Novel Basic Isoflavones as Inhibitors of Bone Resorption

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A number of aminoalkoxy analogues of ipriflavone (= 7-(1-methylethoxy)isoflavone) were prepared and examined for their capacity to inhibit bone resorption induced by bovine parathyroid hormone fragment 1 – 34. Good-to-high activities were found for 7-(aminoalkoxy)isoflavone analogues. Their activity was influenced by a number of structural features, among which the length of the basic side chain, the basicity of the amino group, and the nature and position of substituents on the 3-phenyl ring. 4′-(Aminoalkoxy)ipriflavone derivatives were less active.

Introduction. – A fine-tuned equilibrium between bone formation mediated by osteoblasts and bone resorption mediated by osteoclasts is essential to the growth and maintenance of a healthy skeleton. A major bone disease in elderly persons and, especially, postmenopausal women is osteoporosis, which results from a reduction in bone mass and deterioration in bone architecture. Osteoporosis accelerates senescence by hampering mobility and has a devastating effect on the quality of life.

A number of therapeutic strategies are now used to prevent osteoporosis, among which inhibitors of bone resorption have a prevalent position and are actively explored [1][2]. These agents act either by inhibiting osteoclast activity (biphosphonates and calcitonin) or by reducing osteoclast generation (estrogens). The latter group of drugs is remarkably heterogenous and includes natural and synthetic estrogens, selective estrogen receptor modulators such as raloxifene [3], and phytoestrogens such as genistein [4].

Indeed, there is increasing evidence that some compounds with an isoflavone structure (*e.g.*, genistein = 5,7,4′-trihydroxyisoflavone) may have important effects in various chronic diseases and particularly in preventing postmenopausal bone loss and osteoporosis [5][6]. This activity has been confirmed in a number of pharmacological models such as the suppressive effect of genistein on rat osteoclasts and its activity in maintaining bone mass in ovariectomized rats [7]. The synthetic isoflavone derivative (and marketed drug) Ipriflavone (= 7-(1-methylethoxy)-3-phenyl-4*H*-1-benzopyran-4-one) has also been shown to reduce bone loss in various types of animal models of experimental osteoporosis [8 – 10], providing a rationale for its use in the prevention and treatment of postmenopausal and senile osteoporosis in humans [11][12]. This drug interferes with bone remodeling mainly by inhibiting bone resorption [13 – 15], although some evidence has also suggested a stimulatory effect on bone formation [16 – 18].

These findings have prompted us to initiate a search for new isoflavone (= 3-phenyl-4*H*-1-benzopyran-4-one) derivatives with a basic side chain that could act as bone-sparing agents in biological models. The present paper describes the synthesis and screening of new synthetic isoflavones. Inhibition of bone resorption was assayed *in vitro* using the fetal rat long bones (radius and ulna) in stationary cultures, where bone resorption induced by the calciotrophic bovine parathyroid hormone fragment 1–34 (bPTH 1–34) was measured by assaying the release from bones of previously incorporated ^{45}Ca [19].

Results and Discussion. – *Chemistry.* The products (*Table*) were synthesized according to the general procedures described in *Schemes 1*,*a* and *b*, and *2*. The starting hydroxyisoflavones not commercially available were prepared according to literature methods [20–23].

Compounds **B1**–**8**, **C1**–**15**, **D1**–**3**, **E1**, **G1**–**4** were prepared by alkylation of the corresponding hydroxyisoflavone at the 7-PH group (*Scheme 1*,*a*) or at the 4′-PH group (*Scheme 1*,*b*) with the appropriate dibromo derivative in DMF in the presence of NaH. The monobromo derivatives obtained were treated with an excess of amine in dioxane and gave the final products in moderate-to-good overall yields.

Compounds with a OH group at C(5) (R = OH) could be synthesized as described in *Scheme 1*,*a*, starting from biochanin A (R = OH, R′ = MeO), or from 5,7-dihydroxyisoflavone. We found that the 5-OH group did not react under these conditions, probably due to the formation of a H-bond with the C(4)=O group.

Compounds **C16**, **F1**, and **F3** were prepared by final demethylation in refluxing 48% HBr from the corresponding MeO derivative, in good yields. Reduction with Fe of the NO_2 derivative **C3** gave the NH_2 compound **C17** in 36% yield.

Compounds **E2**, **D4**, **D5**, and **F2** (which have an amide bond) were synthesized by alkylation of the corresponding 7-hydroxyisoflavone with a bromo ester, followed by hydrolysis of the ester to the free acid, activation of the COO group as a *N*-hydroxysuccinimidic ester, and condensation with an excess of piperazine (*Scheme 2*). The final amido derivatives were obtained in 15–30% overall yield.

Effect on Bone Resorption in vitro. Cultures of the fetal rat long bones in the presence of 0.02–10 nM bPTH 1–34, which activates bone resorption by osteoclasts, demonstrated an increase in ^{45}Ca release, resulting in treated over control (T/C) ratios ranging from 1.6 to 4 (not shown). The submaximal concentration of bPTH 1–34 (5 nM) was selected to generate concentration-response curves of the putative bone resorption inhibitors.

In our experimental conditions, three reference compounds, calcitonine, pamidronate, and clodronate (**A1** in the *Table*), showed IC_{50} values (0.2 nM, 2.24 μM and 2.12 μM, resp.) in line with published data [24–26]. We had shown previously [14] that ipriflavone (*ca.* 25% inhibition at 30 μM) and its main *in vivo* metabolites were effective at inhibiting osteoclastic resorption in organ culture. Thus, the ipriflavone metabolites **A3** and **A4** (*Table*) had IC_{50} values of 46 and 17 μM, respectively. Another reference compound in the *Table* is raloxifene (**A2**) [3][27].

To improve the activity of iproflavone, series of aminoalkoxy analogues were prepared and examined, among which 7-(aminoalkoxy)isoflavone analogues (classes **B**–**F**) proved the most promising ones in our screening program. Their antiresorptive

activity (expressed as % of inhibition at 10 μM) is reported in the *Table*, together that of a few 4′-(aminoalkoxy)isoflavone analogues (class **G**).

Qualitative Structure-Activity Relationships. No QSAR studies were carried out with the available results, but a number of trends and qualitative SAR emerge, which may prove useful in further design.

First, compounds **B1** to **B8** (7-*O*-(alkylpiperazinyl)isoflavones) form a homologous series with the $-(CH_2)_n-$ spacer between the 7-O-atom and the piperazine

Table. *Synthesized Compounds and their Activity at Inhibiting Bone Resorption*

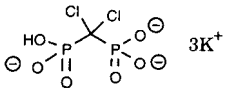
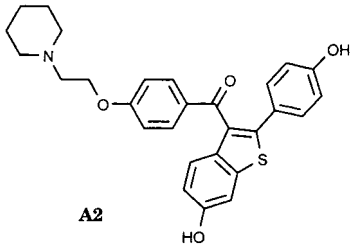
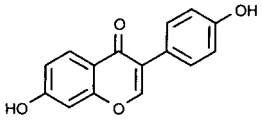
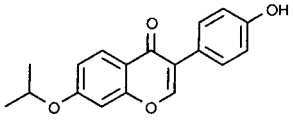
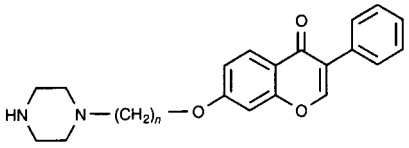
Compounds		% Inhibition at 10 μM ± SD	Number of determinations
Class A (reference compounds)			
 **A1**			
 **A2**			
 **A3**			
 **A4**			
A1	Clodronate	75 ± 5	12
A2	Raloxifene	50 ± 3	12
A3	Daidzein (4′,7-Dihydroxyisoflavone)	9 ± 6	12
A4	4′-Hydroxyipriflavone	31 ± 4	12
Class B			
			
B1	$n = 2$	38 ± 5	12
B2	$n = 3$	57 ± 8	18
B3	$n = 4$	70 ± 8	15
B4	$n = 6$	100	10
B5	$n = 7$	100	12
B6	$n = 8$	84 ± 12	12
B7	$n = 10$	46 ± 2	16
B8	$n = 12$	0	12

Table (cont.)

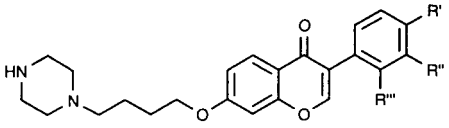
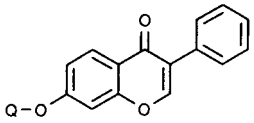
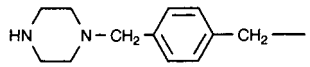
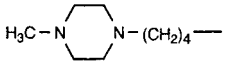
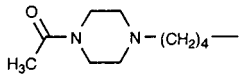
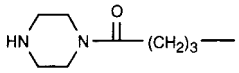
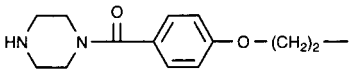
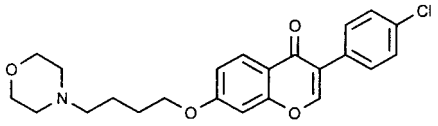
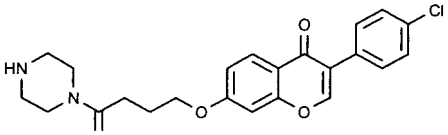
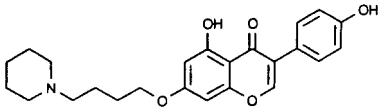
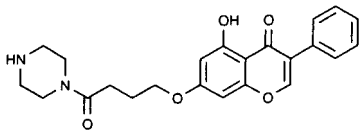
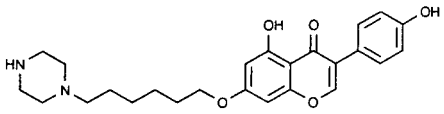
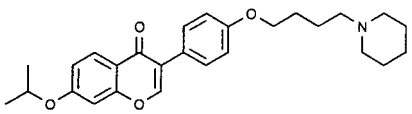
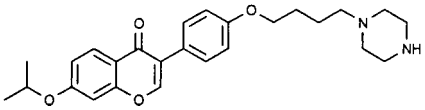
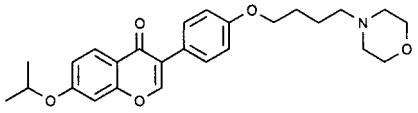
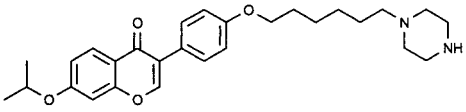
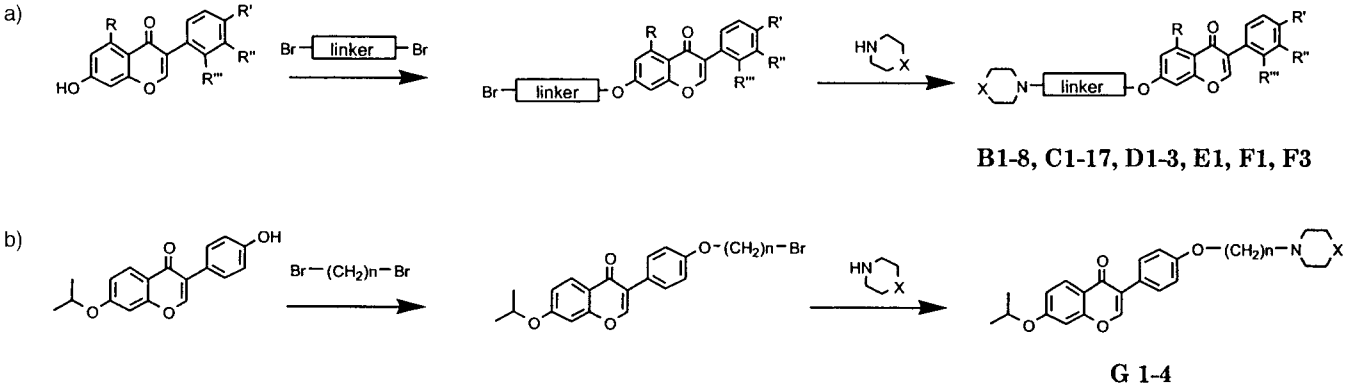
Compounds		% Inhibition at 10 μM ± SD	Number of determinations
Class C			
C1	R′ = MeO; R″ = H; R‴ = H	68 ± 4	15
C2	R′ = CF_3;R″ = H; R‴ = H	81 ± 11	12
C3	R′ = NO_2; R″ = H; R‴ = H	92 ± 16	12
C4	R′ = Me; R″ = H; R‴ = H	73 ± 8	15
C5	R′ = i-Pr; R″ = H; R‴ = H	99 ± 4	12
C6	R′ = *t*-Bu;R″ = H; R‴ = H	89 ± 11	12
C7	R′ = H, R″ – R‴ = benzo	89 ± 15	12
C8	R′ = Ph; R″ = H, R‴ = H	100	15
C9	R′ = F; R″ = H;R‴ = H	75 ± 6	18
C10	R′ = Cl; R″ = H;R‴ = H	100	12
C11	R′ = Br; R″ = H; R‴ = H	84 ± 9	14
C12	R′ = H; R″ = Cl; R‴ = H	66 ± 5	16
C13	R′ = H; R″ = H; R‴ = Cl	64 ± 4	16
C14	R′ = Cl; R″ = Cl; R‴ = H	98 ± 3	16
C15	R′ = Cl; R″ = H; R‴ = Cl	94 ± 6	16
C16	R′ = OH; R″ = H; R‴ = H	82 ± 9	16
C17	R′ = NH_2; R″ = H; R‴ = H	30 ± 4	12
Class D			
D1	Q = 	100	12
D2	Q = 	54 ± 4	12
D3	Q = 	49 ± 2	12
D4	Q = 	39 ± 3	12
D5	Q = 	100	12

Table (cont.)

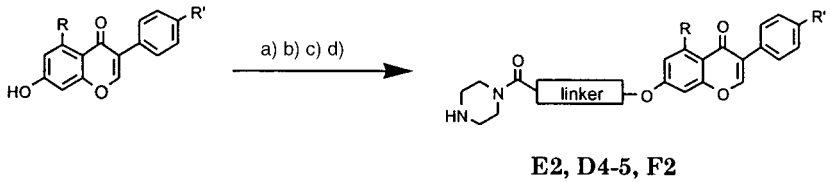
Compounds	% Inhibition at 10 μM ± SD	Number of determinations
Class E		
E1 		
E2 		
E1	76 ± 7	12
E2	82 ± 5	12
Class F		
F1 	95 ± 4	12
F2 	78 ± 6	12
F3 	100	12
Class G		
G1 	77 ± 6	12
G2 	53 ± 5	15
G3 	47 ± 4	15
G4 	81 ± 12	15

[a]) Number of determinations.

Scheme 1



Scheme 2



Scheme 2. a) Ethyl bromoacetate (**E2**, **D4**, **F2**) or ethyl 4-(2-bromoethoxy)benzoate (**D5**), NaH, DMF. b) 37% HCl, reflux. c) *N*-hydroxysuccinimide, DCC, dioxane. d) Piperazine.

ring varying from $n=2$ (% Inh = 38%) to $n=12$ (% Inh = 0). Activity increases in this series to reach a maximum (% Inh = 100%) for $n=6$ and $n=7$, and then drops rapidly beyond $n=8$. An 2-aminoethoxy side chain is present in tamoxifene, raloxifene, and many analogues (see, *e.g.*, [27]). It would be interesting to compare the present series with raloxifene homologues showing the same variation in chain length.

The compounds of series **C** are 7-*O*-(butylpiperazinyl)isoflavones-substituted on the 3-phenyl ring. A variety of 4′-substituents were explored ranging from polar (OH, NH_2) to highly lipophilic (*t*-Bu, Ph, Br). With the exception of the weakly active compound **C17** (4′-NH_2), activities remained in the relatively narrow range of 60–100%, and almost all compounds were more active than the unsubstituted **B3** (% Inh = 70%). No clear trend is apparent in the data. The most active compounds contain a lipophilic substituent (*e.g.*, i-Pr in **C5**, Ph in **C8**, *p*-Cl in **C10**, *m*,*p*-di-Cl in **C14**), but other lipophilic substituents had only a weakly favorable effect (*e.g.*, CF_3 in **C2**, Br in **C11**) or no effect at all (*e.g.*, Me in **C4**, *m*-Cl in **C12**). In one case (**C17**), activity was decreased relative to the unsubstituted compound **B3**. Thus, the 4′-substituent usually increased activity, but this effect remains unexplained.

The series **D** consists in a few heterogeneous 4′-unsubstituted 7-*O*-(spacer)-piperazinyl-isoflavones. Compounds **D2**, **D3** and **D4**, which derive directly from **B3**, indicate that *N*-methylation (**D2**) or amidification of a basic N-atom (**D3** and **D4**) decreased activity. In contrast, inserting a Ph ring in the side-chain (**D1** and **D5**) resulted in highly active compounds, perhaps for reasons of conformational restriction or lipophilicity.

A decrease in the basicity of the piperazinyl group was further examined with **E1** and **E2**, which derive directly from **C10**. Here again, this physicochemical changes decreased activity.

The three compounds in series **F** are characterized by an additional 5-OH group. Compound **F1**, the 5-OH analogue of **C16**, which has also a piperidinyl instead of a piperazinyl ring, was somewhat more active than the latter. Compound **F3**, the 5,4′-dihydroxy analogue of **B4**, retained the very high activity of the latter. A direct comparison (*i.e.*, 5-hydroxylation as the only modification) is afforded by **F2** relative to **D4** (strong increase in activity). Thus, the 5-OH group is certainly a favorable feature in this series.

The **G** series is distinct from all others in this study, being composed of 4′-(aminoalkoxy)ipriflavone derivatives. The basic group here is either a piperidinyl, a morpholinyl, or a piperazinyl ring. Compound **G1** contains in the 4′-position the same side chain as **F1** in the 7-position, and its activity was weaker. The same observation is made when comparing **G2** with **B3**, **G3** with **E1**, and **G4** with **B4**. Thus a basic side-chain in the 7-position appears preferable than in the 4′-position.

Conclusion. – The synthetic isoflavone derivative ipriflavone is available in several countries as a new drug to prevent and treat postmenopausal osteoporosis. Although its mode of action is poorly understood, ipriflavone is known to act on bone cells. In fact, the drug and its main *in vivo* metabolites have been reported to inhibit the proliferation and maturation of human osteoclasts [28]. In addition, ipriflavone may regulate the differentation and biosynthetic properties of human osteoblasts by enhancing the

expression of some important matrix proteins and facilitating the mineralization process [16].

Some of us have previously shown that ipriflavone and its main *in vivo* metabolites (*e.g.*, compounds **A3** and **A4**) are effective at inhibiting osteoclastic resorption in organ culture [14]. With these results in mind, we undertook a search for new isoflavones with potential antiosteoporotic activity. Adding an aminoalkoxy group at C(7) of the isoflavone nucleus produced *in vitro* inhibitors of bone resorption, which were one or two orders of magnitude more potent than ipriflavone and its metabolites.

Further studies in animal models of experimental osteoporosis should clarify whether some of these compounds may become preclinical candidates. Interestingly, some of the above compounds administered at 50 mg/kg/day p.o. prevented bone loss in an ovariectomized rat model of estrogen-deficiency-induced osteopenia, showing efficacy similar to that of raloxifene at 0.1 mg/kg/day p.o.

Experimental Part

1. *Chemistry.* Reference compound **A1** was obtained from *Shanko International*, **A3** from *Apin*; **A2** and **A4** were prepared following literature methods [20–22]. Starting isoflavones employed in *Schemes 1,a*, and *2* were obtained from commercial sources (*Apin*, *Aldrich*, *Acros*) or were prepared according to known methods [23][29]. The reference compound **A4** was used as starting material in *Scheme 1,b*.

Bovine parathyroid hormone fragment 1–34 (bPTH 1–34) and bovine serum albumin RIA grade were from *Sigma Chemical Co.* (St. Louis, MO, USA). BGJ-B Medium *Fitton Jackson* modified without antibiotics was from *GIBCO BRL* (Grand Island, N.Y., USA). Cl_3CCOOH (TCA), $HClO_4$, and DMSO were from *Merck* (Darmstadt, Germany). ^{45}Ca ($CaCl_2 > 10$ Ci/g) was purchased from *New England Nuclear* (Boston, MA, USA).

All reactions were carried out under Ar. Solvents and reagents were used as obtained from commercial sources. Flash chromatographies (FC) were carried out on *Merck* silica gel (40–63 μm). M.p.: *Büchi 535* melting-point apparatus; NMR: *Bruker AC-200* spectrometer. EI-MS (positive mode): *Fision's Trio-2000* spectrometer; ES-MS: *Finnigan SSQ 7000* spectrometer.

General Procedure I (*Scheme 1,a,b*). A soln. of the appropriate 7-hydroxyyisoflavone (10 mmol) and the appropriate dibromo derivative (50 mmol) in DMF (40 ml) was added with 80% NaH (12 mmol) and stirred at r.t. for 3 h. H_2O (20 ml) was cautiously added, the mixture was refrigerated in an ice bath, and the precipitated solid was separated and dissolved in $CHCl_3$. After drying (Na_2SO_4), the solvent was removed under vacuum and the residue purified by flash chromatography (FC; AcOEt/hexane 20:80 to 80:20) to give a white solid in 40–80% yields. The monobromo derivative so obtained (1 mmol) was dissolved in 1,4-dioxane (10 ml), appropriate cyclic amine was added, and the soln. was stirred at 60° for 24 h. The mixture was evaporated to dryness and the residue was triturated in a large amount of H_2O. The solid was filtered, washed with H_2O, dissolved in $CHCl_3$, and the soln. was dried (Na_2SO_4). The solvent was evaporated, and the residue was dissolved in acetone (15 ml/mmol), and a slight excess of 37% HCl was added. The HCl salt separated as crystalline solid. The resulting compounds were checked by TLC with CH_2Cl_2/MeOH/30% NH_4OH 90:10:1 as eluent.

General Procedure II (*Scheme 2*). To a soln. of the appropriate 7-hydroxyisoflavone (10 mmol) and the appropriate bromo ester (12 mmol) in DMF (40 ml) was added 80% NaH (12 mmol), and the mixture was stirred at r.t. for 3 h. H_2O (60 ml) was cautiously added, the mixture was refrigerated in an ice bath, the precipitated solid was suspended in 37% HCl (10 ml/mmol) and refluxed for 4 h. H_2O was added, and the white solid was filtered, washed with H_2O, and dried at 80° under vacuum. The acid so obtained was dissolved in 1,4-dioxane (20 ml/mmol), then stoichiometric amounts of *N*-hydroxysuccinimide and *N,N'*-dicyclohexylcarbodiimide were added. After stirring for 1 h, the precipitated urea was filtered off, a fivefold excess of piperazine was added, and the soln. was stirred for an additional h. The mixture was evaporated to a small volume and poured into H_2O. The precipitate was filtered, washed with H_2O, dissolved in $CHCl_3$, and the soln. was dried (Na_2SO_4). The solvent was evaporated, and the residue was dissolved in acetone (40 ml/mmol), and a slight excess of 37% HCl was added. The HCl salt separated as crystalline solid.

Compounds **B1**–**B8** and **C1**–**C15** were prepared according *General Procedure I*.

*3-Phenyl-7-[2-(piperazin-1-yl)ethoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B1**). Yield 45%. M.p. >260°. ^{1}H-NMR ((D$_6$)DMSO + CF$_3$COOD): 9.9–9.4 (br., NH$_2$, NH); 8.5 (*s*, 1 CH); 8.2–8.0 (*d*, 1 CH, *J* = 10.48); 7.65–7.5 (2*d*, *J* = 9.81, 2 CH), 7.5–7.1 (*m* + 3*d*, 5 CH), 4.6 (*t*, CH$_2$O); 3.9–3.35 (*t* + *m*, 5 CH$_2$N). EI-MS: 350 (M^+).

*3-Phenyl-7-[3-(piperazin-1-yl)propoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B2**). Yield 48%. M.p. >260°. ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., NH); 10.2–9.5 (br., NH$_2$); 8.45 (*s*, CH); 8.15–7.9 (*d*, *J* = 8.17, 1 CH), 7.7–7.5 (2*d*, 2 CH); 7.45–7.3 (*m*, 3 CH); 7.2–7.0 (3*d*, *J* = 1.06, 8.17, 2 CH); 4.25 (*t*, *J* = 10.4, CH$_2$O); 4.0–3.0 (*m*, 5 CH$_2$N), 2.4–2.1 (*m*, 1 CH$_2$). EI-MS: 364 (M^+).

*3-Phenyl-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B3**). Yield 55%. M.p. 252–255°. ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., NH); 10.2–9.5 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.15–7.9 (*d*, *J* = 8.10, 1 CH); 7.7–7.5 (*m*, 2 CH); 7.45–7.3 (*m*, 3 CH); 7.2–7.0 (3*d*, *J* = 0.98, 8.10, 2 CH); 4.1 (*t*, *J* = 11.06, CH$_2$); 3.8–2.9 (*m*, 5 CH$_2$N); 2.1–1.7 (*m*, 2 CH$_2$). EI-MS: 378 (M^+).

*3-Phenyl-7-[[6-(piperazin-1-yl)hexyl]oxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B4**). Yield 53%. M.p. 240–244° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.4–11.4 (br., 1 CH); 10.4–9.7 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.1–7.9 (*d*, *J* = 8.71, 1 CH); 7.7–7.25 (*d* + *m*, 5 CH); 7.15–6.9 (3*d*, *J* = 0.98, 8.71, 3 CH); 4.1 (*t*, *J* = 11.4, CH$_2$O); 3.9–2.9 (*m* + *t*, 5 CH$_2$N); 1.9–1.2 (2*m*, 4 CH$_2$). EI-MS: 406 (M^+).

*3-Phenyl-7-[[7-(piperazin-1-yl)heptyl]oxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B5**). Yield 37%. M.p. 240–242° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., NH); 10.2–9.5 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.15–7.9 (*d*, *J* = 9.01, 1 CH); 7.7–7.5 (2*d*, 2 CH); 7.45–7.3 (*m*, 3 CH); 7.2–7.0 (3*d*, *J* = 1.04, 9.01, 2 CH), 4.1 (*t*, *J* = 12.14, CH$_2$O); 3.8–2.9 (*m*, 5 CH$_2$N); 1.9–1.1 (2*m*, 5 CH$_2$). EI-MS: 420 (M^+).

*3-Phenyl-7-[[8-(piperazin-1-yl)octyl]oxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B6**). Yield 35%. M.p. 230–234° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., NH); 10.2–9.5 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.15–7.9 (*d*, *J* = 9.44, 1 CH); 7.7–7.5 (2*d*, 2 CH); 7.45–7.3 (*m*, 3 CH); 7.2–7.0 (3*d*, *J* = 1.04, 9.44, 2 CH); 4.1 (*t*, *J* = 12.59, CH$_2$O); 3.8–2.9 (*m*, 5 CH$_2$N); 1.9–1.1 (2*m*, 6 CH$_2$). EI-MS: 434 (M^+).

*3-Phenyl-7-[[10-(piperazin-1-yl)decyl]oxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B7**). Yield 28%. M.p. 220–224°. ^{1}H-NMR ((D$_6$)DMSO): 10.5–9.2 (br., NH, NH$_2$); 8.45 (*s*, 1 CH); 8.15–7.9 (*d*, *J* = 9.38, 1 CH); 7.7–7.5 (2*d*, 2 CH); 7.5–7.3 (*m*, 3 CH); 7.2–7.0 (3*d*, *J* = 1.41, 9.38, 2 CH); 4.15 (*t*, *J* = 11.58, CH$_2$O); 3.8–2.9 (*m*, 5 CH$_2$N); 1.9–1.1 (2*m*, 6 CH$_2$). EI-MS: 462 (M^+).

*3-Phenyl-7-[[12-(piperazin-1-yl)dodecyl]oxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**B8**). Yield: 23%. M.p. 200–205° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., 1 NH); 10.2–9.5 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.15–7.9 (*d*, *J* = 10.01, 1 CH); 7.7–7.5 (2*d*, 2 CH); 7.45–7.3 (*m*, 3 CH); 7.2–7.0 (3*d*, *J* = 1.14, 10.01, 2 CH); 4.1 (*t*, *J* = 12.14, CH$_2$O); 3.8–2.9 (*m*, 5 CH$_2$N); 1.9–1.1 (2*m*, 10 CH$_2$). EI-MS: 490 (M^+).

*3-(4-Methoxyphenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C1**). Yield 49%. M.p. 230–232° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 10.7–9.2 (br., NH, NH$_2$); 8.4 (*s*, CH); 8.1–7.95 (*d*, *J* = 10.14, 1 CH); 7.6–7.4 (*d*, *J* = 9.38, 2 CH), 7.2–6.9 (*m* + *d*, 4 CH); 4.2 (*t*, *J* = 11.81, CH$_2$O); 3.7 (*s*, Me); 3.7–3.3 (br., 2 CH$_2$N); 3.3–3.2 (*t*, CH$_2$N); 2.1–1.4 (*m*, 2 CH$_2$). EI-MS: 408 (M^+).

*7-[4-(Piperazin-1-yl)butoxy]-3-[4-(trifluoromethyl)phenyl]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C2**). Yield 28%. M.p. 242–245° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 11.9–11.3 (br., NH); 9.7–9.2 (br., NH$_2$); 8.6 (*s*, 1 CH); 8.15–7.95 (*d*, *J* = 9.33, 1 CH); 7.9–7.7 (*m*, 4 CH); 7.3–7.0 (3*d*, *J* = 1.38, 9.33, 2 CH); 4.15 (*t*, CH$_2$O); 3.9–3.0 (*m*, 5 CH$_2$N); 2.05–1.8 (*m*, 2 CH$_2$). EI-MS: 446 (M^+).

*3-(4-Nitrophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C3**). Yield 15%. M.p. 251–256° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.1–11.6 (br., NH); 10.0–9.5 (br., NH$_2$); 8.75 (*s*, 1 CH); 8.4–8.2 (*d*, *J* = 9.32, 2 CH), 8.15–8.05 (*d*, *J* = 11.41, 1 CH); 8.0–7.8 (*d*, *J* = 9.32, 2 CH); 7.3–7.05 (3*d*, *J* = 1.11, 11.41, 2 CH); 4.2 (*t*, CH$_2$O); 3.9–3.0 (*m*, 5 CH$_2$N); 2.1–1.7 (*m*, 2 CH$_2$). EI-MS: 423 (M^+).

*3-(4-Methylphenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C4**). Yield 33%. M.p. 240–243° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.4–11.5 (br., NH); 10.2–9.6 (br., NH$_2$); 8.45 (*s*, 1 CH), 8.1–7.9 (*d*, *J* = 11.79, 1 CH); 7.7–7.4 (*d*, *J* = 8.25, 2 CH); 7.3–6.9 (4*d*, *J* = 1.06, 8.25, 11.79, 4 CH); 4.2 (*t*, CH$_2$O); 3.9–3.0 (*m*, 5 CH$_2$N); 2.35 (*s*, Me); 2.1–1.7 (*m*, 2 CH$_2$). EI-MS: 392 (M^+).

*3-[4-(1-Methylethyl)phenyl]-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C5**). Yield 22%. M.p. 245–248° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.1–11.6 (br., NH); 10.0–9.5 (br., NH$_2$); 8.5 (*s*, 1 CH); 8.15–8.0 (*d*, *J* = 11.19, 1 CH); 7.6–7.4 (*d*, *J* = 8.85, 2 CH); 7.35–7.25 (*d*, *J* = 8.85, 2 CH); 7.2–7.0 (3*d*, *J* = 1.12, 11.19, 2 CH); 4.2 (*t*, CH$_2$O); 3.9–3.1 (*m*, 5 CH$_2$N); 3.0–2.8 (*m*, Me$_2$C*H*); 2.1–1.7 (*m*, 2 CH$_2$); 1.4–1.1 (*d*, *J* = 6.21, 2 Me). EI-MS: 420 (M^+).

*3-[4-(1,1-Dimethylethyl)phenyl]-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C6**). Yield 15%. M.p. >260°. ^{1}H-NMR ((D$_6$)DMSO): 12.1–11.6 (br., NH); 10.0–9.5 (br., NH$_2$); 8.4 (*s*, 1 CH);

8.25–8.0 (*d*, *J* = 10.76, 1 CH); 7.6–7.4 (*m*, 4 CH); 7.3–7.0 (3*d*, *J* = 1.15, 10.76, 2 CH); 4.2 (*t*, *J* = 11.96, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.1–1.7 (*m*, 2 CH_2); 1.35 (*s*, 3 Me). EI-MS: 434 (M^+).

*3-(Naphthalen-1-yl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C7**). Yield 35%. M.p. 236–239° (dec.). ^{1}H-NMR ((D_6)DMSO): 12.5–11.7 (br., NH); 10.25–9.6 (br., NH_2); 8.4 (*s*, CH); 8.15–7.85 (*m*, 3 CH); 7.75–7.35 (*m*, 5 CH); 7.3–7.0 (3*d*, *J* = 1.21, 10.87, 2 CH); 4.2 (*t*, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.05–1.8 (*m*, 2 CH_2). EI-MS: 428 (M^+).

*3-(1,1′-Biphenyl-4-yl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C8**). Yield 40%. M.p. 248–252° (dec.). ^{1}H-NMR ((D_6)DMSO): 12.3–11.6 (br., NH); 10.2–9.6 (br., NH_2); 8.5 (*s*, CH); 8.2–8.0 (*d*, *J* = 9.32, 1 CH); 7.9–7.6 (*m*, 6 CH); 7.6–7.3 (*m*, 3 CH); 7.25–7.0 (3*d*, *J* = 1.15, 9.32, 2 CH); 4.2 (*t*, CH_2O); 3.9–3.0 (*m* + *t*, 5 CH_2N); 2.1–1.7 (*m*, 2 CH_2). EI-MS: 454 (M^+).

*3-(4-Fluorophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C9**). Yield 55%. M.p. 252–256° (dec.). ^{1}H-NMR ((D_6)DMSO, 340 K): 10.9–9.5 (br., NH, NH_2); 8.45 (*s*, 1 1 CH); 8.1–7.95 (*d*, *J* = 10.42, 1 CH); 7.75–7.5 (*m*, 2 CH); 7.3–7.0 (*m*, 4 CH); 4.2 (*t*, *J* = 13.05, CH_2O); 3.75–3.05 (br. + *t*, 5 CH_2N); 2.05–1.7 (*m*, 2 CH_2). EI-MS: 396 (M^+).

*3-(4-Chlorophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C10**). Yield 60%. M.p. 237–241° (dec.). ^{1}H-NMR ((D_6)DMSO): 12.3–10.6 (br., NH); 10.3–9.6 (br., NH_2); 8.5 (*s*, CH); 8.1–7.9 (*d*, *J* = 8.75, 1 CH); 7.7–7.4 (2*d*, *J* = 7.93, 4 CH); 7.25–7.0 (3*d*, *J* = 1.15, 8.75, 2 CH); 4.15 (*t*, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.1–1.7 (*m*, 2 CH_2). EI-MS: 412 (M^+).

*3-(4-Bromophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C11**). Yield 45%. M.p. >260°. ^{1}H-NMR ((D_6)DMSO): 12.3–11.5 (br., NH); 10.25–9.6 (br., NH_2); 8.5 (*s*, CH), 8.15–7.95 (*d*, *J* = 11.23, 1 CH); 7.7–7.4 (*m*, 4 CH); 7.25–7.0 (3*d*, *J* = 1.15, 11.23, 2 CH); 4.2 (*t*, *J* = 12.4, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.05–1.8 (*m*, 2 CH_2). EI-MS: 456 (M^+).

*3-(3-Chlorophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C12**). Yield 28%. M.p. 240–242° (dec.). ^{1}H-NMR ((D_6)DMSO): 12.1–11.6 (br., NH); 10.0–9.3 (br., NH_2); 8.55 (*s*, 1 CH); 8.15–7.95 (*d*, *J* = 10.68, 1 CH); 7.75–7.65 (*m*, 1 CH); 7.6–7.35 (*m*, 3 CH); 7.25–7.0 (3*d*, *J* = 0.96, 2 CH); 4.2 (*t*, *J* = 11.46, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.1–1.7 (*m*, 2 CH_2). EI-MS: 412 (M^+).

*3-(2-Chlorophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C13**). Yield 25%. M.p. 231–235° (dec.). ^{1}H-NMR ((D_6)DMSO): 12.1–11.6 (br., NH); 10.0–9.5 (br., NH_2); 8.4 (*s*, CH); 8.1–7.9 (*d*, *J* = 8.76, 1 CH); 7.65–7.35 (*m*, 4 CH); 7.3–7.0 (3*d*, *J* = 1.24, 8.76, 2 CH); 4.2 (*t*, *J* = 10.75, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.1–1.7 (*m*, 2 CH_2). EI-MS: 412 (M^+).

*3-(3,4-Dichlorophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C14**). Yield 15%. M.p. >260°. ^{1}H-NMR ((D_6)DMSO): 12.1–11.55 (br., NH); 10.0–9.5 (br., NH_2); 8.6 (*s*, CH); 8.15–8.0 (*d*, *J* = 10.87, 1 CH); 7.95–7.85 (*d*, *J* = 1.15, 1 CH); 7.8–7.5 (3*d*, *J* = 10.01, 1.15, 2 CH); 7.3–7.0 (3*d*, *J* = 1.22, 10.87, 2 CH); 4.2 (*t*, *J* = 11.44, CH_2O); 3.9–3.0 (*m*, CH_2N); 2.05–1.8 (*m*, 2 CH_2). EI-MS: 446 (M^+).

*3-(2,4-Dichlorophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C15**). Yield 18%. M.p. 235–240° (dec.). ^{1}H-NMR ((D_6)DMSO): 12.1–11.6 (br., NH); 10.0–9.5 (br., NH_2); 8.4 (*s*, CH); 8.1–7.9 (*d*, *J* = 8.79, 1 CH); 7.8–7.7 (*d*, *J* = 1.21, 1 CH); 7.6–7.4 (3*d*, *J* = 11.94, 1.21, 2 CH); 7.2–7.0 (3*d*, *J* = 1.15, 8.79, 2 CH); 4.2 (*t*, CH_2O); 3.9–3.0 (*m*, 5 CH_2N); 2.1–1.7 (*m*, 2 CH_2). EI-MS: 446 (M^+).

*3-(4-Hydroxyphenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C16**). Compound **C1** (4.0 g, 8.3 mmol) was refluxed in 48% HBr (40 ml) for 2 h, then the brown soln. was refrigerated to 0° under stirring. The crystalline solid was collected, washed with a little amount of cold H_2O, then with acetone, and finally with Et_2O: **C16** (3.8 g, 6.8 mmol; 82%). White powder. M.p. >260°. ^{1}H-NMR ((D_6)DMSO): 10.8–9.7 (br., NH); 9.5–8.8 (br., NH_2); 8.35 (*s*, 1 CH); 8.1–7.95 (*d*, *J* = 10.15, 1 CH); 7.5–7.3 (*d*, *J* = 9.34, 2 CH); 7.2–7.0 (3*d*, *J* = 1.05, 10.15, 2 CH); 6.9–6.7 (*d*, *J* = 9.34, 2 CH); 4.15 (*t*, *J* = 10.31, CH_2O); 3.7–3.0 (*m*, 5 CH_2N); 2.0–1.7 (*m*, 2 CH_2). EI-MS: 394 (M^+).

*3-(4-Aminophenyl)-7-[4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**C17**). Compound **C3** (500 mg, 1 mmol) was suspended in H_2O (10 ml), heated to reflux and, under stirring, powdered Fe (3 g) was added portionwise. After 3 h, further Fe (3 g) was added, and reflux was continued for additional 3 h. The mixture was poured into H_2O (100 ml) and extracted with CH_2Cl_2 (2 × 100 ml). The org. layers were dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in the minimum amount of acetone and crystallized as the tris[hydrochloride] salt by adding a slight excess of 37% HCl, so obtaining **C17** as a crystalline powder. ^{1}H-NMR ((D_6)DMSO): 10.1–9.3 (br., NH, NH_2, NH_3); 8.5 (*s*, 1 CH); 8.1–7.95 (*d*, *J* = 9.13, 1 CH); 7.7–7.5 (*d*, *J* = 8.85, 2 CH), 7.35–7.0 (4*d*, *J* = 1.15, 8.85, 9.13, 5 CH); 4.2 (*t*, CH_2O); 3.9–3.1 (*m* + *t*, 5 CH_2N); 2.05–1.7 (*m*, 2 CH_2). EI-MS: 393 (M^+).

*3-Phenyl-7-{[4-(piperazin-1-yl)methyl]phenyl}methoxy]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**D1**). Compound **D1** was prepared according to the *General Procedure I*. Yield 38%. M.p. >260°. ^{1}H-NMR

((D$_6$)DMSO): 12.6–11.7 (br., 1 NH); 9.8–9.3 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.2–7.95 (*d*, 1 CH); 7.8–7.0 (*m*, 11 CH); 5.35 (*s*, ArCH_2O); 4.6–4.1 (br., ArCH_2N); 3.9–2.9 (*m*, 4 CH$_2$N). ESI-MS (pos. mode): 427 ([$M+1$]$^+$).

*7-[4-(4-Methylpiperazin-1-yl)butoxy]-3-phenyl-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**D2**). Compound **D2** was prepared according to the *General Procedure I*. Yield 48%. M.p. 240–245° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.7–11.4 (br., 2 NH); 8.45 (*s*, 1 CH); 8.15–7.95 (*d*, $J = 9.15$, 1 CH); 7.65–7.5 (2*d*, $J = 8.13$, 5 CH); 7.5–7.2 (*m*, 3 CH); 7.2–7.0 (3*d*, $J = 1.26, 0.15$, 2 CH); 4.15 (*t*, CH$_2$O); 3.9–3.0 (*m*, 5 CH$_2$N); 2.8 (*s*, Me); 2.05–1.7 (*m*, 2 CH$_2$). EI-MS: 392 (M^+).

*7-[4-(4-Acetylpiperazin-1-yl)butoxy]-3-phenyl-4*H*-1-benzopyran-4-one Hydrochloride* (**D3**). Compound **D3** was prepared according to the *General Procedure I*. Yield 55%. M.p. 242–245° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 11.0–10.3 (br., 1 NH); 8.5 (*s*, 1 CH); 8.15–8.0 (*d*, $J = 9.85$, 1 CH); 7.7–7.5 (2*d*, $J = 9.15$, 2 CH); 7.5–7.3 (*m*, 3 CH); 7.25–7.0 (3*d*, $J = 0.92, 9.85$, 2 CH); 4.5–4.3 (*d*, 1 H, CH$_2$N); 4.2 (*t*, CH$_2$O); 4.1–3.85 (*d*, 1 H, CH$_2$N); 3.7–2.7 (2*m*, 4 CH$_2$N); 2.05 (*s*, Me); 2.0–1.7 (*m*, 2 CH$_2$). ESI-MS (pos. mode): 421 ([$M+1$]$^+$).

*7-[4-Oxo-4-(piperazin-1-yl)butoxy]-3-phenyl-4*H*-1-benzopyran-4-one Hydrochloride* (**D4**). Compound **D4** was prepared according to the *General Procedure II*. Yield 28%. M.p. 258–260° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 9.65–9.25 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.1–7.95 (*d*, $J = 9.71$, 2 CH); 7.7–7.5 (2*d*, $J = 9.54$, 2 CH); 7.5–7.3 (*m*, 3 CH); 7.25–6.95 (3*d*, $J = 1.15, 9.71$, 2 CH); 4.15 (*t*, $J = 12.15$, CH$_2$O); 3.8–3.55 (*m*, 2 CH$_2$N); 3.2–2.9 (br., 2 CONCH$_2$); 2.55 (*t*, NCOCH$_2$); 2.1–1.85 (*m*, CH$_2$). EI-MS: 392 (M^+).

*3-Phenyl-7-(2-[4-[(piperazin-1-yl)carbonyl]phenoxy}ethoxy)-4*H*-1-benzopyran-4-one Hydrochloride* (**D5**). Compound **D5** was prepared according to the *General Procedure II*. Yield 15%. M.p. >260°. ^{1}H-NMR ((D$_6$)DMSO): 9.35–9.1 (br., NH$_2$); 8.5 (*s*, 1 CH); 8.15–7.95 (*d*, $J = 10.20$, 1 CH); 7.7–7.5 (2*d*, $J = 9.85$, 2 CH); 7.5–7.0 (*m*, 9 CH); 4.55–4.35 (*m*, 2 CH$_2$O); 3.85–3.6 (*m*, 2 CONCH$_2$); 3.2–3.0 (*m*, 2 CH$_2$N). EI-MS: 470 (M^+).

*3-(4-Chlorophenyl)-7-[4-(morpholin-4-yl)butoxy]-4*H*-1-benzopyran-4-one Hydrochloride* (**E1**). Compound **E1** was prepared according to the *General Procedure I*. Yield 35%. M.p. 235–237° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 11.9–11.4 (br., NH); 8.45 (*s*, 1 CH); 8.15–7.9 (*d*, $J = 11.37$, 1 CH); 7.7–7.3 (2*d*, $J = 9.75$, 4 CH), 7.2–7.0 (*m*, 2 CH); 4.1 (*t*, $J = 11.78$, CH$_2$O); 4.1–3.8 (*m*, CH$_2$O); 3.6–2.9 (*m*, 3 CH$_2$); 2.1–1.7 (*m*, 2 CH$_2$). EI-MS: 413 (M^+).

*3-(4-Chlorophenyl)-7-[4-oxo-4-(piperazin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Hydrochloride* (**E2**). Compound **E2** was prepared according to the *General Procedure II*. Yield 30%. M.p. 245–248° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 9.65–9.35 (br., NH$_2$); 8.55 (*s*, 1 CH); 8.1–7.95 (*d*, $J = 10.45$, 1 H); 7.7–7.4 (2*d*, $J = 9.90$, 4 CH); 7.25–7.0 (3*d*, $J = 1.15$, 10.45, 2 CH); 4.2 (*t*, $J = 12.05$, CH$_2$O); 3.8–3.6 (*m*, 2 CH$_2$N); 3.2–2.9 (br., 2 CONCH$_2$); 2.6 (*t*, NCOCH$_2$); 2.1–1.85 (*m*, CH$_2$). EI-MS: 426 (M^+).

*5-Hydroxy-3-(4-hydroxyphenyl)-7-[4-(piperidin-1-yl)butoxy]-4*H*-1-benzopyran-4-one Hydrobromide* (**F1**). Compound **F1** was obtained by demethylation of the corresponding MeO derivative (synthesized starting from biochanine, as described in *General Procedure I*) as described for **C16**. Yield 75%. M.p. 240–242° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 13.0 (*s*, OH); 9.7–9.4 (br., OH); 9.35–9.0 (br., NH); 8.4 (*s*, 1 CH), 7.5–7.3 (*d*, $J = 9.45$, 2 CH); 6.9–6.7 (*d*, $J = 9.45$, 2 CH); 6.65 (*d*, $J = 1.15$, 1 CH); 6.45 (*d*, $J = 1.15$, 1 CH); 4.1 (*t*, CH$_2$O); 3.6–2.7 (*m*, 3 CH$_2$N); 2.0–1.3 (*m*, 3 CH$_2$). EI-MS: 409 (M^+).

*5-Hydroxy-7-[4-oxo-4-(piperazin-1-yl)butoxy]-3-phenyl-4*H*-1-benzopyran-4-one Hydrochloride* (**F2**). Compound **F2** was prepared according to the *General Procedure II*. Yield 23%. M.p. 230–232° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.85 (*s*, OH); 9.6–9.2 (br., NH$_2$); 8.5 (*s*, 1 CH); 7.65–7.3 (*m*, 5 CH); 6.7 (*d*, $J = 0.98$, 1 CH); 6.40 (*d*, $J = 0.98$, 1 CH); 4.15 (*t*, $J = 11.95$, CH$_2$O); 3.85–3.55 (*m*, 2 CH$_2$N); 3.2–2.9 (*m*, 2 CONCH$_2$); 2.6 (*t*, NCOCH$_2$); 2.1–1.85 (*m*, CH$_2$). EI-MS: 408 (M^+).

*5-Hydroxy-3-(4-hydroxyphenyl)-7-{[6-(piperazin-1-yl)hexyl]oxy}-4*H*-1-benzopyran-4-one Bis[hydrobromide]* (**F3**). Compound **F3** was obtained by demethylation of the corresponding MeO derivative (synthesized starting from biochanine, as described in the *General Procedure I*) as described for **C16**. Yield 78%. M.p. >260°. ^{1}H-NMR ((D$_6$)DMSO): 12.9 (*s*, OH); 9.8–9.4 (br., OH); 9.3–8.8 (br., NH$_2$); 8.4 (*s*, 1 CH); 7.5–7.3 (*d*, $J = 8.8$, 2 CH); 6.9–6.7 (*d*, $J = 8.8$, 2 CH), 6.6 (*d*, $J = 1.12$, 1 CH); 6.35 (*d*, $J = 1.12$, 1 CH); 4.1 (*t*, CH$_2$O); 3.8–3.0 (*m*, 5 CH$_2$N); 1.9–1.6 (*m*, 2 CH$_2$); 1.6–1.3 (*m*, 2 CH$_2$). EI-MS: 438 (M^+).

Compounds **G1**–**G4** were prepared according to the *General Procedure I*.

*7-(1-Methylethoxy)3-[4-[4-(piperidin-1-yl)butoxy]phenyl]-4*H*-1-benzopyran-4-one Hydrochloride* (**G1**). Yield 30%. M.p. 220–225° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 10.7–10.3 (br., NH); 8.4 (*s*, 1 CH); 8.1–7.9 (*d*, $J = 10.68$, 1 CH); 7.6–7.4 (*d*, $J = 9.34$, 2 CH); 7.2–6.9 (3*d*, $J = 1.15$, 10.68, 4 CH); 5.0–4.7 (*m*, 1 CH); 4.05 (*t*, CH$_2$O); 3.5–3.25 (*m*, CH$_2$N); 3.15–2.7 (2*m*, 2 CH$_2$N); 2.05–1.2 (2*m* + *d*, $J = 6.51$, 12 H, CH$_2$N + Me). EI-MS: 435 (M^+).

*7-(1-Methylethoxy)-3-[4-[4-(piperazin-1-yl)butoxy]phenyl]-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**G2**). Yield 35%. M.p. 223–227° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., NH); 10.2–9.6 (br., NH$_2$); 8.45 (*s*, 1 CH); 8.1–7.9 (*d*, *J* = 10.45, 1 CH); 7.6–7.4 (*d*, *J* = 9.81, 2 CH); 7.2–6.8 (*m*, 4 CH), 5.0–4.7 (*m*, 1 CH); 4.05 (*t*, CH$_2$O); 3.9–2.7 (*m*, 5 CH$_2$N); 2.0–1.65 (*m*, 2 CH$_2$); 1.5–1.2 (*d*, *J* = 6.05, Me). EI-MS: 436 (M^+).

*7-(1-Methylethoxy)-3-[4-[4-(morpholin-4-yl)butoxy]phenyl]-4*H*-1-benzopyran-4-one* (**G3**). Yield 25%. M.p. 212–216° (dec.). ^{1}H-NMR (CDCl$_3$): 8.25–8.1 (*d*, *J* = 10.25, 1 CH); 7.4 (*s*, 1 CH); 7.55–7.4 (*d*, *J* = 10.45, 2 CH); 7.0–6.75 (3*d*, *J* = 1.25, 10.25, 4 CH); 4.75–4.5 (*m*, 1 CH); 4.0 (*t*, CH$_2$O); 3.8–3.6 (*m*, 2 CH$_2$O); 2.5–2.3 (*m*, 3 CH$_2$N); 2.0–1.55 (*m*, 2 CH$_2$); 1.5–1.3 (*d*, *J* = 5.98, 2 Me). EI-MS: 437 (M^+).

*7-(1-Methylethoxy)-3-(4-{[6-(piperazin-1-yl)hexyl]oxy}phenyl)-4*H*-1-benzopyran-4-one Bis[hydrochloride]* (**G4**). Yield 33%. M.p. 230–234° (dec.). ^{1}H-NMR ((D$_6$)DMSO): 12.3–11.4 (br., NH); 10.4–9.7 (br., NH$_2$); 8.35 (*s*, 1 CH); 8.1–7.9 (*d*, *J* = 11.89, 1 CH); 7.6–7.4 (*d*, *J* = 10.96, 2 CH); 7.2–6.8 (*m*, 4 CH); 4.95–4.65 (*m*, 1 CH); 4.0 (*t*, CH$_2$O); 3.9–2.9 (*m* + *t*, 5 CH$_2$N); 1.9–1.15 (2*m* + *d*, *J* = 6.07, 7 CH$_2$). EI-MS: 464 (M^+).

2. *Bone Resorption Assay.* Bone resorption was assayed by measuring the release of previously incorporated ^{45}Ca from fetal rat ulnae and radii [19]. Pregnant *Sprague-Dawley* rats were injected subcutaneously with 0.2 mCi ^{45}Ca on the 18th day of gestation (day of vaginal plug removal equals day 0 of pregnancy). On the following day, the animals were sacrificed, the fetuses removed, and the radius and ulna aseptically excised and dissected free of surrounding soft tissues.

To remove freely exchangeable ^{45}Ca, bones were pre-incubated for 24 h in 48-well polystyrene culture dishes (*Costar*, Cambridge, MA, USA) containing 500 µl of BGJ-B medium in an air-humidified incubator at 37° in 5% CO_2. Bones were then cultured for 5 d in BGJ-B medium containing 1 mg/ml bovine serum albumin in the presence of 5 nM bPTH 1–34, with or without a test compound at 10 µM. The medium was changed after 2 d. The experiment was terminated by washing the bones in 500 µl distilled H_2O and extracting the residual radioactivity with 500 µl of 10% TCA.

bPTH 1–34 was dissolved in BGJ-B medium. All the tested compounds were dissolved in DMSO and added to reach a total DMSO concentration of 0.1%. This amount of DMSO was added to control and stimulate cultures and did not affect resorption.

Calculation of Osteoclastic Resorption. The amount of mobilized mineral was determined by following the release of ^{45}Ca in the media. ^{45}Ca Release was determined as the percentage of initial radioactivity which was calculated as the sum of radioactivity in media obtained at days 2 and 5, as well as in the TCA-solubilized mineral fraction of the bones after culture.

The actual ^{45}Ca release into the medium was measured after correction for physicochemical exchange of radiolabelled mineralized matrix-bound ^{45}Ca with calcium present in the culture medium. Physicochemical exchange represents the amount of ^{45}Ca released into the medium that is not due to osteoclastic resorption. It was determined by culturing the bone explants killed by three cycles of freeze-thawing. This accounts for 5–8% of the total ^{45}Ca incorporated into the explants.

The percent inhibition (% Inh) of the bPTH-induced bone resorption by each tested compound was then calculated using the following formula:

$$\%\ \mathrm{Inh} = 100 \times (\%\ ^{45}\mathrm{Ca}^{\mathrm{PTH}} - \%\ ^{45}\mathrm{Ca}^{\mathrm{test}})/(\%\ ^{45}\mathrm{Ca}^{\mathrm{PTH}} - \%\ ^{45}\mathrm{Ca}^{\mathrm{control}})$$

where % ^{45}Ca$^{\mathrm{PTH}}$ is the % Ca released by bPTH, % ^{45}Ca$^{\mathrm{test}}$ is the % Ca released by bPHT in the presence of the tested compound, and % ^{45}Ca$^{\mathrm{control}}$ is the basal % ^{45}Ca released in the medium.

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