50 mL) maintained at room temperature. After 10 min the reaction product was worked up as usual. The residue was chromatographed on Biosil A (8 g). $CHCl_3$–MeOH (0.7%) eluted pure **3e** (198 mg, 78%), homogeneous on TLC (A, R_f 0.71) and HPLC (t_r = 8.16 min); NMR δ 1.38 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.03 (s, 3 H, Ar OCH_3), 5.23 (m, 1 H, 7-H), 5.50 (m, 1 H, 1′-H), 6.83 (d, J = 8 Hz, 1 H, $NHCOCF_3$), 7.20–8.10 (m, 8 H, aromatic), 13.07 (s, 1 H, phenolic OH), 13.87 (s, 1 H, phenolic OH). Anal. ($C_{36}H_{32}F_3NO_{12}S$) C, H, F, N, S.

Adriamycin 14-Thioacetate (4a). 14-Bromodaunorubicin (**5**; 300 mg, 0.47 mmol) and anhydrous K_2CO_3 (75 mg) in absolute methanol (30 mL) were stirred at room temperature. Thioacetic acid (36 μL, 0.5 mmol) was added and stirring was continued for 10 min. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with pH 10 buffer (2 × 25 mL). The organic extract was dried (Na_2SO_4) and then taken completely to dryness. The residue was chromatographed on Biosil A (9 g). $CHCl_3$–MeOH (5%) eluted pure **4a** (235 mg, 84%), homogeneous on TLC (B, R_f 0.37) and HPLC (t_r = 4.50 min); NMR δ 1.33 (d, J = 6 Hz, 3 H, 5′-CH_3), 2.40 (s, 3 H, $SCOCH_3$), 4.02 (s, 3 H, Ar OCH_3), 5.20 (m, 1 H, 7-H), 5.52 (m, 1 H, 1′-H), 7.20–8.03 (m, 3 H, aromatic). The hydrochloride salt was prepared by adding 1 N HCl (0.38 mL) to **4a** free base (230 mg) at 0 °C in $CHCl_3$–MeOH (1:1, 4 mL), quickly evaporating the solvent on the rotary evaporator, adding 2-propanol (2 × 5 mL) to the residue, and taking this solution to dryness on the rotary evaporator. The residue was then precipitated from MeOH–ether and dried under vacuum (235 mg, 96%). Anal. ($C_{29}H_{31}NO_{11}S{\cdot}HCl{\cdot}1.5H_2O$) C, H, Cl, N; S: calcd, 4.82; found, 4.26.

Adriamycin 14-Thiopropionate (4b). Compound **5** (300 mg, 0.47 mmol) was stirred with anhydrous K_2CO_3 (75 mg) in absolute methanol (30 mL) at room temperature. Thiopropionic acid (42 μL, 0.5 mmol) was added and the stirring was continued for 10 min. The product was worked up as usual and chromatographed on Biosil A (9 g). $CHCl_3$–MeOH (3%) eluted pure **4b**, from which the hydrochloride salt was made as before (245 mg, 80%); homogeneous on TLC (B, R_f 0.37) and HPLC (t_r = 5.13 min); NMR of free base δ 1.20 (t, J = 6 Hz, 3 H, $SCOCH_2CH_3$), 1.31 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.07 (s, 3 H, Ar OCH_3), 5.23 (m, 1 H, 7-H), 5.50 (m, 1 H, 1′-H), 7.25–8.00 (m, 3 H, aromatic). Anal. ($C_{30}H_{33}NO_{11}S{\cdot}HCl{\cdot}H_2O$) C, H, N, S.

Adriamycin 14-Thiobutyrate (4c). To a stirred solution of **5** (300 mg, 0.47 mmol) and anhydrous K_2CO_3 (75 mg) in methanol (30 mL) at room temperature was added thiobutyric acid (100 μL). After the mixture was stirred for 10 min, the product was worked up as before and chromatographed on Biosil A (9 g). $CHCl_3$–MeOH (2%) eluted pure **4c**; the hydrochloride salt (232 mg, 74%), prepared as usual, was homogeneous on TLC (B, R_f 0.40) and HPLC (t_r = 5.61 min); NMR of free base δ 1.02 (t, J = 6 Hz, 3 H, $S\,CO(CH_2)_2CH_3$), 1.33 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.08 (s, 3 H, Ar OCH_3), 5.27 (m, 1 H, 7-H), 5.52 (m, 1 H, 1′-H),

7.20–8.05 (m, 3 H, aromatic). Anal. ($C_{31}H_{35}NO_{11}S{\cdot}HCl{\cdot}0.5H_2O$) C, H, N, S.

Adriamycin 14-Thiovalerate (4d). Thiovaleric acid (100 μL) was added to a stirred mixture of **5** (300 mg, 0.47 mmol) and anhydrous K_2CO_3 (75 mg) in methanol (30 mL) at room temperature. The product, worked up as above, was chromatographed on Biosil A (9 g). $CHCl_3$–MeOH (2%) eluted pure **4d**; the hydrochloride salt (225 mg, 72%) was homogeneous on TLC (B, R_f 0.44) and HPLC (t_r = 6.15 min); NMR of free base δ 0.97 (t, J = 6 Hz, 3 H, S $CO(CH_2)_3CH_3$), 1.32 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.05 (s, 3 H, Ar OCH_3), 5.23 (m, 1 H, 7-H), 5.50 (m, 1 H, 1′-H), 7.20–7.95 (m, 3 H, aromatic). Anal. ($C_{32}H_{37}NO_{11}S{\cdot}HCl{\cdot}0.5H_2O$) C, H, N, S.

Adriamycin 14-Thiobenzoate (4e). Compound **5** (150 mg, 0.24 mmol) and K_2CO_3 (50 mg) were stirred in methanol (20 mL) at room temperature. Thiobenzoic acid (29 μL, 0.27 mmol) was added and stirring was continued for 10 min. The product, worked up as usual, was chromatographed on Biosil A (5 g). $CHCl_3$–MeOH (2%) eluted pure **4e**; hydrochloride salt (121 mg, 74%) homogeneous on TLC (B, R_f 0.38) and HPLC (t_r = 6.19 min); NMR of free base δ 1.32 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.01 (s, 3 H, Ar OCH_3), 5.18 (m, 1 H, 7-H), 5.45 (m, 1 H, 1′-H), 7.25–8.04 (m, 8 H, aromatic). Anal. ($C_{34}H_{33}NO_{11}S{\cdot}HCl{\cdot}2.5H_2O$) C, H, N, S.

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Registry No. **3a**, 101980-69-2; **3b**, 101980-70-5; **3c**, 101980-71-6; **3d**, 101980-72-7; **3e**, 101980-73-8; **4a**, 102045-67-0; **4a**·HCl, 65208-77-7; **4b**, 101980-74-9; **4b**·HCl, 102045-68-1; **4c**, 101980-75-0; **4c**·HCl, 102045-69-2; **4d**, 101980-76-1; **4d**·HCl, 102129-51-1; **4e**, 102045-70-5; **4e**·HCl, 65208-78-8; **5**, 65026-79-1; **6**, 77270-18-9; thioacetic acid, 507-09-5; thiopropionic acid, 1892-31-5; thiobutyric acid, 3931-64-4; thiovaleric acid, 53966-59-9; thiobenzoic acid, 98-91-9.

Adriamycin Analogues. Preparation and Biological Evaluation of Some *N*-(Trifluoroacetyl)-14-*O*-[(*N*-acetylamino)acyl]adriamycin Derivatives[1]

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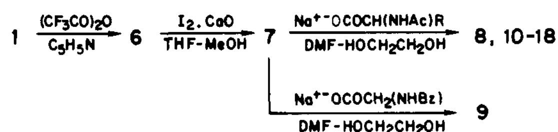
In connection with structure–activity studies related to the novel DNA-nonbinding adriamycin analogues *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32) and *N*-(trifluoroacetyl)adriamycin 14-*O*-hemiadipate (AD 143), we have now prepared a series of *N*-(trifluoroacetyl)adriamycin derivatives with *N*-acylamino acid esters at the 14-carbinol position. Target compounds were made by reaction of *N*-(trifluoroacetyl)-14-iododaunorubicin with the sodium salts of *N*-acylamino acids generally in dimethylformamide–ethylene glycol solvent. Products were evaluated for in vitro growth-inhibitory activity and, to a limited extent, in vivo antitumor activity in the murine P388 leukemia system. ID_{50} values for the target compounds vs. cultured CCRF-CEM cells were generally in the same range as those for the above-mentioned DNA nonbinding adriamycin analogues. Of the four compounds tested for in vivo activity, although none was as effective as *N*-(trifluoroacetyl)adriamycin 14-valerate, all showed significant activity in the P388 assay system, with three of the compounds, at the doses used, being essentially equiactive with an optimal dose of adriamycin. Studies on the rate of esterase-mediated deacylation of the products, in a defined system containing unfractionated mouse serum as the source of enzyme, showed no relationship between the in vitro and in vivo activities of these compounds and the relative ease at which the side-chain ester substituents were hydrolyzed.

A major program in these laboratories directed toward anthracycline chemistry and pharmacology has resulted, among other accomplishments, in the development of certain novel DNA-nonbinding *N*-(trifluoroacetyl)adriamycin 14-*O*-alkanoate esters with therapeutic properties superior in animals and humans to those of the widely used antitumor antibiotics daunorubicin (**1**) and adriamycin (doxorubicin, **2**). Compounds of this class of greatest clinical interest include *N*-(trifluoroacetyl)adriamycin 14-valerate (**3**) and *N*-(trifluoroacetyl)adriamycin 14-*O*-hemiadipate (**4**), as summarized in a recent paper appearing in this Journal.[2]

As part of our effort to explore the unusual properties of these agents, and perhaps identify additional analogues of potential therapeutic value, we have been examining diverse structural variants of **3** and **4**, with the view toward developing structure–activity relationships for this system.[3-9] In this regard, it is known that the initial metabolic processing of **3** and **4** in man, laboratory animals, and cell culture systems involves enzyme-mediated loss of the 14-*O*-acyl function, with consequent formation of *N*-(trifluoroacetyl)adriamycin (**5**), an event brought about by the action of ubiquitously occurring nonspecific serum and tissue esterases.[10-15] The present report concerns the

Scheme I



(1) Work on this project was started at the Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

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