

SYNTHESIS OF POTENTIAL INTERFERON INDUCERS AND DNA INTERCALATORS.
PART I. DERIVATIVES OF 1,8-DIAZAFLUORENE - THE NOVEL ANALOGUES OF
TILORONE

Zdzisław Szulc and Jacek Młochowski

Institute of Organic and Physical Chemistry, Technical University
of Wrocław, 50-370 Wrocław, Poland

Magdalena Fikus

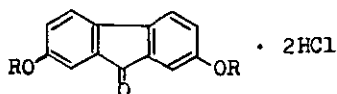
Institute of Biochemistry and Biophysic, Polish Academy of Sciences,
02-532 Warsaw, Poland

Anna D. Inglot

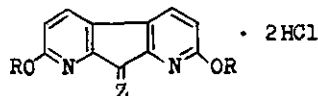
L. Hirszfeld Institute of Immunology and Experimental Therapy,
Polish Academy of Sciences, 53-114 Wrocław, Poland

Abstract - The novel isosteric aza-analogues of tilorone and its congeners, bearing 1,8-diazafluorene nucleus, have been prepared and their biological and biochemical properties are presented.

2,7-Bis[2-(diethylamino)ethoxy]fluoren-9-one dihydrochloride 1, referred in the literature as tilorone, exhibits important biological activities such as virucidal, interferonogenic, antitumor, anti-inflammatory and immunostimulating^{1,2} ones. It seems possible that activity of tilorone is connected with its ability to intercalate DNA, but this problem remains tentative.^{2,3} Many compounds having similar structural elements have been synthesized and their mode of action is under investigation.^{1,4} Nevertheless, despite of these attempts, the compound 1 remains one of the most active among the low-molecular-weight interferon inducers in mice, but unfortunately inactive in humans.^{1c}

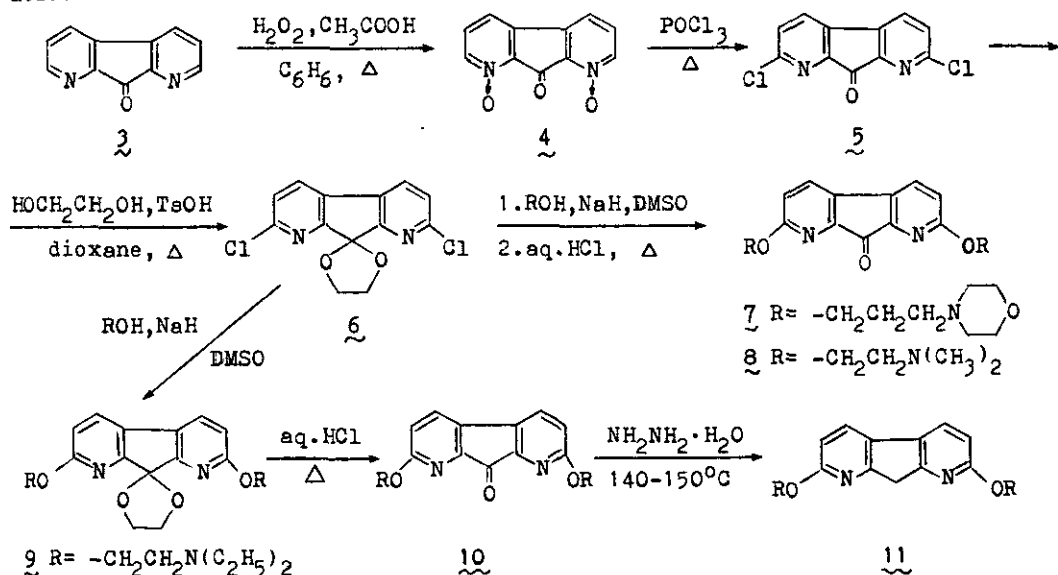


1 R = CH₂CH₂N(C₂H₅)₂



2 R = (CH₂)_nNR'; R' = alkyl
n = 2, 3; Z = O, H₂

The aim of our investigation was a synthesis of compounds 2, new analogues of tilorone and its congeners bearing two nitrogen atoms in both external rings in the peri position to the carbonyl group. The synthetic route involves (except for synthesis of the 1,8-diazafluorenone system^{5a}) three main steps - introduction of two chlorine atoms in the positions 2 and 7, protection of the carbonyl group and replacement of the chlorine atoms with N,N-dialkylaminoalkoxy groups. This method is quite different from the synthesis of tilorone from fluorene, involving intramolecular cyclization of 3-hydroxy-6-(p-hydroxyphenyl)-benzoic acid.^{1a}



1,8-Diazafluorenone (3) was oxidized to di-N-oxide 4^{5b} which was then heated with POCl_3 yielding 2,7-dichloro-1,8-diazafluorenone (5). All attempts of substituting the chlorine atoms in 5, having nonprotected carbonyl group, failed. Compound 5 underwent the usual acid-catalyzed ketalization, yielding the key compound 1,3-dioxolane 6. The nucleophilic displacement of the chlorine atoms in 6 was carried out by using DMSO as the solvent and sodium (N,N-dialkylamino)alkoxylates, generated in situ from the appropriate amino alcohols and NaH. Then, diethers, e.g. 9, obtained were easily converted into desired analogues of tilorone 7, 8 and 10 with diluted HCl. Compound 10 was subsequently reduced with hydrazine^{5c} giving diazafluorene 11. The diethers 7 - 11 were characterized mainly as the hydrochlorides and in this form they were subjected to tests for interferonogenic activity and were studied for ability to interact with DNA. Results are shown in Table.

Table. Biological and biochemical properties of azaanalogues of tilorone

Compound	Toxicity ^{a)} in vivo Mouse LD ₅₀ (mg/kg)	Toxicity ^{b)} in vitro for L cells (μ g/ml)	Interferon ^{c)} induction in the mouse	T _m ($^{\circ}$ C) ^{d)} of calf thymus DNA	% inhibition of E. coli DNA polymerase ^{e)}
7	ND	68	inactive	72	0
8	ND	50	inactive	79	0
9	ND	68	inactive	75	0
10	250	68	weak	80	63
11	ND	34	inactive	76	12
Tilorone	1520	14	strong	84	85
Control	-	-	-	72	0

^{a)} Determined in Balb/c mice after oral administration. LD₅₀ for tilorone is quoted after reference^{1b} ND - not done. ^{b)} Determined in the mouse L cells by micro-assay similar cytotoxicity for several human or bovine cell lines. ^{c)} Determined in Balb/c mice 3, 5, 12 and 24 hrs after oral or intraperitoneal administration. Weak induction means that less than 100 units of interferon were induced and the induction was irregular. ^{d)} T_m - the mid point of thermal transition of calf thymus DNA in 10 mM sodium phosphate buffer, pH 7.0, with ligands added at the molar ratio DNA(P):ligand=5. ^{e)} At 0.5 mM ligand concentration.

The biological assays showed that 10 was almost six times more toxic in vivo than tilorone. However, in vitro tilorone was found to be more toxic than its congeners. Aza analogues of tilorone were inactive or weakly active as interferon inducers. However, 10 interacted with DNA almost to the same extent as tilorone. The mode of interaction of ligands with DNA was also investigated by the electrophoretic method.⁶ It was shown that the complete unwinding of superhelical PM2 DNA was obtained at 0.16, 5.6 and 56 μ g/ml concentration of ethidium bromide, tilorone and 10, respectively. Complete biological and biochemical results will be published separately.

EXPERIMENTAL

2,7-Dichloro-1,8-diazafluorenone (5). A mixture of 4 (6.42 g, 30 mM) and POCl₃ (22 ml) was refluxed for 20 min. An excess of POCl₃ was removed in vacuo. The residue was treated with water (40 ml) and ice (20 g) and the pH was adjusted to 5-6 with 15% aq. K₂CO₃. The crude product was filtered off, washed with cold water and recrystallized from a mixture of EtOH-CH₃COOH (1:3) to give pure 5⁷ in

57% yield as yellow-green crystals, mp 307-309° C. IR (KBr): 1740 cm^{-1} (C=O). NMR (CF_3COOD) δ : 8.12 (2H, d, $J = 8$ Hz, 4-H and 5-H); 7.60 (2H, d, $J = 8$ Hz, 3-H and 6-H). UV (EtOH): λ_{max} 208 nm ($\log \epsilon=4.23$), 250 (4.10), 288 (4.02), 313 (3.97) and 378 (3.30).

Spiro [1',3'-dioxolane-2',9[9H]-2,7-dichloro-1,8-diazafluorene] (6). A mixture of 5 (3.76 g, 15 mM) and ethylene glycol (15 ml) in dioxane (25 ml) containing a catalytic amount of p-TsOH was refluxed for 40 h. After removal of dioxane in vacuo the mixture was cooled to 0° C. The crude product was filtered off, washed with a small amount of cold water, dissolved in hot ethanol and decolorized with charcoal. After removal of the solvent, the product was recrystallized from the mixture of benzene-ethanol (5:1). After drying at 100-105° C for 2 h, white needles of 6 were obtained in 58% yield, mp 285-287° C. NMR (CDCl_3 , 40° C) δ : 7.63 (2H, d, $J = 8$ Hz, 4-H and 5-H); 7.21 (2H, d, $J = 8$ Hz, 3-H and 6-H); 4.50 (4H, s, $-\text{OCH}_2-$). UV (EtOH): λ_{max} 212 nm ($\log \epsilon=4.39$), 248 (4.15), 323 (3.97).

Spiro [1',3'-dioxolane-2',9[9H]-2,7-bis[2''-(N,N-diethylamino)ethoxy]-1,8-diazafluorene] (9). To an oil-free sodium hydride (prepared from 3.10 g of 50% NaH, 64.5 mM), anhydrous $(\text{CH}_3)_2\text{SO}$ (25 ml) and freshly distilled 2-(N,N-diethylamino)-ethanol (8.53 ml, 64.5 mM) were added and the mixture was stirred at 45° C until all sodium hydride dissolved. After cooling to 20° C, 6 (3.17 g, 10.7 mM) was added and the mixture was stirred at room temperature for 12 h and next heated at 40-45° C for 6 h. After cooling, the mixture was poured into water (150 ml) and was allowed to stand in refrigerator. Crude 9 was filtered off, washed with cold water, and dried in the air. Without purification, the base 9 was further dissolved in a mixture of ether-absolute ethanol (3:1) and converted into hydrochloride by means of ethereal HCl. After recrystallization from 95% ethanol and drying at 105-110° C for 2 h, white needles of dihydrochloride of 9 [mp > 235° C (decomp.)] was obtained in 64% yield. IR (KBr): 2600 and 2495 cm^{-1} (NH^+).

NMR (D_2O) δ : 7.90 (2H, d, $J = 8$ Hz, 4-H and 5-H); 6.95 (2H, d, $J = 8$ Hz, 3-H and 6-H); 4.95-4.81 (8H, m, $-\text{OCH}_2-$); 3.93-3.35 (12H, m, $-\text{CH}_2\text{N}(\text{CH}_2)_2-$); 1.56 (12H, t, $J = 7$ Hz, $-\text{CH}_3$). UV (H_2O): λ_{max} 214 nm ($\log \epsilon=4.51$), 292 (4.32), 337 (3.64).

2,7-Bis[2-(N,N-diethylamino)ethoxy]-1,8-diazafluorenone (10). A solution of dihydrochloride of 9 (530 mg, 1 mM) in diluted HCl (2 ml of conc. HCl and 2 ml of water) was heated at 90-95° C for 20 min. After hydrolysis, the solution was cooled and neutralized with concentrated ammonia. The precipitated product was

filtered off, washed with a small amount of cold water, and recrystallized from n-hexane to give pure 10, in 80% yield, as fine carmine plates, mp 93 - 95° C. IR (KBr): 1740 cm^{-1} (C=O). NMR (CCl_4) δ : 7.46 (2H, d, J = 8 Hz, 4-H and 5-H); 6.56 (2H, d, J = 8 Hz, 3-H and 6-H); 4.28 (4H, t, J = 6 Hz, $-\text{OCH}_2-$); 2.68 (4H, t, J = 6 Hz, $-\text{CH}_2\text{CH}_2\text{N}=$); 2.36 (8H, q, J = 7 Hz, $-\text{CH}_2\text{CH}_3$); 0.95 (12H, t, J = 7 Hz, $-\text{CH}_3$). UV (n-hexane): λ_{max} 252 nm ($\log \epsilon=4.63$), 323 (4.30) and 438 (2.62).

Dihydrochloride of 10 was obtained in the same manner as dihydrochloride of 9 as monohydrate [mp > 203° C (decomp.)] in 74% yield. IR (KBr): 2600 and 2490 (NH^+), 1745 cm^{-1} (C=O). NMR (D_2O) δ : 7.63 (2H, d, J = 8 Hz, 4-H and 5-H); 7.06 (2H, d, J = 8 Hz, 3-H and 6-H); 5.00 - 4.66 (4H, m, $-\text{OCH}_2$), 4.08 - 3.46 (12H, m, $-\text{CH}_2\text{N}(\text{CH}_2)_2$); 1.63 (12H, t, J = 7 Hz, $-\text{CH}_3$). UV (H_2O): λ_{max} 256 nm ($\log \epsilon=4.44$), 308 (4.02) and 460 (2.51).

2,7-Bis[2-(N,N-diethylamino)ethoxy]-1,8-diazafluorene 11. The reduction of ketone 10 by hydrazine hydrate was carried out (in autoclave at 140 - 150° C for 14 h) according to the general procedure.^{5c} After recrystallization from n-hexane colorless microcrystalline solid 11 (mp 43 - 44° C) was obtained in 76% yield. NMR (CDCl_3) δ : 7.66 (2H, d, J = 8 Hz, 4-H and 5-H); 6.61 (2H, d, J = 8 Hz, 3-H and 6-H); 4.36 (4H, t, J = 6 Hz, $-\text{OCH}_2-$); 3.73 (2H, s, 9- H_2); 2.78 (4H, t, J = 6 Hz, $-\text{CH}_2\text{CH}_2\text{N}=$); 2.56 (8H, q, J = 7 Hz, $-\text{CH}_2\text{CH}_3$); 0.98 (12H, t, J = 7 Hz, $-\text{CH}_3$). UV (n-hexane): λ_{max} 214 nm ($\log \epsilon=4.53$), 308 (4.56) and 338 (4.38).

Dihydrochloride of 11 was obtained from 11 in the same manner as dihydrochloride of 9. Dihydrochloride of 11 [mp > 177° C (decomp.)] obtained in total 64% yield, was extremely hygroscopic and any contact with moisture should be avoided. NMR (D_2O) δ : 8.35 (2H, d, J = 8 Hz, 4-H and 5-H); 7.35 (2H, d, J = 8 Hz, 3-H and 6-H); 5.10 (9- $\text{H}_2+\text{D}_2\text{O}$, s); 5.05 - 4.90 (4H, m, $-\text{OCH}_2$); 4.08 - 3.94 (4H, m, $-\text{CH}_2\text{N}(\text{CH}_2)_2$); 3.70 (8H, q, J = 7 Hz, $-\text{CH}_2\text{CH}_3$); 1.68 (12H, t, J = 7 Hz, $-\text{CH}_3$). UV (H_2O): λ_{max} 225 nm ($\log \epsilon=4.21$) and 306 (4.22).

2,7-Bis[3-(1-morpholino)propoxy]-1,8-diazafluorenone (7) was obtained from 6 and 3-(1-morpholino)propanol in the same manner as 10. The spiro derivative of 7 as well as 7 itself were extracted with chloroform from the reaction mixture and worked up as described for compounds 9 and 10. Crude dihydrochloride of 7 was recrystallized from a mixture of absolute ethanol-benzene (2:1). After drying at 110 - 115° C for 2 h, pale rose needles of dihydrochloride of 7 [mp > 225° C (decomp.)] as monohydrate with 0.5 equivalent of ethanol were obtained. The

total yield starting from 6 was 30%. IR (KBr): 2600 and 2490 (NH^+), 1740 cm^{-1} (C=O). NMR (D_2O) δ : 7.70 (2H, d, $J = 8$ Hz, 4-H and 5-H); 6.91 (2H, d, $J = 8$ Hz, 3-H and 6-H); 4.66 - 3.80 (12H, m, $-\text{OCH}_2-$); 3.75 - 3.28 (12H, m, $-\text{N}(\text{CH}_2)_3$); 2.58 - 2.19 (4H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2-$). UV (H_2O): λ_{max} 257 nm ($\log \epsilon=4.48$), 308 (4.09) and 4.60 (2.51).

2,7-Bis[2-(N,N-dimethylamino)ethoxy]-1,8-diazafluorenone (8). This compound was obtained from 6 and 2-(N,N-dimethylamino)ethanol in the same way as 7. Crude dihydrochloride of 8 was purified by recrystallization from a mixture of absolute ethanol-benzene (4:1). After drying at 105 - 110° C for 2 h, pale orange needles of dihydrochloride of 8 [$\text{mp} > 215^\circ \text{C}$ (decomp.)] with 0.5 equivalents water and ethanol was obtained in total 49% yield. IR (KBr): 2700 (NH^+), 1740 cm^{-1} (C=O). NMR (D_2O) δ : 7.41 (2H, d, $J = 8$ Hz, 4-H and 5-H); 6.81 (2H, d, $J = 8$ Hz, 3-H and 6-H); 4.71 - 4.60 (4H, m, $-\text{OCH}_2$); 3.83 - 3.55 (4H, m, $-\text{CH}_2\text{N=}$); 3.08 (12H, s, $-\text{CH}_3$). UV (H_2O): λ_{max} 254 nm ($\log \epsilon=4.60$), 306 (4.17) and 440 (2.66).

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