

## Synthesis and structure–activity relationships of analogs of EM-652 (acolbifene), a pure selective estrogen receptor modulator. Study of nitrogen substitution

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### Abstract

EM-652 (acolbifene) analogs have been synthesized as selective estrogen receptor modulators. Substitution on the nitrogen atom of these 2*H*-1-benzopyran derivatives has been studied for its influence on antiestrogenic activity. Binding to the rat estrogen receptor, inhibition of estradiol-stimulated proliferation of T-47D breast cancer cells, as well as antiuterotrophic and uterotrophic activities in ovariectomized mice have been evaluated. 2*H*-1-Benzopyran 1b (EM-343, racemic form of EM-652), which contains a piperidine ring, shows the best pharmacological profile; RBA = 380, IC<sub>50</sub> value = 0.110 nM (in T-47D cells), as well as 63% and 84% antiuterotrophic inhibitions at the 7.5 and 75 nmol doses, respectively.

**Keywords:** SERMs, antiestrogens, EM-652, acolbifene, SAR, 2*H*-1-benzopyran

### Introduction

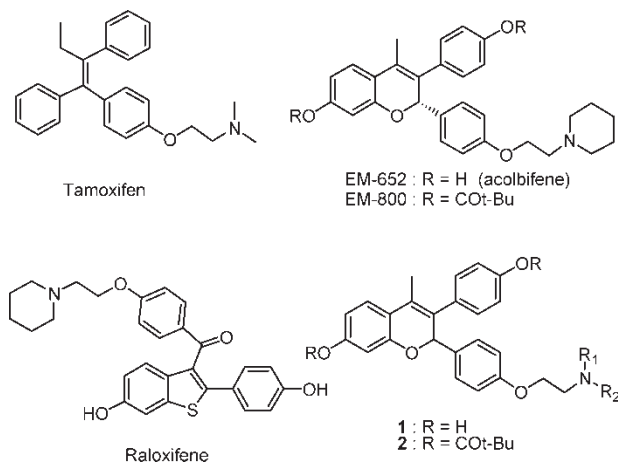
Estrogens have long been recognized to play a key role in the development, growth, and function of female sex organs, and mammary gland [1,2]. Estrogens have also an important role in the skeletal, cardiovascular, and central nervous systems [3–5]. Since estrogens are known to play a predominant role in breast cancer development and growth [6,7], a logical approach for the treatment of estrogen-sensitive breast cancer is the use of antiestrogens which block the interaction of estrogens with their specific receptors. Unfortunately, the available therapies are not efficient in all patients and the positive responses in advanced disease are usually of short duration [8–11]. Tamoxifen, the compound in general use for treatment of breast cancer, possesses mixed agonist-antagonist activities, thus limiting its efficacy as a blocker of estrogen action since it exerts

estrogenic activity at various organs in different species [12–15]. The use of a pure selective estrogen receptor modulator (SERM) as preventive and therapeutic agent should also have positive effects on the skeletal and cardiovascular systems while decreasing the risk as well as treating breast and uterine cancer [16].

We have concentrated our efforts on the synthesis and the biological evaluation of 2*H*-1-benzopyran derivatives. EM-652 (SCH 57068, acolbifene) has been selected for clinical development. We also synthesized EM-800 (SCH 57050), a dipivaloate derivative of EM-652, which plays the role of a prodrug in order to facilitate large scale purification [17]. EM-652·HCl is currently used in our clinical drug development programs. We have shown that EM-652 and EM-800 possess pure and highly potent antiestrogenic activities in all *in vitro* and *in vivo* model systems studied

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[16,18,19]. For example, EM-652 shows the highest affinity for binding to both ER $\alpha$  and ER $\beta$  [17,20,21]. Moreover, EM-652 inhibits both AF1 (ligand-independent) and AF2 (ligand-dependent) of both ER $\alpha$  and ER $\beta$ . EM-652 shows the most potent inhibition of estradiol-stimulated cell proliferation in human breast cancer cells (ZR-75-1, MCF-7, T-47D) and is devoid of any intrinsic estrogenic activity [17,22]. These two compounds are also the most potent antiestrogens to inhibit estrone-stimulated uterine weight in ovariectomized animals (oral and subcutaneous administration) and are devoid of significant intrinsic estrogenic activity on the uterine endometrium [17,23]. Moreover, EM-800 (or EM-652) prevents the development and growth of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat [24–26] and it inhibits the growth of estrone-stimulated human breast cancer xenografts in ovariectomized nude mice [27–33]. Moreover, EM-652 and EM-800 prevent bone loss and lower serum cholesterol and triglyceride levels [16,18,19,24,26,34–36]. Preclinical and clinical data indicate that EM-652 possesses characteristics superior to tamoxifen and raloxifene for breast and uterine cancer prevention and treatment as well as for hormone replacement therapy at menopause [16,18,19].



It is well recognized, that the flexible side chain of SERMs plays a pivotal role in their antiestrogenic activity. The side chain usually contains a terminal tertiary amine which can be considered as a pharmacophore. Recently, the crystal structure of the ligand binding domain (LBD) of the human estrogen receptor (hER $\alpha$ ) in complex with estradiol and raloxifene reinforces this suggestion [37]. The interaction between raloxifene and the ER protein shows a clear hydrogen bonding between the piperidine ring nitrogen and the aspartic acid residue 351 (H3 helix). The H12 helix takes different conformations when bound to estradiol or raloxifene. In the case of raloxifene, it is postulated that the complex precludes the binding of coactivators.

In the present work, we report the synthesis and the structure-activity relationships of analogs of EM-652. In accord with the importance of the nitrogen atom in the structure of the antiestrogen, we have studied the influence of nitrogen substitution in this 2*H*-1-benzopyran family [38]. Consequently, the biological evaluation of compounds **1** and **2** has been the basis for the choice of the EM-652 drug candidate. The use of racemates of **1** and **2** has generated an easier screening program. We also assumed that the (*S*)-enantiomers were more active than the (*R*)-enantiomers [17].

## Materials and methods

### Chemistry

All reagents were purchased from Aldrich Chemical Co. All reactions were carried out in flame-dried glassware under a positive atmosphere of dry Ar. THF was freshly distilled from sodium/benzophenone prior to use. Column chromatography was carried out using silica gel (230–400 mesh) (EM Science). Melting points are uncorrected. <sup>1</sup>H NMR spectra were recorded at 300 MHz on a Bruker WH300 spectrometer. The chemical purity of synthesized and tested compounds was determined by HPLC (Waters system) by using an ultraviolet detector ( $\lambda = 205–325$  nm).

*2,4-Dihydroxy-2'-(4''-hydroxyphenyl)-acetophenone (5)*. A suspension of resorcinol (**3**) (89.2 g, 0.810 mol) and 4-hydroxyphenylacetic acid (**4**) (135.4 g, 0.890 mol) in toluene (240 mL) was treated with boron trifluoride etherate (300 mL, 2.44 mol). The mixture was heated at 100°C for 3 h and then allowed to cool to room temperature. The resulting suspension was stirred overnight with 12% aqueous sodium acetate (400 mL). The precipitate was filtered and washed successively with distilled water (2  $\times$  1 L) and 12% aqueous sodium acetate (400 mL). The solid was again stirred overnight with 12% aqueous sodium acetate (1.2 L). The precipitate was filtered, washed with distilled water (500 mL), and recrystallized (ethanol:water; 0.75:3 L) to yield the trihydroxydeoxybenzoin **5** (160.2 g, 81% yield) which was dried for one week under vacuo, mp 180–183°C (lit. [39] mp 189–191°C); <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>)  $\delta$  4.17 (s, 2H, H-2'), 6.32 (d,  $J = 2.2$  Hz, 1H, Ar), 6.44 (dd,  $J = 2.4$  and 8.8 Hz, 1H, Ar), 6.78 (d,  $J = 8.4$  Hz, 2H, Ar), 7.16 (d,  $J = 8.5$  Hz, 2H, Ar), 7.95 (d,  $J = 8.8$  Hz, 1H, Ar), 8.25 (br s, 1H, OH), 9.50 (s, 1H, OH), 12.76 (s, 1H, OH).

*2-Hydroxy-4-tetrahydropyranloxy-2'-(4''-tetrahydropyranloxyphenyl)-acetophenone (6)*. A suspension of trihydroxydeoxybenzoin **5** (164 g, 0.672 mol) in 3,4-dihydro-2*H*-pyran (600 mL) was treated with

*p*-toluenesulfonic acid monohydrate ( $2 \times 10$  mg) at  $0^\circ\text{C}$ . The reaction mixture was stirred for 1.5 h at  $0^\circ\text{C}$  and then for 1 h after removing the ice bath (the reaction was monitored by TLC, *p*-toluenesulfonic acid monohydrate was added until the starting material and mono-THP ether intermediates disappeared). The clear solution mixture was then treated with saturated sodium bicarbonate (250 mL) and ethyl acetate (1 L). The organic phase was washed with saturated sodium bicarbonate (250 mL) and brine (250 mL), dried over magnesium sulfate, and evaporated under reduced pressure. The crude oil, under high vacuum, gave a solid (overnight). The resulting solid was triturated with hexanes (2 L) for 3 h while stirring. The resulting suspension was left to stand at  $0^\circ\text{C}$  for 5 h and then at  $-20^\circ\text{C}$  for 18 h. The solid was filtered and treated again with hexanes (1 L) with stirring for 1 h to yield the bis-THP ether **6**, which was filtered and dried (190 g, 69% yield), mp  $109\text{--}112^\circ\text{C}$  (lit. [40] mp  $118^\circ\text{C}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.55–2.10 (m, 12H,  $\text{CH}_2$ ), 3.60 (m, 2H,  $\text{CH}_2\text{O}$ ), 3.85 (m, 2H,  $\text{CH}_2\text{O}$ ), 4.15 (s, 2H, H-2'), 5.39 (t,  $J = 3$  Hz, 1H, CH–O), 5.47 (t,  $J = 3$  Hz, 1H, CH–O), 6.55 (dd,  $J = 2.3$  and 8.9 Hz, 1H, Ar), 6.61 (d,  $J = 2.4$  Hz, 1H, Ar), 7.01 (d,  $J = 8.4$  Hz, 2H, Ar), 7.17 (d,  $J = 8.5$  Hz, 2H, Ar), 7.75 (d,  $J = 8.8$  Hz, 1H, Ar), 12.61 (s, 1H, OH).

(2*R,S*)-7-Hydroxy-3-(4'-hydroxyphenyl)-4-methyl-2-(4''-[2'''-(1-piperidino)ethoxy]phenyl)-2*H*-1-benzopyran (**1b**). A solution of bis-THP ether **6** (150 g, 0.364 mol), 4-hydroxybenzaldehyde (recrystallized in distilled water after charcoal treatment) (46 g, 0.377 mol) and piperidine (11 mL, 0.111 mol) in benzene (3.7 L, care-carcinogenic) was stirred and refluxed using a Dean-Stark apparatus for 60 h. After cooling at room temperature, the solvent was removed under reduced pressure to yield quantitatively a mixture of chromanones **7** (3:1 trans/cis ratio) and chalcones **8** (4:1 *Z/E* ratio) at a 2:1 molar ratio. Then, 5% of the crude intermediates (0.018 mol), 1-(2-chloroethyl)piperidine hydrochloride (**9b**) (4.0 g, 0.022 mol), cesium carbonate (14.1 g, 0.0433 mol) and distilled water (2.5 mL) in acetone (200 mL) were stirred and refluxed for 19 h, and then cooled to room temperature. The mixture was filtered and washed with acetone (10 mL). The solvent was then removed under reduced pressure to give a residue which was purified by flash chromatography on silica gel (ethyl acetate to ethyl acetate:methanol; 9:1) to yield chromanones **10b** (~5:1 trans/cis ratio) (7.4 g, 65% yield) which contain about 15% of (*Z*)-chalcone **11b**,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) for chromanone **10b**-(trans)  $\delta$  1.43 (m, 2H,  $\text{CH}_2$ ), 1.59 (m, 10H,  $\text{CH}_2$ ), 1.81 (m, 4H,  $\text{CH}_2$ ), 1.87 (m, 2H,  $\text{CH}_2$ ), 2.48 (m, 4H,  $\text{CH}_2\text{N}$ ), 2.73

(t,  $J = 6$  Hz, 2H,  $\text{NCH}_2$ ), 3.58 (m, 2H,  $\text{OCH}_2$ ), 3.84 (m, 2H,  $\text{OCH}_2$ ), 4.04 (t,  $J = 6$  Hz, 2H,  $\text{OCH}_2$ ), 5.32 (m, 1H, OCH), 5.46 (m, 2H, OCH and H-2), 6.71 (s, 1H, Ar), 6.73 (m, 1H, Ar), 6.76 (d,  $J = 8.5$  Hz, 2H, Ar), 6.88 (d,  $J = 4.4$  Hz, 4H, Ar), 7.13 (dd,  $J = 2.2$  and 8.7 Hz, 2H, Ar), 7.93 (dd,  $J = 2.2$  and 8.7 Hz, 1H, Ar); selected data for chromanone **10b**-(cis)  $\delta$  5.6 (br s, 1H, H-2), 7.94 (dd,  $J = 2.2$  and 8.9 Hz, 1H, Ar); selected data for (*Z*)-chalcone **11b**  $\delta$  6.33 (d,  $J = 8.9$  Hz, 1H, Ar), 6.62 (d,  $J = 2.2$  Hz, 1H, Ar), 6.74 (d, 2H,  $J = 8.7$  Hz, Ar), 6.97 (s, 1H, H- $\beta$ ), 7.01 (d,  $J = 8.8$  Hz, 2H, Ar), 7.20 (d,  $J = 8.7$  Hz, 2H, Ar), 7.34 (d,  $J = 8.8$  Hz, 2H, Ar), 7.49 (d,  $J = 8.9$  Hz, 1H, Ar), 12.6 (s, 1H, OH). To a solution of the above mixture of amines **10b** and **11b** (7.40 g, 0.0118 mol) in dry tetrahydrofuran (150 mL) was added methyllithium (1.4 M solution in ether, 25 mL, 0.036 mol) at  $-78^\circ\text{C}$  for 15 min under argon. The cold bath was removed and the reaction mixture was allowed to warm to room temperature over a 3 h period. The mixture was again cooled to  $-78^\circ\text{C}$ , and treated with saturated ammonium chloride (50 mL). The aqueous solution was extracted with ethyl acetate ( $2 \times 50$  mL). The combined organic phase was washed with brine (50 mL), dried over magnesium sulfate, and evaporated under reduced pressure. The residue was dissolved in a mixture of acetic acid (120 mL) and distilled water (15 mL) and heated at  $90^\circ\text{C}$  for 30 min under a stream of argon after which it was cooled to room temperature and evaporated under reduced pressure to give a residue which was basified with saturated sodium carbonate (90 mL). Decantation gave the crude product which was stirred with a mixture of saturated sodium carbonate (30 mL) and ethyl acetate (50 mL) for 30 min. The aqueous phase was separated and extracted with ethyl acetate (50 mL). The combined organic phase was washed twice with saturated sodium carbonate (30 mL) and brine (50 mL), dried over magnesium sulfate, and evaporated under reduced pressure to give the crude product which was purified by flash chromatography on silica gel (dichloromethane:ethanol; 9:1) to yield 2*H*-1-benzopyran **1b** (3.3 g, 60% yield) as a pink amorphous solid, mp  $132\text{--}137^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  1.47 (m, 2H,  $\text{CH}_2$ ), 1.63 (m, 4H,  $\text{CH}_2$ ), 2.05 (s, 3H,  $\text{CH}_3$ ), 2.54 (br s, 4H,  $\text{NCH}_2$ ), 2.75 (t,  $J = 5.6$  Hz, 2H,  $\text{CH}_2\text{N}$ ), 4.06 (t,  $J = 5.6$  Hz, 2H,  $\text{OCH}_2$ ), 5.80 (s, 1H, H-2), 6.15 (d,  $J = 2.4$  Hz, 1H, Ar), 6.34 (dd,  $J = 2.3$  and 8.9 Hz, 1H, Ar), 6.73 (d,  $J = 8.4$  Hz, 2H, Ar), 6.74 (d,  $J = 8.4$  Hz, 2H, Ar), 7.00 (d,  $J = 8.4$  Hz, 2H, Ar), 7.13 (d,  $J = 8.5$  Hz, 1H, Ar), 7.20 (d,  $J = 8.8$  Hz, 2H, Ar); HPLC chemical purity = 100% (Nova-Pak C18 column, MeOH/THF + 0.1%  $\text{Et}_3\text{N}/\text{H}_2\text{O}$ : 20/20/60,  $\lambda = 240$  nm).

2*H*-1-Benzopyrans **1a**, **1c**, **1d**, **1e**, **1f**, **1k**, **1l**, **1m**, **1n**, **1o**, **1q**, and **1t** were similarly prepared using

the corresponding 1-(2-chloroethyl)dialkylamine **9** as described above for 2*H*-1-benzopyran **1b**.

**2*H*-1-Benzopyran 1a.** (60% and 46% yield for step d and e), mp 131–140 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.76 (m, 4H, CH<sub>2</sub>), 2.01 (s, 3H, CH<sub>3</sub>), 2.61 (m, 4H, NCH<sub>2</sub>), 2.82 (t, *f* = 5.5 Hz, 2H, CH<sub>2</sub>N), 3.96 (t, *f* = 5.5 Hz, 2H, OCH<sub>2</sub>), 5.78 (s, 1H, H-2), 6.14 (d, *f* = 2.5 Hz, 1H, Ar), 6.35 (dd, *f* = 2.5 and 8.5 Hz, 1H, Ar), 6.70 (d, *f* = 8.6 Hz, 2H, Ar), 6.71 (d, *f* = 8.8 Hz, 2H, Ar), 6.98 (d, *f* = 8.6 Hz, 2H, Ar), 7.10 (d, *f* = 8.4 Hz, 1H, Ar), 7.18 (d, *f* = 8.7 Hz, 2H, Ar); HPLC chemical purity = 100% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 35/65 to 60/40 with 10 mM CH<sub>3</sub>CO<sub>2</sub>-NH<sub>4</sub>, λ = 240 nm).

**2*H*-1-Benzopyran 1c.** (72% and 50% yield for step d and e), mp 102–108 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.63 (m, 8H, CH<sub>2</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.79 (m, 4H, NCH<sub>2</sub>), 2.90 (t, *f* = 5.7 Hz, 2H, CH<sub>2</sub>N), 4.03 (t, *f* = 5.7 Hz, 2H, OCH<sub>2</sub>), 5.77 (s, 1H, H-2), 6.12 (d, *f* = 2.5 Hz, 1H, Ar), 6.34 (dd, *f* = 2.4 and 8.3 Hz, 1H, Ar), 6.73 (d, *f* = 8.1 Hz, 2H, Ar), 6.76 (d, *f* = 8.6 Hz, 2H, Ar), 6.98 (d, *f* = 8.6 Hz, 2H, Ar), 7.12 (d, *f* = 8.5 Hz, 1H, Ar), 7.19 (d, *f* = 8.7 Hz, 2H, Ar); HPLC chemical purity = 100% (Nova-Pak C18 column, MeCN + 0.1% Et<sub>3</sub>N/H<sub>2</sub>O: 30/70, λ = 325 nm).

**2*H*-1-Benzopyran 1d.** (83% and 50% yield for step d and e), mp 115–120 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.57 (m, 10H, CH<sub>2</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 2.67 (m, 4H, NCH<sub>2</sub>), 2.82 (t, *f* = 5.9 Hz, 2H, CH<sub>2</sub>N), 3.95 (t, *f* = 5.9 Hz, 2H, OCH<sub>2</sub>), 5.77 (s, 1H, H-2), 6.13 (d, *f* = 2.3 Hz, 1H, Ar), 6.35 (dd, *f* = 2.4 and 8.3 Hz, 1H, Ar), 6.70 (d, *f* = 8.4 Hz, 2H, Ar), 6.72 (d, *f* = 8.3 Hz, 2H, Ar), 6.98 (d, *f* = 8.5 Hz, 2H, Ar), 7.11 (d, *f* = 8.4 Hz, 1H, Ar), 7.18 (d, *f* = 8.6 Hz, 2H, Ar); HPLC chemical purity = 99.5% (Nova-Pak C18 column, MeOH + 0.1% Et<sub>3</sub>N/H<sub>2</sub>O: 60/40, λ = 325 nm).

**2*H*-1-Benzopyran 1e.** (49% and 57% yield for step d and e), mp 118–123 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 2.03 (s, 3H, CH<sub>3</sub>), 2.18 (m, 2H, CH<sub>2</sub>), 2.68 (t, *f* = 5.8 Hz, 2H, NCH<sub>2</sub>), 2.82 (t, *f* = 5.6 Hz, 2H, CH<sub>2</sub>N), 3.06 (m, 2H, NCH<sub>2</sub>), 4.07 (t, *f* = 5.5 Hz, 2H, OCH<sub>2</sub>), 5.69 (m, 2H, vinylic H), 5.77 (s, 1H, H-2), 6.11 (d, *f* = 2.2 Hz, 1H, Ar), 6.34 (dd, *f* = 2.3 and 8.4 Hz, 1H, Ar), 6.70 (d, *f* = 8.5 Hz, 2H, Ar), 6.77 (d, *f* = 8.7 Hz, 2H, Ar), 6.98 (d, *f* = 8.4 Hz, 2H, Ar), 7.12 (d, *f* = 8.4 Hz, 1H, Ar), 7.19 (d, *f* = 8.5 Hz, 2H, Ar); HPLC chemical purity = 99.8% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 95/5 to 90/10 with 20 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>, λ = 240 nm).

**2*H*-1-Benzopyran 1f.** (79% and 40% yield for step d and e), mp 100–108 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 2.02 (s, 3H, CH<sub>3</sub>), 2.53 (m, 4H, NCH<sub>2</sub>), 2.72 (t, *f* = 5.5 Hz, 2H, CH<sub>2</sub>N), 3.66 (m, 4H, CH<sub>2</sub>O), 4.03 (t, *f* = 5.5 Hz, 2H, OCH<sub>2</sub>), 5.77 (s, 1H, H-2), 6.12 (d, *f* = 2.3 Hz, 1H, Ar), 6.34 (dd, *f* = 2.5 and 8.4 Hz,

1H, Ar), 6.72 (m, 4H, Ar), 6.98 (d, *f* = 8.6 Hz, 2H, Ar), 7.11 (d, *f* = 8.3 Hz, 1H, Ar), 7.19 (d, *f* = 8.7 Hz, 2H, Ar); HPLC chemical purity = 98.7% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 95/5 to 90/10 with 20 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>, λ = 212 nm).

**2*H*-1-Benzopyran 1k.** (71% and 80% yield for step d and e), <sup>1</sup>H NMR (acetone-d<sub>6</sub>) δ 1.08 (d, *f* = 6.1 Hz, 6H, CH<sub>3</sub>), 1.30 (m, 2H, CH<sub>2</sub>), 1.54 (m, 4H, CH<sub>2</sub>), ~2.05 (s, 3H, CH<sub>3</sub>), 2.48 (m, 2H, CH(CH<sub>3</sub>)), 2.95 (t, *f* = 7.0 Hz, 2H, CH<sub>2</sub>N), 3.91 (t, *f* = 6.9 Hz, 2H, OCH<sub>2</sub>), 5.87 (s, 1H, H-2), 6.20 (d, *f* = 2.4 Hz, 1H, Ar), 6.41 (dd, *f* = 2.5 and 8.4 Hz, 1H, Ar), 6.79 (d, *f* = 7.9 Hz, 4H, Ar), 7.10 (d, *f* = 8.6 Hz, 2H, Ar), 7.17 (d, *f* = 8.3 Hz, 1H, Ar), 7.26 (d, *f* = 8.7 Hz, 2H, Ar).

**2*H*-1-Benzopyran 1l.** (60% and 70% yield for step d and e), mp 102–108 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 0.92 (s, 6H, CH<sub>3</sub>), 1.23 (m, 2H, CH<sub>2</sub>), 1.59 (m, 2H, CH<sub>2</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.16 (br s, 2H, NCH<sub>2</sub>), 2.41 (m, 2H, NCH<sub>2</sub>), 2.66 (t, *f* = 5.8 Hz, 2H, CH<sub>2</sub>N), 4.03 (t, *f* = 5.9 Hz, 2H, OCH<sub>2</sub>), 5.77 (s, 1H, H-2), 6.12 (d, *f* = 2.3 Hz, 1H, Ar), 6.35 (dd, *f* = 2.4 and *f* = 8.4 Hz, 1H, Ar), 6.70 (d, *f* = 8.5 Hz, 2H, Ar), 6.75 (d, *f* = 8.6 Hz, 2H, Ar), 6.98 (d, *f* = 8.6 Hz, 2H, Ar), 7.12 (d, *f* = 8.5 Hz, 1H, Ar), 7.19 (d, *f* = 8.6 Hz, 2H, Ar); HPLC chemical purity = 99.4% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 55/45 to MeOH with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>, λ = 240 nm).

**2*H*-1-Benzopyran 1m.** (93% and 49% yield for step d and e), mp 120–126 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.02 (s, 12H, CH<sub>3</sub>), 1.39 (m, 4H, CH<sub>2</sub>), 1.52 (m, 2H, CH<sub>2</sub>), 2.00 (s, 3H, CH<sub>3</sub>), 2.81 (t, *f* = 7.7 Hz, 2H, CH<sub>2</sub>N), 3.76 (t, *f* = 7.8 Hz, 2H, OCH<sub>2</sub>), 5.76 (s, 1H, H-2), 6.14 (d, *f* = 2.4 Hz, 1H, Ar), 6.34 (dd, *f* = 2.5 and 8.5 Hz, 1H, Ar), 6.68 (d, *f* = 8.6 Hz, 2H, Ar), 6.69 (d, *f* = 8.5 Hz, 2H, Ar), 6.96 (d, *f* = 8.5 Hz, 2H, Ar), 7.09 (d, *f* = 8.2 Hz, 1H, Ar), 7.16 (d, *f* = 8.6 Hz, 2H, Ar); HPLC chemical purity = 99.4% (Nova-Pak C18 column, THF + 0.1% Et<sub>3</sub>N/MeOH/H<sub>2</sub>O: 40/20/40, λ = 325 nm).

**2*H*-1-Benzopyran 1n.** (63% and 51% yield for step d and e), mp 112–117 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 2.03 (s, 3H, CH<sub>3</sub>), 2.89 (m, 6H, CH<sub>2</sub>), 3.74 (br s, 2H, NCH<sub>2</sub>Ar), 4.15 (t, *f* = 5.6 Hz, 2H, OCH<sub>2</sub>), 5.78 (s, 1H, H-2), 6.12 (d, *f* = 2.5 Hz, 1H, Ar), 6.34 (dd, *f* = 2.3 and 8.3 Hz, 1H, Ar), 6.70 (d, *f* = 8.5 Hz, 2H, Ar), 6.80 (d, *f* = 8.7 Hz, 2H, Ar), 6.97–7.13 (m, 7H, Ar), 7.21 (d, *f* = 8.6 Hz, 2H, Ar); HPLC chemical purity = 97.6% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 55/45 to MeOH with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>, λ = 210 nm).

**2*H*-1-Benzopyran 1o.** (53% and 64% yield for step d and e), mp 140–147 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.2–2.0 (m, 13H, CH and CH<sub>2</sub> (decahydroquinoline)), 2.03 (s, 3H, CH<sub>3</sub>), 2.78 (m, 2H, NCH<sub>2</sub>), 3.04

(m, 3H, NCH and CH<sub>2</sub>N), 4.11 (t,  $\mathcal{J}$  = 5.6 Hz, 2H, OCH<sub>2</sub>), 5.78 (s, 1H, H-2), 6.11 (d,  $\mathcal{J}$  = 2.3 Hz, 1H, Ar), 6.34 (dd,  $\mathcal{J}$  = 2.3 and 8.3 Hz, 1H, Ar), 6.70 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 6.78 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.5 Hz, 2H, Ar), 7.12 (d,  $\mathcal{J}$  = 8.4 Hz, 1H, Ar), 7.21 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar); HPLC chemical purity = 97.7% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 55/45 to MeOH with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 240 nm).

**2H-1-Benzopyran 1q.** (38% and 31% yield for step d and e), mp 129–135°C; <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  2.03 (s, 3H, CH<sub>3</sub>), 2.31 (s, 6H, NCH<sub>3</sub>), 2.73 (t,  $\mathcal{J}$  = 5.4 Hz, 2H, CH<sub>2</sub>N), 4.03 (t,  $\mathcal{J}$  = 5.4 Hz, 2H, OCH<sub>2</sub>), 5.78 (s, 1H, H-2), 6.11 (d,  $\mathcal{J}$  = 2.4 Hz, 1H, Ar), 6.34 (dd,  $\mathcal{J}$  = 2.5 and 8.4 Hz, 1H, Ar), 6.70 (d,  $\mathcal{J}$  = 8.5 Hz, 2H, Ar), 6.78 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 6.98 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.12 (d,  $\mathcal{J}$  = 8.2 Hz, 1H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar); HPLC chemical purity = 98.0% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 55/45 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 240 nm).

**2H-1-Benzopyran 1t.** (50% and 48% yield for step d and e), mp 117–123°C; <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  1.07 (t,  $\mathcal{J}$  = 7.1 Hz, 6H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.67 (q,  $\mathcal{J}$  = 7.1 Hz, 4H, NCH<sub>2</sub>), 2.89 (t,  $\mathcal{J}$  = 5.7 Hz, 2H, CH<sub>2</sub>N), 4.03 (t,  $\mathcal{J}$  = 5.7 Hz, 2H, OCH<sub>2</sub>), 5.78 (s, 1H, H-2), 6.12 (d,  $\mathcal{J}$  = 2.6 Hz, 1H, Ar), 6.34 (dd,  $\mathcal{J}$  = 2.5 and 8.4 Hz, 1H, Ar), 6.70 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 6.77 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 6.98 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.12 (d,  $\mathcal{J}$  = 8.2 Hz, 1H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar); HPLC chemical purity = 98.4% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 55/45 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 240 nm).

(2*R,S*)-7-Trimethylacetoxo-3-(4'-trimethylacetoxyphe-nyl)-4-methyl-2-(4''-[2'''-(1-piperidino)ethoxy]phenyl)-2H-1-benzopyran (**2b**). A solution of 2H-1-benzopyran **1b** (0.536 g, 1.17 mmol) and triethylamine (0.45 mL, 3.3 mmol) in anhydrous dichloromethane (20 mL) was treated with trimethylacetyl chloride (0.35 mL, 2.9 mmol) at 0°C under an argon atmosphere. The cold bath was removed and the reaction mixture was allowed to warm to room temperature over a 2 h period. The mixture was treated with saturated sodium bicarbonate (25 mL). The aqueous solution was extracted with dichloromethane (2 × 25 mL). The combined organic phase was washed with brine (25 mL), dried over magnesium sulfate, and evaporated under reduced pressure to give the desired product which was purified by flash chromatography on silica gel (ethyl acetate:hexanes; 1:1 to ethyl acetate) to yield after recrystallization from isopropanol (35 mL) the 2H-1-benzopyran **2b** (0.482 g, 66% yield), mp 157–159°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.31 (s, 9H, CH<sub>3</sub>), 1.33 (s, 9H, CH<sub>3</sub>), 1.42 (m, 2H, CH<sub>2</sub>), 1.66 (m, 4H, CH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.46 (m, 4H, NCH<sub>2</sub>), 2.72 (t,  $\mathcal{J}$  = 4.9 Hz, 2H,

CH<sub>2</sub>N), 4.02 (t,  $\mathcal{J}$  = 4.9 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.47 (d,  $\mathcal{J}$  = 2.3 Hz, 1H, Ar), 6.64 (dd,  $\mathcal{J}$  = 2.5 and 8.4 Hz, 1H, Ar), 6.74 (d,  $\mathcal{J}$  = 8.5 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.5 Hz, 2H, Ar), 7.14 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 7.8 Hz, 1H, Ar); HPLC chemical purity = 99.9% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 85/15 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 240 nm).

**2H-1-Benzopyrans 2a, 2g, 2h, 2i, 2j, 2p, 2r, and 2s** were similarly prepared using the corresponding diphenol **1** as described above for 2H-1-benzopyran **2b**.

**2H-1-Benzopyran 2a.** (52% yield for step f), mp 147–157°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.31 (s, 9H, CH<sub>3</sub>), 1.34 (s, 9H, CH<sub>3</sub>), 1.78 (m, 4H, CH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.58 (m, 4H, NCH<sub>2</sub>), 2.85 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, CH<sub>2</sub>N), 4.03 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.48 (d,  $\mathcal{J}$  = 2.3 Hz, 1H, Ar), 6.65 (dd,  $\mathcal{J}$  = 2.3 and 8.5 Hz, 1H, Ar), 6.76 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.14 (d,  $\mathcal{J}$  = 8.5 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 7.8 Hz, 1H, Ar); HPLC chemical purity = 95.8% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 75/25 to MeOH with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 205 nm).

**2H-1-Benzopyran 2g.** (36%, 63%, and 76% yield for step d, e, and f respectively), mp 176–178°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.31 (s, 9H, CH<sub>3</sub>), 1.34 (s, 9H, CH<sub>3</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.67 (m, 4H, CH<sub>2</sub>S), 2.80 (m, 6H, CH<sub>2</sub>N), 4.01 (t,  $\mathcal{J}$  = 5.7 Hz, 2H, OCH<sub>2</sub>), 5.86 (s, 1H, H-2), 6.48 (d,  $\mathcal{J}$  = 2.4 Hz, 1H, Ar), 6.65 (dd,  $\mathcal{J}$  = 2.2 and 8.2 Hz, 1H, Ar), 6.74 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.00 (d,  $\mathcal{J}$  = 8.4 Hz, 2H, Ar), 7.15 (d,  $\mathcal{J}$  = 8.4 Hz, 2H, Ar), 7.21 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 8.3 Hz, 1H, Ar); HPLC chemical purity = 99.8% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 85/15 with 10 mM CH<sub>3</sub>-CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 240 nm).

**2H-1-Benzopyran 2h.** (50%, 64%, and 55% yield for step d, e, and f), mp 82–87°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.13 (d,  $\mathcal{J}$  = 6.2 Hz, 3H, CH<sub>3</sub>), 1.3 (m, 2H, CH<sub>2</sub>), 1.31 (s, 9H, CH<sub>3</sub>), 1.34 (s, 9H, CH<sub>3</sub>), 1.63 (m, 4H, CH<sub>2</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.43 (m, 2H, NCH<sub>2</sub>), 2.82 (m, 1H, CH<sub>2</sub>N), 2.96 (m, 1H, NCH), 3.10 (m, 1H, CH<sub>2</sub>N), 4.04 (t,  $\mathcal{J}$  = 6.0 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.48 (d,  $\mathcal{J}$  = 2.1 Hz, 1H, Ar), 6.65 (dd,  $\mathcal{J}$  = 2.1 and 8.5 Hz, 1H, Ar), 6.74 (d,  $\mathcal{J}$  = 8.4 Hz, 2H, Ar), 7.00 (d,  $\mathcal{J}$  = 8.5 Hz, 2H, Ar), 7.15 (d,  $\mathcal{J}$  = 8.3 Hz, 2H, Ar), 7.21 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 8.1 Hz, 1H, Ar); HPLC chemical purity = 98.6% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 75/25 to 90/10 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 220 nm).

**2H-1-Benzopyran 2i.** (46%, 67%, and 80% yield for step d, e, and f), mp 75–79°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.7–0.9 (m, 1H, CH<sub>2</sub>), 0.84 (d,  $\mathcal{J}$  = 5.9 Hz, 3H, CH<sub>3</sub>), 1.2–1.4 (m, 1H, CH<sub>2</sub>), 1.31 (s, 9H, CH<sub>3</sub>), 1.33 (s, 9H, CH<sub>3</sub>), 1.67 (m, 4H, CH<sub>2</sub>), 1.95 (m, 1H, NCH<sub>2</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 2.73 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, CH<sub>2</sub>N), 2.88

(m, 2H, NCH<sub>2</sub>), 4.03 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.48 (d,  $\mathcal{J}$  = 2.3 Hz, 1H, Ar), 6.64 (dd,  $\mathcal{J}$  = 2.3 and 8.4 Hz, 1H, Ar), 6.75 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.15 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 9.1 Hz, 1H, Ar); HPLC chemical purity = 93.3% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 75/25 to 90/10 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 220 nm).

**2H-1-Benzopyran 2j.** (63%, 57%, and 66% yield for step d, e, and f), mp 152–154 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.91 (d,  $\mathcal{J}$  = 6.3 Hz, 3H, CH<sub>3</sub>), 1.26 (m, 2H, CH<sub>2</sub>), 1.31 (s, 9H, CH<sub>3</sub>), 1.33 (s, 9H, CH<sub>3</sub>), 1.62 (m, 3H, CHCH<sub>3</sub> and CH<sub>2</sub>), 2.05 (m, 2H, NCH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.74 (t,  $\mathcal{J}$  = 6.0 Hz, 2H, CH<sub>2</sub>N), 2.92 (m, 2H, NCH<sub>2</sub>), 4.03 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.47 (d,  $\mathcal{J}$  = 2.3 Hz, 1H, Ar), 6.64 (dd,  $\mathcal{J}$  = 2.3 and 8.4 Hz, 1H, Ar), 6.74 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.14 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 8.9 Hz, 1H, Ar); HPLC chemical purity = 99.4% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 75/25 to 90/10 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 220 nm).

**2H-1-Benzopyran 2p.** (51%, 76%, and 52% yield for step d, e, and f), mp 153–154 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.91 (m, 3H, CH<sub>2</sub>), 1.25 (m, 3H, CH<sub>2</sub>), 1.31 (s, 9H, CH<sub>3</sub>), 1.34 (s, 9H, CH<sub>3</sub>), 1.50–1.75 (m, 7H, CH<sub>2</sub>, NCH<sub>2</sub>, and CH), 2.02 (m, 1H, NCH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.73 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, CH<sub>2</sub>N), 2.79 (m, 1H, CH<sub>2</sub>N), 2.96 (m, 1H, CH<sub>2</sub>N), 4.02 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.48 (d,  $\mathcal{J}$  = 2.4 Hz, 1H, Ar), 6.65 (dd,  $\mathcal{J}$  = 2.4 and 8.4 Hz, 1H, Ar), 6.75 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.14 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 7.5 Hz, 1H, Ar); HPLC chemical purity = 99.7% (Nova-Pak C18 column, MeOH/H<sub>2</sub>O: 50/50 to MeOH with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 240 nm).

**2H-1-Benzopyran 2r.** (47%, 67%, and 72% yield for step d, e, and f), mp 153–155 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.90 (t,  $\mathcal{J}$  = 7.2 Hz, 3H, CH<sub>3</sub>), 1.27 (m, 2H, CH<sub>2</sub>), 1.31 (s, 9H, CH<sub>3</sub>), 1.34 (s, 9H, CH<sub>3</sub>), 1.45 (m, 2H, CH<sub>2</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.30 (s, 3H, NCH<sub>3</sub>), 2.41 (t,  $\mathcal{J}$  = 7.5 Hz, 2H, NCH<sub>2</sub>), 2.74 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, CH<sub>2</sub>N), 3.99 (t,  $\mathcal{J}$  = 6.1 Hz, 2H, OCH<sub>2</sub>), 5.85 (s, 1H, H-2), 6.48 (d,  $\mathcal{J}$  = 2.4 Hz, 1H, Ar), 6.65 (dd,  $\mathcal{J}$  = 2.3 and 8.5 Hz, 1H, Ar), 6.75 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 6.99 (d,  $\mathcal{J}$  = 8.3 Hz, 2H, Ar), 7.14 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 6.4 Hz, 1H, Ar); HPLC chemical purity = 99.5% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 80/20 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 215 nm).

**2H-1-Benzopyran 2s.** (43%, 63%, and 46% yield for step d, e, and f), mp 112–118 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.31 (s, 9H, CH<sub>3</sub>), 1.34 (s, 9H, CH<sub>3</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.31 (s, 3H, NCH<sub>3</sub>), 2.76 (t,  $\mathcal{J}$  = 5.9 Hz, 2H, CH<sub>2</sub>N), 3.09 (d,  $\mathcal{J}$  = 6.4 Hz, 2H, NCH<sub>2</sub>), 4.00 (t,

$\mathcal{J}$  = 5.9 Hz, 2H, OCH<sub>2</sub>), 5.15 (m, 2H, vinylic H), 5.85 (s and m, 2H, H-2 and vinylic H), 6.48 (d,  $\mathcal{J}$  = 2.2 Hz, 1H, Ar), 6.65 (dd,  $\mathcal{J}$  = 2.2 and 8.2 Hz, 1H, Ar), 6.76 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.00 (d,  $\mathcal{J}$  = 8.6 Hz, 2H, Ar), 7.14 (d,  $\mathcal{J}$  = 8.4 Hz, 2H, Ar), 7.20 (d,  $\mathcal{J}$  = 8.7 Hz, 2H, Ar), 7.27 (d,  $\mathcal{J}$  = 8.2 Hz, 1H, Ar); HPLC chemical purity = 99.5% (YMC-Pack C4 column, MeOH/H<sub>2</sub>O: 80/20 with 10 mM CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>,  $\lambda$  = 215 nm).

*General procedure for the preparation of 1-(2-chloroethyl)dialkylamines 9.* Under an argon atmosphere, the secondary amine (0.13 mol) was heated at 80 °C, treated with 2-chloroethanol (**12**) (3.4 mL, 0.10 mol), and heated overnight at 100 °C. The reaction mixture was cooled to room temperature, treated with 40% sodium hydroxide (10 mL, 0.10 mol), distilled water (10 mL), and benzene (10 mL, CARE-carcinogenic), and stirred for 30 min. The aqueous phase was extracted with benzene (2 × 10 mL). The combined organic phase was dried over sodium sulfate, filtered, and concentrated. The residue was distilled under high vacuum to give 1-(2-hydroxyethyl)dialkylamine **13**. Under an argon atmosphere, a solution of the obtained alcohol **13** (0.05 mol) in anhydrous 1,2-dichloroethane (40 mL) was treated with a solution of thionyl chloride (3.8 mL, 0.053 mol) in anhydrous 1,2-dichloroethane (10 mL) for 1 h. The reaction mixture (suspension) was refluxed for 1.5 h, cooled to room temperature, filtered (in some cases, ethyl acetate and/or hexanes were added to help salt precipitation), washed with a minimum of 1,2-dichloroethane, and dried in air to give the desired 1-(2-chloroethyl)dialkylamine **9**.

**1-(2-Chloroethyl)heptamethylenimine hydrochloride (9d).** (59% and 33% yield for step a and b), mp 201–203 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.4–2.1 (m, 10H, CH<sub>2</sub>), 3.11 (m, 2H, NCH<sub>2</sub>), 3.36 (q,  $\mathcal{J}$  = 5.8 Hz, 2H, CH<sub>2</sub>N), 3.56 (m, 2H, NCH<sub>2</sub>), 4.08 (t,  $\mathcal{J}$  = 6.7 Hz, 2H, ClCH<sub>2</sub>), 12.55 (m, 1H, NH).

**1-(2-Chloroethyl)-1,2,3,6-tetrahydropyridine hydrochloride (9e).** (52% and 100% yield for step a and b), mp 235–239 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.38 (m, 1H, CH<sub>2</sub>), 2.89 (m, 1H, CH<sub>2</sub>), 3.16 (m, 1H, NCH<sub>2</sub>), 3.50 (m, 4H, NCH<sub>2</sub> and CH<sub>2</sub>N), 4.02 (m, 1H, NCH<sub>2</sub>), 4.14 (t,  $\mathcal{J}$  = 5.7 Hz, 2H, ClCH<sub>2</sub>), 5.71 (m, 1H, vinylic H), 6.03 (m, 1H, vinylic H), 13.22 (m, 1H, NH).

**4-(2-Chloroethyl)thiomorpholine hydrochloride (9g).** (83% and 96% yield for step a and b), mp 213–215 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  2.88 (m, 2H, CH<sub>2</sub>S), 3.18 (m, 2H, CH<sub>2</sub>S), 3.34 (m, 2H, NCH<sub>2</sub>), 3.61 (q,  $\mathcal{J}$  = 6.3 Hz, 2H, CH<sub>2</sub>N), 3.84 (m, 2H, NCH<sub>2</sub>), 4.00 (t,  $\mathcal{J}$  = 6.2 Hz, 2H, ClCH<sub>2</sub>).

*1-(2-Chloroethyl)-2-methylpiperidine hydrochloride (9h)*. (60% and 87% yield for step a and b), mp 186–188°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.38 (d, *J* = 7.0 Hz, ~1H, CH<sub>3</sub>), 1.57 (d, *J* = 6.3 Hz, ~2H, CH<sub>3</sub>), 1.3–2.0 (m, 5.2H, CH<sub>2</sub>), 2.1–2.4 (m, 1.7H, CH<sub>2</sub>), 2.83 (m, 0.5H, CH<sub>2</sub>), 3.07 (m, 0.9H, CH<sub>2</sub>), 3.24 (m, 1.6H, CH<sub>2</sub>), 3.63 (m, 1.5H, CH<sub>2</sub>), 3.9–4.2 (m, 1.8H, CH<sub>2</sub>), 12.53 (m, ~0.5H, NH).

*1-(2-Chloroethyl)-3-methylpiperidine hydrochloride (9i)*. (70% and 91% yield for step a and b), mp 243–250°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.96 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>), 1.08 (m, 1H, CH<sub>2</sub>), 1.89 (m, 2H, CH<sub>2</sub>), 2.36 (m, 3H, CH<sub>2</sub>), 2.63 (m, 1H, CH<sub>2</sub>), 3.32 (d, *J* = 5.2 Hz, 2H, CH<sub>2</sub>N), 3.47 (m, 1H, CH<sub>2</sub>), 3.60 (m, 1H, CH<sub>2</sub>), 4.09 (t, *J* = 6.6 Hz, 2H, ClCH<sub>2</sub>), 12.65 (m, 1H, NH).

*1-(2-Chloroethyl)-4-methylpiperidine hydrochloride (9j)*. (63% and 91% yield for step a and b), mp 144–146°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.02 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub>), 1.4–2.2 (m, 5H, CH<sub>2</sub>), 2.77 (m, 2H, CH<sub>2</sub>), 3.0–3.5 (m, 2H, CH<sub>2</sub>), 3.60 (m, 2H, CH<sub>2</sub>), 4.08 (t, *J* = 6.6 Hz, 2H, ClCH<sub>2</sub>), 12.49 (m, 1H, NH).

*1-(2-Chloroethyl)-cis-2,6-dimethylpiperidine hydrochloride (9k)*. (51% and 74% yield for step a and b), mp 180–185°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.3–1.6 (m, 2H, CH<sub>2</sub>), 1.48 (d, *J* = 6.7 Hz, 3H, CH<sub>3</sub>), 1.58 (d, *J* = 6.2 Hz, 3H, CH<sub>3</sub>), 1.81 (m, 3H, CH<sub>2</sub>), 2.21 (m, 1H, CH<sub>2</sub>), 3.10 (m, 2H, CH<sub>2</sub>), 3.3–3.6 (m, 2H, CH<sub>2</sub>), 3.8–4.0 (m, 2H, CH<sub>2</sub>), 12.22 (m, 1H, NH).

*1-(2-Chloroethyl)-3,3-dimethylpiperidine hydrochloride (9l)*. (74% and 87% yield for step a and b), mp 244–249°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.04 (s, 3H, CH<sub>3</sub>), 1.27 (m, 1H, CH<sub>2</sub>), 1.39 (s, 3H, CH<sub>3</sub>), 1.73 (m, 2H, CH<sub>2</sub>), 2.31 (m, 1H, CH<sub>2</sub>), 2.54 (m, 2H, CH<sub>2</sub>), 3.32 (m, 3H, CH<sub>2</sub>), 3.65 (m, 1H, CH<sub>2</sub>), 4.15 (t, *J* = 6.3 Hz, 2H, ClCH<sub>2</sub>), 12.0 (m, 1H, NH).

*1-(2-Chloroethyl)-2,2,6,6-tetramethylpiperidine hydrochloride (9m)*. (33% and 82% yield for step a and b), mp 212–218°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.35 (s, 6H, CH<sub>3</sub>), 1.5–1.8 (m, 4H, CH<sub>2</sub>), 1.69 (s, 6H, CH<sub>3</sub>), 2.68 (m, 2H, CH<sub>2</sub>), 3.23 (m, 2H, CH<sub>2</sub>N), 4.20 (m, 2H, ClCH<sub>2</sub>), 11.0 (m, 1H, NH).

*2-(2-Chloroethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (9n)*. (37% and 83% yield for step a and b), mp 237–243°C; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 3.24 (m, 2H, CH<sub>2</sub>), 3.72 (m and t, *J* = 6.0 Hz, 4H, CH<sub>2</sub>N), 4.07 (t, *J* = 6.0 Hz, 2H, ClCH<sub>2</sub>), 4.55 (m, 2H, benzylic NCH<sub>2</sub>), 7.22–7.34 (m, 4H, Ar).

*1-(2-Chloroethyl)decahydroquinoline hydrochloride (9o)*. (74% and 66% yield for step a and b), mp 215–217°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.2–2.0 (m, 12H, CH<sub>2</sub>), 2.35 (m, 1H, CH<sub>2</sub>), 2.79 (m, 1H, CH<sub>2</sub>), 3.25 (m, 3H, CH<sub>2</sub>), 3.51 (m, 1H, CH<sub>2</sub>), 4.15 (m, 2H, ClCH<sub>2</sub>).

*2-(2-Chloroethyl)-trans-decahydroisoquinoline hydrochloride (9p)*. (75% and 83% yield for step a and b), mp 244–248°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.87–0.97 (m, 1H, CH<sub>2</sub>), 1.07–1.39 (m, 4H, CH<sub>2</sub>), 1.61–1.78 (m, 5H, CH<sub>2</sub>), 1.90–2.06 (m, 2H, CH<sub>2</sub>), 2.38–2.46 (m, 1H, NCH<sub>2</sub>), 2.71–2.81 (m, 1H, NCH<sub>2</sub>), 3.30 (q, *J* = 5.3 Hz, 2H, CH<sub>2</sub>N), 3.41 (m, 1H, NCH<sub>2</sub>), 3.63 (m, 1H, NCH<sub>2</sub>), 4.09 (t, *J* = 6.5 Hz, 2H, ClCH<sub>2</sub>), 12.69 (m, 1H, NH).

*1-(2-Chloroethyl)-1-methyl-1-butylamine hydrochloride (9r)*. (57% and 68% yield for step a and b), mp 121–124°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.97 (t, *J* = 7.3 Hz, 3H, CH<sub>3</sub>), 1.42 (m, 2H, CH<sub>2</sub>), 1.83 (m, 2H, CH<sub>2</sub>), 2.85 (d, *J* = 4.7 Hz, 3H, NCH<sub>3</sub>), 2.9–3.2 (m, 2H, CH<sub>2</sub>N), 3.31 (m, 1H, CH<sub>2</sub>N), 3.46 (m, 1H, CH<sub>2</sub>N), 4.04 (m, 2H, ClCH<sub>2</sub>), 12.90 (m, 1H, NH).

*1-(2-Chloroethyl)-1-methyl-1-allylamine hydrochloride (9s)*. (46% and 42% yield for step a and b), mp 105–110°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.83 (s, 3H, NCH<sub>3</sub>), 3.29 (m, 1H, CH<sub>2</sub>N), 3.49 (m, 1H, CH<sub>2</sub>N), 3.73 (m, 2H, NCH<sub>2</sub>), 4.04 (m, 2H, ClCH<sub>2</sub>), 5.58 (m, 2H, vinylic H), 6.14 (m, 1H, vinylic H), 13.04 (m, 1H, NH).

## Biology

### Estrogen receptor binding assay

*Tissue preparation.* Female Sprague-Dawley rats (CrI: CD(SD)Br) weighing 200–300 g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats were gonadectomized under general anesthesia (Isoflurane) and killed by cervical dislocation 24–30 h later. The uteri were rapidly removed, dissected free from adhering tissue and frozen on dry-ice. Uteri were kept at –80°C until assayed. All subsequent steps were performed at 0–4°C. Uteri were homogenized in 10 volumes (w/v) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA disodium salt, 10 mM α-monothio glycerol, 10% glycerol, and 10 mM sodium molybdate, pH 7.4), using a Polytron PT-10 homogenizer (Brinkman Instruments, Canada) at a setting of 5 for three periods of 10 s, with intervals of 10 s for cooling. The homogenate was then centrifuged at 105 000 × g for 60 min in a Beckman L5-65 ultracentrifuge (Fullerton, CA).

*Assay.* Estrogen binding was measured using the dextran-coated charcoal adsorption technique as described previously [20,41,42]. Briefly, the radioactive steroid [<sup>3</sup>H]E<sub>2</sub> solubilized in ethanol was diluted into buffer A. Aliquots of uterine cytosol preparation (0.1 mL) were then incubated with 5 nM [<sup>3</sup>H]E<sub>2</sub> (~200 000 cpm, 0.1 mL) in the presence or absence of increasing concentrations of unlabeled compounds (0.1 mL, prepared in buffer A containing 10% ethanol) for 3 h at room temperature. Unbound steroids were then separated by incubation for 15 min

at 0–4°C with 0.3 mL 0.5% Norit-A and 0.05% Dextran T-70 in buffer B (1.5 mM EDTA disodium salt, 10 mM  $\alpha$ -monothioglycerol, and 10 mM Tris-HCl, pH 7.4) and centrifuged at  $3\,000 \times g$  for 15 min. Aliquots of the supernatant (0.3 mL) were removed for radioactivity measurement. Dose-response curves and IC<sub>50</sub> values were calculated using a weighted iterative nonlinear least-squares regression [43]. The IC<sub>50</sub> is the concentration of the antiestrogen that causes a 50% displacement of [<sup>3</sup>H]E<sub>2</sub> and the IC<sub>50</sub> value (or RBA) of estradiol was set at 100.

#### Proliferation of T-47D Cells

**Maintenance of stock cell cultures.** The T-47D human breast cancer cells were obtained from the American Type Culture Collection # HTB 133 at passage 86 and were routinely grown in phenol red-free RPMI-1640 medium supplemented with 1 nM E<sub>2</sub>, 2 mM L-glutamine, 1 mM sodium pyruvate, 15 mM HEPES, 100 IU penicillin/mL, 50  $\mu$ g streptomycin sulfate/mL, and 10% (v/v) FBS, as previously described [14,44]. T-47D cell line was derived from a pleural effusion obtained from a 54-year-old female patient with infiltrating ductal carcinoma. T-47D cells were used between passages 90 and 120 and subcultured weekly.

**Cell proliferation studies.** Cells in their late logarithmic growth phase were harvested with 0.1% pancreatin (Sigma) and resuspended in the appropriate medium containing 50 ng bovine insulin/mL and 5% (v/v) FBS treated twice with dextran-coated charcoal to remove endogenous steroids. Cells were plated in 24-well Falcon plastic culture plates and allowed to adhere to the surface of the plates for 72 h. Thereafter, medium was replaced with fresh medium containing increasing concentrations of compounds diluted from  $1\,000 \times$  stock solutions in 99% redistilled ethanol in the presence of E<sub>2</sub>. Control cells received only the ethanolic vehicle (0.1% EtOH, v/v). Cells were incubated for the specified time intervals with medium changes at 2- or 3-day intervals. Cell number was determined by measurement of DNA content as previously described [44]. Dose-response curves and IC<sub>50</sub> values were calculated using a weighted iterative nonlinear least-squares regression [43]. The IC<sub>50</sub> is the concentration of the antiestrogen giving a 50% inhibition of E<sub>2</sub> action on cell growth.

#### Uterotrophic and antiuterotrophic assays

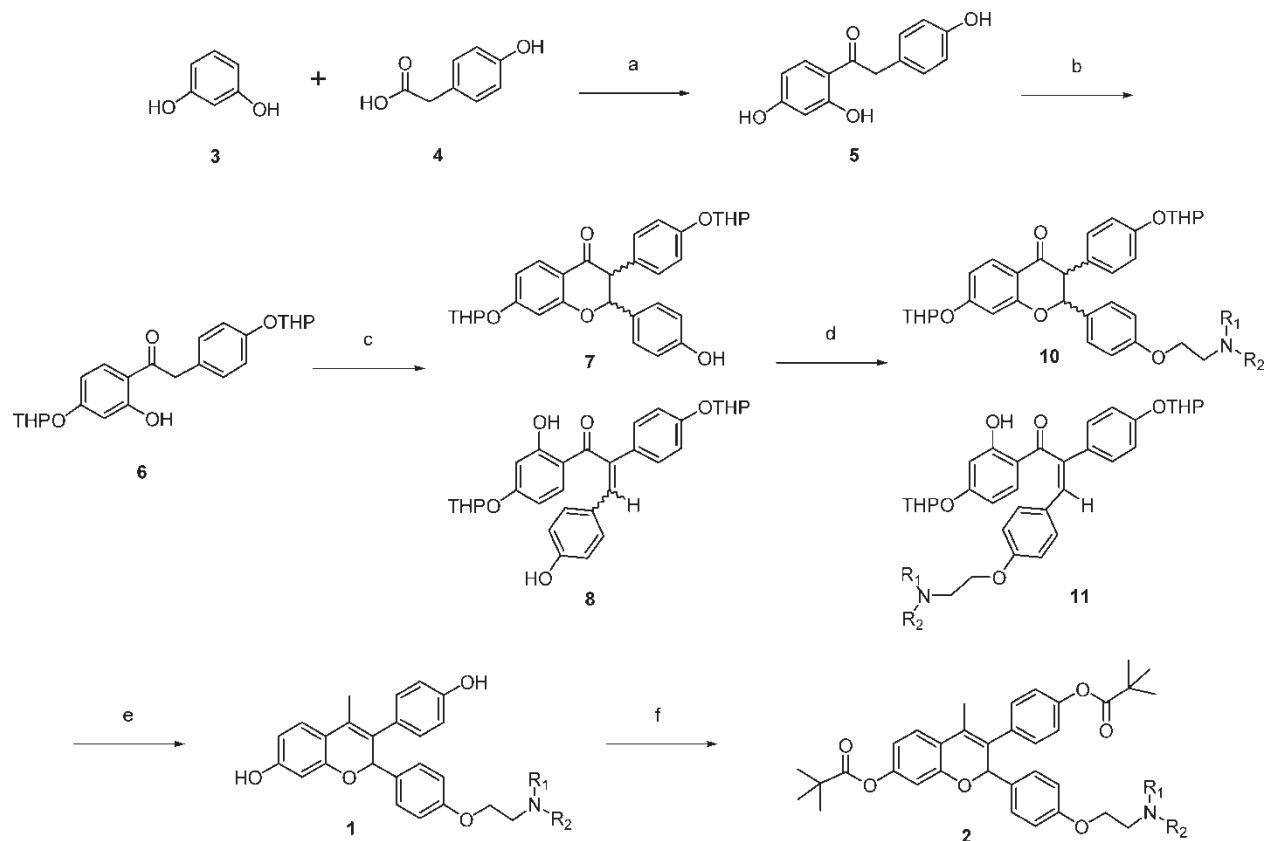
**Animals.** Female BALB/c mice (BALB/cAnNCrIBR) weighing 18–20 g were obtained from Charles-River, Inc. (St-Constant, Quebec, Canada) and housed 4–5 per cage in a temperature ( $23 \pm 1^\circ\text{C}$ )- and light (12 h light/day, lights on at 7:15)- controlled environment. The mice were fed commercial rodent chow and tap water *ad libitum*. On

study day 1 (SD 1), the animals were ovariectomized (OVX) under general anesthesia (Avertin) via bilateral flank incisions and randomly assigned to groups of 8–10 animals.

**Treatments.** To evaluate the estrogenic activity, tested compounds were administered orally by gavage once daily at doses of 7.5 and 75 nmol/animal for 9 days, starting 2 days after ovariectomy (SD 3 to 11). For the antiestrogenic activity, tested compounds were administered as described above but a treatment with estrone (E<sub>1</sub>, 0.06  $\mu$ g, s.c. injection, twice daily) was started 5 days post-ovariectomy and was administered for a 6 day-period (SD 6 to 11). Compounds were dissolved in ethanol (8% final concentration) and administered in 1% (w/v) gelatin – 0.9% NaCl solution. Mice in OVX and OVX + E<sub>1</sub> control groups received the vehicle alone by oral gavage during the 9-day period. The animals were killed by cervical dislocation on SD 12. The uteri were rapidly collected and weighed.

#### Results and discussion

The synthesis of 2*H*-1-benzopyrans **1** and **2** is shown in Scheme 1. The first step was a Friedel-Crafts reaction using BF<sub>3</sub>·Et<sub>2</sub>O as catalyst in toluene [45]. Resorcinol (**3**) was thus acylated with 4-hydroxyphenylacetic acid (**4**) to yield trihydroxydeoxybenzoin **5** in 81% yield. The trihydroxydeoxybenzoin **5** was then protected with DHP, in the presence of TsOH as catalyst, to give the bis-THP ether **6** in 69% yield. The third step was a Knoevenagel reaction of bis-THP ether **6** with 4-hydroxybenzaldehyde, in the presence of piperidine in refluxing benzene which gave quantitatively a mixture of chromanones **7** (3:1 trans/cis ratio) and chalcones **8** (4:1 *Z/E* ratio) at a 2:1 molar ratio. The crude intermediates **7** and **8** were then alkylated with several 1-(2-chloroethyl)dialkylamines hydrochloride **9** in the presence of Cs<sub>2</sub>CO<sub>3</sub> in refluxing acetone–water, to yield the chromanones **10** contaminated with chalcones **11** at 40–90% yields [46]. In the case of the amine **9b**, the Knoevenagel reaction gave the chromatographed chromanones **10b** (~5:1 trans/cis ratio), which contain about 15% of *Z*-chalcone **11b**, in a 65% yield. The chromanones **10** were then alkylated with methylolithium at –78°C to room temperature, to give tertiary alcohol intermediates. The crude alcohols were dehydrated and deprotected in 90% aqueous acetic acid at 90°C to yield 2*H*-1-benzopyrans **1** at 30–80% yields. The 2*H*-1-benzopyrans **1** were amorphous red solids, difficult to purify by flash chromatography, and containing large amounts of residual solvents (around 10% by weight). Consequently, some of the obtained 2*H*-1-benzopyrans **1** were derivatized to improve chemical purity into the corresponding dipivaloates **2** under standard conditions in 50–80% yields. On the other

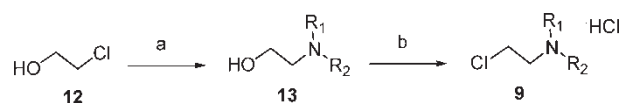


Scheme 1. Synthesis of 2H-1-benzopyrans 1 and 2. Reagents and conditions: (a)  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (3.0 equiv), toluene,  $100^\circ\text{C}$ , 3 h (81% yield); (b) DHP (9.8 equiv), TsOH (catalytic amount),  $0^\circ\text{C}$ , 2.5 h (69% yield); (c) 4-hydroxybenzaldehyde (1.04 equiv), piperidine (0.3 equiv), benzene, reflux, 60 h; (d) 1-(2-chloroethyl)dialkylamine hydrochloride 9 (1.2 equiv),  $\text{Cs}_2\text{CO}_3$  (2.4 equiv), acetone,  $\text{H}_2\text{O}$  (1.3%), reflux, 19 h; (e) (i) MeLi (3.0 equiv), THF,  $-78^\circ\text{C}$  to room temperature, 3 h; (ii) AcOH,  $\text{H}_2\text{O}$  (10%),  $90^\circ\text{C}$ , 0.5 h; (f) PvCl (2.4 equiv),  $\text{Et}_3\text{N}$  (2.8 equiv),  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$  to room temperature, 2 h.

hand, most of the 1-(2-chloroethyl)dialkylamines 9 were not commercially available and were synthesized in two steps according to Scheme 2. 2-Chloroethanol (12) was treated with a small excess of secondary amine to give 1-(2-hydroxyethyl)dialkylamines 13 which were purified by distillation at 35–85% yields [47]. Finally, the alcohols 13, using thionyl chloride, were converted to the crystallized chlorides 9 as hydrochloride salts in 35–100% yields.

2H-1-Benzopyrans 1 and 2 were evaluated in *in vitro* and *in vivo* assays for their antiestrogenic and estrogenic activities (Table I). The estrogen receptor binding of compounds 1 and 2 was measured in rat uterine cytosol. Relative binding affinities (RBA) were calculated from radiolabelled estradiol displacement (RBA of estradiol is set at 100). The compounds in Table I can be divided into four groups ( $\text{NR}_1\text{R}_2$ ), namely ring size (1a to 1d), replacement of the 4'-methylene of the piperidine ring by other chemical functions (1e to 2g), substitution of the piperidine ring (2h to 2p), and dialkylamines (1q to 1t). According to the results in Table I, surprisingly, almost all 2H-1-benzopyrans 1 and 2 had a very good affinity for the estrogen

receptor, except for compounds 2g and 2p. 2H-1-Benzopyrans 1 and 2 have 3–4 fold and 1–1.5 fold higher affinity than estradiol for the estrogen receptor, respectively. The presence of the pivaloate groups in compounds 2 decreases their estrogen receptor affinity by about 2.5 fold (comparison between 1a and 2a, and 1b and 2b). We previously reported, using the same experimental conditions, RBA values of 460 and 3.6 for compounds 1b (EM-343) and 2b (EM-762), respectively.[20] This difference can be explained by variable pivaloate hydrolysis during incubation between different experiments. Moreover, 2H-1-benzopyrans 1 were approximately 4-fold more potent than (*Z*)-4-hydro-



Scheme 2. Synthesis of 1-(2-chloroethyl)dialkylamines 9. Reagents and conditions: (a) (i) secondary amine (1.3 equiv),  $80\text{--}100^\circ\text{C}$ , 18 h; (ii) 40% NaOH (1.0 equiv), room temperature; (b)  $\text{SOCl}_2$  (1.05 equiv),  $\text{ClCH}_2\text{CH}_2\text{Cl}$ , room temperature, 1 h, and reflux, 1.5 h.

Table I. Rat estrogen receptor binding, inhibition of proliferation of T-47D cells, and antiuterotrophic and uterotrophic activity of 2H-1-benzopyran derivatives **1** and **2** in ovariectomized mice.

Compound	NR <sub>1</sub> R <sub>2</sub>	RBA*	T-47D IC <sub>50</sub> (nM)	Antiuterotrophic inhibition (%)		Uterotrophic stimulation (%)	
				Dose <sup>†</sup>		Dose <sup>†</sup>	
				7.5 nmol	75 nmol	7.5 nmol	75 nmol
Tamoxifen		1.8 <sup>‡</sup>	~100 <sup>¶</sup>	35 <sup>§</sup>	16 <sup>§</sup>	252 <sup>§</sup>	385 <sup>§</sup>
4-OH-tamoxifen		93 <sup>‡</sup>	0.522 <sup>¶</sup>	nd	nd	nd	nd
Raloxifene		310	0.382	49	84	36	42
<b>1a</b>		410	0.139	71	79	110	114
<b>2a</b>		140	0.325	60	67	nd	108
<b>1b</b>		380	0.110	63	84	29	25
<b>2b</b>		150	0.258	77	90	38	6
<b>1c</b>		350	0.151	42	72	38	134
<b>1d</b>		370	0.321	28	39	28	276
<b>1e</b>		370	0.372	34	39	190	183
<b>1f</b>		320	1.83	17	30	4	198
<b>2g</b>		10	2.13	13	26	nd	235
<b>2h</b>		120	0.232	30	6	188	365
<b>2i</b>		60	0.343	36	47	65	171
<b>2j</b>		80	0.406	31	20	196	246
<b>1k</b>		350	0.104	40	25	159	nd
<b>1l</b>		450	0.152	42	36	38	328
<b>1m</b>		430	0.408	49	48	75	150
<b>1n</b>		340	0.789	-10	36	-6	119
<b>1o</b>		340	0.314	25	28	8	242
<b>2p</b>		16	1.63	29	9	nd	340
<b>1q</b>		360	0.338	36	37	328	331
<b>2r</b>		110	0.246	13	19	296	311
<b>2s</b>		140	0.349	61	78	69	27
<b>1t</b>		410	0.257	32	36	417	359

\* The RBA of estradiol is set at 100.

<sup>†</sup> nd: not determined.<sup>‡</sup> Data obtained from Ref. [20].<sup>¶</sup> Data obtained from Ref. [22].<sup>§</sup> Data obtained from Ref. [17].

xytamoxifen, the active metabolite of tamoxifen, in their binding to the estrogen receptor. 2*H*-1-Benzopyrans **1** were slightly more potent than raloxifene in their binding to the estrogen receptor.

The antiestrogenic activity of 2*H*-1-benzopyrans **1** and **2** was studied on basal and estradiol-stimulated (0.1 nM) proliferation of T-47D human breast cancer cells. In the absence of added estradiol, compounds **1** and **2** did not alter basal cell proliferation, thus demonstrating the absence of intrinsic estrogenic activity in these compounds. On the other hand, the inhibition of estradiol-stimulated T-47D cell proliferation shows that almost all compounds **1** and **2** were very potent inhibitors (IC<sub>50</sub> = 0.1 – 0.4 nM) of estradiol action. Compounds **1f**, **2g**, **1n**, and **2p** were the least active. Substitution of the 4'-methylene of the piperidine ring by an heteroatom is detrimental for the inhibition of T-47D proliferation (compound **1f**, morpholine; compound **2g**, thiomorpholine). By analogy with the RBA data, 2*H*-1-benzopyrans **2** were 2.5-fold less active than 2*H*-1-benzopyrans **1**. Furthermore, the best 2*H*-1-benzopyrans **1** were approximately 3–4-fold more potent than (*Z*)-4-hydroxytamoxifen and raloxifene in inhibiting breast cancer cell proliferation. The *in vitro* assays for the antiestrogenic evaluation of 2*H*-1-benzopyrans **1** and **2** show that nitrogen substitution has a low degree of discrimination between the analogs studied.

The 2*H*-1-benzopyrans **1** and **2** were next tested for their *in vivo* effects on estrone-stimulated uterine weight in ovariectomized mice as well as when administered alone in ovariectomized animals. All compounds were orally administered with daily (9 days) 7.5 and 75 nmol doses. 2*H*-1-Benzopyrans **1a**, **2a**, **1b**, **2b**, and **2s** led to 60–77% and 67–90% antiuterotrophic inhibitions at the 7.5 and 75 nmol doses, respectively. All other compounds showed lower antiuterotrophic activity due to a significant uterotrophic stimulation (mixed agonist-antagonist activities). 2*H*-1-Benzopyrans **1b** and **2b**, which contain a piperidine ring, had the highest antiuterotrophic inhibitory activity, and a very modest uterotrophic stimulation that could be explained by water imbibition [48] of the myometrium. The pyrrolidine ring (compounds **1a** and **2a**) led to a significant uterotrophic stimulation (108–114%). Consequently, ring size variation (compounds **1a** to **1d**) has an important *in vivo* effect. Tamoxifen, on the other hand, led to only 35% and 16% antiuterotrophic inhibitions while 252% and 385% uterotrophic stimulations were observed at equimolar doses with EM-800 [17]. Raloxifene shows slightly lower antiuterotrophic activity compared to the best 2*H*-1-benzopyrans described here. Other studies have shown that EM-652 ((*S*)-**1b**) and EM-800 ((*S*)-**2b**) have no effect on the height of the endometrial cells of the rat uterus [35] and do not stimulate alkaline phosphatase activity in human

uterine carcinoma Ishikawa cells [17,49] while tamoxifen and raloxifene show persistent estrogenic activity in these biological systems. Moreover, we have previously reported that the (*S*)-enantiomers of compounds **1b** and **2b** are much more potent than the corresponding (*R*)-enantiomers [17]. Since the studied 2*H*-1-benzopyrans **1** and **2** are racemates, we believe that the true biological activities of the (*S*)-enantiomers of the compounds described in this publication are likely to be 2-fold more potent than described above.

In summary, we have synthesized racemic analogs of EM-652 with nitrogen substitutions. The data show that the piperidine ring is the most potent antiestrogenic moiety (NR<sub>1</sub>R<sub>2</sub>) in compounds **1** and **2**. Finally, this work has led to the development of EM-652 ((*S*)-**1b**), a compound having improved characteristics which are potentially useful for the prevention as well as treatment of breast and uterine cancer and for hormone replacement therapy at the menopause.

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