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The Synthesis and Biological Evaluation of A-Ring Substituted Steroidal *p*-Quinones

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Abstract: The preparation of A-ring steroidal 1,4-quinones involves *m*-CPBA / $(BzO)_2O$ / *hv* oxidation of estrone (or estradiol 17-acetate), acid rearrangement of the obtained quinol, and oxidation. A detailed NMR analysis of 1,4-quinones and their derivatives, as well as the results of preliminary antibacterial and cytotoxicity tests is presented. © 1997 Elsevier Science Ltd.

INTRODUCTION

Quinone and hydroquinone-moieties, as parts of complex molecules, are present in cells of all respiring organisms. These moieties are also present in the framework of numerous structurally different natural as well as synthetic products with antibiotic and antitumor properties (e.g. mitomycin C, mytoxantron, avarone, etc.).

Interestingly enough, until recently[1,2] a rational process for the incorporation of a quinoid pharmacophore in a steroid skeleton, such as **1**, has not been developed; consequently, a systematic investigation of the biological activity of this type of compounds could not be efficiently carried out.

In general, the metabolic pathway by which quinones exert cytotoxicity involves either activation yielding an electrophilic agent which alkylates the essential biomolecules, or indirect action *via* semiquinone formation which by electron transfer produces cytotoxic oxygen radical species[3]. The structural complexity of quinoid antitumor drugs currently in clinical use, however, is accompanied by dose-dependant side effects most likely caused by mixed mechanisms of action which are experimentally difficult to follow separately.

Recently, we presented a simple procedure for the preparation of steroidal quinols (and epoxyquinols)[2], the key intermediates in the syntheses of corresponding A-ring substituted 1,4-quinones. Now, an optimised process for the preparation of this type of compounds is presented together with the results of our preliminary investigation of their cytotoxicity and antibacterial activity. The structure of the first set of steroidal quinones synthesised in this work is well defined so that these compounds could represent good models for further structure-activity studies based on reactivity, conformational mobility, redox potentials and related examinations.

RESULTS AND DISCUSSION

Our immediate goal was a simple synthesis of steroidal A ring 1,4-hydroquinones in satisfactory yields so that the preparation of quinones such as **1** and related derivatives **5a–5e** would become feasible. It was anticipated that **1** should be accessible from the corresponding quinol **3a** by dienone-phenol rearrangement and subsequent oxidation (Scheme 1)[4]. The quinols **3** were synthesised earlier,[1,4,5,6] but neither the yields[4,5,6] nor the methods[1a, 5] satisfied our demand for this material.

Now, we report an improved method[2] for efficient (76-90%), large scale (10-50 g) oxidation of steroidal phenols **2** into easily resolvable mixtures of quinols **3** and epoxyquinols **4**; further transformation of quinol **3a** into A-ring substituted quinones **5a–5e**, as well as the results of their preliminary activity tests and detailed spectral proton and carbon assignments for all compounds are also presented.

*Synthesis of quinols **3** and **4***

The peroxyacetic acid, Mg-monoperoxyphtalate (MMPP), *meta*-chloroperoxybenzoic acid (*m*-CPBA) and *para*-nitroperoxybenzoic acid (*p*-NPBA) under various reaction conditions were examined as phenol-to-quinol oxidants. The peroxyacetic acid was ineffective, while MMPP afforded only low yield of desired *p*-quinols. Using a *m*-CPBA / $(BzO)_2$ / hν oxidation system in CH_2Cl_2 / acetone solvent mixture, we were able to optimise the synthesis of quinols **3** up to 57% (Table 1; Run 1) and epoxyquinols **4** up to 54% (Run 8)[7] depending on the light source ap-

TABLE 1. Typical Examples of Oxidation of Steroidal Phenols 2 and Quinols 3 with RCO_3H (*m*-CPBA, MMPP, *p*-NPBA) / $(BzO)_2$[a] / hν System

Run	Substrate	RCO_3H (%)[b]	RCO_3H / substrate (mol)	Light power (W)	Solvent	Reaction time (h)[c]	Yield (%)[d] 3	4
1	**2a**	*m*-CPBA (85)	3	60	A	3.5	57	15
2	**2a**	*m*-CPBA (65)	3	60	A	3	40	32
3	**2a**	*m*-CPBA (85)	3	60	B	3	49	42
4	**2a**	*m*-CPBA (85)	3	250	A	24	-	51
5	**2a**	*p*-NPBA (67)	3.2	60	B	2.5[e]	29	28
6	**2a**	*p*-NPBA (67)	3.2	60	A	5[e]	19	15
7	**2a**	MMPP (85)	3	60	A	6	18	15
8	**2b**	*m*-CPBA (85)	3	250	A	36	-	54
9	**2b**	*m*-CPBA (85)	3	60	A	6	50	22
10	**2b**	*m*-CPBA (85)	3	250	A	8	26	36
11	**2b**	*m*-CPBA (85)	3	60	B	1.5	52	19
12	**3a**	*m*-CPBA (85)	3	60	A	24	-	58
13	**3b**	*m*-CPBA (85)	3	60	A	24	-	62

[a] 0.1 mol per mol of substrate. [b] Determined iodometrically. [c] The reactions were stopped after all starting material was consumed. [d] Yields of isolated products. [e] The peroxyacid was added in three portions: 1.5 mol eqv., 0.5 mol eqv. (after 1 h), and 0.2 mol eqv. (after 2 h). A: CH_2Cl_2 / acetone (4/1), reflux. B: CCl_4/acetone (4/1), reflux.

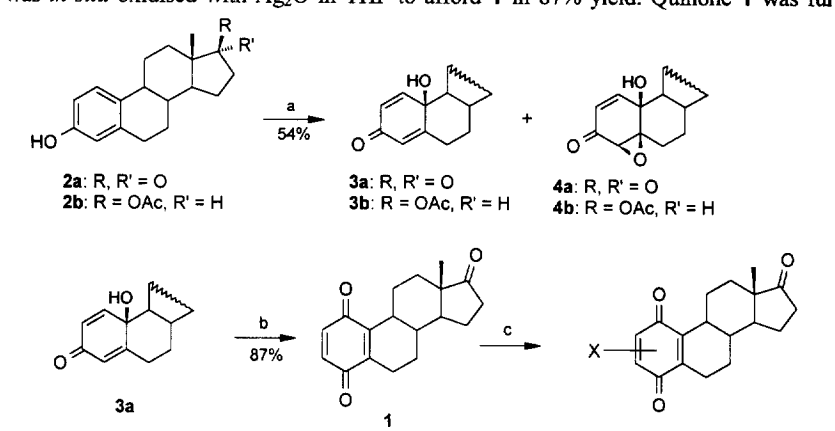
plied. Also, we examined *p*-NPBA but the yield of quinol and epoxyquinol did not exceed 57% (Run 5; **3a**: 29%, **4a**: 28%).

The use of CCl_4 / acetone solvent mixture and 85% *m*-CPBA as an oxidant afforded, as with *p*-NPBA, almost equal product distribution but the overall yield was much better (Run 3; **3a**: 49%, **4a**: 42%), so that less decomposed materiel was formed. Although at lower temperatures (CH_2Cl_2/acetone reflux) oxidation of phenols **2** has been achieved with good selectivity, when a refluxing CCl_4/acetone mixture was used, the reaction has been significantly faster (Run 11 *vs.* 9). Successful oxidation of quinols **3** into epoxyquinols **4** (Runs 12 and 13) confirmed that the former are probable intermediates in the direct oxidation of phenols into epoxyquinols. The epoxidation is site-stereospecific, affording only the *cis*-10-hydroxy-4β,5β-epoxides **4**.

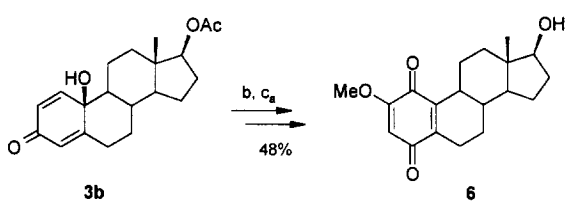
Although at present the mechanism of this reaction is not established, it can be speculated that the radical mechanism is involved, since a radical initiator, benzoyl peroxide, and light are required for the reaction to take place, while the presence of oxygen interrupts the reaction.

Synthesis of quinones ***5***

Quinol **3a** was treated with HCl in AcOH under reflux, yielding the intermediate hydroquinone (not shown) which was *in situ* oxidised with Ag_2O in THF to afford **1** in 87% yield. Quinone **1** was further converted into corresponding substituted quinones (**5a–5e**) using various nucleophiles under acidic (CH_3OH, HCl), neutral (CH_3NH_2) and basic (aziridine, (*S*)-alanine) conditions[8]. Experiments with *N*-nucleophiles were carried out in dark and under argon. In all cases only C(2)-monosubstituted quinones could be isolated, the structure of which (**5a–5c**) has been deduced by extensive NMR measurements (*vide infra*). The regiochemistry of the nucleophilic attack of aziridine and (*S*)-alanine seems to be the same although, it has not been firmly established due to the instability of **5d** and **5e** in most solvents ($CHCl_3$, DMSO, MeOH, acetone, pyridine).



a) *m*-CPBA (85%) / $(BzO)_2$ / CH_2Cl_2 / acetone (4 / 1), rfl., hν, 24 h. b) i) AcOH / HCl / H_2O (7.5 / 2.5 / 1), rfl., 1 h. ii) Ag_2O / THF, r.t., 1 h. c_a) MeOH / H_2SO_4 / $Fe_2(SO_4)_3$, rfl., 30 min; 99%. c_b) i) anh. HCl / $CHCl_3$, 5°C, 2 h. ii) Ag_2O / THF, r.t., 1 h, 67% c_c) $MeNH_2$ x HCl, Py / EtOH / H_2O, Ar, dark, r.t., 24 h; 36%. c_d) C_2H_5N, Py / EtOH, Ar, dark, r.t., 24 h; 30%. c_e) (*S*)-Ala / $NaHCO_3$ (sat.) / EtOH, Ar, dark, r.t., 24 h; 51%.



3b **6**

Scheme 1

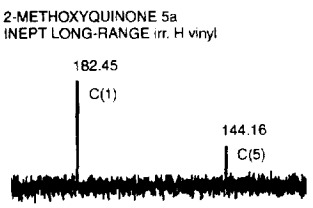
NMR analysis

In order to establish the substitution pattern in quinones **5**, and to determine the position and configuration of the hydroxy group in quinols **3** and **4**, a complete assignation of all spin-active nuclei was necessary. It was achieved by extensive NMR analysis (H,H-COSY, HETCOR, CH coupled carbon, NOE, COLOC, selective INEPT long-range experiments).

*10β-Hydroxyestra-1,4-diene-3,17-dione (**3a**).* The doublet at 7.13 ppm (J = 10.4 Hz) belongs to H-C(1) which is coupled with its "ortho" proton H-C(2). The doublet of doublets at 6.07 ppm (J = 10.4, 2.4 Hz) belongs to H-C(2) and its J-values are measure of scalar coupling with protons H-C(1) and H-C(4). Irregular triplet at 5.92 ppm ($J_{4,2}$ = 2.4, $J_{4,6}$ = 1.2 Hz) belongs to H-C(4), which is coupled with H-C(2) and H_β-C(6). Singlet at 5.67 ppm, exchangeable with D_2O, is assigned to O*H*, while that at 0.84 ppm belongs to angular methyl group. From H,H-COSY spectrum it could be established that signal at 2.67 ppm is correlated with those at 2.33, 2.07 and 1.16 ppm, while from C,H-COSY map, cross-peaks are observed between signals at 31.80 and 2.67 and 2.33 ppm and between the ones at 32.19 and 2.07 and 1.16 ppm. The obtained data indicate that chemical shifts at 2.67, 2.33, 1.16, 2.07, 31.80 and 32.19 ppm can be assigned to H_α-C(6), H_β-C(6), H_α-C(7), H_β-C(7), C(6), and C(7) respectively. The analogous analysis of spectral data affords complete proton and carbon assignments. On the basis of NOE experiment the following results are obtained: irradiation of OH signal at 5.67 ppm causes the enhancement of signals at 7.13 (H-C(1)) and 0.84 ppm (H_3C-C(13)), proving that OH is β-oriented.

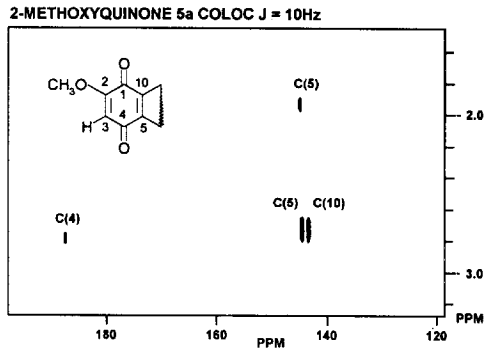
*Estra-2,5(10)-diene-1,4,17-trione (**1**).* AB quartet at 6.71 and 6.73 ppm (J = 10.0 Hz) belongs to two A-ring vinyl protons at C(2) and C(3), and angular methyl group gives singlet at 0.90 ppm. Signal at 2.68 ppm belongs to H_β-C(6) (its σ-orbital is coplanar with A ring). H,H-COSY spectrum shows the connectivity between this signal and those at 2.30, 1.95 and 1.17 ppm which can be assigned to H_α-C(6), H_α-C(7) and H_β-C(7). HETCOR map shows the one-bond correlation between the signal at 24.02, and that at 2.68 and 2.30 ppm, while signal at 23.35 is connected to signals at 1.95 and 1.17 ppm. Based on these data, the chemical shifts of carbons 6 and 7 and of the protons attached to them were determined.

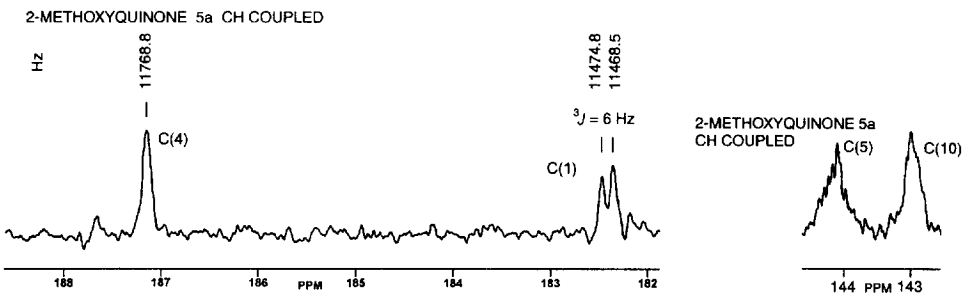
*2-Methoxyestra-2,5(10)-diene-1,4,17-trione (**5a**).* Singlets at 5.86, 3.80 and 0.90 ppm belong to A-ring vinylic proton, methoxy, and angular methyl group, respectively. Chemical shifts were assigned by complementary use of ^1H, ^{13}C, H,H-COSY and HETCOR methods in the same way as with unsubstituted quinone **1**. Chemical shifts of C(1), C(4), C(5) and C(10) were determined by CH coupled, selective INEPT long-range and by two-dimensional COLOC methods. Irradiation of H_β-C(6) in INEPT long-range experiment gives ^{13}C NMR spectrum with three signals in quaternary region at 187.17, 144.16 and 143.00 which belongs to C(4), C(5) and C(10). Irradiation of vinylic proton at 5.86 ppm with selective pulse calibrated to analyse three bond away interactions gives in INEPT long-range spectrum two signals, at 182.45 and 144.16 ppm. Those shifts correspond to C(1) and C(5), respectively, meaning that the irradiated proton is attached to C(3). The obtained results



strongly indicate that the substitution occurred at C(2). Additionally, in a COLOC experiment, cross-peaks for H_β-C(6) reveal its connectivity with C(4), C(5) and C(10).

The most indicative is the C,H coupled spectrum giving additional information of the position of methoxy group in ring A. The doublet at 182.45 ppm ($^3J_{C,H}$ = 6 Hz) and the singlet at 187.14 ppm, prove substitution at C(2)[9]. The broad signal at 144.16 ppm with its fine structure was assigned to C(5), and the signal at 143.00 ppm to C(10), while signal at 159.11 ppm corresponds to C(2). It should be noted that ^{13}C NMR chemical increments are in accordance with those established earlier[10].





Bioassays

Antibacterial effects. Compounds **1**, **3a**, **4a**, **5a-c** and **6** were tested for antibacterial activity against Gram-positive and Gram-negative bacterial strains. Preliminary results indicate that only the 17β-hydroxy-2-methoxyestra-2,5(10)-diene-1,4-dione **(6)** showed moderate activity (MIC = 32 μg/mL) against three different *S. aureus* strains out of nine (see Experimental).

Cytotoxicity. The lowest toxic (LTC, recognisable reduction of cloning efficiency) and lowest lethal concentrations (LLC, no viable cells) in mouse lymphoma L5178Y $tk^{+/-}$cells were recorded for the above set of compounds, with (+S9) and without (-S9) metabolic activation (Table 2). Three of the tested quinones (**3a**, **6** and **5c**) induced very similar cytotoxic effects with and without metabolic activation, and four quinones (**4a**, **1**, **5a** and **5b**) showed slightly lower toxicity with the metabolic activation system. It is remarkable that three compounds (**4a**, **1** and **5b**) showed no signs of acute toxicity directly after the treatment (no reduction of cell counts, normal morphology), but cells were apparently damaged resulting in an inability to form colonies. These three substances were also the most toxic compounds. As can be seen, the range of toxic concentrations varied between the test compounds, with quinol **3a** toxic only at concentrations of 250 μg/mL and higher, whereas chloroquinone **5b** showed signs of toxicity already at 1 μg/mL. The remaining five steroidal quinones have LTCs ranging from 3.9 to 30 μg/mL without metabolic activation.

Table 2. Lowest Toxic and Lowest Lethal Concentration of Steroidal Quinones with and without Metabolic Activation

Compound	Tested conc. [μg / mL]	LTC [μg / mL]		LLC [μg / mL]	
		(-S9)	(+S9)	(-S9)	(+S9)
3a	7.8 - 2000	250	250	500	500
4a	7.8 - 2000	<7.8	62.5	7.8	125
1	3.9 - 1000	<3.9	7.8	3.9	31.3
5a	0.03 - 250	10	30	30	250
6	0.1 - 600	30	30	100	100
5c	0.03 - 150	25	25	150	150
5b	0.03 - 300	1	10	1	30

LTC - lowest toxic concentration; LLC - lowest lethal concentration.

The above results show that the presented synthesis is a rational approach for the preparation of steroidal A-ring 1,4-quinones. From the presented NMR analysis it can be concluded that in general, the assignment of the exact position of the substituent in monosubstituted A-ring steroidal quinones can easily be achieved by complementary use of CH coupled and COLOC, or CH coupled and INEPT experiments.

Also, the preliminary results of bioassays justify our further attempts to expand the diversity of quinone moiety containing compounds and their biological evaluation. Current investigations of the possible mechanism by which the steroidal quinones exert biological effects indicate, as in the case of avarone derivatives,[8] that cytotoxicity is roughly related to the respective redox potentials (to be published). The epoxyquinols **4**, prepared in one-pot oxidation of phenols with *m*-CPBA / hν / $(BzO)_2O$ system, represent a structurally different type of compounds and are the subject of separate chemical and biochemical investigation.

EXPERIMENTAL SECTION

General. Melting points were determined on a Boetius PMHK apparatus and were not corrected. Specific rotations were measured on a Perkin-Elmer 141 MC and Karl Zeiss Polamat A polarimeters at the given temperatures. IR spectra were recorded on Perkin-Elmer spectrophotometer FT-IR 1725X. UV spectra were recorded on a Beckman DU-420 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AM-600, Bruker AM-250 and Varian Gemini-200 (at 600, 250 and 200 MHz respectively) spectrometers. 2D and ^{13}C NMR spectra were recorded on a Bruker AC-250 spectrometer (at 62.9 and 250 MHz) in the indicated solvent using TMS as internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. Mass spectra were taken on a Finnigan-MAT 8230 spectrometer.

Chemistry

Oxidation of estrone by *m*-CPBA / $(BzO)_2$ / hν system: 10β-hydroxyestra-1,4-diene-3,17-dione (3a) and 10β-hydroxy-4β,5β-epoxyestr-1-ene-3,17-dione (4a). A stirred solution of estrone (**2a**; 10.00 g, 37.0 mmol),

m-CPBA (22.53 g, 111.0 mmol; 85%, Jansen Chimica) and $(BzO)_2$ (900 mg, 3.70 mmol) in 2 L mixture of CCl_4 / Me_2CO (4 / 1) was heated to reflux for 3 h while irradiated with 60 W tungsten lamp. Upon evaporation of the solvent, extraction with $CHCl_3$ (3 × 200 mL), washing with saturated $NaHCO_3$ (2 × 100 mL) and H_2O (100 mL), and drying over anh. Na_2SO_4, the residue was chromatographed on SiO_2 column. Elution with PhMe / EtOAc (1 / 1 and 7 / 3, respectively) and crystallisation from benzene gave 5.19 g (49%) of quinol **3a** and 4.70 g (42%) of epoxyquinol **4a**, both as colourless needles. **3a:** mp = 219-221°C (benzene). Lit.[4] = 215-217 °C. $[\alpha]_{546}^{24.0}$ = + 62; $[\alpha]_{578}^{24.0}$ = + 68 (c = 1.32, chl.). UV: λ_{max}^{MeOH} = 229 nm (15500). IR (KBr): 3359s, 2941m, 1736s, 1664s, 1622s, 1601m cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): 7.13 (d, J = 10.4 Hz, H-C(1)), 6.07 (dd, J = 10.4, 2.4 Hz, H-C(2)), 5.92 (irreg. t, $J_{4,2}$ = 2.4, $J_{4,6\beta}$ = 1.2 Hz, H-C(4)), 5.67 (s, H-O, exchangeable with D_2O), 2.67 (tdd, J = 15.2, 6.4, 1.2 Hz, H_β-C(6)), 2.47-2.30 (H_α-C(6)), 1.97-1.83 (m, H_β-C(8) and H_β-C(11) - from NOE DIFF. spectrum), 1.30-1.18 (m, H_α-C(11)), 0.97 (s, H_3C-C(13)). ^{13}C NMR (62.9 MHz, DMSO-d_6): 220.33 (C(17)), 185.53 (C(3)), 165.09 (C(5)), 150.25 (C(1)), 128.30 (C(2)), 123.09 (C(4)), 70.10 (C(10)), 51.18 (C(9)), 50.10 (C(14)), 47.75 (C(13)), 35.62 (C(16)), 34.58 (C(8)), 32.19 (C(7)), 31.80 (C(6)), 31.03 (C(11)), 22.00 (C(12)), 21.90 (C(15)), 13.73 (C(18)). MS (EI, *m/z*): 286(M^+, 84), 268(M^+ - H_2O, 39), 150(68), 145(100), 124(76), 107(50), 91(50), 79(54), 55(60). Anal. Calcd. for $C_{18}H_{22}O_3$ (286.37): C, 75.50; H, 7.74. Found: C, 75.41; H, 7.76. **4a:** mp = 203-205°C (benzene). Lit.[7a] = 201.5-203°C. $[\alpha]_{546}^{23.5}$ = + 317; $[\alpha]_{578}^{23.5}$ = +283 (c = 1.04, chl.). UV : λ_{max}^{MeOH} = 229 (3500), 208 nm (5000). IR (KBr): 3328 s, 2863m, 1718s, 1686s, 1620w, 1392m, 1084m, 1054m, 1007m, 840s cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): 6.68 (d, J = 10.6 Hz, H-C(1)), 5.79 (s, H-O, exchangeable with D_2O), 5.77 (dd, J = 10.6, 2.2 Hz, H-C(2)), 3.33 (d, J = 2.2 Hz, H_α-C(4)), 2.45-2.25 (m, 2H). ^{13}C NMR (62.9 MHz, DMSO-d_6): 219.54 (C(17)), 195.14 (C(3)), 153.06 (C(1)), 122.33 (C(2)), 71.74 (C(10)), 64.59 (C(5)), 59.56 (C(4)), 54.19 (C(9) or C(14)), 48.99 (C(9) or C(14)), 46.93 (C(13)), 35.12 (C(16)), 33.62 (C(8)), 30.53 (C(15)), 28.15 (C(6)), 27.89 (C(12)), 21.45 (C(7)), 20.55 (C(11)), 13.30 (C(18)). MS (EI, *m/z*): 302(M^+, 100), 256(38), 199(37), 107(36), 93(41), 91(55), 81(41), 79(54), 55(85), 41(59). Anal. Calcd. for $C_{18}H_{22}O_4 \times 2/3\ C_6H_6$ (354.45): C, 74.55; H, 7.39. Found: C, 74.61; H, 7.12.

Selective synthesis of 10β-hydroxy-4β,5β-epoxyestr-1-ene-3,17-dione (4a).

i : A stirred solution of estrone (**2a**; 10.00 g, 37.0 mmol), *m*-CPBA (22.53 g, 111.0 mmol; 85%, Jansen Chimica) and $(BzO)_2$ (900mg, 3.70 mmol) in 2 L mixture of CH_2Cl_2 / Me_2CO (4 / 1) was heated to reflux for 24 h while irradiated with 250 W tungsten lamp. After usual work-up and SiO_2 column chromatography, 5.40 g (51 %) of epoxyquinol **4a** was obtained.

ii : A stirred solution of quinol (**3a**; 5.00 g, 17.4 mmol), *m*-CPBA (10.60 g, 52.2 mmol; 85%, Jansen Chimica) and $(BzO)_2$ (420mg, 1.74 mmol) in 250 mL mixture of CH_2Cl_2 / Me_2CO (4 / 1) was heated to reflux for 24 h while irradiated with 60 W tungsten lamp. Usual work-up and SiO_2 column chromatography gave 3.06 g (58 %) of epoxyquinol **4a**.

Compounds **3b** and **4b** were synthesised by analogy from estradiol monoacetate **2b**. Oxidation of estradiol monoacetate **2b** afforded 50% of 10β-hydroxy-17β-acetoxyestra-1,4-dien-3-one (**3b**) and 22% of 10β-hydroxy-17β-acetoxy-4β,5β-epoxyestr-1-en-3-one (**4b**), while selective synthesis provided 54% (from **2b**; **i**) and 62% (from **3b**; **ii**) of epoxyquinol **4b**. **3b:** mp = 179-181°C (colourless needles, MeOH). $[\alpha]_{546}^{23.5}$ = +317 ; $[\alpha]_{578}^{23.5}$ = +283 (c = 1.0, chl). UV : λ_{max}^{MeOH} = 204 (12800), 247 nm (25300). IR (KBr): 3474s, 3039m, 2933m, 1733s,

1665s, 1614s, 1603s, 1238s cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): 7.08 (d, J = 10.2 Hz, H-C(1)), 6.16 (dd, J = 10.2, 1.8 Hz, H-C(2)), 5.98 (t, $J_{4,2}$ = 1.8, $J_{4,6}$ = 1.5 Hz, H-C(4)), 4.58 (dd, J = 8.6, 7.1 Hz, H_α-C(17)), 2.85-2.66 (m, H_β-C(6)), 2.40-2.27 (m, 2H), 2.05 (s, AcO-C(17)), 0.90 (s, H_3C-C(13)). MS (EI, *m/z*): 330(M^+, 60), 288(14), 270(M^+-AcOH, 42), 255(8), 147(100), 124(25), 43(4). Anal. Calcd. for $C_{20}H_{26}O_4$ (330.42): C, 72.70; H, 7.93. Found: C, 72.80; H, 7.80. **4b:** mp = 177-179°C (colourless prisms, petrol ether / acetone). $[\alpha]_{589}^{24.0}$ = + 103.8 (c = 0.8, chl). UV : λ_{max}^{MeOH} = 232 nm (5500). IR (KBr): 3443s, 2925s, 2853m, 1729s, 1688s, 1594w, 1385m, 1251s cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): 6.64 (d, J = 10.7 Hz, H-C(1)), 5.83 (dd, J = 10.7, 2.1 Hz, H-C(2)), 4.58 (dd, J = 7.5, 1.6 Hz, H_α-C(17)), 3.31 (d, J = 2.1 Hz, H_α-C(4)), 2.50-2.35 (m, 2H), 2.05 (s, AcO-C(17)), 0.88 (s, H_3C-C(13)). ^{13}C NMR (62.9 MHz, DMSO-d_6): 194.66 (C(3)), 171.20 (CH_3COO), 150.76 (C(1)), 123.37 (C(2)), 82.41 (C(17)), 72.88 (C10)), 64.83 (C(5)), 61.34 (C(4)), 53.56 (d), 49.44 (d), 42.43 (t), 36.11 (t), 34.33 (CH_3COO), 28.86 (t), 28.70 (t), 27.22 (t), 23.49 (t), 21.11 (d), 21.04 (t), 11.87 (C(18)). MS (EI, *m/z*): 348(M^+, 21), 318(16), 317(21), 286(32, M-AcOH), 268(50). Anal. Calcd. for $C_{20}H_{26}O_5$ (330.42): C, 69.34; H, 7.56. Found: C, 69.52; H, 7.39.

Estra-2,5(10)-diene-1,4,17-trione (1). A stirred solution of 10β-hydroxy-1,4-estradiene-3,17-dione (**3a**; 1.00 g, 3.43 mmol) in 45.1 mL mixture of AcOH / H_2O / conc. HCl (15 /2 / 5) was heated to reflux for 60 min. Then, most of the solvent was evaporated, residue was diluted with H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with sat. $NaHCO_3$ (2 × 100 mL) and H_2O (50 mL), dried over anh. Na_2SO_4, filtered and evaporated to dryness. The obtained oil was triturated with ether and dissolved in 15 mL THF. Powdered Ag_2O (840 mg, 3.64 mmol) was then added and the suspension was vigorously stirred at r.t. for 1 h. Filtering through SiO_2 column (PhMe / EtOAc (8 / 2)) afforded 860 mg (87 %) of estra-2,5(10)-diene-1,4,17-trione (**1**) as yellow prisms. **1:** mp = 173 °C (petroleum ether), lit.[4] = 173.8-174.4 °C. $[\alpha]_{546}^{25.2}$ = – 262; $[\alpha]_{578}^{25.2}$ = + 248 (c = 1.0, EtOH). UV : λ_{max}^{MeOH} = 204 (3700), 249 (14000), 338 nm (1000). IR (KBr): 2926m, 1731s, 1650s, 1549m cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): 6.71, 6.63 (AB, J = 10.0 Hz, H-C(2) and H-C(3)), 2.68 (m, H_β-C(6)), 2.63 (m, H_α-C(11)), 2.42 (m, H_α-C(16)), 2.30 (m, H_α-C(6)), 2.22 (m, H_α-C(9)), 2.10 (m, H_β-C16)), 2.05 (m, H_α-C(15)), 1.95 (m, H_β-C(7)), 1.83 (m, H_β-C(12)), 1.63 (m, H_β-C(15)), 1.57 (m, H_α-C(14)), 1.50 (m, H_β-C(8)), 1.48 (m, H_α-C(12)), 1.22 (m, H_β-C(11)), 1.17 (m, H_α-C(7)), 0.90 (s, H_3C-C(13)). ^{13}C NMR (62.9 MHz, $CDCl_3$): 219.76 (C(17)), 187.83 (C(4)), 187.32 (C(1)), 144.84 (C(10)), 143.40 (C(5)), 137.44 (C(2)), 135.41 (C(3)), 49.73 (C(13)), 49.72 (C(14)), 43.34 (C(9)), 38.34 (C(8)), 35.57 (C(16)), 31.90 (C(12)), 25.37 (C(11)), 24.02 (C(6)), 23.55 (C(7)), 21.43 (C(15)), 14.17 (C(18)). MS (EI, *m/z*): 286(M^+ +2, 92), 284(M^+, 42), 266(12), 219(18), 111(24), 97(38), 83(47),71(54), 57(100), 43(75). Anal. Calcd. for $C_{18}H_{20}O_3$ (284.36): C, 76.03; H, 7.09. Found: C, 76.17; H, 7.19.

2-Methoxyestra-2,5(10)-diene-1,4,17-trione (5a). A suspension of quinone **1** (200 mg, 0.70 mmol) and $Fe_2(SO_4)_3$ (560 mg, 1.40 mmol) in dry MeOH (35 mL) and conc. H_2SO_4 (2.20 mL) was heated to reflux for 1 h. The cooled reaction mixture was diluted with H_2O (50 mL), extracted with CH_2Cl_2 (3 × 15 mL), dried over anh. Na_2SO_4 and evaporated to dryness. Chromatography on SiO_2 column using PhMe / EtOAc (7 / 3) as eluent, and crystallisation from EtOH afforded 2-methoxyestra-2,5(10)-diene-1,4,17-trione (**5a**; 219 mg, 99%) as yellow

needles. **5a:** mp = 220-224°C (dec). $[\alpha]_{546}^{28.5}$ = + 272; $[\alpha]_{578}^{28.5}$ = + 247 (c = 0.9, chl.). UV: λ_{max}^{MeOH} = 203 (8000), 271 (13000), 361 (280), 373 nm (270). IR(KBr): 2964m, 1735s, 1671s, 1638s, 1599s, 1266s cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): 5.86 (s, H-C(3)), 3.80 (s, H_3CO), 2.70 (m, H_β-C(6)), 2.68 (m, H_α-C(11)), 2.50 (m, H_α-C(16)), 2.35 (m, H_α-C(6)), 2.08 (m, H_β-C(16)), 2.00 (m, H_α-C(15)), 1.90 (m, H_β-C(7)), 1.82 (m, H_β-C(12)), 1.60 (m, H_β-C(15)), 1.53 (m, H_α-C(14)), 1.52 (m, H_α-C(12)), 1.52 (m, H_α-C(9)), 1.52 (m, H_β-C(8)), 1.20 (m, H_β-C(11)), 1.16 (m, H_α-C(7)), 0.93 (s, H_3C-C(13)). ^{13}C NMR (62.9 MHz, $CDCl_3$): 219.96 (C(17)), 187.17 (C(4)), 182.45 (C(1)), 159.11 (C(2)), 144.16 (C(5)), 143.00 (C(10)), 106.28 (C(3)), 56.21 (OCH_3), 49.70 (C(14)), 48.25 (C(13)), 43.17 (C(9)), 38.50 (C(8)), 35.60 (C(16)), 31.91 (C(12)), 25.14 (C(11)), 24.22 (C(6)), 23.61 (C(7)), 21.47 (C15), 14.82 (C(18)).. MS (EI, *m/z*): 316(M^++2, 9), 314(M^+, 100), 297(14), 257(17), 225(18), 218(32), 204(21), 158(30), 91(22), 69(28). Anal. Calcd. for $C_{19}H_{22}O_4$ × 1/2 EtOH (337.42): C, 71.19; H, 7.47. Found: C, 71.44; H, 7.30.

2-Chloroestra-2,5(10)-diene-1,4,17-trione (5b). Anh. HCl was bubbled trough ice-cooled stirred solution of quinone **1** (150 mg, 0.53 mmol) in $CHCl_3$ (20 mL) within 2 h. The crude product was washed with saturated $NaHCO_3$ (2 × 20 mL) and H_2O (20 mL), dried over anh. Na_2SO_4, and evaporated to dryness. The obtained chloro hydroquinone was oxidised with Ag_2O as previously described. Chromatography on SiO_2 column (eluent: PhMe / EtOAc (8 / 2)) and crystallisation from hexane afforded 112.7 mg (67%) of 2-chloroestra-2,5(10)-diene-1,4,17-trione (**5b**) as yellow needles. **5b:** mp = 159-163°C. $[\alpha]_{546}^{24.2}$ = + 343; $[\alpha]_{578}^{25.2}$ = + 344 (c = 0.68, chl.). UV : λ_{max}^{MeOH} = 214 (6600), 264 (10000), 348 nm (360). IR(KBr): 2927m, 1734s, 1667s, 1653s, 1594s cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): 6.92 (s, H-C(3)), 2.75 (m, H_β-C(6)), 2.68 (m, H_α-C(11)), 2.51 (m, H_α-C(16)), 2.38 (m, H_α-C(9)), 2.28 (m, H_α-C(6)), 2.13 (m, H_β-C(16)), 2.04 (m, H_α-C(15)), 1.98 (m, H_β-C(7)), 1.87 (m, H_β-C(12)), 1.66 (m, H_β-C(15)), 1.61 (m, H_α-C(14)), 1.54 (m, H_β-C(8)), 1.52 (m, H_α-C(12)), 1.25 (m, H_β-C(11)), 1.15 (m, H_α-C(7)), 0.93 (s, H_3C-C(13)). ^{13}C NMR (62.9 MHz, $CDCl_3$): 219.78 (C(17)), 185.00 (C(4)), 179.92 (C(1)), 145.15 (C(10)), 144.36 (C(2)), 144.04 (C(5)), 132.67 (C(3)), 49.65 (C(14)), 48.23 (C(13)), 43.80 (C(9)), 38.51 (C(8)), 35.38(C(16)), 31.90 (C(12)), 25.35 (C(11)), 24.14 (C(6)), 23.42 (C(7)), 21.45 (C(15)), 14.17 (C(18)). MS (EI, *m/z*): 320(M^+ +2, 52), 318(M^+, 100), 300(40), 261(38), 222(44), 209(48), 182(40), 91(40), 55(48), 43(88). Anal. Calcd. for $C_{18}H_{19}O_3Cl$ (318.80): C, 67.82; H, 6.01; Cl, 11.12. Found : C, 67.44; H, 5.96; Cl, 10.61.

2-Methylaminoestra-2,5(10)-diene-1,4,17-trione (5c). A solution of quinone **1** (300 mg, 1.06 mmol), $MeNH_2$ × HCl (1.72 g, 25.5 mmol) and pyridine (3.45 mL, 43.1 mmol) in EtOH / H_2O (1 / 1, 600 mL) was stirred in dark for 24 h, at room temperature under argon. After evaporation of EtOH, the residue was extracted with CH_2Cl_2 (3 × 30 mL), washed with H_2O (50 mL), dried over anh. Na_2SO_4, and evaporated to dryness. Chromatography on SiO_2 column using PhMe / EtOAc (6 / 4) as eluent and crystallisation from hexane / acetone afforded 120 mg (36%) of 2-methylaminoestra-2,5(10)-diene-1,4,17-trione (**5c**) as dark red plates. **5c**: mp = 209-211°C. $[\alpha]_{546}^{28.5}$ = + 109 , $[\alpha]_{578}^{28.5}$ = + 109 (c= 0.88, MeOH). UV: λ_{max}^{MeOH} = 227.5 (17800), 287.5 (18200), 479 nm (2700). IR (KBr): 3250m, 2923s, 1734s, 1664s, 1628s, 1583s cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): 5.62 (br s, H-N), 5.39 (s, H-C(3)), 2.85 (m, H_β-C(6)), 2.84 (d, J = 6.4 Hz, CH_3-N), 2.70 (m, H_α-C(11)), 2.44 (m, H_α-

C(16)), 2.40 (m, H_α-C(6)), 2.20 (m, H_α-C(9)), 2.17 (m, H_β-C(16)), 2.00 (m, H_α-C(15)), 1.92 (m, H_β-C(7)), 1.82 (m, H_β-C(12)), 1.68 (m, H_β-C(15)), 1.57 (m, H_α-C(14)), 1.50 (m, H_β-C(8)), 1.48 (m, H_α-C(12)),1.21 (m, H_β-C(11)), 1.18 (m, H_α-C(7)), 0.95 (s, H_3C-C(13)). ^{13}C NMR (62.9 MHz, $CDCl_3$): 220.21 (C(17)), 185.16 (C(4)), 183.79 (C(1)), 148.13 (C(10)), 147.49 (C(5)), 140.15 (C(2)), 97.05 (C(3)), 49.78 (C(14)), 48.30 (C(13)), 43.17 (C(9)), 38.55 (C(8)), 35.62(C(16)), 31.94 (C(12)), 30.30 (CH_3-N), 25.14 (C(11)), 24.89 (C(6)), 23.76 (C(7)), 21.47 (C(15)), 14.22 (C(18)). MS (EI, *m/z*): 313(M^+, 10), 286(8), 285(26), 130(36), 129(34), 128(26), 91(31), 85(44), 71(62), 57(100), 43(88). Anal. Calcd. for $C_{19}H_{23}NO_3$ (313.40): C, 72.82; H, 7.40; N, 4.47. Found: C, 73.16; H, 7.64; N, 4.68.

2- (or 3-) Aziridinoestra-2,5(10)-diene-1,4,17-trione (5d) : A solution of quinone **1** (300 mg, 1.05 mmol) and aziridine (82 μL, 2.45 mmol) in EtOH (50 mL) was stirred in dark for 24 h, at room temperature under argon. After evaporation of EtOH, the residue was chromatographed on Al_2O_3 (basic) column. Elution with PhMe / EtOAc (7 / 3) afforded 103 mg (30%) of 2- (or 3-) aziridinoestra-2,5(10)-diene-1,4,17-trione (**5d**) as orange oil. **5d:** $[\alpha]_{546}^{25.4}$ = + 351; $[\alpha]_{578}^{25.4}$ = + 296 (c = 0.81, MeCN). UV: λ_{max}^{MeCN}= 222 (5500), 288 (6600), 337 (1500), 401 nm (800). IR (KBr): 2928m, 1739s, 1663s, 1640s, 1593s, 1070w cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD): 5.92 (s, 1 H, vinylic), 2.70-2.58 (m, 2 H) 2.42 (dd, *J* = 19.2, 8.5 Hz, 2 H), 2.12 (dd, *J* = 18.4, 6.5 Hz, 4 H, azirid.), 0.88 (s, H_3C-C(13)). MS (CI, *m/z*): 326(MH^+, 98), 301(100), 300(10), 285(6), 222(6).

(*S*)-*N*-(Estra-2,5(10)- diene-1,4,17-trione-2- (or 3-) yl) alanine, sodium salt (5e): A solution of (*S*)-Ala (1.29 g, 14.5 mmol) in sat. $NaHCO_3$ (130 mL) was added to the solution of quinone **1** (300 mg, 1.05 mmol) in EtOH (130 mL). Reaction mixture was stirred in dark for 24 h, at room temperature under argon. After evaporation of EtOH, the residue was extracted with *n*-BuOH (3 × 15 mL), dried over anh. Na_2SO_4, and evaporated to dryness. Chromatography on Lobar B column RP-18 using MeOH / H_2O (1 / 1) as eluent, and crystalization from MeOH / PhH afforded 910 mg (51%) of *N*-(Estra-2,5(10)-diene-1,4,17-trione-2- (or 3-) yl) alanine, sodium salt (**5e**) as dark red plates. **5e:** mp > 300 °C (dec.). $[\alpha]_{546}^{23.0}$ = + 163; $[\alpha]_{578}^{23.0}$ = + 163 (c = 0.54, MeOH). UV: λ_{max}^{MeOH} = 218 (11500), 289 (5500), 343 (1900), 481 nm (1400). IR (KBr): 3650-3300s, 2927s, 1739s, 1715-1560s, 1587s cm^{-1}. ^{1}H NMR (600 MHz, D_2O): 5.10 (s, 1 H, vinylic), 3.68 (q, *J* = 7.0 Hz, CH_3CH(NH-)COONa), 2.50-2.36 (m, 3 H), 1.32 (d, *J* = 7.0 Hz, CH_3CH(NH-) COONa), 0.80 (s, H_3C-C(13)). MS (FAB, NaI, *m/z*): 416 (M^++Na, 5), 393 (M^+, 24), 370 (M^+-Na, 13), 326(M^+-CO_2Na, 100), 325(35), 299(17), 255(18). Anal. Calcd. for $C_{21}H_{24}NO_5Na$ (393.42): C, 64.11; H, 6.40; N, 3.56. Found: C, 63.38; H, 6.64; N, 3.38.

17β-Hydroxy-2-methoxyestra-2,5(10)-diene-1,4 -dione (6) was prepared by analogy from quinol **3b**. **6:** mp = 113-115°C (yellow needles, cyclohexane). $[\alpha]_{546}^{17.3}$ = + 48; $[\alpha]_{578}^{17.3}$ = + 43 (c = 0.64, EtOH). UV : λ_{max}^{MeOH}= 210 (2800), 222 (2900), 273 (2900), 374 (67) nm. IR(KBr) : 3409(m), 2922(m), 1670(s), 1637(s), 1593(s), 1225(s) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 5.80 (s, H-C(3)), 3.77 (s, H_3C-O), 3.72 (t, *J* = 8.5 Hz, H_α-C(17)), 2.72-2.64 (m, 1H), 2.55 (dd, *J* = 13.2, 3.3, 1H), 2.32-2.21 (m, 1 H), 2.18-2.04 (m, 3 H), 0.76 (s, H_3C-C(13)). ^{13}C NMR (125 MHz, $CDCl_3$): 187.37, 182.87, 159.14, 144.22, 143.59, 106.27, 81.69, 56.16, 49.44, 43.73, 43.25, 38.93, 37.12, 30.45, 26.90, 25.65, 24.31, 23.07, 11.41. MS (EI, *m/z*) : 318(M^++2, 30), 316(M^+, 100), 298(M^+-H_2O, 71), 258(12), 244(38), 204(21), 203(18), 178(17), 153(10).

Bioassays

Antibacterial tests. Seven steroidal quinones were tested for antibacterial activity in the following series of Gram-positive and Gram-negative bacterial strains: *Escherichia coli* 25922, *E. coli* B, *E. coli* H560, *Klebsiella pneumoniae* NCTC 418, *Pseudomonas aeruginosa* 799/61, *Acinetobacter sp.* 5I-156, *Stenotrophomonas maltophilia* 1AC 739, *Staphylococcus aureus* 25923, *S. aureus* 25923/CYC-R-1/3, *S. aureus* 25923/CYC-R-5/2, *S. aureus* 25923/CYC-R, *S. aureus* 25923/NOVO-R, *S. aureus* 25923/COUM-R, *S. aureus* Smith, *S. aureus* QR-54, *S. aureus* H19982, *S. aureus* 744, *Staphylococcus epidermidis* 16/2, *S. epidermidis* ATCC 14990, *Streptococcus pyogenes* b15 and *Enterococcus faecalis* 6. For reference a third generation cephalosporin, Ceftriaxone, was included in the susceptibility tests. Minimum inhibitory concentrations, MICs (μg/mL), were determined on Mueller-Hinton agar (Difco). The test compounds were dissolved in a small volume of DMSO, diluted in water and incorporated in two-fold serial dilutions into the agar. The inoculum was prepared from overnight cultures of the strain, diluted and applied to the agar surface by using a multipoint inoculator. The size of inoculum was approximately 104 colony-forming units per spot. The plates were incubated for 20 hrs at 35°C. The MICs were determined as the lowest concentration that prevented visible growth, disregarding less than 3 colonies or faint haze. 17β-Hydroxy-2-methoxyestra-2,5(10)-diene-1,4-dione **(6)** showed moderate antibacterial activity against *Staphylococcus aureus* 25923, *S. aureus* 25923/CYC-R and *S. aureus* QR-54 with a MIC of 32 (Ceftriaxone: SA 25923: 2, SA 25923/CYC-R: – and SA QR-54: >32). All other tested quinones showed MICs of >32 for all strains.

Cytotoxicity. Seven steroidal quinones were evaluated for cytotoxicity in mouse lymphoma cells using a fluctuation protocol. Cytotoxicity was determined by measuring the ability of the cells to proliferate and form colonies (relative viability = RV). For this cell concentrations were adjusted to 8 cells / mL and 0.2 mL was plated into 96 microtitre wells. After incubation at 37°C in a humidified incubator gassed with 5% (v/v) CO_2 in air for at least 5 days, the number of wells containing viable clones were counted under a microscope or by eye. Additionally cell counts were measured directly after the treatment period (relative cell counts = RCC). The experiments were conducted both with (+S9) and without (-S9) metabolic activation. The post-mitochondrial fraction (S9) of the livers of phenobarbital / β-naphtoflavone induced rats was used as metabolic activation system. The exposure period was 3 h for both test conditions. All seven test compounds were dissolved in DMSO to get appropriate stock solutions. These stock solutions were diluted 1:100 with treatment medium (RPMI 1640 medium with 5 % horse serum) yielding treatment suspensions (cells, medium and S9 or KCl) of various test compound concentrations. The lowest toxic concentration (LTC, recognisable reduction of cloning efficiency) and the lowest lethal concentration (LLC, no more viable cells) were listed in Table 2.

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7. Further transformation of epoxyquinols **4** will be published elsewhere.

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