Smythies, R. J. Bradley, V. S. Johnston, F. Benington, R. D. Morin, and L. C. Clark, Jr., *Psychopharmacologia*, **10**, 379 (1967).
(3) A. T. Shulgin, T. Sargent, and C. Naranjo, *Nature (London)*, **221**, 537 (1969).
(4) (a) B. T. Ho, W. M. McIsaac, R. An, L. W. Tansey, K. E. Walker, L. F. Englert, Jr., and M. B. Noel, *J. Med. Chem.*, **13**, 26 (1970); (b) L. E. Hollister, M. F. Macnicol, and H. K. Gillespie, *Psychopharmacologia*, 14, 62 (1969); (c) L. A. Faillace, S. H. Snyder, and A. Weingartner, *J. Nerv. Ment. Dis.*, **150**, 119 (1970).
(5) (a) A. T. Shulgin, *Experientia*, **20**, 366 (1964); (b) J. R. Smythies, V. S. Johnston, R. J. Bradley, F. Benington, R. D. Morin, and L. C. Clark, Jr., *Nature (London)*, **216**, 128 (1967); (c) C. Mitoma, *Proc. Soc. Exp. Biol. Med.*, **134**, 1162 (1970).
(6) (a) J. Huang and B. T. Ho, *J. Pharm. Pharmacol.*, **24**, 657 (1972); (b) S. H. Snyder, L. Faillace, and L. Hollister, *Science*, **158**, 669 (1967); (c) E. D. Hendley and S. H. Snyder, *Nature (London)*, **229**, 264 (1971); (d) B. E. Leonard, *Proc. Brit. Pharmacol. Soc.*, 165P (1972).
(7) (a) B. T. Ho, V. Estevez, L. W. Tansey, L. F. Englert, P. J. Creaven, and W. M. McIsaac, *J. Med. Chem.*, 14, 158 (1971); (b) B. T. Ho and L. W. Tansey, *ibid.*, 14, 156 (1971); (c) B. T. Ho, V. Estevez, and G. E. Fritchie, *Brain Res.*, **29**, 166 (1971).
(8) (a) A. S. V. Burger and L. L. Iversen, *Brit. J. Pharmacol. Chemother.*, **25**, 34 (1965); (b) M. D. Fairchild and G. A. Alles, *J. Pharmacol. Exp. Ther.*, **158**, 135 (1967); (c) C. K. Nielsen, M. P. Magnussen, K. Kampmann, and H.-H. Frey, *Arch. Int. Pharmacodyn. Ther.*, **170**, 428 (1967); (d) K. E. Moore, *J. Pharmacol. Exp. Ther.*, **142**, 6 (1966); (e) G. Moruzzi and H. W. Magoun, *Electroencephalog. Clin. Neurophysiol.*, 1, 455 (1949).
(9) (a) A. T. Shulgin, *J. Pharm. Pharmacol.*, **25**, 271 (1973); (b) F. Benington, R. D. Morin, J. Beaton, J. R. Smythies, and R. J. Bradley, *Nature, New Biol.*, **242**, 185 (1973).
(10) (a) R. L. Furner, J. S. McCarthy, R. E. Stitzel, and M. W. Anders, *J. Pharmacol. Exp. Ther.*, **169**, 153 (1969); (b) H. Keberle, K. Hoffman, and R. Bernhard, *Experientia*, **18**, 105 (1962); (c) K. S. Marshall and N. Castagnoli, Jr., *J. Med. Chem.*, **16**, 266 (1973); (d) K. H. Palmer, M. S. Fowler, M. E. Wall, L. S. Rhodes, W. J. Waddell, and B. Baggett, *J. Pharmacol. Exp. Ther.*, **170**, 355 (1969); (e) T. Meshi, M. O. Tsuka, and Y. Sato, *Biochem. Pharmacol.*, **19**, 2937 (1970); (f) L. G. Dring, R. L. Smith, and R. T. Williams, *Biochem. J.*, **16**, 425 (1970); (g) H. R. Sullivan, S. E. Emits, S. L. Due, R. E. Booker, and R. E. McMahon, *Life Sci.*, **11**, 1093 (1972).
(11) (a) L. M. Gunne, *Biochem. Pharmacol.*, **16**, 863 (1967); (b) A. H. Beckett and M. Rowland, *J. Pharm. Pharmacol.*, **17**, 628 (1965).
(12) (a) M. Goldstein and B. Anagnoste, *Biochim. Biophys. Acta*, **107**, 166 (1965); (b) M. Goldstein, M. R. McKereghan, and E. Lauber, *Biochim. Biophys. Acta*, **89**, 191 (1964).
(13) S. B. Matin, M. Rowland, and N. Castagnoli, Jr., *J. Pharm. Sci.*, **62**, 821 (1973).
(14) T. A. Montzka, T. L. Pindell, and J. D. Matiskella, *J. Org. Chem.*, **33**, 3993 (1968).
(15) D. E. Nichols, C. F. Barfnecht, D. B. Rusterholz, F. Benington, and R. D. Morin, *J. Med. Chem.*, **16**, 480 (1973).
(16) H. E. Smith, S. L. Cook, and N. E. Warren, Jr., *J. Org. Chem.*, **29**, 2265 (1964).
(17) A. T. Shulgin, *J. Med. Chem.*, **9**, 445 (1966).
(18) H. C. Brown and N. M. Yoon, *J. Amer. Chem. Soc.*, **88**, 1464 (1966).
(19) Reference deleted in revised proof.
(20) H. B. Hucker, B. M. Michniewicz, and R. E. Rhodes, *Biochem. Pharmacol.*, **20**, 2123 (1971).
(21) Jh. Sadeh, Z. Pelah, and A. Kalir, *Isr. J. Chem.*, **5**, 44 (1967).
(22) J. Tagg, D. M. Yasuda, M. Tanabe, and C. Mitoma, *Biochem. Pharmacol.*, **16**, 143 (1967).
(23) W. Michaelis, J. H. Russel, and O. Schindler, *J. Med. Chem.*, **13**, 497 (1970).
(24) A. A. R. Sayigh, H. Ulrich, and M. Green, *J. Chem. Soc.*, 2483 (1964).
(25) B. Munson, *Anal. Chem.*, **43**, 28A (1971).
(26) (a) G. F. Phillips and R. J. Mesley, *J. Pharm. Pharmacol.*, **21**, 9 (1969); (b) A. T. Shulgin, U. S. Patent 3,547,999 (1970).

Bis-Basic-Substituted Polycyclic Aromatic Compounds. A New Class of Antiviral Agents.[1,2] 2. Tilorone and Related Bis-Basic Ethers of Fluorenone, Fluorenol, and Fluorene

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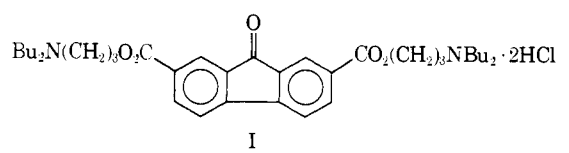
Tilorone hydrochloride, 2,7-bis[2-(diethylamino)ethoxy]-9*H*-fluoren-9-one (11), was found to prolong survival of mice infected with lethal challenges of encephalomyocarditis (EMC) virus. It was effective by oral as well as subcutaneous administration. It showed broad-spectrum antiviral activity and was later found to induce interferon in mice. Tilorone was selected from a series of congeners that was synthesized to determine structure–activity correlations. These indicated that fluorenol and fluorene analogs were much less effective than fluorenones, that thioethers showed less activity than corresponding ethers, that the 2,6- and 2,5-substituted isomers of tilorone were also active, and that elongation of the side chains and increase of molecular weight of the dialkylamine substituent led to decreased oral activity. Monoalkamine ethers showed very little or no activity.

In the first paper of this series,[2] discovery of the antiviral activity of bis(3-dibutylaminopropyl) 9-oxo-9*H*-fluorene-2,7-dicarboxylate dihydrochloride (I) and related bisalkamine esters of fluorenone-, fluorenol-, and fluorenedicarboxylic acids was reported. It also contains an introduction to this series of papers with a general discussion of our extensive synthetic work on bis-basic-substituted polycyclic aromatic compounds prepared to determine those structural features that give optimum biological properties to members of this new class of antiviral agents. Several presentations on this subject have been given.[1,3,4]

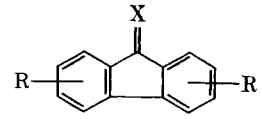


I

In this paper, we wish to report the synthesis and evaluation of the antiviral activity of a series of bis-basic ethers of fluorenone, fluorenol, and fluorene. This series includes tilorone hydrochloride (11), the first member of this class of antiviral agents to be reported.[5,6]

Chemistry. Tilorone and related bisalkamine ethers (Table I) were prepared from 2,7-dihydroxy-9*H*-fluoren-9-one (VI) as shown in Scheme I. By the method of Cour-

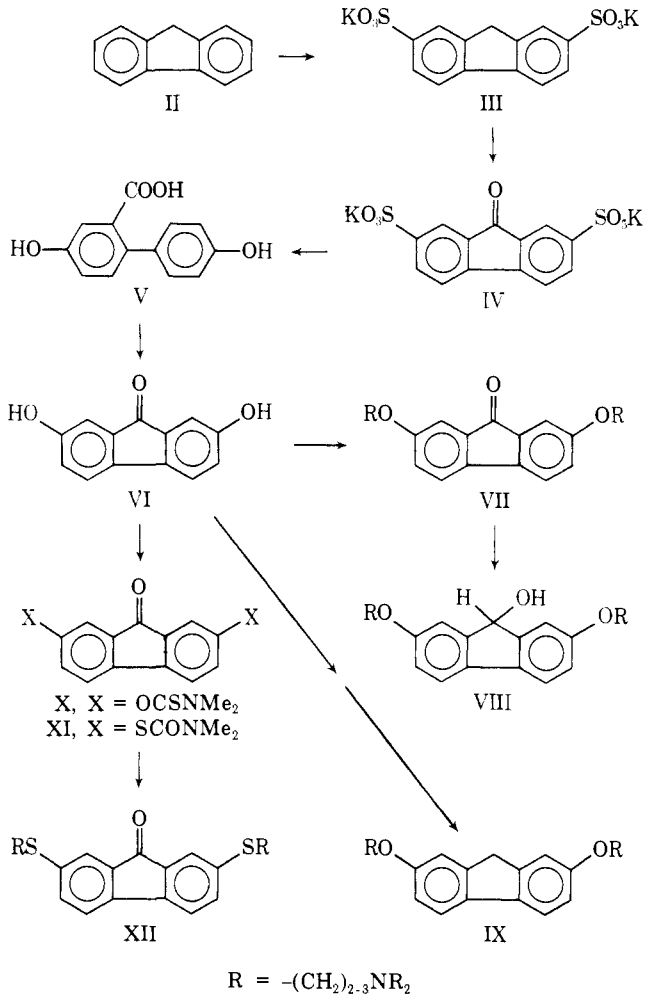
Table I. Chemical and Antiviral Properties of Bis-Basic Ethers of Fluorenone, Fluorenol, and Fluorene



Compd	R	X	Mp, °C[a]	Yield,[b] %	Recrystn solvent[c]	Formula[d]	STR *vs.* EMC virus in mice at various doses, mg/kg[e]						
							Subcutaneous admin				Oral admin		
							250	50	10	2	250[f]	50	10
1	2,7-$OCH_2CH_2N(C_2H_5)_2$	H_2	213–216	52	A	$C_{25}H_{36}N_2O_2 \cdot 2HCl$	Lethal	1.22	1.02	1.06	0.98	1.00[f]	
2	2,7-$OCH_2CH_2CH_2N(C_2H_5)_2$	H_2	217–220	61	A	$C_{27}H_{40}N_2O_2 \cdot 2HCl$	Lethal	1.42	1.06	0.94	0.96	0.89[f]	
3	2,7-$OCH_2CH_2CH_2N(C_4H_9)_2$	H_2	170–172	36	A	$C_{35}H_{36}N_2O_2 \cdot 2HCl$	1.21[g]	1.15	1.13		1.02	1.02	1.00
4	2,7-$OCH_2CH_2N(CH_3)_2$	H, OH	264–266	74	B	$C_{21}H_{28}N_2O_3 \cdot 2HCl$	Lethal	0.87	1.09		0.95[g]	0.94	
5	2,7-$OCH_2CH_2N(C_2H_5)_2$	H, OH	196–197	27	B	$C_{25}H_{36}N_2O_3 \cdot 2HCl$	Lethal	1.11[g]	1.09[g]		1.02[g]	0.96	
6	2,7-$OCH_2CH_2N(CH_3)C_2H_5$	H, OH	201–204	67	B	$C_{23}H_{32}N_2O_3 \cdot 2HCl$		0.74[g]	0.94		1.05	0.90	
7	2,7-$OCH_2CH_2N(CH_3)_2$	O	278–280 dec	43	C	$C_{21}H_{26}N_2O_3 \cdot 2HCl$		1.85	1.43	1.34	2.26	1.98	1.14
8	2,7-$SCH_2CH_2N(CH_3)_2$	O	272–274 dec	47	C	$C_{21}H_{26}N_2OS_2 \cdot 2HCl$		1.41	0.98		1.00	1.26	
9	2,5-$OCH_2CH_2N(C_2H_5)_2$	O	238–240	43	B	$C_{25}H_{34}N_2O_3 \cdot 2HCl \cdot 0.5H_2O$	2.15	1.98	1.22		2.09	1.83	
10	2,6-$OCH_2CH_2N(C_2H_5)_2$	O	247–249	38	B	$C_{25}H_{34}N_2O_3 \cdot 2HCl \cdot H_2O$	1.70[g]	1.43	1.04		2.11	1.41	
11[h]	2,7-$OCH_2CH_2N(C_2H_5)_2$	O	235–236 dec	72	B	$C_{25}H_{34}N_2O_3 \cdot 2HCl$	Lethal	1.95	1.37	1.21	2.27	1.83	1.26
12	2,7-$SCH_2CH_2N(C_2H_5)_2$	O	228–229 dec	70	B	$C_{25}H_{34}N_2OS_2 \cdot 2HCl$		1.04	1.00		0.96	0.98	
13	2,7-$OCH_2CH_2N(C_3H_7)_2$	O	194–197	58	A	$C_{29}H_{42}N_2O_3 \cdot 2HCl$	1.38[g]	1.26	1.06		1.09	0.98	
14	2,7-$OCH_2CH_2N[CH(CH_3)_2]_2$	O	238–239	20	D	$C_{29}H_{42}N_2O_3 \cdot 2HCl$[i]	2.02[g]	1.55	1.09		1.89	1.66	
15	2,7-$OCH_2CH_2N(C_4H_9)_2$	O	165–167	10	A	$C_{33}H_{50}N_2O_3 \cdot 2HCl$	1.43	0.98	0.92		0.88	0.96	
16	2,7-$OCH_2CH_2N(CH_3)C_2H_5$	O	245–247	54	A	$C_{23}H_{30}N_2O_3 \cdot 2HCl$		1.47[f]			2.44	1.44[f]	
17	2,7-$OCH_2CH_2N(CH_3)C_4H_9$	O	240–243	39	D	$C_{27}H_{38}N_2O_3 \cdot 2HCl$	1.30[g]	1.04	1.02		1.17	1.00	
18	2,7-$OCH_2CH_2N(CH_2C_6H_5)_2$	O	87–92	8	E	$C_{45}H_{42}N_2O_3$	1.05	1.05	1.02		1.05	1.05	
19	2,7-Pyrrolidinoethoxy	O	275–278	15	C	$C_{25}H_{30}N_2O_3 \cdot 2HCl$	Lethal	0.78[g]	1.12		1.70	1.28	
20	2,7-Piperidinoethoxy	O	304–306	28	C	$C_{27}H_{34}N_2O_3 \cdot 2HCl$		Lethal	1.09			1.26[g]	1.20
21	2,7-Morpholinoethoxy	O	291–293	43	F	$C_{25}H_{30}N_2O_3 \cdot 2HCl$	0.77[g]	1.13	1.02		0.90	0.83	
22	2,7-$OCH_2CH_2CH_2N(CH_3)_2$	O	282–283	65	C	$C_{23}H_{30}N_2O_3 \cdot 2HCl$	1.70	1.93	1.33		1.09[g]	1.04	1.00
23	2,7-$OCH_2CH_2CH_2N(C_2H_5)_2$	O	256–257	36	A	$C_{27}H_{38}N_2O_3 \cdot 2HCl$	Lethal	1.89	1.51	1.06	1.15	1.00[f]	1.00[f]
24	2,7-$OCH_2CH_2CH_2N(C_4H_9)_2$	O	176–179	51	A	$C_{35}H_{54}N_2O_3 \cdot 2HCl$	1.44	1.93	1.24	1.04	1.02	1.11	
25	2,7-Piperidinopropoxy	O	279.5–280.5	57	A	$C_{29}H_{38}N_2O_3 \cdot 2HCl$	Lethal	1.55	1.27		1.23	1.00[f]	
26	2,7-$OCH_2CH(CH_3)CH_2N(CH_3)_2$	O	263–265	8	G	$C_{25}H_{34}N_2O_3 \cdot 2HCl$	1.21[g]	1.25	0.98		1.15	0.90	
27	2-$OCH_2CH_2N(C_2H_5)_2$	O	207–210	76	A	$C_{19}H_{21}NO_2 \cdot HCl$	0.63[g]	1.21	1.21		1.05	1.00	
28	4-$OCH_2CH_2N(C_2H_5)_2$	O	217–220	58	A	$C_{19}H_{21}NO_2 \cdot HCl$	1.27	1.00	0.98		0.92	1.08	

[a] Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. [b] Yields refer to analytically pure product obtained in the final step of preparation of VII, VIII, IX, or XII. No effort was made to optimize yields, except for compound **11**. [c] A = 2-butanone–MeOH; B = *i*-PrOH–MeOH; C = MeOH; D = EtOH; E = pentane; F = MeOH–EtOH–H_2O; G = *i*-PrOH–EtOH. [d] All compounds were analyzed for the elements C, H, and Cl (or N). Unless otherwise indicated, microanalytical results were within ±0.4% of calculated values. Neutralization equivalents were determined on all hydrated compounds and were within 1% of calculated values. Method: nonaqueous titration with $HClO_4$ in AcOH with $Hg(OAc)_2$ added and Crystal Violet or *p*-naphtholbenzein as indicator [USP XVIII, 836(1970)]. [e] STR, survival time ratio, is defined in the description of the test method in the Experimental Section. Unless otherwise indicated, test compound was administered in four doses 28, 22, and 2 hr before and 2 hr after inoculation with virus. [f] Single dose administered 22–28 hr before virus inoculation. [g] Early deaths observed at specified dose. [h] Tilorone hydrochloride. [i] *Anal.* Calcd: C, 64.55; H, 8.22; Cl, 13.14. Found: C, 64.00; H, 8.22; Cl, 12.86.

Scheme I



tot,[7] fluorene (II) was sulfonated with hot concentrated sulfuric acid and the resulting 9*H*-fluorene-2,7-disulfonic acid was neutralized to the dipotassium salt III, which was oxidized with potassium permanganate to 9-oxo-9*H*-fluorene-2,7-disulfonic acid dipotassium salt (IV). Sodium hydroxide fusion of IV at 300° gave 4,4-dihydroxybiphenyl-2-carboxylic acid (V), which was cyclized to VI with $ZnCl_2$ as described by Agrawal.[8] Reaction of VI with appropriate dialkylaminoalkyl halides in the presence of base gave tilorone and related compounds VII. Catalytic hydrogenation of VII over palladium-on-charcoal catalyst gave the fluorenol analogs VIII. The fluorene analogs IX were prepared from 2,7-dihydroxy-9*H*-fluorene obtained by Wolff-Kishner reduction of VI.[8,9] 2,6- and 2,5-Dihydroxy-9*H*-fluoren-9-one were obtained from the corresponding dinitro compounds by reduction, diazotation, and hydrolytic displacement as described by Barker and Barker for the 3,6 isomer.[10] The thioethers XII were obtained *via* 2,7-bis(dimethylcarbamoylthio)-9*H*-fluoren-9-one (XI) obtained by pyrolysis of the dimethylthiocarbamoyl derivative X by the method of Newman and Karnes.[11] Examples of these syntheses are given in the Experimental Section.

Biological Activity. The compounds listed in Table I were evaluated for their effectiveness in protecting mice against encephalomyocarditis (EMC) virus infections. As defined in the Experimental Section, antiviral activity is expressed as survival time ratio (STR). By our definition, an STR of 1.30 or greater indicates high activity.

Several bis(dialkylaminoalkoxy)fluorenones were highly effective by subcutaneous administration. These included compounds **7**, **9**, **11**, **14**, **22**, **23**, and **24**. Each had a high degree of activity, gave a good dose-response effect, and showed little or no evidence of lethal effects at higher dose levels.

These seven bisethers are all fluorenones. The corresponding fluorenes **1–3** were less active and the fluorenols **4–6** showed little or no activity in this test system. Of the three position isomers **9–11**, in which the two side chains are attached at the 2,5, 2,6, and 2,7 positions, the 2,6 isomer **10** had the lowest and the 2,7 isomer **11** (tilorone) the highest activity at the lower doses. The two thioether analogs **8** and **12** showed much less activity than the corresponding ethers **7** and **11**, respectively. The length of the alkylene chain separating O and N atoms appears to have less effect on parenteral activity than the nature of the amine function. However, both of these features have a marked effect on oral activity.

Oral activity was determined by the same dosage regimen used with subcutaneous administration; the test compound was administered 28, 22, and 2 hr before and 2 hr after virus inoculation. In addition, the effect of a single oral dose of 250 mg/kg, administered 22–28 hr prior to infection, was evaluated. Of the seven compounds that showed high activity on subcutaneous administration, only **7**, **9**, **11**, and **14** showed correspondingly high oral activity. Compounds **10**, **16**, and **19** were also orally active. All of these compounds have two-carbon side chains with low molecular weight dialkylamino (Me_2N to i-Pr_2N) or pyrrolidino terminal groups. Compounds with higher molecular weight dialkylamino, dibenzylamino, and heterocyclic amino substituents, and those compounds with three-carbon side chains, lacked oral activity. Monoalkamine ethers (**27** and **28**) showed very little or no activity.

Compound **11** (tilorone hydrochloride) was selected from this series for extended biological, toxicological, and clinical evaluation.

Tilorone Hydrochloride. Tilorone hydrochloride showed oral antiviral activity also against Semliki Forest virus, an RNA virus of the arbovirus group (STR 1.70); vesicular stomatitis virus (STR 1.80); Mengo virus (STR 1.82); herpes simplex, a DNA virus (STR 1.18); and the RNA myxoviruses, influenza B (Massachusetts, STR 1.22), influenza A/equine-1 (Prague, STR 1.22), and influenza A_2 (Jap/305, STR 1.25). It also reduced tail lesions induced in mice by nonlethal doses of vaccinia, a DNA virus.[5] This broad spectrum of antiviral activity has been confirmed in other laboratories.[12,13] Optimum oral activity against subcutaneous challenges of encephalomyocarditis (EMC) or Semliki Forest virus was found when tilorone was given 24 hr before virus inoculation.[5,14]

Tilorone hydrochloride was found to induce high blood titers of interferon in mice.[6] This finding has been confirmed in other laboratories.[12,15,16] A good correlation of interferon induction and antiviral activity has been established in mice against encephalomyocarditis and vesicular stomatitis viruses.[6,12] In other species (rats, rabbits), no serum interferon levels were observed at doses that still afforded protection against viral infection.[15,†]

Tilorone hydrochloride has been reported to have antitumor activity[17-19] and to ameliorate Friend virus leukemia.[19,20] It has been proposed that tilorone hydrochloride inhibits nucleic acid transcriptases associated with oncogenic viruses.[21,‡] Tilorone hydrochloride was found to stimulate the primary immune response induced in mice by sheep red blood cells[19,22] but to inhibit the cell-mediated immune response induced in rats by allergic en-

†G. D. Mayer, Merrell-National Laboratories, Cincinnati, Ohio, personal communication, 1973.

‡M. Green, Institute for Molecular Virology, St. Louis University, St. Louis, Mo., personal communication, 1973.

cephalomyelitis.[23] *Mycobacterium butyricum* induced adjuvant arthritis in rats was completely inhibited after 14 consecutive days of treatment with 100 mg/kg po of tilorone hydrochloride.[23] Pathologic-toxicologic evaluations of tilorone hydrochloride have been reported.[24-26] Clinical evaluation of tilorone hydrochloride is in progress.

Experimental Section

Antiviral Evaluation Method. The anti-EMC activity of compounds in this study was determined in CF-1 male mice, 15–17 g each, at the several dose levels indicated in Table I. Ten mice were used for each dose level of a compound, and the control group for each compound was 20–30 untreated mice. The test compound was dissolved or suspended in 0.15% hydroxyethylcellulose in H_2O and injected subcutaneously in the nape of the neck or administered orally by gavage. For each dose level, the indicated dose was given 28, 22, and 2 hr before and 2 hr after inoculation with virus. In oral evaluations, the 250 mg/kg dose was a single dose administered 22–28 hr prior to virus infection. The EMC virus was administered subcutaneously in the groin at infective doses in the range of 4–62 LD_{50}. In each test, treated and control mice were infected with the same viral challenge. The mice were observed for 10 days after inoculation. Deaths were recorded twice daily and the mean day of death of each group was determined. A score of 11 was assigned to each survivor and used in determining the mean. A survival time ratio (STR), which is the mean day of death of the treated group divided by the mean day of death of the control group, was calculated for each dose level.

The mean day of death (MDD) of control mice infected with EMC virus varies little between 4 LD_{50} (MDD 4.7–5.0) and 100 LD_{50} (MDD 4.1–4.3). Since the viral challenge in each test is the same for treated and control mice, the effect of the challenge on the STR is not significant for active compounds. In tests with tilorone hydrochloride (250 mg/kg orally), the following results were obtained.

EMC virus (LD_{50})	Control MDD	Treated MDD	STR
5	4.8	10.4	2.14
32	4.3	9.9	2.30
100	4.1	10.4	2.54

Activity is interpreted on the basis of parameters derived from standard deviations of the mean of control groups. An STR of less than 0.90 indicates that early deaths were observed; a ratio of 0.90–1.09 indicates that there was no activity; a ratio of 1.10–1.19 indicates low or weak activity (p = 0.2–0.05 by Student's t test); a ratio of 1.20–1.29 indicates medium activity (p = 0.1 to <0.001); and a ratio of 1.30 or greater indicates high activity (p = 0.05 to <0.001).

4,4′-Dihydroxybiphenyl-2-carboxylic Acid (V). Fluorene (200 g) was added to 800 g of concentrated H_2SO_4 at 100°. After 5 min of vigorous stirring, the solution was allowed to cool and ice was added until a volume of 2 l. was obtained. The solution was filtered and heated, and 400 g of KCl was added. The potassium salt precipitated on cooling was collected and recrystallized from 7 l. of H_2O to which 700 g of K_2CO_3 was added to neutralize excess acid. 9*H*-Fluorene-2,7-disulfonic acid dipotassium salt (III) was obtained, 398.8 g (82%). It was dissolved in 7 l. of H_2O, and a hot solution of 208 g of $KMnO_4$ in 3.5 l. of H_2O was added over 1 hr while a reaction temperature of 20–30° was maintained. MnO_2 that was formed was removed by filtration. The filtrate was concentrated to about 4.5 l. and cooled (5°). 9-Oxo-9*H*-fluorene-2,7-disulfonic acid dipotassium salt (IV) precipitated and was collected. A second crop was obtained from the mother liquor concentrated to 1.5 l. to give a total of 366.3 g (89%).

To 210 g of NaOH fused at 300–325° in a stainless steel beaker 105 g of IV was added in portions during 1 hr with occasional stirring with a stainless steel rod. The mixture was then allowed to cool, was dissolved in 1.5 l. of H_2O, filtered and cooled in an ice bath, and then acidified by addition of 0.5 l. of concentrated HCl. The resulting product V was collected and dried: 57.9 g (95%); mp 270–273° (lit.[27] mp 281–282°). This material was sufficiently pure for preparation of 2,7-dihydroxy-9*H*-fluoren-9-one (VI) by the method described by Agrawal.[8]

2,7-Bis[2-(diethylamino)ethoxy]-9*H*-fluoren-9-one Dihydrochloride (11, Tilorone Hydrochloride). To a stirred mixture of 31.8 g (0.15 mol) of VI, 103.2 g (0.60 mol) of 2-diethylaminoethyl chloride hydrochloride, 100 ml of H_2O, and 450 ml of toluene, 66.0 g (1 mol) of KOH pellets in 100 ml of H_2O was added. This mixture was stirred vigorously at reflux temperature for 20 hr. The mixture was allowed to cool; the toluene layer was separated, washed with 20% KOH and saturated NaCl solution, and then dried ($MgSO_4$). After filtration and evaporation of solvents, the residue was dissolved in *i*-PrOH and acidified with ethanolic HCl. The orange product (55.6 g) was recrystallized from *i*-PrOH–MeOH to give 51.6 g (72%) of 11, mp 235–236° dec.

This procedure was also used for preparation of compounds 14, 16–21, 25, and 26, while compounds 15, 22–24, 27, and 28 were prepared in anhydrous toluene with $NaOCH_3$ as base. Yields and physical properties are given in Table I.

2,7-Bis[2-(dimethylamino)ethoxy]-9*H*-fluoren-9-one Dihydrochloride (7). A mixture of 21.2 g (0.10 mol) of VI, 16.0 g (0.296 mol) of NaOMe, 350 ml of C_6H_5Cl, and 60 ml of MeOH was stirred and heated in such a way as to allow the MeOH to distill off until the reaction mixture reached a temperature of 130°. The mixture was then cooled below 100° and a solution of 32.6 g (0.30 mol) of 2-dimethylaminoethyl chloride in 100 ml of C_6H_5Cl was added. The mixture was heated to reflux for 18 hr. The C_6H_5Cl layer was separated, washed (H_2O), and dried ($MgSO_4$), and the solvent was evaporated. The residue was dissolved in MeOH and acidified with ethanolic HCl. The resulting product was recrystallized twice from MeOH to give 18.5 g (43%) of 7 (Table I). Compounds 9, 10, and 13 were also prepared by this procedure.

2,7-Bis[2-(diethylamino)ethoxy]-9*H*-fluorene Dihydrochloride (1). To 4.9 g (0.025 mol) of 2,7-dihydroxy-9*H*-fluorene [obtained by Wolff–Kishner reduction of VI as described by Agrawal,[8] mp 261–269° (lit.[8] mp 269–270°)] and 2.7 g (0.05 mol) of NaOMe in 200 ml of toluene (dried over molecular sieves) was added 2-diethylaminoethyl chloride, obtained from 12.9 g (0.075 mol) of its hydrochloride salt, in dry toluene. The mixture was stirred at the reflux temperature for 3 hr and cooled, and the NaCl that had precipitated was collected by filtration. The filtrate was washed (H_2O), dried ($MgSO_4$), and acidified with ethereal HCl. The resulting precipitate was recrystallized once from 2-butanone–MeOH to give 5.8 g (52%) of 1 (Table I). Compounds 2 and 3 were prepared by the same procedure.

2,7-Bis[2-(dimethylamino)ethoxy]-9*H*-fluoren-9-ol Dihydrochloride (4). A solution of 8.6 g (0.02 mol) of 7 in 105 ml of MeOH and 45 ml of H_2O over 2.0 g of 10% palladium-on-charcoal catalyst was hydrogenated in a Parr shaker at room temperature for 30 min. The catalyst was removed by filtration and the filtrate evaporated to dryness. The residue was recrystallized twice from *i*-PrOH–MeOH to give 6.4 g (74%) of 4 (Table I). Compounds 5 and 6 were prepared in a similar manner.

2,7-Bis(2-dimethylthiocarbamoyl)-9*H*-fluoren-9-one (X). By the general procedure of Newman and Karnes,[11] 32.0 g (0.80 mol) of a 60% dispersion of NaH in mineral oil was added in small portions to a cooled solution of 84.9 g (0.40 mol) of VI in 450 ml of $HCONMe_2$. After hydrogen evolution had ceased, the solution was cooled to 10° in an ice bath and 100.0 g (0.80 mol) of dimethylthiocarbamoyl chloride was added. The ice bath was removed and the mixture was heated to 80° for 5 hr. The mixture was then poured onto 1.5 kg of ice. The product was collected and recrystallized three times from $HCONMe_2$–H_2O to give 83.5 g of material of mp 202–205°. This material was finely pulverized and washed with MeOH to give 50.3 g (33%) of X, mp 235–237°. *Anal.* ($C_{19}H_{18}N_2O_3S_2$) C, H, N.

2,7-Bis(dimethylcarbamoylthio)-9*H*-fluoren-9-one (XI). The dimethylthiocarbamoyl derivative X (50.3 g) was pyrolyzed in a round-bottom flask submerged and rotated in an oil bath heated to 240° for 45 min. The melt was cooled, dissolved in $HCONMe_2$, and precipitated by addition of H_2O to give 46.1 g (92%) of XI, mp 222–224°. *Anal.* ($C_{19}H_{18}N_2O_3S_2$) C, H, N.

2,7-Bis[2-(diethylamino)ethylthio]-9*H*-fluoren-9-one Dihydrochloride (12). A mixture of 2,7-bis(2-dimethylcarbamoylthio)-9*H*-fluoren-9-one (XI) (18.0 g, 0.047 mol), MeOH (100 ml), 50% NaOH (50 ml), and H_2O (100 ml) was refluxed for 24 hr. C_6H_5Cl (350 ml) was added and the mixture was heated and stirred in such a way as to allow the MeOH and H_2O to distill off until the reaction mixture reached a temperature of 125°. The mixture was then cooled below 100° and a solution of 2-diethylaminoethyl chloride (20.1 g, 0.149 mol) in C_6H_5Cl (450 ml) was added. This mixture was stirred at reflux temperature for 24 hr. Water (300 ml) and 2 *N* NaOH (200 ml) were added, the C_6H_5Cl layer was separated, washed (H_2O), and dried ($MgSO_4$), and the solvent was evaporated. The residue was dissolved in *i*-PrOH, ethereal HCl was added, and the resulting precipitate was recrystallized twice from *i*-PrOH–MeOH to give 16.9 g (70%) of 12 (Table I). Compound 8 was prepared by the same method.

2,6-Dihydroxy-9*H*-fluoren-9-one. Following the procedure of Barker and Barker,[10] nitrosylsulfuric acid, prepared from 60.0 g (0.086 mol) of $NaNO_2$ and 120 ml of H_2SO_4, was added dropwise to a cooled (0–5°) H_2SO_4 solution of 9.0 g (0.043 mol) of 2,6-diamino-9*H*-fluoren-9-one, mp 203–204° (lit.[28] mp 202–203°), prepared by $SnCl_2$–HCl–AcOH reduction of 2,6-dinitro-9*H*-fluoren-9-one. The mixture was stirred at 0–5° for 45 min and then poured onto 600 g of ice. Excess HNO_2 was destroyed by addition of 8.4 g of sulfamic acid and the solution was warmed slowly to boiling. The solution was cooled and the precipitate that separated was collected. It was dissolved in aqueous alkali, filtered, and reprecipitated by addition of dilute HCl. The product was recrystallized from aqueous EtOH to give 6.5 g (71%) of 2,6-dihydroxy-9*H*-fluoren-9-one, mp 247–249°. *Anal.* Calcd for $C_{13}H_8O_3$: C, 73.58; H, 3.80. Found: C, 73.03; H, 3.79.

This sample was used to prepare **10**. 2,5-Dihydroxy-9*H*-fluoren-9-one was similarly prepared from 2,5-dinitro-9*H*-fluoren-9-one; the crude material, mp 299–301°, was used to prepare **9**.

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References

(1) Presented in part at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, Abstract MEDI 18.
(2) A. D. Sill, W. L. Albrecht, E. R. Andrews, R. W. Fleming, S. W. Horgan, E. M. Roberts, and F. W. Sweet, *J. Med. Chem.*, **16**, 240 (1973) (paper 1).
(3) W. L. Albrecht, E. R. Andrews, A. A. Carr, R. W. Fleming, J. M. Grisar, S. W. Horgan, A. D. Sill, F. W. Sweet, and D. L. Wenstrup, paper presented at the 13th National Medicinal Chemistry Symposium of the American Chemical Society, Iowa City, Iowa, June 1972.
(4) R. F. Krueger, G. D. Mayer, K. P. Camyre, and S. Yoshimura, paper presented at the International Colloquium on Interferon and Interferon Inducers, Leuven (Louvain), Belgium, Sept 1971.
(5) R. F. Krueger and G. D. Mayer, *Science*, **169**, 1213 (1970).
(6) G. D. Mayer and R. F. Krueger, *Science*, **169**, 1214 (1970).
(7) C. Courtot, *Ann. Chim. (Paris)*, **14**, 5 (1930).
(8) K. C. Agrawal, *J. Med. Chem.*, **10**, 99 (1967).
(9) C. D. Nenitzescu and M. Avram, *Acad. Repub. Pop. Rom., Stud. Cercet. Chim.*, 4, 57 (1956); *Chem. Abstr.*, 51, 3535 (1957).
(10) A. Barker and C. C. Barker, *J. Chem. Soc.*, 870 (1954).
(11) M. S. Newman and H. A. Karnes, *J. Org. Chem.*, 31, 3980 (1966).
(12) E. De Clercq and T. C. Merigan, *J. Infec. Dis.*, **123**, 190 (1971).
(13) J. F. Niblack, "23rd International Congress of Pure and Applied Chemistry. Special Lectures," Vol. 3, Butterworths, London, 1971, pp 111–131.
(14) R. F. Krueger, G. D. Mayer, S. Yoshimura, and K. A. Ludwig, *Antimicrob. Agents Chemother.*, 486 (1971).
(15) D. J. Giron, J. P. Schmidt, and F. F. Pindak, *Antimicrob. Agents Chemother.*, 1, 78 (1972).
(16) D. A. Stringfellow and L. A. Glasgow, *Antimicrob. Agents Chemother.*, **2**, 73 (1972).
(17) R. H. Adamson, *J. Nat. Cancer Inst.*, **46**, 431 (1971).
(18) D. Roye, A. Rhoads, H. P. Morris, and W. L. West, *Pharmacologist*, **13**, 260 (1971).
(19) A. E. Munson, J. A. Munson, W. Regelson, and G. L. Wampler, *Cancer Res.*, **32**, 1397 (1972).
(20) A. D. Barker, M. S. Rheins, and H. E. Wilson, *Proc. Soc. Exp. Biol. Med.*, **137**, 981 (1971).
(21) P. Chandra, F. Zunino, and A. Götz, *FEBS Lett.*, **22**, 161 (1972).
(22) P. F. Hoffman, H. W. Ritter, and R. F. Krueger, *Advan. Antimicrob. Antineoplastic Chemother.*, 217 (1972).
(23) H. Megel, A. Raychaudhuri, S. Goldstein, C. R. Kinsolving, I. Shemano, and J. G. Michael, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **32**, 1021 (1973); *Proc. Soc. Exp. Biol. Med.*, in press.
(24) M. W. Rohovsky, J. W. Newberne, and J. P. Gibson, *Toxicol. Appl. Pharmacol.*, **17**, 556 (1970).
(25) M. W. Rohovsky, J. W. Newberne, and J. P. Gibson, *Toxicol. Appl. Pharmacol.*, **19**, 415 (1971).
(26) G. Zbinden and E. Emch, *Acta Haematol.*, **47**, 49 (1972).
(27) C. Courtot and R. Geoffroy, *C. R. Acad. Sci., Paris*, **180**, 1665 (1925).
(28) N. Ishikawa and M. Hayashi, *Yuki Gosei Kagaku Kyokaishi*, **14**, 80 (1956); *Chem. Abstr.*, **51**, 8058 (1957).

Bis-Basic-Substituted Polycyclic Aromatic Compounds. A New Class of Antiviral Agents.[1-4] 3. 2,7-Bis(aminoacyl)fluorenes and -fluorenones

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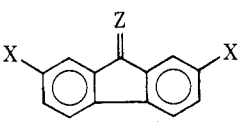
The synthesis and antiviral activity of a number of 2,7-bis(aminoacyl)fluorenes and -fluorenones are described. The structural features investigated include the alkylene chain, the terminal amine function of fluorene and fluorenone bis-basic ketones, and reduction of the carbonyl function in the side chain. Several compounds have broad spectrum antiviral activity, induce interferon, and are effective by oral as well as parenteral administration. 1,1′-(9*H*-Fluorene-2,7-diyl)bis[2-(diethylamino)ethanone]dihydrochloride (**2**, RMI 11002 DA) was selected for clinical trial.

It was previously reported from this laboratory that bisalkamine esters of fluorenone-, fluorenol-, and fluorenedicarboxylic acids I are potent antiviral agents.[1] In these compounds the two basic-substituted side chains are attached to the fluorene moiety by an ester linkage. To explore the importance of this linkage, analogous compounds were prepared in which the side chains are linked through ether bonds, as in II. This led to antiviral agents effective on oral as well as subcutaneous administration including tilorone hydrochloride, the first member of this class of antiviral agents to be reported.[5,6] This series is described in the preceding paper.[2] In this paper, we are reporting the synthesis and antiviral properties of a series of compounds III in which the basic-substituted side chains are linked to the fluorene moiety by carbon–carbon bonds. Of these, 2,7-bis(aminoacyl)fluorenes and -fluorenones (III, Y = O) showed favorable antiviral properties and several members were found to be active on oral administration.



I, X = $-C(=O)O(CH_2)_nNR_2$ Z = O; H, OH; H, H
II, X = $-O(CH_2)_nNR_2$ Z = O; H, OH; H, H
III, X = $-C(=Y)(CH_2)_nNR_2$ Z = O; H, H
Y = O: H, OH; H, H; NOH