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Diphenyl Quinolines and Isoquinolines: Synthesis and Primary Biological Evaluation

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Abstract—The synthesis of a series of 35 substituted 3,4-diphenyl quinolines and isoquinolines is described. The majority of these molecules differ from all other triphenylethylene based antiestrogens by a different spatial location of the aminoalkyl side chain. The binding affinity of the most representative molecules (**8**, **9**, **19**, **20**, **21**, **23** and **25**), including analogues **8** and **21** without the side chain, for the estrogen receptor α (ER)[†] was determined. The ability of these molecules to induce the progesterone receptor was also studied. Antiproliferative activity was evaluated on MCF-7 human breast cancer cells, while intrinsic cytotoxic/cytostatic properties resulting from interaction with other targets than ER were assayed on L1210 murine leukemia cells. Introduction of an aminoalkylamino side chain at carbon 2 confers strong cytotoxic properties to diphenylquinolines **9** and **10** as well as pure antiestrogenic activities. However, cytotoxicity is so high with respect to antiestrogenicity that the latter was clearly observable only in one case (**9b**). The structure of compound **9b** was determined by X-ray crystallography. Molecular modeling of its docking within the hormone-binding domain of the receptor was subsequently undertaken. According to our results, the design of molecules with the side chain bound to the ethylene part of the triphenyl ethylene skeleton might generate compounds of potential pharmacological interest.

Introduction

Tamoxifen **1** is one of the most widely prescribed anticancer drugs (for reviews, see refs 1, 2). This triarylethylene compound, which elicits partial antiestrogenic activity, is today the drug of choice for palliative therapy of advanced breast cancer.[3,4] It is also an efficient adjuvant therapy for early stage disease.[1] Furthermore, clinical trials are in progress to evaluate the use of tamoxifen in chemoprevention for women at high risk of breast cancer.[5–7]

Unfortunately tamoxifen resistance can develop which limits its efficiency.[8] Side effects, including endometrial carcinoma, are also sometimes observed as a major adverse consequence of drug treatment.[9–11] In response to this situation, the search for antiestrogens without residual estrogenic activity has developed considerably over the last 10 years. In this context, we recently described the synthesis of a series of 3,4-diphenyl isoquinolines,[12] while Kihara and his group investigated a series of 3,4-diphenyl 1,2-dihydroisoquinolines **2**.[13,14]

Several studies have clearly shown that tamoxifen inhibits mammary tumor cell growth mainly through binding to their estrogen receptor α (ER). However, intrinsic

Abbreviations: ER, estrogen receptor α; HBD, hormone binding domain; E_2, estradiol; DES, diethylstilbestrol; PgR, progesterone receptor; OH-Tam, 4-hydroxytamoxifen; NMR, nuclear magnetic resonance.

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[†]Two forms of ER have been identified (α and β). The present paper refers to ERα since experiments were conducted with MCF-7 cells which are known to solely express high amounts of this receptor form.

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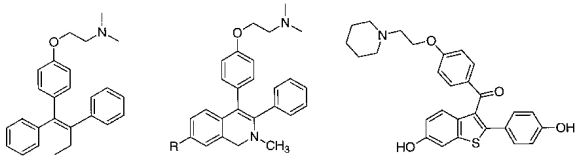
toxic properties, detected in both ER-positive and ER-negative tumors, may also contribute to the antitumor potency of the drug. Indeed, interaction with targets of lower binding affinity may lead to cell death (cytotoxic effect).[15] Estrogen secretion and/or administration abrogate ER-mediated effects of tamoxifen while maintaining fully its cytotoxicity.

The basic aminoalkyl side chain of tamoxifen (i.e., $-OCH_2CH_2N(Me)_2$) plays a major role in its anti-estrogenic/antitumoral activity. Indeed, analogues in which this side chain is absent do not inhibit the growth of mammary tumor cells in monolayer culture. Recent structural studies concerning the interaction between ER and 4-hydroxytamoxifen (OH-Tam), a metabolite showing a ~100-fold higher binding affinity for ER, provide the first explanation for this phenomenon.[16] Transcriptional activation of the receptor is mediated by separate regions ('activation functions' AF-1 and AF-2), of which AF-2 is solely responsive to ligand binding.[17] Estrogenic ligands trigger AF-2 expression, while OH-Tam does not, although as recently revealed by crystal structure analysis and mutants selection, they bind at the same site within the core of the hormone binding domain (HBD).[16,18–20] Data relative to both the unliganded and the estradiol (E_2)/diethylstilbestrol (DES) saturated HBD indicate that estrogenic hormones induce a dramatic repositioning of the HBD helix 12, leading to an AF-2 surface appropriate for specific interactions with co-activators.[19] OH-Tam[16] (as well as the antiestrogen raloxifene **3**[18]) impedes such a displacement of helix 12, maintaining the receptor in an inactive status.

The key residues Glu-353, Arg-394 and Hist-524 in the HBD are essential for the binding of E_2, DES and the estrogenic stilbene and stilbene-like core of OH-Tam and raloxifene.[18,19] Specific interactions of the aminoalkyl side chain of these antiestrogens with residues in a channel located at the entrance of the HBD (Asp-351, Thr-347, Ala-350 and Trp-383) have also been established.[16] Certain of these contacts, including the interaction of Asp-351 with the nitrogen atom of the terminal dimethylamino group, are thought to be responsible for the antiestrogen properties. Since the orientation of this side chain within this channel is governed mainly by its presence at a specific position on the triphenylethylene core, it may be anticipated that its attachment at any other position may induce a dramatic change in the endocrinological profile. This hypothesis is analyzed in the present study.



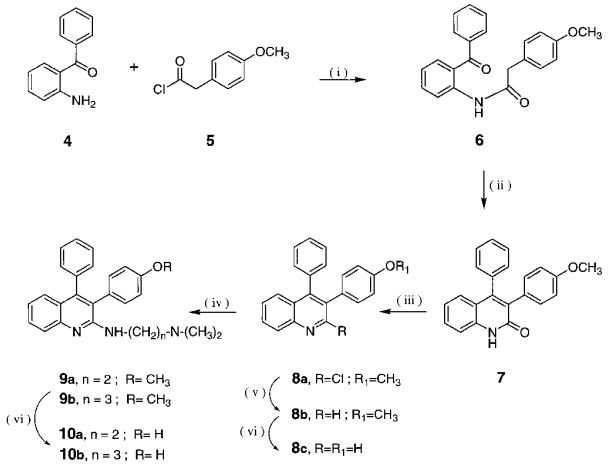
1 2 3

Herein, we describe the preparation and evaluation of a series of 35 substituted 3,4-diphenyl quinolines and isoquinolines. The majority of these molecules can be distinguished from other triphenylethylenic antiestrogens by a different spatial location of their aminoalkyl side chain in respect to the aromatic part of the molecule (i.e., this chain is situated on the nitrogen heterocycle bearing the ethylene group, rather than on one of the phenyl groups). As a consequence, it is expected that upon binding to ER they would confer an alternate structural conformation to the HBD. The potential influence of such a change on the endocrinological/antitumoral activity was assessed. The binding affinity for ER of compounds **9a**, **9b**, **19a**, **19c**, **19d**, **19g**, **19h**, **20c, 20e**, **23** and **25b**, as well as compounds **8b**, **8c** and **21a–c** without the crucial aminoalkyl side chain, was determined. The capacity to activate ER (i.e. induction of progesterone receptor), as well as antiproliferative activity of these compounds on MCF-7 breast cancer cells, was also analyzed. Potential cytotoxic activity resulting from interaction with targets other than ER was evaluated on L1210 cells.

Chemistry

3,4-Diphenyl quinoline. As shown in Scheme 1, *N*-acylation of 2-amino-benzophenone **4** through reaction with *p*-methoxyphenylacetyl chloride **5** provided compound **6** in 80% yield. Cyclization of this intermediate under alkaline conditions gave diaryl quinolone **7**,[21] which was converted to the chloro derivative **8a** by reaction with phosphorus oxychloride. By refluxing **8a** with an excess of the appropriate diamine, the substituted diaryl quinolines **9a** and **9b** were obtained. *O*-Demethylation to the phenol derivatives **10a**,**b** was achieved by refluxing **9a**,**b** in HBr for 6 h under a nitrogen atmosphere. Reductive dechlorination of **8a** (Zn, acetic acid, H_2O, reflux) afforded **8b**, which was subsequently demethylated to **8c**.

3,4-Diaryl isoquinolines. The synthesis of the mono- and dimethoxy1-1-chloro-3,4-diarylisoquinolines **18a–c** was previously described.[12] In essentially the same manner, the preparation of trimethoxy derivative **18d** was achieved

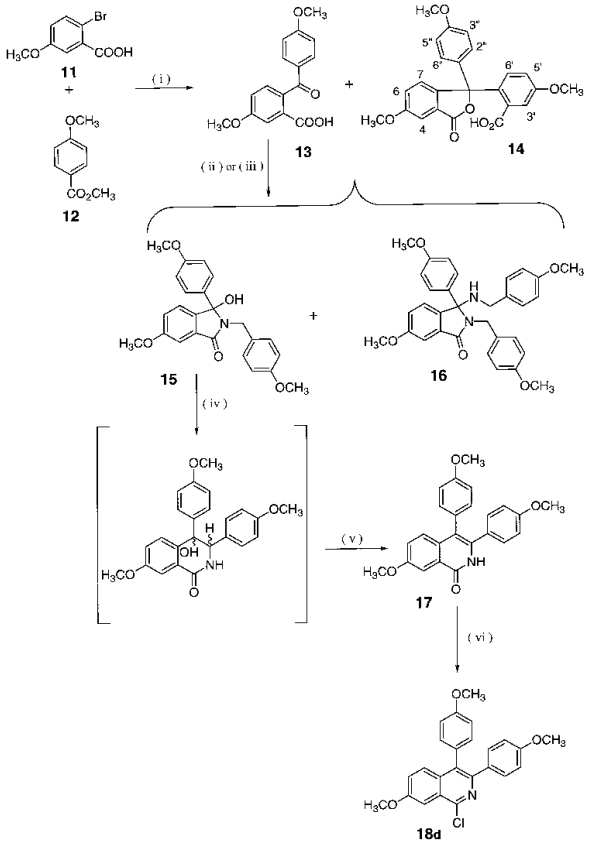


Scheme 1. Reagents and conditions: (i) CH_2Cl_2, 0 °C, 3 h; (ii) C_2H_5-OH, KOH, reflux; (iii) $POCl_3$; (iv) diamine, reflux; (v) AcOH, Zn, 75 °C, 1 h; (vi) HBr, reflux.

(Scheme 2). Thus, the metallation of 2-bromo-5-methoxybenzoic acid **11** using two molar equivalents of *n*-butyllithium at −100 °C followed by condensation with 4-methoxymethyl benzoate **12** afforded 2-(4-methoxybenzoyl)-5-methoxybenzoic acid **13**.[22] Low temperature was an important factor in this reaction since at more elevated temperatures self-condensation occured,[23] leading after acidic work up to a mixture of **13** and phthalate **14**, which are difficult to separate.

Under classical amide forming conditions, the acid chloride obtained from **13** ($SOCl_2$ at room temperature) was reacted with *p*-methoxybenzylamine to afford a mixture of the expected compound *N*-(4-methoxybenzyl)-1-hydroxy-1-(4-methoxyphenyl)-isoindol-3-one **15** and 6-methoxy-2-(4-methoxybenzyl)-3-(4-methoxyphenyl)-3-(4-methoxybenzylamino)-2,3-dihydro-isoindol-1-one **16** in moderate yield (21 and 22%, respectively).

A more synthetically useful yield of **15** (89%) was obtained when the transformation was carried out using diphenyl phosphoryl azide (DPPA) in DMF at 0 °C.[24] As described earlier,[12] the phthalimide salt generated by the reaction of **15** with 4 equiv of lithium di-isopropylamide in THF at room temperature underwent pyrrole ring opening followed by subsequent closure to a mixture of *cis*/*trans* 3,4-diaryl-tetrahydro-4-hydroxy-2*H*-isoquinolin-1-ones. These isomeric products were directly dehydrated (refluxing formic acid, 0.5 h) to isoquinolin-1-one **17**. Compound **17** was converted to the chloro derivative **18d**.

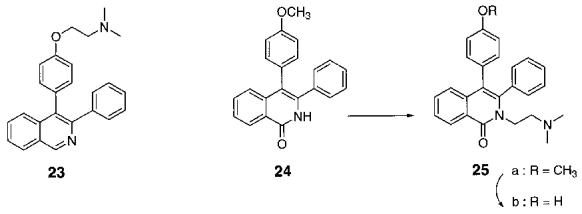


Scheme 2. Reagents and conditions: (i) *n*-BuLi, 2 equiv THF, −100 °C; (ii) CH_2Cl_2, $SOCl_2$, 4-methoxybenzylamine; (iii) DMF, 4-methoxybenzylamine, DPPA, $(C_2H_5)_3N$, −20 °C, 6 h; (iv) THF, LDA 4 equiv, −78 °C; (v) HCO_2H, reflux, 0.5 h; (vi) $POCl_3$, reflux, 3 h.

Conversion of chloro compounds **18a–d** to products **19a–i** was achieved by refluxing with an excess of the required diamine. Subsequent reductive dechlorination of chloroisoquinolines **18a–d** using Zn in warm acetic acid gave the diaryl isoquinolines **21a–d**. Demethylation of **19c–i** and **21b–d** was accomplished as for **9a**,**b** affording **20c–i** and **22b–d**. (Scheme 3).

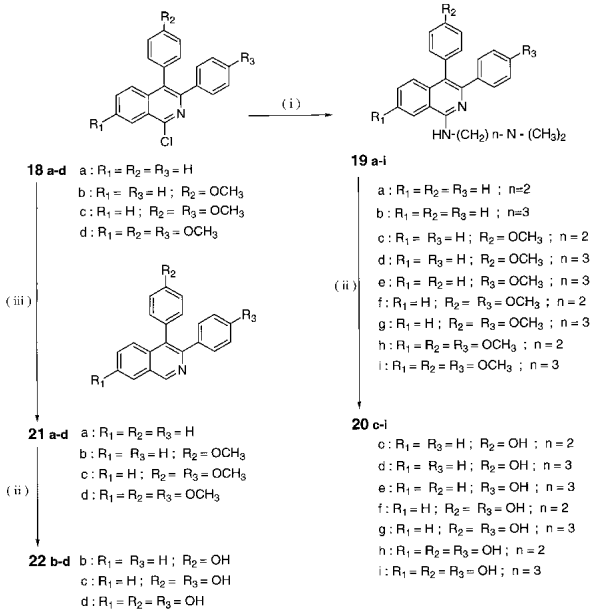
Compound **23**, bearing the tamoxifen side chain, was obtained by *O*-alkylation of **22b** with 2-dimethylaminoethyl chloride.[25]

Condensation of diphenyl isoquinolin-1-one **24** with dimethylaminoethylamine in DMF, in the presence of K_2CO_3, gave **25a**, which was demethylated under classical conditions to **25b**.



Biological evaluation

Binding affinity for ER. At high concentration (10 μM), the methoxy substituted compounds **8b**, **9b**, **19h** and **21b,c** as well as the chloroisoquinoline **21a** produced a significant (30–70%) inhibition of $[^3H]E_2$ (0.005 μM) binding to cytosolic ER from MCF-7 cells. At lower concentrations, they were without effect, suggesting a



Scheme 3. Reagents and conditions: (i) diamine, reflux; (ii) HBr, reflux; (iii) Zn, AcOH/H_2O, reflux.

weak binding affinity. All the other compounds investigated (see Table 1), and in particular those with the corresponding free OH group, failed to display any binding ability. The positioning of the oxygen substituent on the phenyl group attached at position 3 of the quinoline (or isoquinoline) nucleus, which is essentially opposite to that for the hydroxyl group in typical antiestrogens, is most probably at the origin of this difference in activity. Indeed, the 4-OH group in OH-Tam occupies effectively the same space as the 3-OH group in E_2, whereas the OCH_3 groups in our molecules correlate best with the 17β-OH in E_2. In E_2, it is well established that transfer of the acidic 3-OH proton to the protein plays an important role in binding.

Estrogenic/antiestrogenic activity

The influence of these representative compounds on ER and PgR levels from MCF-7 cells was assessed. The weak binding affinity of compounds **8b** and **21a–c** for ER was reflected by their ability, at 1 μM, to decrease its concentration (down-regulation) and to induce PgR.[26] Compound **8c**, devoid of significant binding affinity for ER (15% inhibition of [^{3}H]E_2 binding at 10 μM), behaved similarly. At 0.1 μM, **8b**, **21a** and **21c** still induced PgR (36, 41 and 18% of optimal induction produced by E_2, respectively), but at 0.01 μM they were ineffective.

Compounds **9b** and **19h**, which also weakly bind to ER, decreased its level without any induction of PgR. Such behavior suggested an antiestrogenic activity. Interestingly, compound **19g** behaved similarly, while it failed to significantly bind to ER (22% inhibition of [^{3}H]E_2 binding at 10 μM). These observations are also summarized in Table 1. For additional comparison, note that at 0.1 μM OH-Tam invariably eliminated ER and induced PgR by ~50% of the optimal potency of E_2.

Table 1. Influence of 1 μM of compounds on ER and PgR concentration in MCF-7 cells

Compound	ER % of controls	PgR[a] % of optimal induction produced by E_2
E_2 (0.01 μM)	0	100
OH-Tam (0.1 μM)	0	50
8b	18	56[1]
8c	6	61[1]
9a	47	1[2]
9b	48	0[2]
19a	90	0[2]
19c	134	2[3]
19d	107	1[3]
19g	40	2[3]
19h	45	0[3]
20c	114	1[3]
20e	123	4[3]
21a	17	93[3]
21b	27	60[4]
23	116	65
25b	104	0[5]

[a]5 independent experiments. PgR induction by E_2 (% of controls) : 1 ≈ 301 ; 2 ≈ 792 ; 3 ≈ 540 ; 4 ≈ 520 ; 5 ≈ 205.

The weak estrogenic activity of several of these compounds was reflected by their ability to stimulate MCF-7 cell growth at 1 μM (% of 0.01 μM E_2: **8b** = 102; **8c** = 85; **21a** = 73; **21b** = 92; **21c** = 82). At 10 μM this stimulatory effect was either largely diminished or totally absent. With the exception of **9b**, which inhibited growth by 40%, none of the other compounds displayed any significant activity at 1 μM, while only a few cells were still adhering to the culture dishes at 10 μM, due to a high cytotoxicity (see below).

The tamoxifen analogue **23** increased PgR concentration without affecting ER, which distinguishes its behavior from that of the parent compound (see above). It stimulated the growth of MCF-7 cells at 1 μM (40% of optimal stimulatory effect of E_2), but displayed an IC_{50} of 10 μM. It should therefore be considered as a weak antiestrogen with residual estrogenic properties. Evaluated in parallel, OH-Tam (0.01 μM) displayed a conventional inhibitory potency (50% growth inhibition).

Antiproliferative activity

The cytostatic and/or cytotoxic properties of these molecules were further evaluated on two cell lines: the L1210 murine leukemia, which can be considered as a good model for exploring the intrinsic toxicity of the drugs, and the MCF-7 human mammary carcinoma, which might give some specific response to molecules interfering with the estrogen dependent growth process. The IC_{50} (drug concentration inhibiting cell growth by 50%) data are presented in Table 2.

In the quinoline series (compounds **8**–**10**), the majority of the tested substances had an IC_{50} in the micromolar range (4 to 10 μM). Introduction of an aminoalkyl side chain as well as replacing the OCH_3 group by a free OH residue did not significantly improve either the growth inhibition or the cytotoxic properties. Even **9b**, which appeared to have a slight binding affinity for ER and some antiestrogenic properties, elicited a moderate growth inhibiting effect on MCF-7 cells. No significant difference was observed for the effect on L1210 and MCF-7 lines excepted for compounds **8b** and **8c**, which have, respectively, a 2 and 3 times higher IC_{50} on L1210 than on MCF-7.

In the isoquinoline series (compounds **19**–**22**), those molecules without the aminoalkylamino side chain (**21a–c** and **22b**,**c**) were weakly cytostatic in the L1210 assay, with IC_{50}s ranging from about 25 μM to more than 100 μM, and relatively highly cytotoxic toward MCF-7 cells. However, in this study, at low doses (0.1 to 1 μM), compounds **21b** and **21c**, and to a lesser extent **21a** and **22b**, increased significantly the growth of this cell line (data not shown), thus acting as effective estrogenic molecules.

Introduction of a dimethylaminoalkylamino side chain at the C1 position of the isoquinoline ring generally led to an increase in the cytotoxic properties in both cell lines with IC_{50} ranging from 2 to 10 μM. The trihydoxy derivatives **20h,i** were exceptions to this trend, having IC_{50}s

Table 2. Cytotoxicity on L1210 murine leukemia and on MCF-7 human mammary carcinoma cells in vitro. Results are expressed as IC_{50} in μmol

Ref.	L1210	MCF-7
8b	10.9	5.6
8c	17	4.3
9a	10	16.6
9b	4.1	7.1
10a	nt[a]	8
10b	5	6.5
19a	3.6	4.3
19b	2.7	3.1
19c	5.1	3
19d	2.4	3.1
19e	2	2.4
19f	2.3	2.4
19g	2.5	2.4
19h	nt	6.4
19i	nt	5.9
20c	7	3.4
20d	6.2	3.2
20e	4.2	6
20f	9.1	7.5
20g	3.5	7.4
20h	110	37
20i	140	33
21a	> 100	9
21b	26	8
21c	33	6
22b	35	9.7
22c	> 100	31.5
22d	nt	82
23	nt	9.5
25b	nt	> 100
Mitomycin	0.04	nt
OH-Tam	nt	0.08
Tamoxifen	4.5	7.5 (4 in ref 31)

[a]nt: not tested.

between 30 and 40 μM for MCF-7 cells. Surprisingly, in this series, the hydroxy derivatives appeared to be less toxic than the corresponding methoxylated compounds.

Antiestrogenic profile of 9b

The antiestrogen activity observed for compound **9b** led us to characterize it more fully. As shown in Table 3, at 1 μM, it produced a weak growth inhibition of MCF-7 cells. This effect was partly suppressed by 0.01 μM E_2. At the same concentration tamoxifen induced a significant cytostatic activity which was inhibited by the hormone. At 10 μM, **9b** appeared more cytotoxic than tamoxifen, since its growth inhibition could not be antagonized by E_2. In RTx6 cells (a tamoxifen resistant clone from MCF-7 cells[27]), compound **9b** had a growth inhibiting profile comparable to that observed with MCF-7 cells. On the other hand, tamoxifen appeared less effective, confirming the higher toxicity of **9b**. A low binding affinity of **9b** may explain this behavior.

Structure of compound 9b

The X-ray crystal structure for **9b** revealed that the two phenyl rings at C3 and C4 are respectively tilted by 110 and 105 degrees with respect to the quinoline system. The chain substituted at C2 is disordered, existing predominantly in two possible six-membered ring 'chair forms', generated by a folding of the C24, C25, C26 atoms to permit H-bonding between the H atom of N23 with N27. This chair type six-membered ring structure is nearly co-planar to the quinoline ring (dihedral angle of 20° and 7.8° for the two forms of the chain respectively; Figure 1). This configuration appears to be a general characteristic of the three-carbon chain since the ^1H NMR signal for their NH is shifted slightly downfield (0.5 to 0.8 ppm) with respect to the two-carbon chain.

Starting from the recently published X-ray structures of ER co-crystallized with either OH-Tam[16] or raloxifene,[18] we carried out docking experiments of **9b** within the HBD of the receptor. Comparison of both raloxifene and OH-Tam/ER structures shows how the HBD pocket might accommodate the ligand. The raloxifene/ER complex structure appeared more appropriate for docking **9b** because of its larger binding cavity. On this basis, molecular modeling of the docking of **9b** within the receptor pocket was carried out using the protein core of the raloxifene/ER complex.

Whereas superposition of the side chains of **9b** and raloxifene (tail–tail orientation; Figure 2(a)) led to a high affinity complex, superposition of the triphenyl ethylene skeleton (head–tail orientation; Figure 2(b)) resulted in far higher binding energies ($\Delta E \approx 21$ kcal/mol). Indeed, the more stable complexes in the head–tail orientation have the amino side chain in a hydrophobic pocket. In contrast, the tail–tail orientation places the polar side chain toward the outside of the cavity. It is therefore unambiguous that the tail–tail orientation is far more accurate. From this result, it is suggested that a biological effect might be observed for a three–carbon side chain. The lower growth inhibition potency of the two–carbon analogue **9a** supports this view.

From these data we can conclude that **9b** fits the receptor pocket of ER. However, the OH group position of OH-

Table 3. Comparison of growth inhibitory potency of **9b** and tamoxifen (% of control growth in the absence or presence of 0.01 μM estradiol[a,b])

	MCF-7 cells				RTx6 cells			
	9b	+ E_2	Tamoxifen	+ E_2	**9b**	+ E_2	Tamoxifen	+ E_2
1 μM	73	77b	36	73	82	75	66	92
10 μM	4	5	9	29	27	9	29	28

[a]Assessment of DNA amounts after 120 h of culture,[32] culture performed in quadruplicate (μg DNA in controls, MCF-7 = 35.9±1.7, RTx6 = 21.5±2.4).
[b]Values after corrections for stimulatory effect of E_2.

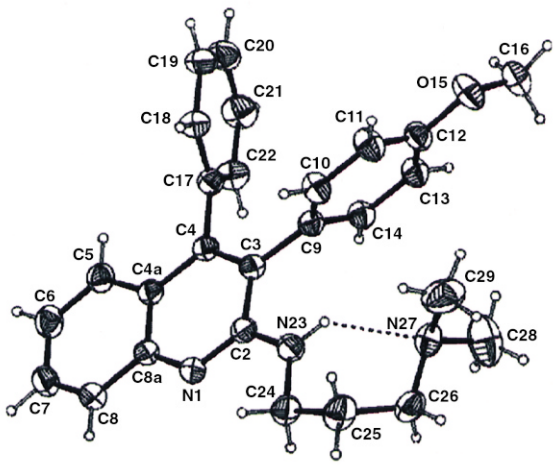


Figure 1. Computer-drawn ellipsoid representation of compound **9b** from X-ray data. The benzene solvent molecule linking two molecules in the cell was omitted.

Tam is opposite to that of the OCH_3 of **9b**. This fact may explain the low affinity of **9b** for ER as well as its antiestrogenic property, free of residual estrogen activity. This hypothesis is partly confirmed by the lack of difference in the binding affinity of **9b** and its corresponding OH analogue **10b** (data not shown).

Discussion

Although they bind to ER with a very low binding affinity, diphenyl quinolines and isoquinolines which incorporate the triphenyl ethylene structural motif can interfere with estrogen dependent physiological processes. In the absence of aminoalkylamine substitution, biologically active compounds of this study display weak estrogenic properties. Introduction of an aminoalkylamino side chain appears to confer strong cytotoxicity, as well as pure antiestrogenic properties. However, cytotoxicity is so high that antiestrogenicity was detectable in only one case (compound **9b**). This compound appears more toxic than tamoxifen, possibly due to its lower binding affinity to ER. Interestingly, it failed to overcome resistance to tamoxifen (RTx6 cells), indicating that this phenomenon is relevant to (a) target(s) non-related to ER dependent systemic toxicity. On the other hand, compound **23**, bearing an aminoalkyl side chain at the same position as tamoxifen, displayed an antiestrogenic activity (growth inhibition of MCF-7 cells) without total loss of estrogenic activity (PgR induction). This behavior is reminiscent of that of conventional antiestrogens (tamoxifen, nafoxidine, raloxifene). Thus, positioning of the side chain at the ethylene part of the triphenyl ethylene skeleton might be a convenient way to attain antiestrogenic molecules, provided that the potency issue can by addressed.

Modeling of the binding of OH-Tam and raloxifene to ER revealed a specific orientation of their side chain with respect to a channel located at the entrance of the HBD.[16,18] According to our data, this channel would also accept the side chain of **9b**, although the latter is attached on the opposite side of the triphenyl ethylene-like core of the molecule. Hence, interaction of the amine function of this side chain with (a) residue(s) of the channel (i.e., Asp 351[18]) would compensate for the absence of the phenyl ring usually bearing this side chain. Association with ER and thereby estrogen antagonism would, however, be dramatically affected. Hydrophobic substituents linked at position 11β of E_2, which more or less mimic this benzene ring, are indeed known to strongly increase binding affinity to ER.[28,29] Although the observed decrease of binding affinity may hamper the design of 'reversed' antiestrogens such as **9b**, one may assume that the introduction of small hydrophobic substituent in their side chain may overcome the present deficiency. E_2 derivatives in which the 11β or 7α hydrogen (corresponding position for **9b**) is substituted by a hydrophobic group or the side chain of tamoxifen are, indeed, almost equivalent in terms of binding to ER.[30] Such reversed antiestrogens may differ from those presently available in terms of cytotoxicity, metabolism and clearance. Therefore, one may suggest that linkage of a side chain at the non-aromatic part of the ethyl-triphenyl-ethylene skeleton may generate compounds of potential pharmacological interest. Efforts in this direction are currently being made.

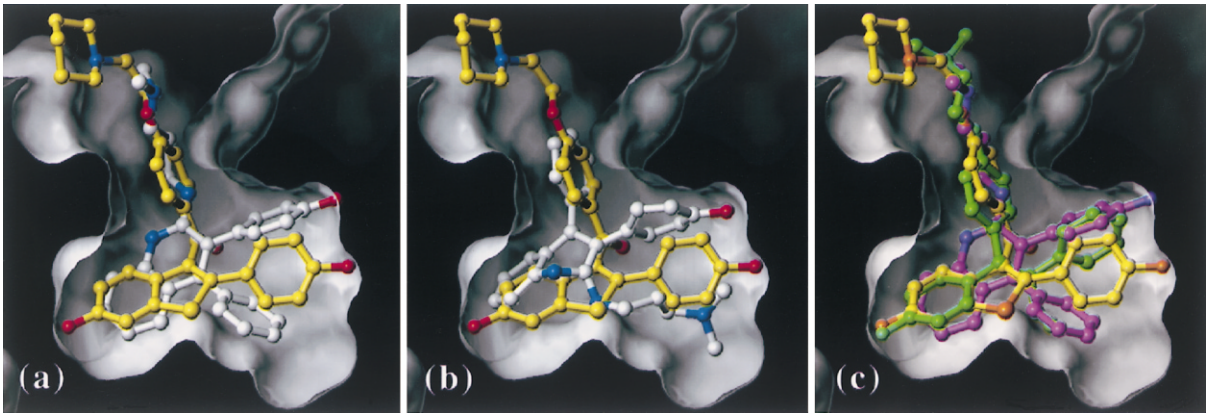


Figure 2. Superposition of raloxifene (yellow) and **9b** (gray) within the HBD domain of ER: (a) best docking hit in the tail–tail orientation and (b) best docking hit in the head–tail orientation. Panel c shows the superposition of raloxifene (yellow), OH-Tam (green) and **9b** (purple, tail–tail orientation), under their lowest energy of interaction.

Experimental

Chemistry

^1H and ^{13}C NMR spectra were performed on Bruker 200 or 400 AC spectrometers operating at respectively 200 or 400 MHz (^1H) and 50 or 100 MHz (^{13}C). Chemical shifts are reported in δ (ppm) with the following abbreviations: singlet (s), doublet (d), doublet doublet (dd), triplet (t), multiplet (m), br s (broad singlet); Ph refers to phenyl rings at positions 3 and 4 of the heterocyclic rings. Melting points were determined on Reichert hot stage microscope and are uncorrected. Microanalytical results (combustion analysis) were obtained from CNRS, Institut des substances naturelles, Gif sur Yvette; values for the mentioned elements are within ±0.4% of the theoretical values.

***N*-(2-Benzoylphenyl)-2-(4-methoxyphenyl)-acetamide (6).** To a solution of 2-aminobenzophenone (4.0 g, 20 mmol) in CH_2Cl_2 (100 mL), 4-methoxyphenyl acetyl chloride (4.4 g, 24 mmol) was added dropwise at 0 °C with stirring. The reaction mixture was allowed to warm to rt, stirred for 3 h and quenched with a saturated $NaHCO_3$ solution. After partitioning the organic layer was dried over $MgSO_4$ and evaporated to a crude residue (5.6 g, 80%) which was used directly in the next step. An analytical sample was purified by column chromatography on silica gel eluting with CH_2Cl_2 to give pure **6** as yellow oil. ^1H NMR (DMSO-d_6) δ 10.26 (s, 1H, NH); 7.72–7.47 (m, 8H); 7.42 (dd, 1H, J = 1. 5, 7.61 Hz, H3); 7.34 (m, 1H, H4); 7.07 (d, 2H, J = 8.7 Hz, H2′ H6′); 6.84 (d, 2H, H3′ H5′); 3.74 (s, 3H, OCH_3); 3.41 (s, 2H, CH_2).

3-(4-Methoxyphenyl)-4-phenyl-1*H*-quinolin-2-one (7). A mixture of **6** (5.1 g, 15 mmol) and KOH (2.5 g, 44 mmol) in absolute ethanol (150 mL) was refluxed for 1 h. On cooling, the resultant precipitate was collected by filtration, washed with further ethanol and dried. Recrystallization from ethanol afforded pale yellow needles (4.3 g, 89%), mp > 320 °C. ^1H NMR (DMSO-d_6) δ 12.07 (s, 1H, NH); 7.57–7.44 (m, 1H, H8 or H5); 7.44–7.13 (m, 4H); 7.2–7.08 (m, 4H) ; 7.04 (d, 2H, J = 8.6 Hz, H2 H6 Ph3); 6.73 (d, 2H, H3 H5 Ph3); 3.70 (s, 3H, OCH_3). Anal. ($C_{22}H_{17}NO_2 + 1/4\ C_2H_5OH$) C, H, N.

5-Methoxy-2-(4-methoxybenzoyl)-benzoic acid (13). Lithium *O*-lithiobenzoate was prepared from 2-bromo-5-methoxybenzoic acid **11** (5.78 g, 25 mmol) in dry THF (100 mL) by addition of *n*-BuLi (25 mL of 2 M solution in cyclohexane, 50 mmol). The mixture was protected under nitrogen and the temperature was controlled by a liquid nitrogen–diethyl ether bath maintained below −95 °C over 1 h. 4-Methoxymethylbenzoate (4.15 g, 25 mmol) in THF (50 mL) was then added dropwise, the mixture stirred for 2 h whereupon it was warmed to rt. The reaction was quenched on pouring into 5% aqueous HCl (100 mL) and extracted with CH_2Cl_2. The organic layer was washed with H_2O and extracted with 10% NaOH solution. The aqueous layer was then acidified, extracted with CH_2Cl_2 and the organic layer dried ($MgSO_4$) before evaporation in vacuo. Crystallization from toluene gave **13** as a colorless powder (3 g, 43%); mp 179 °C; ^1H NMR ($CDCl_3$) δ 7.71 (d, 2H, J = 8.6 Hz, H2′ H6′); 7.55 (d, 1H, J = 2, 4 Hz, H6); 7.32 (d, 1H, J = 8.6 Hz, H3); 7.13 (dd, 1H, J = 2.4, 8.4 Hz, H4); 6.89 (d, 2H, J = 8.6 Hz, H3′ H5′); 3.90 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3). Anal. ($C_{16}H_{14}O_5$) C, H, O.

Where the temperature was allowed to rise above −90° self-condensation led to another product, namely 5-methoxy-2-[5-methoxy-1-(4-methoxyphenyl)-3-oxo-1,3-dihydro-isobenzofuran-1-yl]-benzoic acid (**14**) as colorless powder, mp 188 °C (toluene); ^1H NMR ($CDCl_3$) δ 7.59 (d, 1H, J = 8.5 Hz, H7); 7.35 (d, 1H, J = 2.2 Hz, H3′); 7.27–7.18 (m, 3H, H4 H4′ H5′); 7.11 (s, 1H, H1); 7.13 (d, 2H, J = 8.5 Hz, H2″ H6″); 6.90 (q, 1H, J = 2.6, 8.8 Hz, H6); 6.78 (d, 2H, H3″ H5″); 3.86 (s, 3H, OCH_3 5); 3.82 (s, 3H, OCH_3 5′); 3.73 (s, 3H, OCH_3 4″). Anal. ($C_{24}\ H_{20}O_7$) C, H.

3-Hydroxy-6-methoxy-2-(4-methoxybenzyl)-3-(4-methoxyphenyl)-2,3-dihydro-isoindol-1-one (15). To a solution of acid **13** (5.7 g, 20 mmol) and 4-methoxybenzylamine (3.28 g, 24 mmol) in dry DMF (200 mL) at 0 °C under nitrogen, freshly distilled DPPA (12.65 g, 46 mmol, 10 mL) was added. The reaction mixture was cooled to −20° under stirring over 10 min, when thiethylamine (10 mL, 7.2 g, 70 mmol) was added dropwise. After 6 h the reaction was warmed to rt and DMF removed in vacuo. Water was added and the mixture extracted with CH_2Cl_2, which was then washed with water, dried and evaporated. Crystallization from toluene gave **15** as colorless microcrystals (6.9 g, 89%); mp 174 °C. ^1H NMR ($CDCl_3$) δ 7.16 (m, 6H, H4 H7, H2′ H6′, H2 H6 benzyl); 6.95 (dd, 1H, J = 2.4, 8 Hz, H5); 6.76 (d, 2H, J = 8.9 Hz, H3′ H5′); 6.66 (d, 2H, J = 8.6 Hz, H3 H5 benzyl); 4.65 (d, 1H, CH_2); 4.02 (d, 1H, J = 14.8 Hz, CH_2); 3.80 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.69 (s, 3H, OCH_3); 3.07 (s, 1H, OH). Anal. ($C_{24}H_{23}NO_5$) C, H, N.

When the method previously described[12] was employed, **15** was obtained (21%) and a second compound which was isolated by chromatography on silica gel, eluting with CH_2Cl_2 and identified as 6-methoxy-2-(4-methoxybenzyl) - 3 - (4 - methoxybenzylamino) - 3 - (4 - methoxyphenyl)-2,3-dihydroisoindol-1-one (**16**) (22%). Crystallization from toluene gave colorless microcrystals of mp 128 °C. ^1H NMR ($CDCl_3$) δ 7.38 (d, 1H, J = 2.2 Hz, H7); 7.07 (d, 1H, J = 8.5 Hz, H4); 6.96 (dd, 1H, J = 2.2, 8.5 Hz, H5); 7.32 (d, 2H, J = 8.5 Hz, H2 H6 phenyl); 7.27 (d, 2H, J = 8.3 Hz, H2 H6 benzyl); 6.73 (d, 2H, J = 8.3 Hz, H2 H6 benzylamine); 6.81 (d, 2H, J = 8.5 Hz, H3 H5 phenyl); 6.75 (d, 2H, J = 8.5 Hz, H3 H5 benzyl); 6.69 (d, 2H, J = 8.5 Hz, H3 H5 benzylamine); 5.07 (d, 1H, J = 15 Hz, CH_2 phenyl) 3.80 (d, 1H, J = 15 Hz, CH_2 benzyl); 3.84 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); 3.74 (s, 6H, $2OCH_3$); 2.84 (t, 1H, J = 12 Hz and 9 Hz, CH_2 benzylamine); 2.70 (d, 1H, J = 12 Hz, CH_2 benzylamine); 1.92 (d,1H, J = 9 Hz, NH). ^{13}C NMR ($CDCl_3$) δ 168 (C=O), 45 (CH_2 benzylamine), 42 (CH_2 benzyl). Anal. ($C_{24}H_{23}NO_5$) C, H, N.

7-Methoxy-3,4-bis(4-methoxyphenyl)-2*H*-isoquinolin-1-one (17). A solution of LDA mono tetrahydrofuran (6.6 mL, 1.5 M in cyclohexane, 9.8 mmol) was added dropwise to a stirred solution of **15** (1.0 g, 2.47 mmol) in dry THF (30 mL) at −78 °C under an argon atmosphere,

a highly blue color appearing immediately. The mixture was allowed to warm to rt and left stirring overnight, whereupon it was quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$ before solvent removal in vacuo. The resultant residue was dehydrated on refluxing in formic acid over 30 min. After cooling, the mixture was evaporated under reduced pressure. After partitioning between H_2O and CH_2Cl_2, the organic layer was washed with H_2O, dried and evaporated. The resultant crude quinolone **17** was crystallized from toluene as colorless needles (480 mg, 50%), mp 233 °C. ^{1}H NMR ($CDCl_3$) δ 9.14 (br s, 1H, NH); 7.83 (d, 1H, J = 2.4 Hz, H8); 7.30–7.95 (m, 6H); 6.82 (d, 2H, J = 8.4 Hz, H3 H5 Ph3); 6.73 (d, 2H, J = 8.7 Hz, H3′ H5′ Ph4); 3.91 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3). Anal. ($C_{24}H_{21}NO_4$) C, H, N.

1-Chloro-5-methoxy-3,4-bis(4-methoxyphenyl)-isoquinoline (18d). A mixture of **17** (2.38 g, 6.2 mmol) in $POCl_3$ (20 mL) was refluxed for 3 h. After cooling, excess $POCl_3$ was evaporated in vacuo, and the residue poured into ice-water to which was added a saturated aqueous K_2CO_3 solution. The mixture was extracted with CH_2Cl_2, the organic layer washed twice with H_2O, dried and evaporated. The crude chloro compound was purified by chromatography on silica gel (eluting with CH_2Cl_2) and crystallized from cyclohexane as colorless needles (2.1 g, 84%), mp 138 °C. ^{1}H NMR ($CDCl_3$) δ 7.57 (m, 2H, H5 H8); 7.29 (d, 2H, J = 8.9 Hz, H2 H6 Ph3); 7.26 (m, 1H, H6); 7.12 (d, 2H, J = 8.8 Hz, H2 H6 Ph4) 6.91 (d, 2H, J = 8.8 Hz, H3′ H5′ Ph4); 6.73 (d, 2H, J = 8.9 Hz, H3 H5 Ph3); 3.99 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3). Anal. ($C_{24}H_{20}$ Cl NO_3) C, H, N.

2-Chloro-3-(4-methoxyphenyl)-4-phenylquinoline (8a). Using the procedure described above, **8a** was obtained from quinolone **7** as pale yellow needles (74%), mp 199 °C. ^{1}H NMR ($CDCl_3$) δ 8.10 (1H, J = 8 Hz, H8); 7.72 (m, 1H, H7); 7.49 (m, 2H, H6 H5); 7.26–7.08 (m, 5H Ph4); 7.02 (d, 2H, J = 8.8 Hz, H2 H6 Ph3); 6.75 (d, 2H, H3 H5 Ph3); 3.96 (s, 3H, OCH_3). Anal. ($C_{22}H_{16}ClNO$) C, H, N.

General procedure for amino substituted quinolines and isoquinolines (9a,b, 19a–i). A mixture of the relevant chloro derivative (1.5 mmol) and the appropriate amine (15 mL, large excess) was refluxed under nitrogen for 24 h. Evaporation of the diamine under reduced pressure provided a residue which was recrystallized from either ethanol or methanol. Trimethoxy derivatives were prepared in an autoclave at 160 °C over 3 days and the residues obtained after the evaporation of the diamine were purified by chromatography on alumina and recrystallized from cyclohexane. Physical data are reported in Table 4.

General procedure for diaryl quinoline and isoquinolines (8b and 21a–d). The relevant chloro compounds, **8a** and **18a–d** (1.5 mmol), were dissolved in a mixture of acetic acid (20 mL) and water (1.7 mL) and heated to 75°, when powdered zinc (6 mmol) was added After 1 h the reaction was quenched on addition of water and the mixture made basic on addition of aqueous NaOH (40% w/v) This solution was then extracted with CH_2Cl_2 and the organic layer washed with H_2O, dried ($MgSO_4$), evaporated and the residue purified by chromatography. Physical data are reported in Table 5.

General procedure for demethylation. The appropriate ethers **8b**, **9a,b**, **19c–i** and **21b,c** (1.5 mmol) were refluxed in HBr (10 mL) under nitrogen for 6 h (24 h for the trimethoxy derivatives) and, after cooling, the mixture was basified with NH_4OH and the crude products purified by recrystallization from ethanol or methanol. Physical data are reported in Table 6.

4-[4-(2-Dimethylaminoethoxy)phenyl]-3-phenylisoquinoline (23). Compound **22b** (0.8 g, 2.7 mmol) was gradually added with stirring to a suspension of NaH, freshly washed with hexane (0.105 mg as 60% w/w mineral oil dispersion, 3.8 mmol) in DMF (5 mL) at rt and allowed to stand for 1.5 h. 2-Chloroethyldimethylamine (0.32 g, 3 mmol) (obtained by treatment of the corresponding hydrochloride with 10% NaOH solution) was then added and, following 2 h stirring at 100 °C, the reaction mixture was cooled and evaporated under reduced pressure. The resultant residue was extracted with CH_2Cl_2 and the organic layer washed with 2N HCl. The aqueous layer was adjusted to pH 10 on addition of a 10% NaOH solution and extracted with CH_2Cl_2, dried, evaporated and purified by chromatography (Al_2O_3) eluting with CH_2Cl_2. Compound **23** was obtained as white needles on recrystallization from ethanol (200 mg, 20%), mp 115 °C. ^{1}H NMR ($CDCl_3$) δ 9.36 (s, 1H, H1); 8.08 (m, 1H, H8); 7.72 (m, 1H, H5); 7 62 (m, 2H, H6 H7); 7.39 (m, 2H, H2 H6 Ph3); 7.22 (m, 3H, H3 H5 Ph3); 7.15 (d, 2H, J = 8.7 Hz, H2 H6 Ph4); 6.93 (d, 2H, H3 H5 Ph4); 4.11 (t, 2H, O-CH_2); 2.77 (t, 2H, N-CH_2); 2.37 (s, 6H, $N(CH_3)_2$). Anal. ($C_{25}H_{24}N_2O$) C, H, N.

2-(2-Dimethylaminoethyl)-4-(4-hydroxyphenyl)-3-phenylisoquinolin-1-one (25b). 4-(4-Methoxyphenyl)-3-phenylisoquinolin-1-one **24** (1.0 g, 3 mmol) was dissolved in dry DMF (36 mL) at 60 °C. After cooling to rt the mixture was sequentially treated with potassium carbonate (5.0 g, 36 mmol) and diethylaminoethylamine dropwise (0.45 g, 35 mmol), obtained from alkali treatment of diethylaminoethylamine hydrochloride. The reaction mixture was stirred at 40 °C over 24 h, whereupon it was cooled, filtered and DMF removed under reduced pressure. The residue was extracted with CH_2Cl_2 and washed with water, dried and evaporated. The solid was recrystallized from ethanol to yield **25a** as colorless needles (0,6 g, 50%), mp 179 °C. ^{1}H NMR ($CDCl_3$) δ 8.55 (m, 1H, H8); 7.51 (m, 2H, H6 H7); 7.30–7.20 (m, 3H, H6, H2 H6 Ph3); 7.21–7.14 (m, 3H, H5, H3 H5 Ph3); 6.97 (d, 2H, J = 8.4 Hz, H2 H6 Ph4); 6.73 (d, 2H, H3 H5 Ph4); 4.04 (m, 2H, $CH_2\alpha$); 3.75 (s, 3H, OCH_3); 2.52 (m, 2H, $CH_2\beta$); 2.07 (s, 6H, N-$(CH_3)_2$). Anal. ($C_{26}H_{26}N_2O_2$) C, H, N.

Demethylation was performed as described above and yielded the hydrobromide of hydroxy compound **25b** (yield 30%) as a colorless powder from methanol, mp 261 °C. ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1H, HBr); 9.32 (br s, 1H, OH); 8.41 (m, 1H, H8); 7.85–7.55 (m, 1H, H6 H7); 7.38 (s, 5H, Ph3); 7.15 (m, 1H, H5); 6.92 (d, 1H,

Table 4. Physical data for methoxy derivatives **9a**,**b**, **19a–i**

Compound no.	R_1	R_2	R_3	*n*	Formula[a]	Yield (%)	Mp (°C) Solvent	NMR δ_H ($CDCl_3$)
9a	H	H	OCH_3	2	$C_{26}H_{27}N_3O$	87	139 Methanol	7.78 (m, 1H, H8); 7.50 (m, 1H, H6); 7.31–7.17 (m, 4H, H5, H7, H2H6 Ph4); 7.12–7.05 (m, 3H, H3H4H5 Ph4); 7.04 (d, 2H, J = 8.6 Hz, H2H6 Ph3); 6.76 (d, 2H, H3H5 Ph3); 5.06 (t, 1H, NH); 3.74 (s, 3H, OCH_3); 3.64 (q, 2H, J = 6.3 and 11.6 Hz, $CH_2\alpha$); 2.47 (t, 2H, J = 6.3 Hz, $CH_2\beta$); 2.19 (s, 6H, $N(CH_3)_2$).
9b	H	H	OCH_3	3	$C_{27}H_{29}N_3O$	73	137 Ethanol	7.76 (m, 1H, J = 8.2 Hz, H8); 7.48 (m, 1H, J = 1.5 Hz, 6.8 Hz, H6); 7.25–7.17 (m, 4H, H5H7, H2H6 Ph4); 7.10 (m, 5H, H3H4H5 Ph4, H2H6 Ph3); 6.76 (d, 2H, J = 8.75 Hz, H3H5 Ph3); 5.83 (t, 1H, NH); 3.74 (s, 3H, OCH_3), 3.63 (m, 2H, $CH_2\alpha$); 2.29 (t, 2H, J = 6.3 Hz, $CH_2\gamma$); 1.97 (s, 6H, $N(CH_3)_2$); 1.72 (m, 2H, $CH_2\beta$).
19a	H	H	H	2	$C_{25}H_{25}N_3$	72	119 Methanol	7.90 (m, 1H, H8); 7.59–7.12 (m, 13H); 6.10 (t, 1H, NH); 3.77 (q, 2H, J = 10.5 and 6.3 Hz, $CH_2\alpha$); 2.67 (t, 2H, J = 6.3 Hz, $CH_2\beta$); 2.33 (s, 6H, $N(CH_3)_2$).
19b	H	H	H	3	$C_{26}H_{27}N_3$	82	160	7.77 (m, 1H, H8); 7.51–7.12 (m, 14H, HAr, NH); 3.79 (m, 2H, $CH_2\alpha$); 2.56 (t, 2H, J = 6 Hz, $CH_2\gamma$); 2.36 (s, 6H, $N(CH_3)_2$); 1.91 (m, 2H, $CH_2\beta$).
19c	H	OCH_3	H	2	$C_{26}H_{27}N_3O$	91	164 Ethanol	7.89 (m, 1H, H8); 7.60–7.40 (m, 5H, H5H6H7, H2H6 Ph3); 7.20–7.08 (m, 5H, H3H4H5 Ph3, H2H6 Ph4); 6.88 (d, 2H, J = 8.7 Hz, H2H6 Ph4); 6.05 (t, 1H, NH); 3.82 (s, 3H, OCH_3); 3.76 (m, 2H, $CH_2\alpha$); 2.66 (t, 2H, J = 5.9 Hz, $CH_2\beta$); 2.32 (s, 6H, $N(CH_3)_2$).
19d	H	OCH_3	H	3	$C_{27}H_{29}N_3O$	78	158 Ethanol	7.75 (m, 1H, H8); 7.54–7.37 (m, 6H, H5H6H7, H2H6 Ph3, NH); 7.21–7.08 (m, 5H, H3H4H5 Ph3, H2H6 Ph4); 6.86 (d, 2H, J = 8.7 Hz, H3H5 Ph4); 3.82 (s, 3H, OCH_3); 3.80 (m, 2H, $CH_2\alpha$); 2.55 (t, 2H, J = 6 Hz, $CH_2\gamma$); 2.35 (s,6H, $N(CH_3)_2$); 1.90 (m, 2H, $CH_2\beta$).
19e	H	H	OCH_3	3	$C_{27}H_{29}N_3O$	87	154 Methanol	7.74 (m, 1H, H8); 7.52–7.15 (m, 11H, 10 HAr, NH); 6.68 (m, 2H, J = 8.8 Hz, H3H5 Ph3); 3.79 (m, 2H, $CH_2\alpha$) ,3.74 (s, 3H, OCH_3); 2.55 (t, 2H, $CH_2\gamma$); 2.35 (s, 6H, $N(CH_3)_2$); 1.90 (m, 2H, $CH_2\beta$).
19f	H	OCH3	OCH3	2	$C_{27}H_{29}N_3O_2$	78	162 Methanol	7.89 (m, 1H, H8); 7.50–7.40 (m, 5H, H7H6H5, H2H6 Ph3); 7.13 (d, 2H, J = 8.7 Hz, H2H6 Ph4); 6.90 (d, 2H, H3H5 Ph4); 6.72 (d, 2H, J = 8.8 Hz, H3H5 Ph3); 6.09 (m, 1H, NH); 3.84 (s, 3H, OCH_3); 3.79 (m, 2H, $CH_2\alpha$); 3.76 (s, 3H, OCH_3); 2.78 (t, 2H, J = 6 Hz, $CH_2\beta$); 2.33 (s, 6H, $N(CH_3)_2$).
19g	H	OCH_3	OCH_3	3	$C_{28}H_{31}N_3O_2$	77	142 Methanol	7.73 (m, 1H, H8); 7.50–7.34 (m, 6H, H5H6H7, H2H6 Ph3, NH); 7.12 (d, 2H, J = 8.6 Hz, H2H6 Ph4); 6.88 (d, 2H, H3H5 Ph4); 6.70 (d, 2H, J = 8.7 Hz, H3H5 Ph3); 3.83 (s, 3H, OCH_3); 3.79 (m, 2H, $CH_2\alpha$); 3.75 (s, 3H, OCH_3); 2.55 (t, 2H, J = 6 Hz, $CH_2\gamma$); 2.35 (s, 6H, $N(CH_3)_2$); 1.90 (m, 2H, $CH_2\beta$).
19h	OCH_3	OCH_3	OCH_3	2	$C_{28}H_{31}N_3O_3$	65	168 Cyclohexane	7.57 (d, 1H, J = 9 Hz, H5); 7.47 (d, 2H, J = 8.9 Hz, H2H6 Ph3); 7.27 (m, 4H, H8H6, H2H6 Ph4); 6.98 (d, 2H, J = 8.7 Hz, H3H5 Ph4); 6.80 (d, 2H, H3H5 Ph3); 5.91 (t, 1H, NH); 4.04 (s, 3H, OCH_3); 3.93 (s, 3H, OCH_3); 3.89 (m, 2H, $CH_2\alpha$); 3.85 (s, 3H, OCH_3); 2.77 (t, 2H, J = 5.8 Hz, $CH_2\beta$); 2.42 (s, 6H, $N(CH_3)_2$).
19i	OCH_3	OCH_3	OCH_3	3	$C_{29}H_{33}N_3O_3$	64	138 Cyclohexane	7.65 (m, 1H, H8); 7.48 (t, 1H, NH); 7.35–7.19 (m, 4H, H5H6, H2H6 Ph3); 7.10 (d, 2H, J = 8.2 Hz, H2H6 Ph4); 6.97 (d, 2H, H3H5 Ph4); 6.75 (d, 2H, J = 8.8 Hz, H3H5 Ph3); 3.94 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 3.73 (s, 3H, OCH_3); 3.55 (m, 2H, $CH_2\alpha$); 2.39 (m, 2H, $CH_2\gamma$); 2.20 (s, 6H, $N(CH_3)_2$); 1.90 (m, 2H, $CH_2\beta$).

[a]Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values.

J = 8.4 Hz, H2 H6 Ph4); 6.65 (d, 1H, H3 H5 Ph4); 4.10 (t, 2H, $CH_2\alpha$); 3.22 (t, 2H, $CH_2\beta$); 2.75 (s, 6H, $N(CH_3)_2$). Anal. ($C_{25}H_{24}N_2O_2$; HBr) Br.

Biological studies

MCF-7 cells used for receptor assays and growth stimulation were from the Michigan Cancer Foundation (Detroit, MI). They were maintained in monolayer culture at 37 °C in Earle's base MEM containing 10% heat inactivated (56 °C, 1 h) fetal calf serum (FCS).

Tamoxifen resistant RTx6 cells (from Dr. J. C. Faye INSERM U 168, Toulouse, France) were maintained under the same conditions in RPMI 1640 in the presence of 1 μM tamoxifen, which was removed before each experiment.

Table 5. Physical data for derivatives **8b, 21a–c**

Compound no.	R_1	R_2	R_3	Formula[a]	Yield (%)	Mp (°C) Solvent	NMR δ_H ($CDCl_3$)
8b	H	H	OCH_3	$C_{22}H_{17}NO$	62	162	8.98 (s,1H, H2); 8.17 (m, 1H, H8); 7.74–7.65 (m, 2H, H5H6); 7.46 (m, 1H, H7); 7.41–7.15 (m, 5H, Ph4); 7.10 (d, 2H, $J=8.9$ Hz, H2H6 Ph3); 6.77 (d, 2H, H3H5 Ph3); 3.77 (s, 3H, OCH_3).
21a	H	H	H	$C_{22}H_{17}N$	45	155	G. Berti[33]
21b	H	OCH_3	H	$C_{22}H_{17}NO$	40	123	9.34 (s, 1H, H1); 8.03 (m, 1H, H8); 7.75–7.52 (m, 3H, H5H6 H7); 7.42–7.32 (m, 2H, H2H6 Ph3); 7.28–7.18 (m, 3H, H3H4H5 Ph3) 7.15 (d, 2H, $J=8.8$ Hz, H2H6 Ph4); 6.90 (d, 2H, H3H5Ph4); 3.83 (s, 3H, OCH_3).
21c	H	OCH_3	OCH_3	$C_{23}H_{19}NO$	39	167	9.31 (s, 1H, H1); 8.00 (m, 1H, H8); 7.72–7.53 (m, 3H, H5H6H7); 7.32 (d, 2H, $J=8.9$ Hz, H2H6 Ph3); 7.16 (d, 2H, $J=8.7$ Hz, H2H6 Ph4); 6.92 (d, 2H, H3H5 Ph4); 6.75 (d, 2H, H3H5 Ph3); 3.85 (s, 3H, OCH_3 Ph4); 3.77 (s, 3H, OCH_3 Ph3).
21d	OCH_3	OCH_3	OCH_3	$C_{23}H_{19}NO$	53	158	9.02 (s, 1H, H1); 7.58 (d, 1H, $J=8$ Hz, H8); 7.35–7.20 (m, 4H, H5H6, H2H6 Ph3); 7.14 (d, 2H, $J=8.7$ Hz, H2H6 Ph4); 6.91 (d, 2H, H3H5 Ph4); 6.74 (d, 2H, $J=8.9$ Hz, H3H5 Ph3); 3.96 (s, 3H, OCH_3 7); 2.34 (s, 3H, OCH_3 Ph4); 2.26 (s, 3H, OCH_3 Ph3).

[a]Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values.

Cells were cultured at 37 °C in a water-jacketed CO_2 incubator (5% CO_2) with L-glutamine, penicillin, streptomycin and gentamicin at the usual concentrations (all materials from Gibco, Life Technologies, Cergy-Pontoise, France).

Measurement of binding affinity of compounds for ER, modulation of ER and PgR levels as well as growth stimulation were performed according to experimental protocols previously described.[26,32,34]

Measurement of binding affinity for ER

Cytosol of MCF-7 cells was overnight incubated at 0–4 °C with 5 nM [^{3}H]E_2 in the absence or presence of increasing amounts of either unlabeled E_2 (control) or the investigated compound (1 nM to 10 μM). Unbound ligands were then removed by a dextran-coated charcoal (DCC) treatment and the residual radioactivity measured by scintillation counting.

Effect on ER and PgR levels[26]

Measurements were carried out on cytosols from MCF-7 cells incubated for 3 days in the absence (control) or presence of increasing amounts (range 0.01 to 1 μM) of the investigated compound.

Cytosolic ER levels were measured by Scatchard plot analysis according to the procedure established by the EORTC Receptor Group[35] using [^{3}H]E_2 as the labeling agent. Progesterone receptors (PgR) were measured according to this procedure using [^{3}H]ORG-2058 as labeled ligand.

Effect on cell growth

Effect of the investigated compounds on breast cancer cell lines was assessed after 120 h of culture.[32] Cells were plated in 25 mm Petri dishes (MCF-7: 2×10^4 cells/mL; RTx6: 4×10^4 cells/mL) in 10% DCC treated serum (DCC-FCS in MEM or RPMI). After 24 h, tested compound was added to the medium and 48 h later the medium was replaced. Cells were harvested 72 h later and their growth evaluated by measuring their DNA content by the diphenyl amine method. Each culture was performed in quadruplicate.

Cytotoxicity (measurement of IC_{50} values) was determined in L1210 and MCF-7 cells by an investigator (DC) ignorant of the growth data established on MCF-7 cells by another investigator (GL). Murine leukemia L1210 cells (ATCC-CCL 219) were cultivated in Dulbecco's MEM supplemented with 10% FCS. Cells were seeded at 10^5 cells/mL in 1-mL multiwell plates. After 24 h (usually 3 to 4×10^5 cells/mL), tested compounds were added in duplicate at various concentrations and the plates incubated for 24 h before counting with a Coulter-Counter ZM (Coultronics Inc.). The dose inhibiting the growth by 50% (IC_{50}) was extrapolated from regression curves obtained with experimental points free of significant toxicity. MCF-7 cells (ATCC HTB 22) were cultivated in the same medium. Cells were seeded in 1-mL multiwell plates (2×10^5 cells/well). After 24 h, tested compounds were added in triplicate at various concentrations and cells were incubated for 7 days. Cell density was then determined using the MTT assay.[36] Absorbance at 540 nm was measured with a Bio-Rad microplate reader (Model 450). Survival was expressed as % of untreated controls and IC_{50} was extrapolated from regression curves obtained from experimental points.

X-ray crystal structure determination of 9b. Colorless crystal ($0.13\times0.16\times0.53$ mm) (benzene): $C_{27}H_{29}N_3O$, 0.5 C_6H_6, $M_w=450.59$, triclinic system, space group P-1, $Z=2$, $a=9.971$ (6), $b=10.213$ (8), $c=13.643$ (12) Å, $\alpha=106.94$ (6), $\beta=89.04$ (5), $\gamma=103.42$ (4)°, $V=1290.8$ Å^3, $d_c=1.16$ g cm^{-3}, $F(000)=482$, λ (Cu K_α) = 1.5418 Å, $\mu=0.55$ mm^{-1}; 4913 intensities measured on a Nonius CAD-4 diffractometer ($-11\leqslant h\leqslant 11$, $-12\leqslant k\leqslant 11$, l: 0–16) of which 4694 unique (Rint = 0.022) and 3681 observed with $Fo=4\sigma(Fo)$. The structure was solved by direct methods using SHELXS-86[37] and refined by full-matrix least squares based upon a unique Fo^2 with SHELXL-93.[38] The *N*,*N*-dimethyldiamine chain substituent at C2 was found to be disordered, existing in

Table 6. Physical data for hydroxy compounds

Compound no.	R_1	R_2	R_3	*n*	Formula[a]	Yield (%)	Mp (°C) Solvent	NMR δ_H (DMSO)
8c	H	H	OH	—	$C_{21}H_{15}NO$	63	239	9.53 (br s, 1H, OH); 8.96 (s, 1H, H2); 8.14 (m, 1H, H8); 7.80 (m, 1H, H6); 7.65–7.50 (m, 2H, H5H7); 7.51–7.20 (m, 5H, Ph4); 7.05 (d, 2H, *J* = 8.6 Hz, H2H6 Ph3); 6.68 (d, 2H, H3H5 Ph3).
10a	H	H	OH	2	$C_{25}H_{25}N_3O$ HBr	55	235	9.46 (s, 1H, OH); 7.7 (m, 1H, H8); 7.58 (m, 1H, H6); 7.35–7.22 (m, 3H, H5, H2H6 Ph4); 7.17–7.07 (m, 4H, H7, H3H4H5 Ph4); 6.95 (d, 2H, *J* = 8.4 Hz, H2H6 Ph3); 6.68 (d, 2H, H3H5 Ph3); 6.00 (t, 1H, NH); 3.77 (m, 2H, $CH_2\alpha$); 2.95 (s, 6H, $N(CH_3)_2$); 2.53 (m, 2H, $CH_2\beta$).
10b	H	H	OH	3	$C_{26}H_{27}N_3O$ HBr	64	203	9.41 (s, 1H, OH); 7.63 (m, 1H, *J* = 8.2 Hz, H8); 7.50 (m, 1H, H6); 7.38–7.20 (m, 3H, H5, H2H6 Ph4); 7.20-7.00 (m, 4H, H7, H3H4H5 Ph4); 6.93 (d, 2H, *J* = 8.6 Hz, H2H6 Ph3); 6.67 (d, 2H, H3H5 Ph3); 6.27 (t, 1H, NH); 3.52 (m, 2H, $CH_2\alpha$); 2.53 (s, 6H, $N(CH_3)_2$); 2.30 (t, 2H, $CH_2\gamma$); 1.66 (m, 2H, $CH_2\beta$).
20c	H	OH	H	2	$C_{26}H_{25}N_3O$ HBr	65	263	10.09 (br s, 1H, HBr); 9.48 (s, 1H, OH); 8.39 (m, 1H, H8); 7.97 (t, 1H, NH); 7.75–7.55 (m, 2H, H5H6); 7.52–7.35 (m, 3H, H7, H2H6 Ph3); 7.32–7.08 (m, 3H, H3H4H5 Ph3); 6.97 (d, 2H, *J* = 8.4 Hz, H2H6 Ph4); 6.79 (d, 2H, H3H5 Ph4); 3.93 (m, 2H, $CH_2\alpha$); 3.46 (t, 2H, $CH_2\beta$); 2.83 (s, 6H, $N(CH_3)_2$).
20d	H	OH	H	3	$C_{26}H_{27}N_3O$ HBr	70	261	9.46 (s, 1H, OH); 8.32 (m, 1H, H8); 7.78 (t, 1H, NH); 7.70–7.55 (m, 2H, H5H6); 7.52-7.37 (m, 3H, H7, H2H6 Ph3); 7.36–7.20 (m, 3H, H3H4H5 Ph3); 6.97 (d, 2H, *J* = 8.4 Hz, H2H6 Ph4); 6.77 (d, 2H, H3H5 Ph4); 3.66 (m, 2H, $CH_2\alpha$); 3.09 (m, 2H, $CH_2\gamma$); 2.58 (s, 6H, $N(CH_3)_2$); 2.06 (m, 2H, $CH_2\beta$).
20e	H	H	OH	3	$C_{26}H_{27}N_3O$ HBr	57	272	9.96 (br s, 1H, HBr); 9.48 (s, 1H, OH); 8.33 (m, 1H, *J* = 7.7 Hz, H8); 7.85 (t, 1H, NH); 7.70–7.15 (m, 10H); 6.61 (d, 2H, *J* = 8.6 Hz, H3H5 Ph3); 3.70 (m, 2H, $CH_2\alpha$); 3.16 (t, 2H, $CH_2\gamma$); 2.61 (s, 6H, $N(CH_3)_2$); 2.10 (m, 2H, $CH_2\beta$).
20f	H	OH	OH	2	$C_{25}H_{25}N_3O_2$ HBr	75	257	9.40 (br s, 2H, OHPh3, Ph4); 8.24 (m, 1H, *J* = 7.6 Hz, H8); 7.60–7.32 (m, 4H, H5H6H7, NH); 7.23 (d, 2H, *J* = 8.6 Hz, H2H6 Ph3); 6.96 (d, 2H, *J* = 8.4 Hz, H2H6 Ph4); 6.80 (d, 2H, H3H5 Ph4); 6.57 (d, 2H, H3H5 Ph3); 3.69 (m, 2H, $CH_2\alpha$); 2.62 (m, 2H, $CH_2\beta$); 2.26 (s, 6H, $N(CH_3)_2$).
20g	H	OH	OH	3	$C_{26}H_{27}N_3O_2$ HBr	70	296	10.07 (br s, 1H, HBr); 9.48 (s, 1H, OH Ph4); 9.46 (s, 1H, OH Ph3); 8.33 (m, 1H, *J* = 7.8 Hz, H8); 7.82 (t, 1H, NH); 7.70–7.40 (m, 3H, H5H6H7); 7.23 (d, 2H, *J* = 8.6 Hz, H2H6 Ph3); 6.96 (d, 2H, *J* = 8.2 Hz, H2H6 Ph4); 6.80 (d, 2H, H3H5 Ph4); 6.64 (d, 2H, H3H5 Ph3); 3.70 (m, 2H, $CH_2\alpha$); 3.15 (t, 2H, $CH_2\gamma$); 2.59 (s, 6H, $N(CH_3)_2$); 2.08 (m, 2H, $CH_2\beta$).
20h	OH	OH	OH	2	$C_{25}H_{25}N_3O_3$	50	274	10.72 (br s, 1H, HBr); 9.93 (s, 1H, OH7); 9.43 (s, 1H, OH Ph4); 9.39 (s, 1H, OH Ph3); 7.60 (t, 1H, NH); 7.52 (m, 1H, H8); 7.38-7.12 (m, 4H, H5H6, H2H6 Ph3); 6.93 (d, 2H, *J* = 8.2 Hz, H2H6 Ph4); 6.77 (d, 2H, H3H5 Ph4); 6.62 (d, 2H, *J* = 8.5 Hz, H3H5 Ph3); 3.84 (m, H, $CH_2\alpha$); 3.37 (m 2H, $CH_2\beta$); 2.74 (s, 6H, $N(CH_3)_2$).
20i	OH	OH	OH	3	$C_{26}H_{27}N_3O_3$	39	248	12.04 (br s, 1H, HBr); 10.59 (br s, 1H, OH7); 9.64 (br s, 2H, OH Ph4, Ph3); 9.35 (br s, 1H, NH); 7.97 (m, 1H, H8); 7.55–7.31 (m, 2H, H5H6); 7.15 (d, 2H, *J* = 8.6 Hz, H2H6 Ph3); 6.94 (d, 2H, *J* = 8.5 Hz, H2H6 Ph4); 6.77 (d, 2H, H3H5 Ph4); 6.72 (d, 2H, H3H5 Ph3); 3.80 (m, 2H, $CH_2\alpha$); 3.22 (m, 2H, $CH_2\gamma$); 2.84 (s, 6H, N $(CH_3)_2$); 2.15 (m, 2H, $CH_2\beta$).
22b	H	OH	H	—	$C_{21}H_{15}NO$	63	259	9.61 (s, 1H, OH); 9.44 (s, 1H, H1); 8.24 (m, 1H, H8); 7.83–7.61 (m, 3H, H5H6H7); 7.43–7.22 (m, 5H, Ph3); 7.06 (d, 2H, *J* = 8.4 Hz, H2H6 Ph4); 6.82 (d, 2H, H3H5 Ph4).
22c	H	OH	OH	—	$C_{21}H_{15}NO_2$	65	> 260	9.39 (s, 1H, H1); 8.19 (m, 1H, H8); 7.84–7.53 (m, 3H, H5H6H7); 7.22 (d, 2H, *J* = 8.4 Hz, H2H6 Ph3); 7.05 (d, 2H, *J* = 8.2 Hz, H2H6 Ph 4); 6.85 (d, 2H, H3H5 Ph4); 6.64 (d, 2H, H3H5 Ph3); 6.17 (br s for exangeable protons HBr and OH).
22d	OH	OH	OH	—	$C_{21}H_{15}NO_3$	59	> 260	10.00 (br s, 1H, OH7); 9.50 (br s, 2H, OH); 9.16 (s, 1H, H1); 7.46 (d, 1H, *J* = 9 Hz, H5); 7.34 (d, 1H, *J* = 1.9 Hz, H8); 7.28 (dd, 1H, *J* = 9 and 1.9 Hz, H6); 7.16 (d, 2H, *J* = 8.5 Hz, H2H6 Ph3); 7.02 (d, 2H, *J* = 8.4 Hz, H2H6 Ph4); 6.82 (d, 2H, H3H5 Ph4); 6.61 (d, 2H, H3H5 Ph3).

[a]Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values.

two alternate chair-like structures (2/3:1/3 occupancy for atoms C24 to C29). Atoms of weight 1/3 (C24′–C29′) were refined only isotropically. Indeed, the splitting results in a 'flip-flap' motion of atoms C25 and C26 around the pivot atoms C24 and N27, as shown by deviations of these atoms (C25···C25′ = 0.68, C26···C26′ = 0.91 Å) larger than those of the other atoms of the chain (C24···C24′ = 0.18, N27···N27′ = 0.36, C28···C28′ = 0.37, C29···C29′ = 0.57 Å). Each folding of the chain allows formation of a strong intramolecular hydrogen bond between nitrogen atoms N23 and N27 (mean distances N23···N27 = 2.884(4), H_{N23}···N27 = 2.20 Å, angle N-H···N = 136°). This H-bonding renders the chain rigid. In addition, a benzene solvent molecule was found around the symmetry center at (0, 1/2, 1/2), linking the two molecules in the cell. All the H atoms

were located and fitted at idealized positions, except those of the minor chain (calculated). All hydrogens were assigned an isotropic displacement parameter equivalent to that of the bonded atom, plus 20% (or 30% for those of the methyl groups). Thus, refinement converged to $R_1(F) = 0.0574$ for the 3681 observed Fo, and $wR_2(F^2) = 0.1861$ for all the 4693 data with 'goodness-of-fit' $S = 1.070$. The residual electron density was found between -0.20 and $0.45\,e\,Å^{-3}$ in the final difference map, near the benzene molecule. In the crystal, only van der Waals contacts are observed. Full crystallographic results have been deposited at the Cambridge Crystallographic Data Centre (CCDC), UK, as Supplementary Material (CIF file).

Molecular modeling calculations

Molecular modeling calculations were carried out using SYBYL (SYBYL 6.5.2, Tripos Associates, Inc., St. Louis, MO) on a Silicon Graphics workstation. Compound **9b** geometry issued from X-ray crystal structure data was built from a standard fragment library, and its geometry was optimized using Tripos Force Field[39] without electrostatic terms. Minimization was carried out using Powell's method until the gradient value was smaller than 0.01 kcal/mol·Å. Conformational search was then performed to identify lowest energy conformations using random search technique and the following options: maximum hits = 10, RMS threshold = 0.2 Å, and energy cut-off = 10 kcal/mol. Each of the 149 conformers was then superposed onto X-ray data extracted from the raloxifene study, using the FIT option of SYBYL with centroids of cycles A and C, and the side chain's nitrogen as template (tail–tail orientation). After Gasteiger–Hückel charges were added, the 37 conformations having a superposition RMS less than $1.2\,Å^2$ were then docked within the estrogen receptor α raloxifene binding site. The best 11 hits (binding energy within 10 kcal/mol) were subsequently minimized treating the receptor as flexible within an 8 Å sphere around the ligand.

In parallel, the 149 conformations were superposed to raloxifene using A, C and D aromatic centroïds (head–tail). All conformations were docked manually within the raloxifene-binding site, 85 of the conformations were rejected and the 64 conformations left were minimized keeping the receptor as rigid body. The 7 best hits (binding energy within 20 kcal/mol) were subsequently minimized treating the receptor as flexible within an 8 Å sphere around the ligand.

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