the interaction of the thymine and phosphoraziridine moieties. Therefore, the corresponding carbamate derivatives will be synthesized and investigated.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. NMR spectra were obtained on Varian A-60 and T-60 spectrometers in deuterated chloroform solution, using tetramethylsilane as internal standard. Infrared spectra were taken in a Beckman IR-8 spectrometer. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

5′-(Dichlorophosphinyl)-3′-acetylthymidine (5). To a solution of $POCl_3$ (4.904 g, 32 mmol) in $(MeO)_3PO$ (10 mL), cooled in an ice bath, was added 3′-acetylthymidine (4;[10] 4.548 g, 16 mmol). The reaction mixture was stirred in a cold room (4–7 °C) for 16 h. The excess $POCl_3$ and the HCl formed were removed under high vacuum (0.5–1 mmHg) at room temperature for 4 h. The resulting solution was immediately used for the next reaction step.

5′-[Bis(1-aziridinyl)phosphinyl]-3′-acetylthymidine (6). To a three-neck round-bottom flask (100 mL, equipped with thermometer, drying tube, and pressure equalized additional funnel) was introduced a solution of aziridine (1.724 g, 50 mmol) and triethylamine (4.048 g, 40 mmol) in 40 mL of 1,2-dimethoxyethane (DME). The container was cooled to 0–5 °C on an ice bath. The solution of 5 (16 mmol) in 10 mL of $(MeO)_3PO$ (see above) was added dropwise. The reaction temperature was maintained below 9 °C. After the addition was completed, the reaction mixture was stirred in a cold room (4–7 °C) for 42 h. The precipitated solids were collected by filtration and washed with DME (20 mL) and then with ether (20 mL). The precipitate was extracted with DME (300 mL), and the extracted solution was concentrated to 10 mL at room temperature under reduced pressure. The residue was chilled overnight in the refrigerator to obtain 2.05 g (overall yield, 31%) of 6 as white crystals: mp 183–185 °C; NMR ($CDCl_3$) δ 7.54 (br, 1 H), 6.30 (q, J = 6 Hz, 1 H), 5.31 (m, 1 H), 4.38 (m, 2 H), 4.15 (m, 1 H), 2.25, 2.07 (d, J = 15 Hz, 13 H, methylene protons of aziridine rings, 2′-CH_2 of thymidine and CH_3 of acetyl), 1.93 (d, J = 1, Hz, CH_3); IR (KBr) 1730 (ester), 1675 (amide), 830 cm^{-1} (P-N); $[\alpha]^{25}_D$ +4.45° (c 1.12, $CHCl_3$). The analytical sample was recrystallized from DME. Anal. ($C_{16}H_{23}N_4O_7P$) C, H, N, P.

5′-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-3′-acetylthymidine (7). This compound was synthesized by the same method as described for the preparation of 6. From 4.54 g (16 mmol) of compound 4 was obtained 3.4 g (7.2 mmol, 45%) of 7: mp 171–173 °C; NMR ($CHCl_3$) δ 7.61 (br, 1 H), 6.43 (q, J = 6 Hz, 1 H), 5.35 (m, 1 H), 4.30 (m, 3 H), 2.21 (d, J = 14 Hz, 6 H; methylene protons of aziridines and 2′-CH_2 of thymidine), 2.12 (s, 3 H; acetyl), 1.95 (br, 3 H), 1.46 (s, 12 H; methyl protons of aziridine rings; IR (KBr) 1732 (ester), 1680 (amide), 1390, 1380 [$C(CH_3)_2$], 1270 (P=O), 955 (PN) cm^{-1}; $[\alpha]^{25}_D$ +7.14° (c 0.98, $CHCl_3$). The analytical sample was recrystallized from DME.

Anal. ($C_{20}H_{31}N_4O_7P$) C, H, N, P.

5′-[Bis(1-aziridinyl)phosphinyl]thymidine (8). To 15 mL of absolute MeOH saturated with ammonia (10 mL) in an ice bath was added compound 6 (800 mg, 1.9 mmol). The solution was warmed up to room temperature, stirred for 4 h, and then evaporated to dryness. The residue was dissolved in $CHCl_3$ (10 mL), and dry ether (10 mL) was added until precipitation was observed. The solution was chilled in the refrigerator overnight; the precipitate was collected and dried under vacuum to give 0.50 g of 8 (1.3 mmol, 67%) as a foamy solid: mp 61–64 °C; NMR ($CDCl_3$) δ 7.47 (s, 1 H), 6.35 (t, J = 6 Hz, 1 H), 4.0–4.7 (two broad bands, 4 H), 2.32 (d, J = 15 Hz, 8 H, CH_2), 1.90 (s, 3 H, CH_3); IR (KBr) 1675 (CONH), 1266 (P=O), 930 (PN) cm^{-1}. The product was highly hygroscopic and difficult to analyze; therefore, its identification was based on the NMR spectra. The latter showed well-resolved peaks (see above) which integrated satisfactorily in the case of the freshly prepared sample; however, the spectra became blurred after a few days storage (even at refrigeration temperature), indicating polymerization.

5′-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]thymidine (9). Compound 7 was deacetylated in the same manner as described for the preparation of 8. From 800 mg (1.7 mmol) of compound 7 was obtained 0.433 g (yield 57%) of the deblocked compound 9: mp 50–53 °C; NMR ($CDCl_3$) δ 7.46 (s, 1 H), 6.36 (t, 1 H), 4–4.6 (br m, 4 H, 3′-CH, 4′-CH, 5′-CH_2), 2.17 (d, J = 14 Hz, 6 H, CH_2), 1.92 (s, 3 H, CH_3), 1.42 (s, 12 H, CH_3); IR (KBr) 1680 (CONH), 1389, 1387 [$C(CH_3)_2$], 1270 (P=O), 960 (PN) cm^{-1}. The compound was highly hygroscopic and difficult to handle. Anal. ($C_{18}H_{29}N_4O_6P \cdot H_2O$) C, H, N.

Assays of Cholinesterase Inhibitory Activity. The in vitro inactivation of horse serum cholinesterase, with procaine hydrochloride as substrate, was measured by a slight modification of the method of Lalka and Bardos.[7] The enzyme solution, 20 mL, 5.0 units/mL in 0.066 M NaH_2PO_4 buffer, pH 7.4, was made 1.4×10^{-4} M with respect to the inhibitor, at time zero, by adding 1.0 mL of a 2.94×10^{-3} M solution of the inhibitor in the same buffer. The resulting solution containing the enzyme and the inhibitor were placed in a 37 °C shaker bath, and 1.5-mL aliquots were withdrawn at various times and cooled to 24 °C in tap water. The substrate solution, 1.5 mL of 5.6×10^{-5} M procaine hydrochloride in the same buffer, was added to each tube as well as to a control containing no inhibitor, and the initial rate of the decrease of absorbance at 300 nm was measured as described previously.[7]

(Phenylthio)phenylamine Derivatives as Potential Antiinflammatory Compounds

A. Marcincal-Lefebvre,*[1a] J. C. Gesquiere,[1a] C. Lemer,[1b]

Laboratoire de Chimie Organique, Faculté de Pharmacie, 59045 Lille Cédex, France

and B. Dupuis

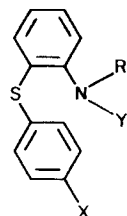*Laboratoire de Pharmacologie, Faculté de Médecine, 59045 Lille Cédex, France. Received August 6, 1980*

A series of (phenylthio)phenylamines and related compounds was prepared as potential antiinflammatory agents. Among them, N-[(diethylamino)acetyl]-4-[(4-chlorophenyl)thio]phenylamine and N-[(diethylamino)acetyl]-2-[(4-chlorophenyl)thio]phenylamine showed good antiinflammatory activity in the carrageenin assay on rats.

In a previous work, we reported the synthesis of 2-aminonicotinic acids, N-substituted by different diphenyl sulfide groups. Among these products, an analogue of compound III (Table I), with X = Cl, Y = H, and R =

Table I. (Phenylthio)phenylamines and Derivatives

IV (40–42) V (43–45) VI (46, 47)

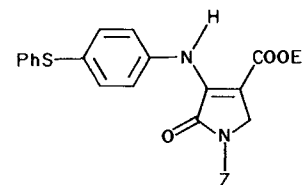| no.	X	R	Y	parent amine	meth-od[a]	mp, °C	recrystn solvent	yield,[b] %	formula[c]	antiinflammatory act. vs. carrageenin				
										dose, mg/kg	% redn, 1 h	% redn, 2 h	% redn,[d] 3 h	ca. LD_{50}[e] in mice, mg/kg
1	H	H	H			96–96.5[f]	EtOH	90						
2	Cl	H	H			58–60[g]	95% EtOH	90						
3	H	Me	H			39.5–40.5[g]	i-PrOH	60						
4	Cl	Me	H			65	i-PrOH	60	$C_{13}H_{12}NSCl$					
5	Cl	Me	CHO			72	c-C_6H_{12}	73[j]	i					
6	H	Me	$COCH_2Cl$	3	A	121–122	i-PrOH	64[j]	$C_{15}H_{14}NOSCl$					
7	H	H	$COCH_2Cl$	1	A	121–121.5[h]	i-PrOH	89[j]	h					
8	Cl	H	$COCH_2Cl$	2	A	145	PrOH	84[j]	i					
9	Cl	Me	$COCH_2Cl$	4	A	93	95% EtOH	65[j]	$C_{15}H_{13}NOSCl_2$					
10	H	Me	$COCH_2NMe_2$	3	B	178.5	PrOH	78	$C_{17}H_{20}N_2OS \cdot C_2H_2O_4$	5 ip	18	14	22	50 iv
11	H	Me	$COCH_2NEt_2$	3	B	104–106	Et_2O[k]	65	$C_{19}H_{24}N_2OS \cdot C_2H_2O_4 \cdot$ 0.25H_2O	5 ip	53	50	52*	30 iv[t]
12	H	Me	$COCH_2N(CH_2)_4$[l]	3	B	159–160	PrOH	78	$C_{19}H_{22}N_2OS \cdot C_2H_2O_4$	5 ip	18	18	23	35 iv
13	H	Me	$COCH_2N(CH_2)_5$[m]	3	B	170.5	PrOH	73	$C_{20}H_{24}N_2OS \cdot C_2H_2O_4$	5 ip	40	29	34*	45 iv
14	H	Me	$COCH_2NC_4H_8O$[n]	3	B	179.5	PrOH	68	$C_{19}H_{22}N_2O_2S \cdot C_2H_2O_4$	10 ip[r]	55	45	43*	75 iv
15	H	Me	$COCH_2NC_4H_8NMe$[o]	3	B	208	MeOH	70	$C_{20}H_{25}N_3OS \cdot 2C_2H_2O_4$	10 ip[s]	45	30	38*	75 iv
16	H	H	$COCH_2NMe_2$	1	B	164.5	PrOH	81	$C_{16}H_{18}N_2OS \cdot C_2H_2O_4$					
17	H	H	$COCH_2NEt_2$	1	B	163.5–165	i-PrOH	50	$C_{18}H_{22}N_2OS \cdot C_2H_2O_4$	25 ip	30	47	22	45 iv[u]
18	H	H	$COCH_2N(CH_2)_4$[l]	1	B	106	i-PrOH	86	$C_{18}H_{20}N_2OS$					
19	H	H	$COCH_2NC_4H_8NMe$[o]	1	B	208	90% EtOH	43	$C_{19}H_{23}N_3OS \cdot 2C_2H_2O_4$					
20	Cl	H	$COCH_2NMe_2$	2	B	92.5	70% EtOH	68	$C_{16}H_{17}N_2OSCl$					
21	Cl	H	$COCH_2NEt_2$	2	B	166.5–167	80% EtOH	49	$C_{18}H_{21}N_2OSCl \cdot C_2H_2O_4$	25 ip	56	74	72*	45 iv[v]
22	Cl	H	$COCH_2N(CH_2)_4$[l]	2	B	193	PrOH	38	$C_{18}H_{19}N_2OSCl \cdot C_2H_2O_4$[y]					
23	Cl	H	$COCH_2N(CH_2)_5$[m]	2	B	76.5–77.5	i-PrOH	43	$C_{19}H_{21}N_2OSCl$					
24	Cl	H	$COCH_2NC_4H_8O$[n]	2	B	195.5	95% EtOH	48	$C_{18}H_{19}N_2O_2SCl \cdot C_2H_2O_4$					
25	Cl	H	$COCH_2NC_4H_8NMe$[o]	2	B	214	i-PrOH	38	$C_{19}H_{22}N_3OSCl \cdot 2C_2H_2O_4$					
26	Cl	Me	$COCH_2NMe_2$	4	B	185	i-PrOH	53	$C_{17}H_{19}N_2OSCl \cdot C_2H_2O_4$					
27	Cl	Me	$COCH_2NEt_2$	4	B	oil	p	30	p	10 ip	13	15	0	45 iv[w]
28	Cl	Me	$COCH_2NC_4H_8NMe$[o]	4	B	224.5	80% EtOH	79	$C_{20}H_{24}N_3OSCl \cdot 2C_2H_2O_4$					
29	H	Me	4-ClC_6H_4CO	3	C	101–101.5	PrOH	58	$C_{20}H_{16}NOSCl$	500 po	65	60	48*	>1000 po
30	H	Me	2-$AcOC_6H_4CO$	3	D	102.5	95% EtOH	47	$C_{22}H_{19}NO_3S$	500 po	59	55	32*	>1000 po

Scheme I

Scheme II

2-nicotinic acid,[2] had, in the rat paw carrageenin test, significant antiinflammatory activity relative to the standard indomethacin and was less toxic (LD_{50} to mice 10 times ED_{50} to rats). In recent years, a number of nonacidic antiinflammatory agents with an amino or an amido group have been reported, like fluoroalkanesulfonanilides[3a] or some aminopyrimidines.[3b] In addition, in a few cases the reported compounds had an ether structure, like I,[4] or a thioether structure, like cinanserin II.[5] In the
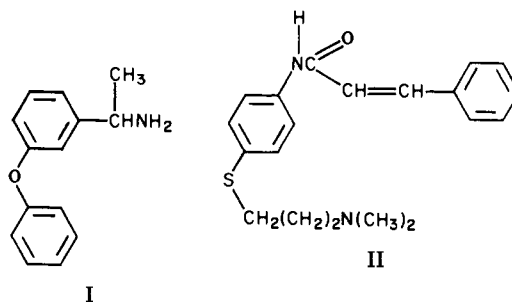
present work, we describe the synthesis and the antiinflammatory activity of amides with diphenyl sulfide groups III–V (Table I). Table I lists the compounds prepared, together with the physical data and biological activity.

Chemistry. The starting materials required for the synthesis were 4-(phenylthio)phenylamine (1),[6] 4-[(4-chlorophenyl)thio]phenylamine (2),[6] 2-[(4-chlorophenyl)thio]phenylamine (40),[2] N-methyl-4-(phenylthio)phenylamine (3),[6] and N-methyl-4[(4-chlorophenyl)thio]phenylamine (4). Compound 4 was prepared from 2 and trimethyl orthoformate, via the formyl derivative 5[6] (Scheme I).

Syntheses of compounds 43–47 were performed by using 4-carbethoxy-2,3-dioxopyrrolidines which were prepared by a literature procedure.[7,8] Reaction of 4-carbethoxy-

Table I

| No.	R	R'	acyl / Z group	mp (°C)	recrystn solvent	method[a]	formula[c]	% yield[b]	dose	act.	act.	act.[d]	LD₅₀[e]
31	Me	H	c-C₃H₅CO[z]	46–48	petr ether	C	C₁₇H₁₇NOS	53	500 po	57	48	37*	>1000 po
32	H	H	4-ClC₆H₄CO	193–193.5	i-PrOH	C	C₁₉H₁₄NOSCl	71					
33	H	H	2-AcOC₆H₄CO	110.5–111.5	c-C₆H₁₂/EtOH	D	C₂₁H₁₇NO₃S	46					
34	H	H	c-C₃H₅CO[z]	151	80% EtOH	C	C₁₆H₁₅NOS	75					
35	H	Cl	4-ClC₆H₄CO	226–227	EtOH	C	C₁₉H₁₃NOSCl₂	42					
36	H	Cl	2-AcOC₆H₄CO	134–135	toluene	D	C₂₁H₁₆NO₃SCl	30					
37	H	Cl	c-C₃H₅CO[z]	181–182	MeCOEt	C	C₁₆H₁₄NOSCl	40					
38	Me	Cl	4-ClC₆H₄CO	92	95% EtOH	C	C₂₀H₁₅NOSCl₂	40					
39	Me	Cl	2-AcOC₆H₄CO	96.5–97	i-PrOH	D	C₂₂H₁₈NO₃SCl	28					
40	H	Cl	H	32[q]	70% EtOH			80					
41	H	Cl	COCH₂Cl	127–128	PrOH	A		76	30 ip	65	63	68*	55 iv[x]
42	H	Cl	COCH₂NEt₂	149.5–150.5	95% EtOH	B	C₁₈H₂₁N₂OSCl·C₂H₂O₄	50					
43	Me	H	Z = Me	179–180	xylene	E	C₁₉H₁₈N₂O₃S	38	200 po	36	0	0	>1000 po
44	Me	H	Z = i-Pr	162–163	xylene	E	C₂₁H₂₂N₂O₃S	48	200 po	30	26	18	>1000 po
45	Me	H	Z = Ph	186.5–187	xylene	E	C₂₄H₂₀N₂O₃S	29	200 po	36	5	0	>1000 po
46	H	H	Z = Me	105–106	EtOH	F	C₂₀H₂₀N₂O₃S	72	200 po	22	30	30	>1000 po
47	H	H	Z = i-Pr	101–103	EtOH	F	C₂₂H₂₄N₂O₃S	61					
indomethacin									10 ip	61	65	67*	>1000 po

[a] The letter refers to general procedure given in the Experimental Section. [b] The yield of analytically pure compounds is given, unless otherwise noted, and, in most cases, no attempt was made to optimize the yields. [c] The compounds were analyzed, unless otherwise noted, for C, H, N, S and, where present, Cl and O; results are within ±0.04% of theoretical values. [d] An asterisk indicates that the result is significant on Student's t test at $p < 0.01$. [e] LD_{50}, approximative values, ten animals per dose. [f] Previously described, refs 6 and 11. [g] Previously described, ref 6. [h] Lit.[11] mp 115 °C. [i] Compound was not analyzed but used without further purification. [j] Crude yield. [k] The compound crystallized by trituration with Et₂O. [l] N(CH₂)₄ = pyrrolidino. [m] N(CH₂)₅ = piperidino. [n] NC₄H₈O = morpholino. [o] NC₄H₈NCH₃ = 4-methylpiperazino. [p] See Experimental Section. [q] Lit.[2] bp (0.05 mm) 135–140 °C. [r] 5 mg ip, 24, 31, and 33% reduction. [s] 5 mg ip, 24, 27, and 32% reduction. [t] LD_{50} ip = 140 mg ± 40. [u] LD_{50} ip = 275 mg ± 25. [v] LD_{50} ip = 275 mg ± 25. [w] LD_{50} ip = 175 mg ± 45. [x] LD_{50} ip = 350 mg ± 50. [y] Analyzed only for CHNO. [z] c-C₃H₅ = cyclopropyl.

(1) (a) Chemistry. (b) Pharmacology.
(2) A. Marcincal-Lefebvre, J. C. Gesquiere, G. Bethegnies, B. Dupuis, A. Vincent, and C. Lemer, Ann. Pharm. Fr., 38, 243 (1980).
(3) (a) J. K. Harrington, J. E. Robertson, D. C. Kvam, R. R. Hamilton, K T. McGurran, R. J. Trancik, K. F. Swingle, G. G. I. Moore, and J. F. Gerster, J. Med. Chem., 13, 137 (1970). (b) S. A. Lang, Jr., and E. Cohen, J. Med. Chem., 18, 623 (1975).
(4) W. S. Marshall, British Patent (to Eli Lilly and Co.) 1 377 480 (1974).
(5) R. C. Millonig, B. J. Amrein, J. Kirschbaum, and S. M. Hess, J. Med. Chem., 17, 772 (1974).
(6) D. Bar, A. Marcincal-Lefebvre, and J. C. Gesquiere, Ann. Pharm. Fr., 38, 161 (1980).

2,3-dioxopyrrolidines (Scheme II) with *N*-methyl-4-(phenylthio)phenylamine (3) gave us amides 43 (Z = Me), 44 (Z = *i*-Pr), and 45 (Z = Ph). In our hands, using the primary amine 4-(phenylthio)phenylamine (1) did not give us amides but rather enamines 46 and 47. Other compounds were prepared by standard methods of organic chemistry. Further details are available in Table I.

Pharmacology. Compounds were tested for LD_{50} and for their antiinflammatory activity using the carrageenin-induced paw edema method[9,10] (see Experimental Section and results in Table I).

We first tested aminoacetyl compounds 10–15 (III, R = Me), the activity of which could not be attributed to the presence of an acidic hydrogen. Modification of the amino group changed the activity: the best results were obtained with a morpholino group (14), and especially with a diethylamino group (11) (55% edema inhibition at a dose of 5 mg/kg ip; i.e., one-thirtieth of LD_{50} ip). Then, other compounds with diethylamino groups were tested, to give us the following results: the loss of the methyl group of 11 to give 17, which possesses an amidic hydrogen, caused a slight decrease in activity and, paradoxically, introduction of a chlorine atom on the structure 11 gave us the nearly inactive product 27, whereas chlorination of the non-methylated compound 17 gave us 21 that, like its ortho isomer 42, exhibited a good dose–response (one-fifteenth of LD_{50} ip) with an activity comparable with the standard indomethacin. Compounds 21 and 42, which exhibited the best activity when injected intraperitoneally, were tested when administered by gavage, but they did not exhibit any activity (at 50, 100, and 200 mg/kg po). Among insoluble compounds 29–31, 43–45, and 47, only 29 exhibited a significant activity. Other compounds were not tested. For active compounds, experimental results were controlled by a Student's *t* test.

Experimental Section

All melting points were determined using a Tottoli melting point apparatus and are uncorrected. The structures of all compounds (as free bases) were consistent with their IR and NMR spectra. Purity of all products was established by demonstrating a single spot on TLC. Reactions were monitored by TLC, using Merck 60 F 254 silica gel plastic sheets. Preparations of compounds 1–3[6] and 40[2] have been reported.

N-**Formyl-*N*-methyl-4-[(4-chlorophenyl)thio]phenylamine (5).** A mixture of 20 g (0.08 mol) of 4-[(4-chlorophenyl)thio]phenylamine (2), 12.8 g (0.12 mol) of trimethyl orthoformate, and 0.26 mL of concentrated sulfuric acid was heated by an oil bath to 120 °C and then the MeOH produced was distillated. Trimethyl orthoformate (5 mL) was added twice, while heating was continued, until there was no primary amine in the mixture (TLC). The still head was then replaced by a refluxing column, 5 mL of trimethyl orthoformate was added, and the temperature was raised to 170 °C. After heating for 3 h, the excess of ortho ester was distillated under reduced pressure, to give crude 5 which will be used to prepare compound 4. An analytical sample can be prepared by recrystallization from cyclohexane.

N-**Methyl-4-[(4-chlorophenyl)thio]phenylamine (4).** The crude 5 was added to 5 mL of concentrated HCl and 20 mL of H$_2$O and heated to reflux during 4 h. The cooled reaction mixture was treated with 15% NaOH until basic and extracted with Et$_2$O. The ether solution was washed, dried (Na$_2$SO$_4$), and added to a

(7) P. L. Southwick and R. T. Crouch, *J. Am. Chem. Soc.*, 75, 3413 (1953).

(8) P. L. Southwick, E. P. Previc, J. Casanova, Jr., and E. H. Carlson, *J. Org. Chem.*, 21, 1087 (1956).

(9) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).

(10) C. A. Winter, *Rhumatologie*, 16, 405 (1964).

(11) E. Fournier, L. Petit, J. Pichon-Gouot, and M. Dursin, *Bull. Soc. Chim. Fr.*, 1754 (1966).

solution of an excess of oxalic acid in Et$_2$O. The precipitated oxalic salt was recrystallized from 95% EtOH. Compound 4 was obtained as a free base from its oxalic salt and 15% NaOH.

N-**(Chloroacetyl)-4-[(4-chlorophenyl)thio]phenylamine (8). Procedure A.** To a solution of 9.4 g (0.04 mol) of 2, in 30 mL of AcOH, was added dropwise 4 mL (0.05 mol) of chloroacetyl chloride in 4 mL of AcOH. After 60 min, the resulting precipitate was collected and washed thoroughly with water to give 10.5 g of 8 (84%), mp 142 °C, which was used without further purification in subsequent reactions. An analytical sample was prepared by recrystallization from PrOH, mp 145 °C.

Other substituted chloroacetyl derivatives (6, 7, 9, and 41) were obtained in the same way from the appropriate amines and chloroacetyl chloride.

N-**[(Diethylamino)acetyl]-4-[(4-chlorophenyl)thio]phenylamine (21). Procedure B.** A mixture of 1.9 g (0.006 mol) of 8 and 10 mL of diethylamine was stirred with refluxing for 2 h and then at room temperature overnight. After evaporation under reduced pressure, the residual oil was added to 50 mL of Et$_2$O. The resulting solution was washed three times with H$_2$O, dried (Na$_2$SO$_4$), and added dropwise to a stirred solution of an excess of oxalic acid in Et$_2$O. The white, flocculent crystals were filtered, washed with Et$_2$O, and recrystallized from EtOH–H$_2$O to give 0.9 g of 21 (oxalic salt) (49%).

Compounds 18, 20, and 23, after evaporating the reaction mixture, were obtained as crystallized free base products; purification was then continued by recrystallizing them as free bases.

Compound 27 gave us an oily free base and a pastly oxalic salt. It was purified by performing the sequence free base/oxalic salt/free base three times to give us a product, the purity of which was established by TLC and IR and NMR spectra.

N-**(4-Chlorobenzoyl)-4-(phenylthio)phenylamine (32). Procedure C.** To a stirred solution of 2 g (0.01 mol) of 1 in 15 mL of AcOH was added dropwise a solution of 1.75 g (0.01 mol) of 4-chlorobenzoyl chloride in 15 mL of AcOH. After 15 min the precipitated product was collected by filtration, washed three times with AcOH, and recrystallized from *i*-PrOH to give 2.4 g of 32 (71%), mp 193–193.5 °C.

This procedure was followed to prepare compounds 29, 35, and 38 from the appropriate amines and 4-chlorobenzoyl chloride, while compounds 31, 34, and 37 were obtained using cyclopropylcarbonyl chloride.

N-**[2-(Acetyloxy)benzoyl]-4-(phenylthio)phenylamine (33). Procedure D.** A solution of 3.5 g (0.018 mol) of acetylsalicyloyl chloride in 25 mL of Et$_2$O was added dropwise to a stirred solution of 3 g (0.015 mol) of 1 and 1.2 mL (0.015 mol) of pyridine in 40 mL of Et$_2$O. After 2 h, the solid product was collected by filtration and recrystallized, once in EtOH, once in EtOH–cyclohexane, and twice in *i*-PrOH to give 2.5 g of 33 (46%), mp 111 °C.

Compounds 30, 36, and 39 were prepared in the same way, using acetylsalicyloyl chloride and appropriate amines.

N-**Methyl-*N*-[(4-phenylthio)phenyl]-2,3-dioxo-1-methylpyrrolidine-4-carboxamide (43). Procedure E.** To a boiling mixture of 2 g (0.011 mol) of 4-carbethoxy-2,3-dioxo-1-methylpyrrolidine in 10 mL of xylene was added dropwise a solution of 2.36 g (0.011 mol) of 3 in 10 mL of boiling xylene, and refluxing was continued for 1 h. As the solution was heating, the solvent was slowly removed by means of a still head and xylene was added occasionally, in order to maintain a volume greater than 15 mL. The mixture was then poured into 10 mL of petroleum ether and cooled. A precipitate formed which was filtered, dried, and recrystallized from xylene to give 1.5 g (40%) of 43, mp 179–180 °C.

Compounds 44 and 45 were similarly obtained from 3 and appropriate 1-substituted 4-carbethoxy-2,3-dioxopyrrolidines. Treating the same amount of the primary amine 1 gave us enamines 46 and 47 in poor yields.

4-Carbethoxy-1-methyl-2-oxo-3-[[4-(phenylthio)phenyl]amino]-3-pyrroline (46). Procedure F. A stirred solution of 2 g (0.011 mol) of 4-carbethoxy-2,3-dioxo-1-methylpyrrolidine and 2.2 g (0.011 mol) of 1 in 50 mL of EtOH was refluxed for 48 h. The resulting mixture was then concentrated under reduced pressure to 25 mL. After the mixture cooled, the solid material was collected by filtration, dried, and recrystallized from EtOH to give 2.9 g of 46 (72%), mp 105–106 °C.

Secondary arylalkylamines, like 3, did not react in our hands to give formation of enamines.

Pharmacological Methods. Antiinflammatory activity was measured by the method of Winter. Fifteen male Wistar rats weighing 170–220 g were used for each group. Compounds having amino groups in their structures were injected intraperitoneally as their soluble oxalic salts. Insoluble compounds were administered by gavage. Thirty minutes later, carrageenin (0.05 mL, 1%) in physiological saline was injected subcutaneously under the plantar skin of the hind paw. The volume of the injected paw was measured just before (T_0) and 1, 2, and 3 h after the injection of carrageenin for calculation of percent inhibition (Table I).

Nucleosides. 114. 5'-O-Glucuronides of 5-Fluorouridine and 5-Fluorocytidine. Masked Precursors of Anticancer Nucleosides[1]

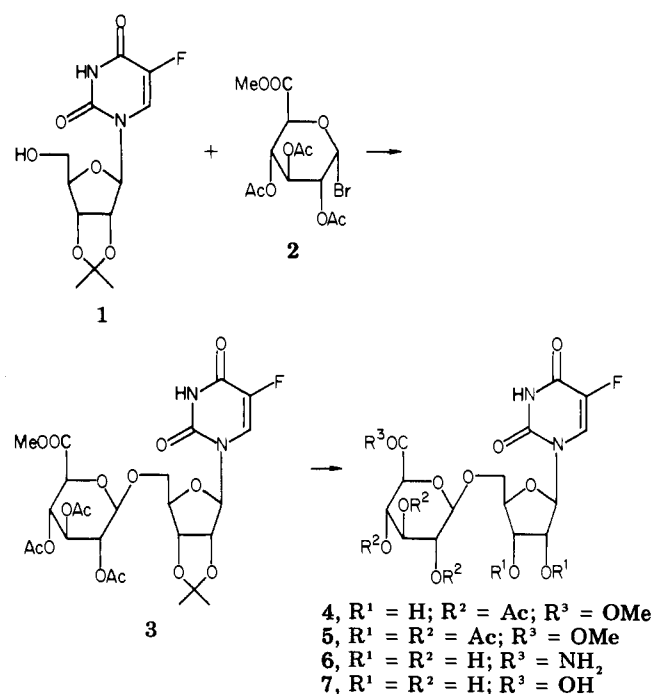K. A. Watanabe,* A. Matsuda, M. J. Halat, D. H. Hollenberg, J. S. Nisselbaum, and J. J. Fox

Laboratories of Organic Chemistry and Applied and Diagnostic Biochemistry, Sloan-Kettering Institute of Cancer Research, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021. Received February 17, 1981

5'-O-Glucuronides of anticancer nucleosides, 5-fluorouridine and 5-fluorocytidine, were synthesized by three different methods. The best preparative procedure was the one starting from benzyl 5-O-(methyl 2',3',4'-tri-O-acetyl-β-D-glucopyranosyluronate)-2,3-O-isopropylidene-β-D-ribofuranoside (15) that was obtained almost quantitatively by condensation of benzyl 2,3-O-isopropylidene-β-D-ribofuranoside (8) with methyl (2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide)uronate (2). After de-O-isopropylidenation of 15, the crystalline product, benzyl 5-O-(methyl 2',3',4'-tri-O-acetyl-β-D-glucopyranosyluronate)-β-D-ribofuranoside (16), was de-O-benzylated catalytically to 5-O-(methyl 2',3',4'-tri-O-acetyl-β-D-glucopyranosyluronate)-D-ribofuranose (17). Compound 17 was acetylated to crystalline 5-O-(methyl 2',3',4'-tri-O-acetyl-β-D-glucopyranosyluronate)-1,2,3-tri-O-acetyl-β-D-ribofuranose (18) and condensed with trimethylsilylated 5-fluorouracil of 5-fluorocytosine in the presence of SnCl₄ to afford the corresponding protected nucleosides 5 and 19 in good yields. Saponification of these compounds gave 5'-O-β-D-glucuronides of 5-fluorouridine and 5-fluorocytidine (20 and 21) isolated as their crystalline Na salts. These glucuronides were substrates of both bacterial and bovine β-glucuronidase. They were, as expected, much less toxic against several leukemic cell lines in tissue culture.

Elevated β-glucuronidase activity in malignant human tumors was first reported by Fishman et al.[2] Subsequently, many reports up to 1966 were reviewed by Levvy and Conchie[3] showing that the activity of this enzyme in human cancer tissues is extraordinarily high, relative to normal tissues. Sweeney et al.[4] later showed that mycophenolic acid, a compound which inhibits mecca lymphosarcoma and CA-755 mammary carcinoma, is converted into the glucuronide as the only metabolite. They also showed[4] that, in general, the tumors most responsive to mycophenolic acid are those in which β-glucuronidase activity is highest, while it is the lowest in nonresponsive tumors. Conners et al.[5] demonstrated that the N,N-bis-(2-chloroethyl)aniline is oxidized in vivo to the corresponding 4-hydroxy derivative, which is then converted into its glucuronide. This aromatic nitrogen mustard is uniquely effective against the mouse ADJ/PCS plasma cell tumor which exhibits particularly high levels of β-glucuronidase activity.[6]

5-Fluorouracil (FU) is a potent agent presently used in the treatment of certain solid tumors.[7] The corresponding

Scheme I

4, R¹ = H; R² = Ac; R³ = OMe
5, R¹ = R² = Ac; R³ = OMe
6, R¹ = R² = H; R³ = NH₂
7, R¹ = R² = H; R³ = OH

ribonucleoside (FUR)[8] and deoxyribonucleoside (FUDR)[9] are also active. These compounds are converted in vivo into FUDR-5'-P, which then interferes with thymidylate

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services (Grants CA-08748 and 18601).
(2) Fishman, W. H. *Science* 1947, *105*, 646. Fishman, W. H.; Anlyan, A. J. *Cancer Res.* 1947, *7*, 808.
(3) Levvy, G. A.; Conchie, J. In "Glucuronic Acid"; Dutton, G. J., Ed.; Academic Press: New York, 1966; p 301.
(4) Sweeney, M. J.; Hoffman, D. H.; Poore, G. A. *Cancer Res.* 1971, *31*, 477. Sweeney, M. J. *Jpn. J. Antibiot.* 1977, *30*-(Suppl), 85.
(5) Conners, T. A.; Farmer, P. B.; Foster, A. B.; Gilsenan, A. M.; Jarman, M.; Tisdale, M. J. *Biochem. Pharmacol.* 1973, *22*, 1971.
(6) Conners, T. A.; Whisson, M. E. *Nature (London)* 1965, *206*, 609. *Ibid.* 1966, *210*, 866.

(7) Heidelberger, C. In "Cancer Medicine", Holland, J. F.; Frei, E., Eds.; Lea and Febiger: Philadelphia, 1973; p 768.
(8) Yung, N. C.; Burchenal, J. H.; Fecher, R.; Duschinsky, R.; Fox, J. J. *J. Am. Chem. Soc.* 1961, *83*, 4060.
(9) Hoffer, M.; Duschinsky, R.; Fox, J. J.; Yung, N. C. *J. Am. Chem. Soc.* 1959, *81*, 4112.