# DERIVATIVES OF BENZO(*c*)FLUORENE: II. SYNTHESIS AND BIOLOGICAL EFFECT OF BASIC ETHERS OF 7-OXO-7*H*-BENZO(*c*)FLUORENE*

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Alkylation of phenols *IIa—IIc* with ω-(N,N-dialkylamino)alkyl chlorides *IIIa—IIId* in an aqueous and/or a two-phase (toluene and aqueous potassium hydroxide) medium, and reactions of phenols *IId—IIf* with isopropylamine, gave rise to ethers *IV—XIX*. Compounds *IV* and *V* were used to prepare derivatives *XX—XXVI*. Reduction of the 7-oxo group in the compound *V* by the action of sodium borohydride produced the 7-hydroxy derivatives *XXIV—XXVI*, whereas reduction under conditions of the Wolf–Kishner reaction led to derivatives *XXVII* and *XXVIII*, respectively, which were also obtained by alkylation of *XXIX* with *IIIa* in an anhydrous medium. The compound *XXIX* was prepared by reduction of compound *IIa*. Most the of compounds prepared had marked antineoplastic effects, particularly compounds *IV* and *V*. Compounds *V*, *VII*, *IX* and *XVIII* exhibited antibacterial effects, and compounds *XVI*, *XVIII* and *XXIII* were as efficacious in subcutaneous application against encephalomyocarditis virus as Tiloron, used as standard.

Our preceding communication[1] describes syntheses of benzo(*c*)fluorene derivatives, with randomly chosen substituents attached to positions 5, 6 and 7. The study was undertaken with a view to getting some preliminary knowledge about the effect of substitution on the aromatic skeleton upon the biological activity. The compounds prepared had a narrow spectrum of antiviral activity and no antineoplastic effect. Since the fluorene skeleton is a structural unit occurring in some compounds exhibiting antiviral and antineoplastic effects[2–9], we assumed that substitution of suitable groups for hydrogens on the benzo(*c*)fluorene skeleton might give rise to compounds of higher biological efficacy. From the viewpoint of antiviral, interferonogenic, immunostimulating and antitumorous activities, much attention has been given in the literature to Tiloron (*I*), which is a fluorenone derivative with two basic 2-(N,N-diethylamino)ethyl groupings bound *via* oxygen atoms to the fluorene nucleus[4]. The ω-(N,N-dialkylamino)alkyl grouping, bound *via* oxygen or nitrogen to various structural matrices, occurs in a number of substances with potential antineoplastic effects. We have, therefore, prepared a number of benzo(*c*)fluorene derivatives with ethereal links carrying an ω-(N,N-dialkylamino) alkyl residue or the

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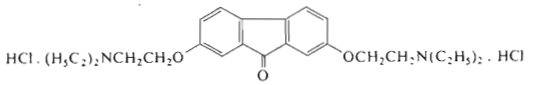
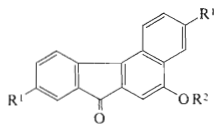
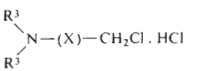
more hydrophilic 1-isopropylamino-2-propanol residue (compounds *IV–XIX*, Table I). To ascertain the effect of substitution in position 7 on the biological activity of the benzo(*c*)fluorene skeleton having a basic substituent, *viz.* a 2-(N,N-dimethylamino)ethyl group, bound *via* oxygen to position 5 (this substituent had proved the best for biological activity), we synthetized ethers *XX–XXVIII* (Table II)




Tiloron

*I*




*IIa*: $R^1$, $R^2$ = H
*IIb*: $R^1$ = $CH_3$, $R^2$ = H
*IIc*: $R^1$ = $C_2H_5$, $R^2$ = H
*IId*: $R^1$ = H, $R^2$ = $CH_2$—$C_2H_3O$-cyclic
*IIe*: $R^1$ = $CH_3$, $R^2$ = $CH_2$—$C_2H_3O$-cyclo
*IIf*: $R^1$ = $C_2H_5$, $R^2$ = $CH_2$—$C_2H_3O$-cyclo

*IIIa*: X = $CH_2$, $R^3$ = $CH_3$
*IIIb*: X = $CH_2$, $R^3$ = $C_2H_5$
*IIIc*: X = $CH_2CH_2$, $R^3$ = $CH_3$
*IIId*: X = $CH_2C(CH_3)_2$, $R^3$ = $CH_3$

Syntheses of the compounds *IV–XIX* started from 5-substitution derivatives of 7-oxo-7*H*-benzo(*c*)fluorene and its 3,9-dialkyl analogues (*IIa–IIf*, refs[1,10]), and consisted in alkylation of the 5-hydroxy group in compounds *IIa–IIc* with ω-(N,N-dialkylamino)alkyl chlorides *IIIa–IIId* (ethers *IV–XV*) or in opening the oxirane ring in compounds *IId–IIf* by the action of isopropylamine (ethers *XVI–XIX*).

The compounds *IIa–IIc* were alkylated either in an anhydrous medium, using sodium methoxide in an aromatic solvent (method *A*), or in a two-phase medium composed of toluene and aqueous alkali hydroxide (method *B*). In the alkylation according to method *B* the compounds *IIIa–IIId* were used in the form of hydrochlorides, whereas in method *A* they were added in the form of bases, liberated from their hydrochlorides by aqueous sodium hydroxide and taken to an organic solvent immiscible with water, used as the reaction medium for the alkylation. The alkylation with chloride *IIIa* gave good yields in method *A* only. Compound *XIII* was also obtained by alkylation of *IIc* with *IIIa* in the presence of triethylbenzylammonium chloride as phase-transfer catalyst. Reactions of compounds *IId–IIf* with isopropylamine proceeded readily and in good yields by heating in toluene to 140 to 150°C in an autoclave.

TABLE I

Substitution derivatives of 5-hydroxy-7-oxo-7*H*-benzo[*c*]fluorene

| Compound | $R^2$ | Formula | M.p., °C | Calculated/Found | | | |
|---|---|---|---|---|---|---|---|
| $R^1$ | X | (mol.mass) | Solvent | % C | % H | % N | % Cl |
| *IV* | $CH_3$ | $C_{21}H_{19}NO_2$ | 91—93 | 79·50 | 6·01 | 4·41 | — |
| H | — | (317·4) | (chloroform–hexane) | 79·51 | 6·06 | 4·26 | — |
| *V*[a] | $CH_3$ | $C_{21}H_{20}ClNO_2$ | 268—269 | 71·28 | 5·69 | 3·95 | 10·02 |
| H | — | (353·9) | (ethanol) | 70·98 | 5·48 | 3·72 | 9·82 |
| *VI*[b] | $CH_3$ | $C_{27}H_{27}NO_9$ | 165—167 | 64·48 | 5·22 | 2·68 | — |
| H | — | (521·5) | (ethanol) | 64·07 | 5·40 | 2·49 | — |
| *VII*[a] | $C_2H_5$ | $C_{23}H_{24}ClNO_2$ | 248—250 | 72·34 | 6·33 | 3·67 | 9·28 |
| H | — | (381·9) | (ethanol) | 72·19 | 6·24 | 3·67 | 9·25 |
| *VIII* | $CH_3$ | $C_{22}H_{21}NO_2$ | 81—82 | 79·73 | 6·38 | 4·22 | — |
| H | $CH_2$ | (331·4) | (ethanol) | 79·47 | 6·46 | 4·06 | — |
| *IX*[a] | $CH_3$ | $C_{22}H_{22}ClNO_2$ | 241—243 | 71·82 | 6·03 | 3·81 | 9·64 |
| H | $CH_2$ | (367·9) | (ethanol) | 71·84 | 5·91 | 3·76 | 9·62 |
| *X*[a] | $CH_3$ | $C_{24}H_{26}ClNO_2$ | 216—218 | 72·80 | 6·61 | 3·53 | 8·95 |
| H | $C(CH_3)_2$ | (395·9) | (methanol) | 73·02 | 6·74 | 3·44 | 8·74 |
| *XI*[c] | $CH_3$ | $C_{23}H_{26}ClNO_3$ | 250—252 | 69·07 | 6·55 | 3·52 | 8·86 |
| $CH_3$ | — | (399·9) | (chloroform) | 68·91 | 6·62 | 3·51 | 9·80 |
| *XII*[a] | $C_2H_5$ | $C_{25}H_{28}ClNO_2$ | 251—253 | 73·24 | 6·88 | 3·42 | 8·65 |
| $CH_3$ | — | (409·9) | (ethanol) | 72·97 | 7·04 | 3·36 | 8·53 |
| *XIII* | $CH_3$ | $C_{25}H_{27}NO_2$ | 88—90 | 80·39 | 7·29 | 3·75 | — |
| $C_2H_5$ | — | (373·5) | (hexane) | 80·67 | 7·02 | 3·53 | — |
| *XIV*[a] | $CH_3$ | $C_{25}H_{26}ClNO_2$ | 243—245 | 73·60 | 6·42 | 3·43 | 8·69 |
| $C_2H_5$ | — | (407·9) | (chloroform–ethanol) | 73·76 | 6·62 | 3·45 | 8·55 |
| *XV*[a] | $C_2H_5$ | $C_{26}H_{32}ClNO_2$ | 190—192 | 74·04 | 7·36 | 3·20 | 8·10 |
| $C_2H_5$ | — | (438·0) | (ethanol) | 73·86 | 7·39 | 3·29 | 8·10 |
| *XVI* | i-$C_3H_7$ | $C_{23}H_{23}NO_3$ | 156—157 | 76·43 | 6·41 | 3·88 | — |
| H | CH(OH) | (361·4) | (benzene) | 76·33 | 6·72 | 4·13 | — |
| *XVII* | i-$C_3H_7$ | $C_{25}H_{27}NO_3$ | 173—175 | 77·09 | 6·98 | 3·59 | — |
| $CH_3$ | CH(OH) | (389·5) | (chloroform) | 77·10 | 6·86 | 3·41 | — |
| *XVIII* | i-$C_3H_7$ | $C_{27}H_{31}NO_3$ | 144—145 | 77·69 | 7·48 | 3·35 | — |
| $C_2H_5$ | CH(OH) | (417·6) | (chloroform) | 77·57 | 7·25 | 3·29 | — |
| *XIX* | i-$C_3H_7$ | $C_{27}H_{32}ClNO_3$ | 218—220 | 71·43 | 7·10 | 3·08 | 7·80 |
| $C_2H_5$ | CH(OH) | (454·0) | (methanol) | 71·07 | 7·00 | 3·02 | 8·04 |

[a] Hydrochloride; [b] citrate; [c] monohydrate hydrochloride.

To synthetize compounds *XX*–*XXVII*, the 7-oxo group in compounds *IV* and *V* was substituted and reduced, respectively. Reaction of compounds *V* with phenylhydrazine in ethanol gave the phenyl hydrazone *XX*, and, analogously, with thiosemicarbazide the thiosemicarbazone *XXII*. Semicarbazone *XXI* was obtained from compound *IV* by the procedure described previously[1]. Analogously, reaction of compound *V* with aminoguanidine hydrogen carbonate in aqueous ethanol afforded compound *XXIII*.

To prepare derivatives of 7-hydroxy-7*H*-benzo(*c*)fluorene, *XXIV*, compound *V* was reduced with sodium borohydride in ethanol at room temperature. Column chromatography of the reaction mixture gave the compound *XXIV* and a compound *XXV*, which was characterised spectrally as a boran complex of the compound *XXIV*. The derivatives *XXIV* and *XXV* were rather unstable and during storage in solid state under normal conditions they gradually went orange, as a result of oxidation

TABLE II

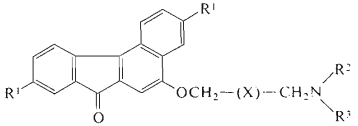
7-Substitution derivatives of 5-(2-(N,N-dimethylamino)ethoxy)-7-oxo-7*H*-benzo[*c*]fluorene

| Compound | Z | Formula (mol.mass) | M.p., °C Solvent | Calculated/Found | | | |
|---|---|---|---|---|---|---|---|
| | | | | % C | % H | % N | % Cl(S) |
| *XX* | $NNHC_6H_5$ | $C_{27}H_{28}ClN_3O_2$ (462·0) | 155–157 (chloroform) | 70·19<br>70·10 | 6·11<br>6·04 | 9·09<br>8·81 | 7·67<br>7·90 |
| *XXI* | $NNHCONH_2$ | $C_{22}H_{23}ClN_4O_2$ (410·9) | 245–246 (ethanol) | 64·30<br>64·51 | 5·64<br>5·42 | 13·63<br>13·81 | 8·63<br>8·42 |
| *XXII*[a] | $NNHCSNH_2$ | $C_{22}H_{22}N_4OS$ (390·5) | 112–113 (chloroform–ethanol) | 67·66<br>67·92 | 5·67<br>5·56 | 14·35<br>14·19 | (8·21)<br>(8·19) |
| *XXIII* | $NNHC(NH)NH_2$ HCl | $C_{22}H_{25}Cl_2N_5O$ (446·4) | 270–272 (ethanol) | 59·19<br>58·91 | 5·64<br>5·71 | 15·69<br>15·47 | 15·88<br>15·60 |
| *XXIV*[a] | OH, H | $C_{21}H_{21}NO_2$ (319·4) | 152–155 (ethanol) | 78·97<br>78·88 | 6·63<br>6·65 | 4·38<br>4·51 | —<br>— |
| *XXVI* | OH, H | $C_{21}H_{22}ClNO_2$ (355·9) | 226–230 (ethanol) | 70·88<br>71·04 | 6·23<br>6·55 | 3·94<br>3·83 | 9·96<br>10·02 |
| *XXVII*[a] | H, H | $C_{21}H_{21}NO$ (303·4) | 64–66 (chloroform) | 83·13<br>82·80 | 6·97<br>6·81 | 4·62<br>4·38 | —<br>— |
| *XXVIII* | H, H | $C_{21}H_{22}ClNO$ (339·8) | 257–259 (ethanol) | 74·20<br>74·06 | 6·52<br>6·47 | 4·12<br>4·10 | 10·43<br>10·48 |

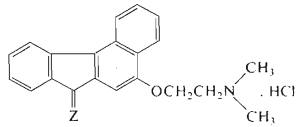
[a] Base.

of the 7-hydroxy group to a 7-oxo group (compound *IV*). The oxidation rate was much enhanced (analysis by TLC) by heating the compounds *XXIV* and *XXV* in ethanol and an aqueous-ethanolic solution of NaOH respectively; after 1 h heating compound *V* was isolated after conversion into its hydrochloride. For biological tests the compound *XXIV* was converted into the more stable hydrochloride *XXVI*.

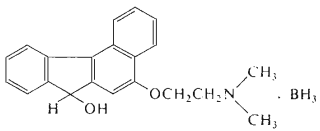
To prepare the benzo(*c*)fluorene derivative *XXVII* we either reduced compound *IV* according to the Wolf–Kishner method (in analogy to the reduction of a derivative of fluorenone[4,9]), or reduced compound *IIa* to 5-hydroxy-7*H*-benzo(*c*)fluorene *XXIX*, which was alkylated with *IIIa* by method *A*. Compound *XXVII* was converted into the hydrochloride *XXVIII*.



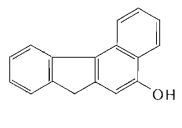

*XV*: $R^2 = R^3$
*XVI–XIX*: $R^3 = H$




*XX–XXIV*,
*XXVI–XXVIII*




*XXV*




*XXIX*

The structures of selected compounds were corroborated by spectral analysis. The presence of the carbonyl group in compounds *IV–XIX* was demonstrated by the IR band at 1 700–1 720 $cm^{-1}$, corresponding to a conjugated five-membered ketone. The disappearance of this band suggested substitution or reduction of the oxo group in position 7 (*XX–XXVIII*). The structure of derivative *XXV* was demonstrated by the hydroxy group band at 3 600 $cm^{-1}$ in the IR spectrum and by the presence of a proton at 5·35$\delta$ in the NMR spectrum, belonging to the hydrogen atom in position 7. The borane complex $R_3N—BH_3$ was inferred from the presence, in the IR spectrum, of two stretching vibration bands at 2 285 $cm^{-1}$ ($\nu$ sym.) and 2 380 $cm^{-1}$ ($\nu$ asym.), in contrast to $BH_4^-$, where $\nu_S$ B—H is not active in the IR spectrum[11]. The structure of the benzo(*c*)fluorene derivative *XXVII* was verified by the band of two protons at 3·90$\delta$, corresponding to two hydrogen atoms in position 7.

The compounds prepared were tested for antibacterial, antiviral and antineoplastic effects, always in the form of hydrochlorides. In the screening for antibacterial efficacy on seven bacterial strains and two kinds of yeast *in vitro*, positive results were observed with the bacteria only. The antibacterial effects were most marked with compounds *V*, *VII*, *XVIII* and *IX* against *Streptococcus pyogenes* CI, *Streptococcus faecalis*, *Staphylococcus pyogenes aureus*, *Escherichia coli*, and *Mycobacterium tuberculosis* H 37R$_v$, the minimum inhibitory concentration being 0·7–6·25 μg/ml, depending on the bacterial strain. In the antiviral screening, compounds *XVI*, *XVIII* and *XXIII*, administered *s.c.*, exhibited marked effects against the encephalomyocarditis virus, and compound *XXIII* even against the vaccinia virus, comparable with that of Tiloron as standard (for the method of evaluation see ref.[4]). In the screening for antitumorous action in animals with experimental tumours (murine tumours: the Crocker sarcoma 180, tumour HK, sarcoma Sa 37 and the Krebs tumour Kr 2, rat Yoshida tumour) statistically significant antitumorous effects were observed with compounds *IV*, *V*, *VII*, *IX*, *X*, *XIV*, *XVI*, *XVIII* and *XIX*; for the technique of the screening assay see, *e.g.*[12]. Compounds *IV* and *V*, referred to as Benfluron, were selected for a more detailed pharmacological study, since in a screening extended to include a wider spectrum of tumours they exhibited activity in animals with the given tumours, and also in animals with the solid form of the Ehrlich carcinoma. Data on the activity found in animals with experimental leukemias LA and L 1210 are given elsewhere[13]. Compound *V in vitro*, at concentrations higher than $10^{-6}$ mol $l^{-1}$ retarded the incorporation of thymidine, uridine and L-amino acids into cells of an Ehrlich ascitic carcinoma, which demonstrates interference with the syntheses (and reparation) of DNA, RNA and proteins of tumour cells, and explains the mechanism of the cytostatic action.

## EXPERIMENTAL

The melting points, determined on the Kofler block, are not corrected. Samples for elemental analyses were dried at temperatures adequate to their melting points over phosphorus pentoxide at a pressure of 70 Pa. Homogeneity of the samples and composition of the reaction mixtures were tested by TLC on reflex foils Silufol $UV_{254}$ (Kavalier), followed by quenching of UV light at 254 nm. The components of the reaction mixtures were separated by column chromatography on Kieselgel 60 reinst (Merck), a 30-fold weight of the sample being used. $^1$H NMR spectra were measured with an apparatus Tesla BS487C (80 MHz) in deuteriochloroform or deuterated acetone, in 10% solutions, with tetramethylsilane as internal standard. IR spectra were recorded with a spectrometer Perkin–Elmer 577 in 5% solutions in chloroform or in KBr pellets. Mass spectra were measured in an apparatus MS-9.

Alkylation of Compounds *IIa*–*IIc* with ω-(N,N-dialkylamino)alkyl Chlorides *IIIa*–*IIId*

*Method* A: To a solution of 2–3 mol equivalents of sodium methoxide in methanol was added chlorobenzene or toluene and 1 mol equivalent of *IIa*, *IIb* or *IIc*. The mixture was brought to the boil and methanol was allowed to distil off. After all the methanol had been removed,

along with part of the aromatic solvent, a solution of 2 to 4 mol equivalents of *IIIa*,*IIIb*,*IIIc* or *IIId* in chlorobenzene or toluene (liberated from the corresponding hydrochlorides with aqueous KOH, taken to an aromatic solvent and dried with solid KOH) were added to the boiling mixture, which was kept refluxing for 4 to 12 h. After cooling down, the mixture was repeatedly extracted with 20% KOH (to remove the unreacted starting compound), with an equal volume of water and with a saturated solution of sodium chloride. The organic layer was dried with anhydrous magnesium sulphate and the aromatic hydrocarbon was distilled off in a vacuum rotary evaporator. The residue was purified by crystallization or column chromatography (bases *IV*, *VIII*, *XIII*), or dissolved in ethanol, acidified with an ethanolic solution of hydrogen chloride to approximately pH 3, and concentrated to crystallize (hydrochlorides *V*—*VII*, *IX*—*XII*, *XIV*, *XV*).

*Method* B: To a mixture of toluene and water (5 : 1) were added 6 mol equivalents of potassium hydroxide, 1 mol equivalent of *IIa*, *IIb* or *IIc*, and 2·2 mol equivalents of hydrochlorides of *IIIa*—*IIId*, and the mixture was boiled under a reflux condenser for 5—16 h. Then the toluene layer was separated and the aqueous layer was extracted with an equal volume of toluene. The toluene extracts were combined and worked up as in method *A*.

*IV*: Method *A*, 36·9 g (0·15 mol) of *IIa*, 6·9 g (0·45 mol) of sodium, 115 ml of methanol, 375 ml of chlorobenzene, 32·3 g (0·3 mol) of base *IIIa* in 150 ml of chlorobenzene, reflux for 4·5 h. Yield 39·7 g (83%). Method *B*, 4·9 g (0·02 mol) of *IIa*, 6·6 g of potassium hydroxide, 6·34 g (0·044 mol) of *IIIa*, 40 ml of water, 200 ml of toluene, reflux for 9 h. Yield after crystallization 2·3 g (36%). IR spectrum (chloroform): 1 560, 1 610 (Ar), 1 720 $cm^{-1}$ (conjugated ketone). Mass spectrum: *m/e* 317 ($M^+$, $C_{21}H_{19}NO_2$). $^1H$ NMR spectrum (deuteriochloroform): $\delta$ 7·00—8·40 (m, 8 H, ArH), 6·89 (s, 1 H, $H_{(6)}$), 4·20 (t, $J$ = 6·0 Hz, 2 H, $OCH_2$), 2·84 (t, $J$ = 6·0 Hz, 2 H, $CH_2N$), 2·35 (s, 6 H, $NCH_3$).

*V*: Base of compound *IV* (3·17 g, 0·01 mol) was dissolved in 30 ml of ethanol and acidified with a solution of hydrogen chloride in ethanol, saturated at room temperature, to *c.* pH 3 and the solution was concentrated to crystallize; yield 3·1 g (87%). IR spectrum(KBr): 1580, 1 608 (Ar), 1 710 (C=O, conjugated ketone), 2 400 $cm^{-1}$ ($NH^+$).

*VI*: Base of compound *IV* (1·06 g, 0·003 mol) was dissolved in 50 ml of ethanol and 1·92 g (0·01 mol) of citric acid was added. After 24 h standing in a refrigerator at −5°C the separated compound was collected on a filter and recrystallized; yield 1·29 g (75%). $^1H$ NMR spectrum (hexadeuteroacetone): $\delta$ 7·15—8·50 (m, 8 H, ArH), 7·05 (s, 1 H, $H_{(6)}$), 4·45 (bt, 2 H, $OCH_2$), 3·25 (bt, 2 H, $CH_2N^+$), 2·68 (s, 4 H, $CH_2COO^-$), 2·81 (s, 6 H, $N^+CH_3$).

*VII*: Method *B*, *IIa* (2·46 g, 0·01 mol), hydrochloride *IIIb* (3·74 g, 0·022 mol), 3·3 g of potassium hydroxide, 100 ml to toluene, 20 ml of water, reflux for 14 h. Crystallization of the crude product gave a yield of 3·2 g (84%). IR spectrum (KBr): 1 520, 1 580, 1 605, 1 620 (Ar), 1 710 (C=O, conjugated ketone), 2 460, 2 570 $cm^{-1}$ ($NH^+$).

*VIII*: Method *A*, *IIa* (12·3 g, 0·05 mol), sodium (2·3 g, 0·1 mol), 37 ml of methanol, 125 ml of toluene, *IIIc* (12·1 g, 0·1 mol) in 120 ml of toluene, reflux for 12 h; yield 10·2 g (62%). $^1H$ NMR spectrum (deuteriochloroform): $\delta$ 7·06—8·40 (m, 8 H, ArH), 6·92 (s, 1 H, $H_{(6)}$), 4·18 (t, $J$ = = 6·0 Hz, 2 H, $OCH_2$), 2·55 (t, $J$ = 6·0 Hz, 2 H, $CH_2N$), 2·30 (s, 6 H, $NCH_3$), 2·10 (m, 2 H, $CH_2$).

*IX*: Method *B*, *IIa* (4·92 g, 0·02 mol), *IIIc* (6·96 g, 0·044 mol), potassium hydroxide (6·6 g, 0·12 mol), 40 ml of water, 200 ml of toluene, reflux for 8 h; yield 4·4 g (60%) after crystallization. IR spectrum (KBr): 1 585, 1 610 (Ar), 1 715 (C=O, conjugated ketone), 2 490, 2 610 $cm^{-1}$ ($NH^+$). Mass spectrum: *m/e* 331 ($M^+$, $C_{22}H_{21}NO_2Cl$).

*X*: Method *B*, *IIa* (4·92 g, 0·02 mol), *IIId* (8·2 g, 0·044 mol), potassium hydroxide (6·6 g, 0·12 mol), 40 ml of water, 200 ml of toluene, reflux for 9 h; yield after crystallization 4·0 g (50%). IR spectrum (KBr): 720, 760 (*ortho*-disubstituted benzene), 1 360, 1 380 $(C/CH_3)_2$, 1 580, 1 612 (Ar), 1 705 (C=O, conjugated ketone), 2 700 $cm^{-1}$ ($NH^+$). Mass spectrum: *m/e* 358 ($M^+$, $C_{24}H_{25}NO_2$), $^1H$ NMR spectrum (hexadeuterioacetone): $\delta$ 10·60 (bs, 1 H, $NH^+$), 7·10–8·60 (m, 8 H, ArH), 7·00 (s, 1 H, $H_{(6)}$), 4·20 (bs, 2 H, $OCH_2$), 3·35 (bs, 2 H, $CH_2N^+$), 2·85 (s, 6 H, $N^+CH_3$), 1·35 (s, 6 H, $CCH_3$).

*XI*: Method *A*, *IIb* (5·48 g, 0·02 mol), sodium (0·9 g, 0·04 mol), 20 ml of methanol, 50 ml of chlorobenzene, base *IIIa* (4·3 g, 0·04 mol), in 10 ml of chlorobenzene, reflux for 8 h; yield 6·8 g (85%). IR spectrum (KBr): 1 580, 1 622 (Ar), 1 710 (C=O, conjugated ketone), 2 460, 2 600 $cm^{-1}$ ($NH^+$). Mass spectrum: *m/e* 345 ($M^+$, $C_{23}H_{23}NO_2$).

*XII*: Method *B*, *IIb* (2·74 g, 0·01 mol), *IIIb* (3·74 g, 0·022 mol), 3·3 g (0·06 mol) of potassium hydroxide, 20 ml of water, 100 ml of toluene, reflux for 8 h; yield 2·1 g (51%). IR spectrum (KBr): 1 580, 1 628 (Ar), 1 710 (C=O, conjugated ketone), 2 500, 2 580 $cm^{-1}$ ($NH^+$). Mass spectrum: *m/e* 373 ($M^+$, $C_{25}H_{27}NO_2$).

*XIII*: Method *A*, *IIc* (0·9 g, 0·003 mol), sodium (0·13 g, 0·006 mol), 2 ml of methanol, 8 ml of chlorobenzene, base *IIIa* (0·64 g, 0·006 mol) in 5 ml of chlorobenzene, reflux for 8 h. The crude product was purified by column chromatography (chloroform–benzene, 1 : 1 as eluant); yield 0·8 g (71%). IR spectrum (chloroform): 1 570, 1 610 (Ar), 1 720 $cm^{-1}$ (C=O, conjugated ketone). $^1H$ NMR spectrum (deuteriochloroform): $\delta$ 7·00–8·20 (m, 6 H, ArH), 6·90 (s, 1 H, $H_{(6)}$), 4·32 (bm, 2 H, $OCH_2$), 3·05 (bm, 2 H, $CH_2N$), 2·72 (q, $J$ = 7·0 Hz, 2 H, $ArCH_2$), 2·64 (q, $J$ = = 7·0 Hz, 2 H, $ArCH_2$), 2·52 (s, 6 H, $NCH_3$), 1·28 (t, $J$ = 7·0 Hz, 3 H, $CH_2—CH_3$), 1·20 (t, $J$ = 7·0 Hz, 3 H, $CH_2—CH_3$). Mass spectrum: *m/e* 373 ($M^+$, $C_{25}H_{27}NO_2$).

*XIV*: Base *XIII* (0·37 g, 0·001 mol) was dissolved in 5 ml of ethanol and acidified with an ethanolic solution of hydrogen chloride to *c.* pH 3. The hydrochloride separated during standing in a refrigerator and was purified by crystallization; yield 0·35 g (85%).

*XV*: Method *B*, *IIc* (0·30 g, 0·001 mol), *IIIb*; (0·52 g, 0·003 mol), potassium hydroxide (0·33 g, 0·006 mol), 2 ml of water, 10 ml of toluene, reflux for 16 h; yield 0·3 g/68%). IR spectrum (KBr): 725, 785 (1,2,3-trisubstituted Ar), 840 (1,2,4-trisubstituted Ar), 1 605, 1 565 (Ar), 1 710 (C=O, conjugated ketone), 2 400 $cm^{-1}$ ($NH^+$).

3,9-Diethyl-5-(2-N,N-dimethylamino)ethoxy)-7-oxo-7*H*-benzo(*c*)fluorene (*XIII*)

To a solution of potassium hydroxide (0·99 g, 0·018 mol) in 5 ml of water were added 10 ml of benzene, *IIc* (0·90 g, 0·003 mol), *IIIa* (0·95 g, 0·066 mol) and 0·09 g of triethylbenzylammonium chloride, and the heterogeneous mixture was stirred and boiled under a reflux condenser for 7 h. The benzene layer was separated and the aqueous layer was extracted with two 10 ml portions of benzene. The benzene layer and the benzene portions were combined, washed with two 10 ml portions of 10% potassium hydroxide, once with 10 ml of water and dried. The organic phase was concentrated and purified by column chromatography, with chloroform–benzene (1 : 1) as eluant. The corresponding fractions were combined and crystallized; yield 0·25 g (21%) of compound *XIII*.

Reaction of Compounds *IId*–*IIf* with Isopropylamine

To a mixture of toluene (30 ml) and isopropylamine (10 ml) in a steel autoclave, (volume 100 ml) was added 10 mmol of compound *IId* (3·02 g), *IIe* (3·30 g) or *IIf* (3·58 g). The contents were

stirred and the autoclave was closed and submerged into a bath of 140—150°C, where it was kept for 6 h with occasional stirring of the contents. After cooling down the contents were taken to dryness and the crude product was purified by column chromatography (eluant chloroform–benzene, 1 : 1) and crystallization; yields 2·9 g (80%) of compound *XVI*, 3·0 g (77%) of *XVII*, and 3·5 g (84%) of *XVIII*. *XVI*: Mass spectrum: *m/e* 361 ($M^+$, $C_{23}H_{23}NO_3$). *XVII*: IR spectrum (KBr): 725, 780 (1,2,3-trisubstituted Ar), 822 (1,2,4-trisubstituted Ar), 1 565, 1 605 (Ar), 1 710 (C=O, conjugated ketone), 3 440 $cm^{-1}$ (OH, NH).

*Hydrochloride of compound* XVIII: To a solution of the base *XVIII* (0·42 g, 0·001 mol) in 5 ml of ethanol was added an ethanolic solution of hydrogen chloride until the pH had decreased to 2, and the mixture was allowed to crystallize by cooling to −5°C. Recrystallization from methanol gave 0·2 g (44%) of compound *XIX*.

Reaction of Compound *V* with Phenylhydrazine

To a solution of compound *V* (1·77 g, 0·005 mol) in ethanol (25 ml) was added freshly distilled phenylhydrazine (0·54 g, 0·005 mol), and the mixture was refluxed for 6 h. After cooling to room temperature the separated precipitate was collected on a filter, washed with 5 ml of ethanol and recrystallized from chloroform; yield 1·85 g (80%). IR spectrum (KBr): 1 590 (Ar), 3 300 $cm^{-1}$ (NH). $^1$H NMR spectrum (hexadeuterioacetone, 80°C): $\delta$ 6·90—8·70 (m, 14 H, ArH), 4·70 (bt, 2 H, $OCH_2$), 3·70 (bt, 2 H, $CH_2N$), 2·85 (s, 6 H, $NCH_3$).

Reaction of Compound *IV* with Semicarbazide Hydrochloride

Semicarbazide hydrochloride (2·0 g) was triturated with sodium acetate (2·0 g) in a mortar and the paste was transferred into ethanol (20 ml), briefly boiled and filtered hot through sintered glass (S3). The filtrate was mixed with compound *IV* (0·32 g, 0·001 mol) and the mixture was refluxed for 1·5 h. The precipitate that separated after cooling was collected on a filter and recrystallized from 80% ethanol in water; yield 0·26 g (63%). IR spectrum (KBr): 1 665 $cm^{-1}$ (C=O, amide). $^1$H NMR spectrum (hexadeuterioacetone): $\delta$ 10·60 (bs, 1 H, NHCO), 7·12 (bs, 2 H, $NH_2CO$), 7·25—8·70 (m, 9 H, ArH), 4·35 (t, $J$ = 6·0 Hz, 2 H, $OCH_2$), 2·80 (t, $J$ = 6·0 Hz, 2 H, $CH_2N$), 2·28 (s, 6 H, $NCH_3$).

Reaction of Compound *V* with Thiosemicarbazide

To a solution of compound *V* (0·35 g, 0·001 mol), in ethanol (10 ml) were added 3 drops of acetic acid and 0·5 g of thiosemicarbazide, and the mixture was refluxed for 10 h. The precipitate that separated in the course of the reaction and after cooling to room temperature was collected on a filter, and suspended in 20 ml of water. The mixture was brought to approx. pH 10 with concentrated ammonium hydroxide and the organic portion was taken into chloroform (3 × × 10 ml) and concentrated. Column chromatography (chloroform with 2% of ethanol as eluant) and recrystallization of the combined fractions gave 0·27 g (69%) of compound *XXII*. IR spectrum (KBr): 1 590 (Ar), 3 160, 3 300, 3 400 $cm^{-1}$ (NH, $NH_2$), $^1$H NMR spectrum (hexadeuterioacetone): $\delta$ 7·20—8·70 (m, 9 H, ArH), 4·38 (t, $J$ = 6·0 Hz, 2 H, $OCH_2$), 2·87 (t, $J$ = 6·0 Hz, 2 H, $CH_2N$), 2·31 (s, 6 H, $NCH_3$).

Reaction of Compound *V* with Aminoguanidine]

To a solution of compound *V* (0·35 g, 0·001 mol) in ethanol (50 ml) was added aminoguanidine hydrogen carbonate (0·39 g, 0·003 mol) and a solution of sodium hydroxide (0·12 g, 0·0028 mol)

in water (30 ml). The mixture was refluxed for 10 h and taken to dryness. The residue was dissolved in chloroform and purified by column chromatography with chloroform containing 20% of ethanol as eluant. The corresponding fractions were combined, acidified with an ethanolic solution of hydrogen chloride to *c.* pH 2, and concentrated to crystallize. Recrystallization from ethanol gave 0·26 g (58%) of compound *XXIII*. $^1$H NMR spectrum (hexadeuterioacetone): $\delta$ 8·80 (bs, NH + $NH_2$), 7·20–8·60 (m, 9 H, ArH), 4·75 (bt, 2 H, $OCH_2$), 3·72 (bt, 2 H, $CH_2N$), 3·00 (s, 6 H, $NCH_3$).

Reduction of Compound *V* with Sodium Borohydride

To a suspension of compound *V* (3·54 g, 0·01 mol) in ethanol (120 ml) was gradually added sodium borohydride (9·0 g, 0·23 mol) in the course of 2 h. The mixture was stirred at room temperature for 5 h, then left standing overnight. The orange solution was taken to dryness, the residue was stirred up in water (100 ml), and the solution was brought to pH 4 with concentrated hydrochloric acid. The undissolved portion was collected on a filter, washed with water, dried, taken into chloroform (300 ml) and extracted with a 5% solution of sodium carbonate (150 ml). The chloroform layer was dried, concentrated and subjected to column chromatography (chloroform as eluant). The first fractions contained compound *XXV*, which was purified by crystallization from benzene (m.p. 152–155°C); yield 0·93 g (25%). It was not possible to prepare this compound analytically pure. IR spectrum (chloroform): 1 590 (Ar), 2 285 (νsym.), 2 380 (ν asym.) ($R_3N$—$BH_3$), 3 600 $cm^{-1}$ (OH). $^1$H NMR spectrum (deuteriochloroform): $\delta$ 7·10–8·70 (m, 8 H, ArH), 7·02 (s, 1 H, $H_{(6)}$), 5·35 (bs, 1 H, CH—OH), 4·48 (t, $J$ = 6·0 Hz, 2 H, $OCH_2$), 3·25 (t, $J$ = 6·0 Hz, 2 H, $CH_2N$), 2·71 (s, 6 H, $NCH_3$), 2·30 (bs, $R_3N$—$BH_3$+OH). Elution of the column with methanol and crystallization of the combined fractions gave 0·32 g (10%) of compound *XXIV*. 1·0 g (0·003 mol) of this product was dissolved in 10 ml of ethanol, the solution was brought to pH 2 with hydrogen chloride in ethanol, concentrated and left standing in a refrigerator to crystallize; yield 0·5 g (47%) of compound *XXVI*.

5-(2-(N,N-Dimethylamino)ethoxy)-7*H*-benzo(*c*)fluorene (*XXVII*)

a) *Wolf–Kishner reduction of compound* IIa: The procedure was analogous to that described in refs[4,9]; to a suspension of *IIa* (3·7 g, 0·015 mol) in triethylene glycol was added an 80% solution of hydrazine hydrate (15 ml) and the mixture was heated to 120°C for 1 h. Then potassium hydroxide (6·0 g) was added and the mixture was heated to 200°C for 2 h. After cooling, dilution with water (40 ml) and acidification with concentrated hydrochloric acid to pH 2, the precipitate was collected on a filter, washed with water and recrystallized from 80% aqueous ethanol; yield 2·32 g (67%) of compound *XXIX*, m.p. 180–181°C. For $C_{17}H_{12}O$ (232·3) calculated 87·90% C, 5·20% H; found: 87·52% C, 5·09% H. $^1$H NMR spectrum (hexadeuterioacetone): $\delta$ 10·50 (s, 1 H, OH), 7·20–8·90 (m, 8 H, ArH), 7·20 (s, 1 H, $H_{(6)}$), 3·98 (s, 2 H, $ArCH_2Ar$).

*b) Alkylation of compound* XXIX: To a solution of sodium (0·09 g, 0·004 mol) in methanol (1·5 ml) was added toluene (5 ml) and compound *XXIX* (0·46 g, 0·002 mol). The mixture was boiled, while the methanol and part of toluene (*c.* 2·5 ml) distilled off. To the boiling suspension was added a solution of base *IIa* (0·43 g, 0·004 mol); the mixture was refluxed for 4 h and worked up by procedure *A*. The product was purified by column chromatography, with chloroform containing 2% of ethanol being used as eluant. The corresponding fractions were combined and crystallized from chloroform; yield 0·4 g (66%) of compound *XXVII*. $^1$H NMR spectrum (hexadeuterioacetone): $\delta$ 7·20–8·70 (m, 8 H, ArH), 7·15 (s, 1 H, $H_{(6)}$), 4·28 (t, $J$ = 6·0 Hz, 2 H, $OCH_2$), 3·90 (s, 2 H, $ArCH_2Ar$), 2·98 (t, $J$ = 6·0 Hz, 2 H, $CH_2N$), 2·40 (s, 6 H, $NCH_3$).

c) *Reduction of compound* V (*XXVIII*): To a suspension of compound *V* (1·6 g, 0·005 mol) in triethylene glycol (40 ml) was added an 80% solution of hydrazine hydrate (10 ml) and the mixture was heated to 120°C for 4 h. Potassium hydroxide (4·0 g) was then added and the mixture was heated to 200°C for 2 h. After cooling and dilution with water (30 ml), the mixture was acidified with concentrated hydrochloric acid, concentrated and left standing overnight in a refrigerator to crystallize. The product was purified by recrystallization; yield 1·1 g (65%) of compound *XXVIII*.

*The elemental analyses were performed by Mrs J. Komancová, Analytical Department (head Dr J. Körbl), antibacterial activity was assessed by Mrs M. Pivoňková, Microbiological Department (head Dr J. Turinová), antiviral effects by Dr F. Šmejkal (head of the Virological Department). The mass spectra were measured by Dr M. Ryska, Physico-Chemical Department (head Dr B. Kakáč). Skilled technical assistance by Mrs J. Seligerová, Fourth Department of Organic Synthesis, is also appreciated.*

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