A New Class of Nonsteroidal Aromatase Inhibitors: Design and Synthesis of Chromone and Xanthone Derivatives and Inhibition of the P450 Enzymes Aromatase and 17α-Hydroxylase/C17,20-Lyase

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Aromatase (P450arom) is a target of pharmacological interest for the treatment of breast cancer. In this paper, we report the design, synthesis, and in vitro biological evaluation of a series of new (di)benzopyranone-based inhibitors of this enzyme. The design of the new compounds was guided by a CoMFA model previously developed for a series of nonsteroidal aromatase inhibitors. Both the chromone and the xanthone nuclei were taken as molecular skeletons, and the functions supposed to be critical for binding to the aromatase active site − a heterocyclic ring (imidazole or 1,3,4-triazole) linked to the aromatic moiety by a methylene unit and an H-bond accepting function (CN, NO_2, Br) located on the aromatic ring at a suitable distance from the heterocyclic nitrogen carrying the lone pair − were attached to them. The chromone, xanthone, and flavone derivatives were prepared by conventional synthetic methods from the appropriate methyl analogues. Aromatase inhibitory activities were determined by the method of Thompson and Siiteri, using human placental microsomes and [1β,2β-^{3}H]testosterone as the labeled substrate. All the compounds were also tested on 17α-hydroxylase/C17,20-lyase (P450 17), an enzyme of therapeutic interest for the treatment of prostatic diseases. The goal to find new potent inhibitors of aromatase was reached with the xanthone derivatives **22d,e** (IC_{50} values 43 and 40 nM, respectively), which exceeded the potency of the known reference drug fadrozole and also showed high selectivity with respect to P450 17. Moreover, compounds **22g**−**i** based on the same xanthonic nucleus showed fairly high potency as P450 17 inhibitors (IC_{50} values 220, 130, and 42 nM, respectively). Thus, they might be new leads for the development of drug candidates for androgen-dependent diseases.

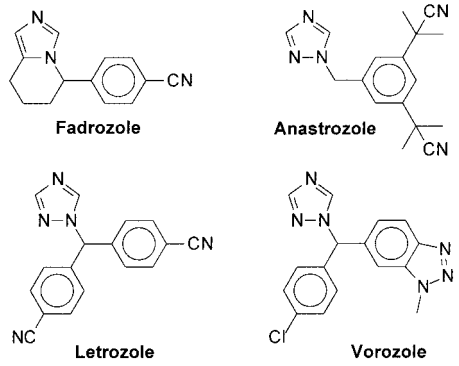
Introduction

The role of endogenous estrogens in the development of hormone-dependent breast cancer has long been recognized,[1] and two main approaches have been devised to antagonize the action of these hormones. It is possible either to act directly at the estrogen receptor by means of antagonists, among which tamoxifen is the most popular one,[2] or to block the biosynthesis of the hormones, by inhibiting a key enzyme of the process. In the latter strategy, since the early days, the research efforts have been focused on aromatase,[3] a cytochrome P450 enzyme that catalyzes the conversion of androgens into estrogens by aromatization of the steroid A ring. Recently, however, reports showing the therapeutic interest of other enzymes of the estrogen biosynthetic pathway, like estrone sulfatase and estrone sulfotransferase, have appeared.[4]

Aromatase inhibitors can be both steroidal and nonsteroidal compounds.[5] The latter class evolved from the first marketed drug aminoglutethimide to the potent and selective azoles, some of which have already been marketed (fadrozole,[6] anastrozole,[6] letrozole[7]) or are in phase III clinical trial (vorozole[8]) (Chart 1).

Most of the nonsteroidal aromatase inhibitors of therapeutic importance act by binding to the enzyme via a competitive mechanism that involves the coordination of the heme iron. These compounds cause a bathochromic shift in the UV absorption spectrum Soret band of the heme deriving from the coordination of the iron contained in the porphyrin ring by a heteroatom (usually N, but also S or O) of the inhibitor.[9]

Chart 1



With the aim to design a new class of aromatase inhibitors, we relied on (*S*)-fadrozole[10] as the reference compound and on some recently described comparative molecular field analysis (CoMFA) models accounting for the three-dimensional structure−activity relationships (SAR) of a rather large series of nonsteroidal aromatase inhibitors.[11] As the molecular scaffolds on which to insert the pharmacophoric functions typical of the azole-

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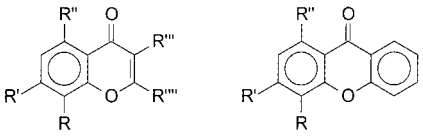
Chart 2



type compounds (see below), we chose both the benzopyran-4-one (chromone) molecule and its benzo analogue, xanthone (Chart 2). Actually, it is known that other benzopyranone derivatives, namely flavones, are able to inhibit human aromatase.[12] Moreover, in a number of previous cases, we showed the versatility of the benzopyranone moiety as a carrier of pharmacophoric groups in as many different therapeutic fields as those of analeptics,[13] adrenergic β-blocking agents,[14] clofibrate-like hypolipemic drugs,[15] bradycardic[16] and antitumor[17] agents, and acetylcholinesterase inhibitors.[18]

An important criterion for assessing the possible clinical usefulness of aromatase inhibitors in the treatment of breast cancer is their selectivity with respect to other P450 enzymes of physiological importance. On the other hand, recently, it has been shown that some aromatase inhibitors based on the benzocycloalkene structure are able to inhibit 17α-hydroxylase/C17,20-lyase (P450 17) as well.[19] A finding like this is critical and must be taken into consideration when designing new analogues in view of SAR studies and also when planning the development of the lead compound(s). In fact, inhibitors of P450 17 have great therapeutic interest, as they might be an alternative to antiandrogens in the treatment of androgen-dependent diseases such as prostate cancer.[20]

In this paper, we report the design, synthesis, and in vitro biological evaluation of a series of new (di)-benzopyranonic aromatase inhibitors, together with a discussion of their SARs. Based on the above considerations, all the newly designed aromatase inhibitors were also tested in a P450 17 inhibition assay, to obtain a preliminary biological profile of the series indicating which compounds might be worthy of further investigation and in which direction the optimization efforts must be addressed.

Inhibitor Design

In previous studies, we attempted the rationalization of the SARs of a rather large series of nonsteroidal aromatase inhibitors in terms of their three-dimensional properties.[11] CoMFA analyses were carried out on series of inhibitors belonging to two different structural classes: one related to (*S*)-fadrozole and one related to (*E*)-2-(4-pyridylmethylene)-1-tetralone.[21] A comprehensive CoMFA model was obtained accounting for the three-dimensional SAR of the whole series and leading to the individuation of regions in the space around the molecules where their steric or electrostatic characteristics cause variations of the inhibitory activity. The design of the series presented here was guided by these theoretical models, which, however, were not considered as definitive and unmodifiable, such that some choice was determined by classical SAR considerations.

It is known that the CoMFA analysis can provide a quantitative description in terms of steric and electrostatic properties of both favorable and unfavorable zones in the inhibitor space, after the compounds have been aligned onto a template molecule on the basis of some pharmacophoric hypothesis.[22] As regards the nonsteroidal aromatase inhibitors, the alignment was suggested by a hypothesis of Furet et al.,[10] who recognized the possibility for (*S*)-fadrozole to bind to the enzyme in a way similar to that of some steroidal competitive inhibitors. According to this hypothesis, the imidazolic N coordinates the heme iron and the CN function acts as an H-bond acceptor in the same way as the 17-carbonyl group of steroids does. Given that the clearest (and statistically most significant) indication coming from the CoMFA models for the nonsteroidal aromatase inhibitors was the existence of a wide favorable steric zone located around the phenyl ring of fadrozole-type inhibitors,[11] the consequent operative conclusion was to design molecules able to fill this area.

In Table 1, the structures of the molecules studied as aromatase inhibitors in the present paper are reported. They were designed by taking the chromone and the xanthone nuclei as molecular skeletons and by inserting on them the functions supposed to be critical for the binding to the aromatase active site. These functions are (a) a heterocyclic ring (imidazole or 1,3,4-triazole) linked to the aromatic moiety by a methylene unit and (b) an H-bond accepting function (CN, NO_2, Br) located on the aromatic ring at a suitable distance from the heterocyclic nitrogen atom carrying the lone pair.[23]

Compounds **22a**−**f** are characterized by the presence of an imidazole moiety linked through a methylene bridge to position 8 of the chromone nucleus (**22a**−**c**) or, correspondingly, to position 4 of the xanthone nucleus (**22d**−**f**) (substituents R, Chart 2). These compounds carry also an H-bond acceptor group or atom (R″) in a position para to the former substituent. In compounds **22g**−**i**, the positions of the two functions on the aromatic nucleus of xanthone were inverted, to investigate whether the presence of the ethereal oxygen or, in turn, of the carbonyl group close to the electron-attracting function could modulate the affinity for the target enzyme.

Conformational analyses were carried out on all the compounds with the aim to find an energetically accessible conformation of each molecule that could allow an overlapping of the two pharmacophoric functions on the corresponding ones of (*S*)-fadrozole. It resulted that, for the molecules in their putative active conformation, all or part of both the chromone and xanthone moieties can occupy the favorable CoMFA steric region (green); moreover, no part of the molecules touches the unfavorable region (yellow), as shown in Figure 1a for compound **22d**.

In compounds **22j**−**m**, the position of the five-membered heterocycle on the (di)benzopyranonic ring was again varied, and the possibility that the nuclear carbonyl function could act as an H-bond acceptor was explored. We verified that the introduction of the coordinating heterocycle both in position 7 of the chromone nucleus (R′ in **22j,k**) and in the corresponding position 3 of the xanthone ring (R′ in **22l**,**m**) allowed for the alignment of the carbonyl group of these molecules onto the CN function of (*S*)-fadrozole. In these cases too, part of each molecule was engulfed in the

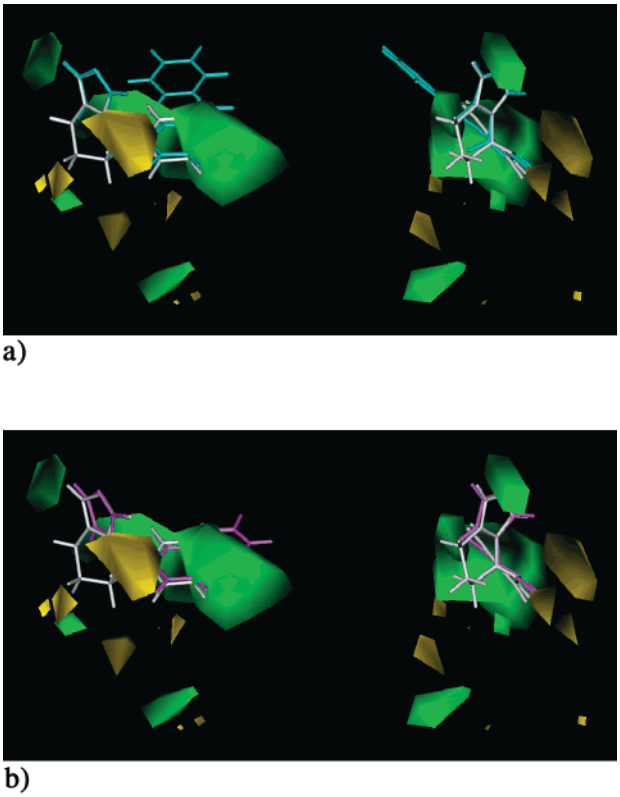


Figure 1. Orthogonal views of the fit of (a) compound **22d** (cyan) and (b) compound **22j** (magenta) into the CoMFA model used for inhibitor design; the reference compound (*S*)-fadrozole (white) is also shown superimposed to the new derivatives. The occupancy of the large sterically favorable green volume by part of the xanthone ring of **22d** (a, left) and by the chromone ring of **22j** (b, left) is evident; the yellow sterically forbidden volumes are not touched by any part of the inhibitors (a,b, right).

favorable CoMFA volumes, thus satisfying the steric requirement (Figure 1b, compound **22j**).

Compounds **22n**,**o** were prepared in order to probe the possibility that flavones fit the CoMFA model for the inhibition of aromatase. In our extended CoMFA model,[11b] a series of pyridyltetralones lacking a suitably located H-bond acceptor function was aligned in an alternative way with respect to (*S*)-fadrozole. In the design hypothesis, compounds **22n**,**o** mimic the relative orientation of the tetralones in the CoMFA model, possessing the coordinating heterocycle but lacking the suitably located H-bonding group.

The triazole analogues **22k**,**m**,**o** were prepared with the aim of exploring the effects of the variation of the aza heterocycle. It has been reported that the replacement of the imidazolic moiety with a triazolic one can lead to better in vivo activity of the compound.[23]

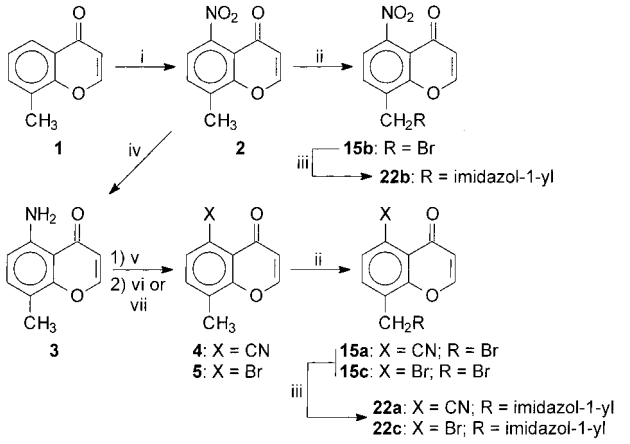
Chemistry

The chromone, xanthone, and flavone derivatives of Table 1 were prepared according to Schemes 1−4 from the appropriate methyl analogues that were obtained by conventional synthetic methods. To prepare the xanthone derivatives **22d**−**f**, an alternative route was followed with respect to the corresponding chromones.

8-Methyl-4*H*-benzopyran-4-one (**1**) was nitrated with HNO_3 in H_2SO_4 to give 8-methyl-5-nitro-4*H*-benzopyran-4-one (**2**), which was brominated with *N*-bromosuccinimide and condensed with imidazole to give compound **22b** (Scheme 1). Reduction of compound **2** with H_2/Pd/$CaCO_3$ afforded 5-amino-8-methyl-4*H*-benzopyran-4-one (**3**) that was treated with an aqueous solution of $NaNO_2$, keeping the temperature below 5 °C: the obtained diazonium salt was then cautiously added to a boiling solution of NaCN/CuCN to give 5-cyano-8-methyl-4*H*-benzopyran-4-one (**4**) or to a solution of CuBr to yield 5-bromo-8-methyl-4*H*-benzopyran-4-one (**5**). Compounds **22a**,**c** were obtained by bromination of intermediates **4** and **5** followed by condensation with imidazole.

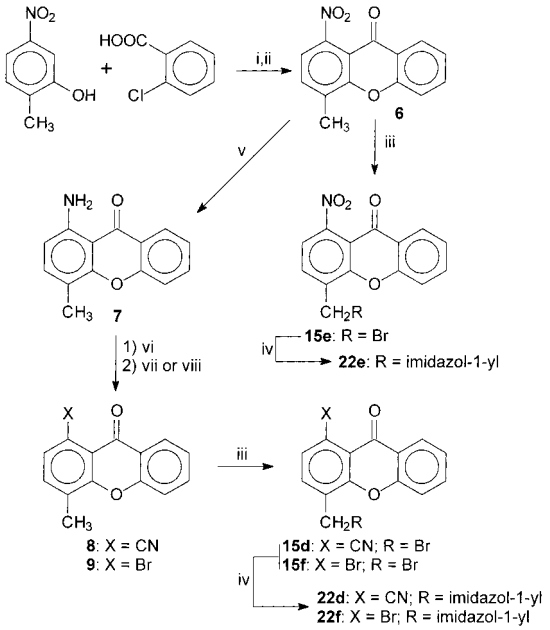
Since several attempts to obtain 4-methyl-1-nitro-9*H*-9-xanthenone (**6**) by nitration of 4-methyl-9*H*-9-xanthenone failed, this compound was synthesized from 2-methyl-5-nitrophenol, through reaction with *O*-chlorobenzoic acid in nitrobenzene affording the correspond-

Scheme 1[a]



[a] Reagents: (i) HNO_3/H_2SO_4, 0−5 °C; (ii) *N*-bromosuccinimide, CCl_4, reflux 5 h; (iii) imidazole, CH_3CN, reflux 6 h under N_2; (iv) H_2, Pd/$CaCO_3$; (v) $NaNO_2$, HCl, 0−5 °C; (vi) NaCN/CuCN, 100 °C, 20 min; (vii) CuBr, rt, 12 h.

Scheme 2[a]



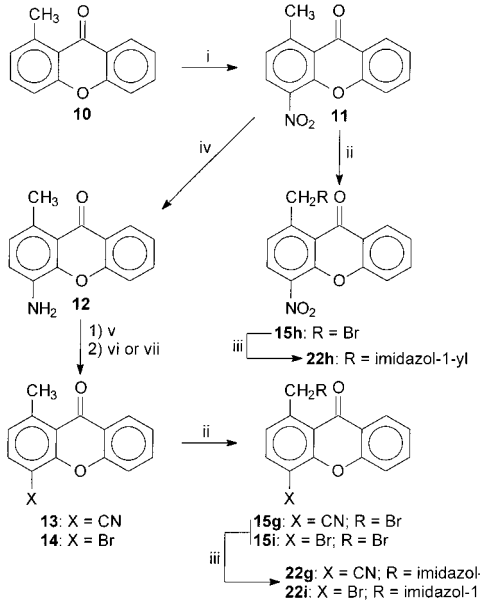
[a] Reagents: (i) Cu, nitrobenzene, K_2CO_3, 170 °C, 6 h; (ii) PPA; (iii) *N*-bromosuccinimide, CCl_4, reflux, 5 h; (iv) imidazole, CH_3CN, reflux 6 h under N_2; (v) H_2, Pd/$CaCO_3$; (vi) $NaNO_2$, HCl, 0−5 °C; (vii) NaCN/CuCN, 100 °C, 20 min; (viii) CuBr, rt, 12 h.

Table 1. Structure and Analytical Data of the Chromone (**22a**−**c,j,k,n,o**) and Xanthone (**22d**−**i,l,m**) Derivatives

no.	scaffold	R	R′	R″	R‴	R⁗	formula[a]	mp (°C)	yield (%)
22a	chromone	CH_2Im[b]	H	CN	H	H	$C_{14}H_9N_3O_2$	192−5[c]	50
22b	chromone	CH_2Im	H	NO_2	H	H	$C_{13}H_9N_3O_4$	207−8[c]	55
22c	chromone	CH_2Im	H	Br	H	H	$C_{13}H_9BrN_2O_2$	141−3[c]	60
22d	xanthone	CH_2Im	H	CN	H	H	$C_{18}H_{11}N_3O_2$	255−7[c]	40
22e	xanthone	CH_2Im	H	NO_2	H	H	$C_{17}H_{11}N_3O_4$	234−6[c]	55
22f	xanthone	CH_2Im	H	Br	H	H	$C_{17}H_{11}BrN_2O_2$	218−20[c]	40
22g	xanthone	CN	H	CH_2Im	H	H	$C_{18}H_{11}N_3O_2$	172−4[d]	40
22h	xanthone	NO_2	H	CH_2Im	H	H	$C_{17}H_{11}N_3O_4$	152−4[d]	45
22i	xanthone	Br	H	CH_2Im	H	H	$C_{17}H_{11}BrN_2O_2$	211−4[d]	40
22j	chromone	H	CH_2Im	H	H	H	$C_{13}H_{10}N_2O_2$	216−8[d]	50
22k	chromone	H	CH_2Tri[e]	H	H	H	$C_{12}H_9N_3O_2$	198−200[d]	40
22l	xanthone	H	CH_2Im	H	H	H	$C_{17}H_{12}N_2O_2$	158−62[d]	40
22m	xanthone	H	CH_2Tri	H	H	H	$C_{16}H_{11}N_3O_2$	235−6[d]	40
22n	chromone	H	OMe	H	CH_2Im	Ph	$C_{20}H_{16}N_2O_3$	188−9[d]	45
22o	chromone	H	OMe	H	CH_2Tri	Ph	$C_{19}H_{15}N_3O_3$	191−3[d]	40

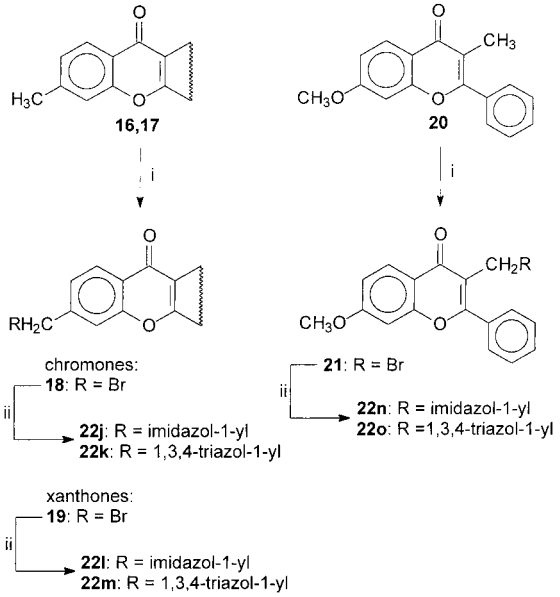
[a] C, H, and N analyses were within ±0.4% of the theoretical values. [b] CH_2Im: imidazol-1-ylmethyl. [c] Crystallizing solvent: petroleum ether. [d] Crystallizing solvent: ethanol. [e] CH_2Tri: 1,3,4-triazol-1-ylmethyl.

Scheme 3[a]



[a] Reagents: (i) HNO_3/H_2SO_4, 0−5 °C; (ii) *N*-bromosuccinimide, CCl_4, reflux 5 h; (iii) imidazole, CH_3CN, reflux 6 h under N_2; (iv) H_2, $Pd/CaCO_3$; (v) $NaNO_2$, HCl, 0−5 °C; (vi) NaCN/CuCN, 100 °C, 20 min; (vii) CuBr, rt, 12 h.

Scheme 4[a]



[a] Reagents: (i) *N*-bromosuccinimide, CCl_4, reflux 5 h; (ii) imidazole/1,3,4-triazole, CH_3CN, reflux 6 h under N_2.

ing diphenyl ether, and a subsequent cyclization by means of polyphosphoric acid, as described in Scheme 2. Bromination with *N*-bromosuccinimide, followed by reaction with imidazole, afforded compound **22e**.

The nitro derivative **6** was hydrogenated to 1-amino-4-methyl-9*H*-9-xanthenone (**7**) and treated, as previously described, with $NaNO_2$ in the presence of NaCN/CuCN or CuBr to give 4-methyl-9-oxo-9*H*-1-xanthenecarbonitrile (**8**) or 1-bromo-4-methyl-9*H*-9-xanthenone (**9**), respectively. Derivatives **8** and **9** were brominated, and the resulting bromomethyl derivatives **15d,f** were condensed with imidazole to afford **22d**,**f**, respectively (Scheme 2).

The synthesis of compounds **22g**−**i** was accomplished as illustrated in Scheme 3, using the same procedure described for compounds **22a**−**c** (Scheme 1) and starting from 1-methyl-9*H*-9-xanthenone (**10**).

Compounds **22j**−**o** were prepared as shown in Scheme 4: the selected methyl derivative (**16**, **17**, or **20**) was brominated with *N*-bromosuccinimide in refluxing CCl_4 to yield the corresponding bromomethyl derivative **18**, **19**, or **21**, respectively, which was then condensed with imidazole or 1,3,4-triazole to give the desired compounds.

Biological Properties

Inhibition of P450arom. The method of Thompson and Siiteri, i.e., human placental microsomes and [1β,2β-^{3}H]testosterone, was used for the determination of inhibitory activities.[24] In Table 2, IC_{50} values and inhibitory potencies of the compounds relative to aminoglutethimide are shown; the IC_{50} value of fadrozole tested in our test system[25] is also reported for comparison.

Considering the inhibitors carrying the *N*-imidazolylmethyl substituent (R, Chart 2) both in position 8 of the chromone ring (compounds **22a**−**c**) and in position 4 of the xanthone nucleus (compounds **22d**−**f**), it is evident that the xanthone-based molecules are much better aromatase inhibitors than the chromone-based ones. As R″ substituents, CN and, in case of the xanthones, NO_2 turned out to be superior to Br. Thus, compounds **22d**,**e** are extremely potent aromatase inhibitors exceeding the

Table 2. Inhibition of the Steroidogenic P450 Enzymes Aromatase and P450 17 by Chromone (**22a**−**c,j,k,n,o**) and Xanthone (**22d**−**i,l,m**) Derivatives

	human aromatase[c]			human 17α-hydroxylase[d]	
no.	% inhib (25 μM)	IC_{50}[a] (μM)	rel pot[b]	% inhib (2.5 μM)	IC_{50}[a] (μM)
22a	98	0.24	76	3	
22b	95	1.1	17	5	
22c	94	2.1	9	26	
22d	99	0.043	430	14	
22e	99	0.040	463	4	
22f	99	0.089	208	38	
22g	93	2.3	8	92	0.22
22h	95	0.97	19	94	0.13
22i	95	0.94	20	98	0.042
22j	98	0.10	185	9	
22k	85	3.7	5	5	
22l	97	0.39	47	33	
22m	27			8	
22n	94	0.55	33	44	
22o	21			14	
fadrozole	99	0.052	359		

[a] The given values are mean values of at least three experiments. The deviations were within ±5%. [b] Relative potency calculated with respect to aminoglutethimide (IC_{50} = 18.5 μM). [c] Human placental microsomes, concentration of testosterone (substrate): 2.5 μM; [1β,2β-^{3}H]testosterone: 0.225 μCi. [d] Human testicular microsomes, concentration of progesterone (substrate): 2.5 μM.

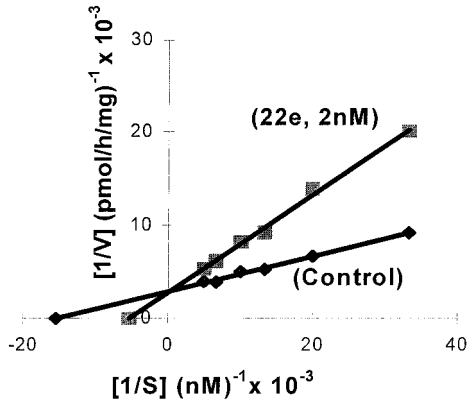


Figure 2. Lineweaver−Burk plot for the inhibition of aromatase activity by compound **22e**. Initial rates (*V*) are expressed as pmol/h/mg of ^3H_2O formed in the aromatization of the tritiated substrate and were obtained as described in ref 26.

enzyme inhibitory activity of the well-known nonsteroidal inhibitor fadrozole: their relative potencies with respect to fadrozole are 1.2 and 1.3, respectively. The exchange of the *N*-imidazolylmethyl substituent for the electron-withdrawing group (R against R″) dramatically decreases the enzyme inhibitory activity (compounds **22g**−**i**). Derivatives bearing the *N*-imidazolylmethyl group as R′ substituent show an increase in aromatase inhibition in the case of the chromone scaffold (**22j**) and a decrease in the xanthone series (**22l**). The 3-imidazolylmethyl-substituted (R‴, Chart 2) chromone **22n** shows mediocre aromatase inhibition. The replacement of imidazole with 1,3,4-triazole in compounds **22j**,**l**,**n** strongly decreases the inhibitory activity (compounds **22k**,**m**,**o**, respectively).

The mode of aromatase inhibition was investigated more in depth for the most potent compound **22e**. By performing difference spectroscopy experiments, we found that this derivative shows a characteristic type II UV−vis difference spectrum (minimum at 390 nm, maximum at 420 nm) indicating the formation of a coordination bond between the imidazole nitrogen and the heme iron of P450arom. A study of the steady-state kinetics of **22e** was then performed, using a microsomal aromatase preparation derived from human placenta and the tritiated water method.[26] The xanthone derivative exhibited an inhibition constant (K_i) of 1.05 nM obtained from a Lineweaver−Burk plot (Figure 2) that revealed also a typical competitive type of inhibition. The corresponding K_m and V_{max} values were 65.1 nM and 353.2 pmol/h/mg, respectively.

Inhibition of P450 17. The assay was performed as recently described by us, using human testicular microsomes as source of the enzyme and nonlabeled progesterone as substrate.[27] Percent inhibition values at an inhibitor concentration of 2.5 μM (Table 2) indicate that, with the exception of compounds **22g**−**i**, all other compounds do not significantly inhibit the enzyme or show only marginal inhibitory activity. The xanthone-derived compounds **22g**−**i** with the "inverted" position of the substituents are rather strong inhibitors of P450 17, being 3−18 times (compounds **22g**,**i**, respectively) more potent than the reference compound ketoconazole (IC_{50} = 0.74 μM[27]).

Discussion

The series of compounds whose structure and biological activity are presented in Tables 1 and 2, respectively, shows that it is possible to obtain potent inhibitors of the P450arom and P450 17 by suitably modifying the (di)benzopyran-4-one nucleus.

Our first goal was to find new potent inhibitors of aromatase, and it was reached with compounds **22d,e** that exceeded the potency of the marketed drug fadrozole (Afema). However, depending on the substitution pattern, the aromatase inhibitory activity varies substantially, and the reasons thereof might be tentatively looked for in some physicochemical properties of the compounds. The most striking evidence is the dramatic loss of activity that occurs upon replacement of the imidazole ring of compounds **22j**,**l**,**n** with the 1,3,4-triazole in compounds **22k**,**m**,**o**. This observation was also made with other inhibitors of P450arom[19c] and P450 17,[28] and an explanation advanced for this finding involved the different atomic charges of the heterocyclic nitrogens responsible for the interaction with the heme iron.[28] The HOMO for compounds **22l**,**m** calculated with the SYBYL program using the AM1 method[29] is shown in Figure 3a,b, respectively. It appears evident that the molecular orbital involved in the eventual coordination bond with the iron atom is located on the imidazole ring of compound **22l**, but it is spread over the xanthone nucleus in the case of **22m**. The relative unavailability of the nitrogen electron pair of the latter compound explains reasonably well the low inhibitory activity of the 1,3,4-triazol-1-yl-substituted derivatives. Regarding the effects of different patterns of structural modification, it appears from the data of Table 2 that the combination xanthone, imidazol-1-ylmethyl, and strong H-bond acceptor (CN or NO_2) is the best one for an efficient aromatase inhibition (compounds **22d**−**f**). This is true, considering both the xanthone/chromone replacement (compounds **22a**−**c**) and the inversion of the

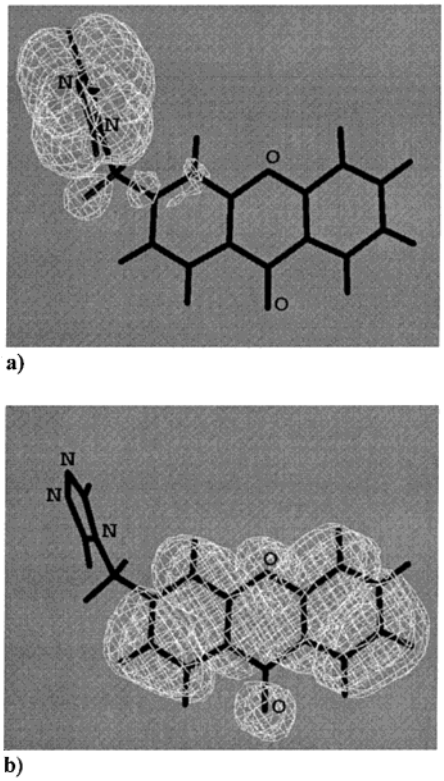


Figure 3. HOMO of compounds **22l** (a) and **22m** (b), showing the localization of the orbital over the imidazole ring and over the xanthone nucleus, respectively.

positions of the imidazolylmethyl and electron-attracting groups on the xanthone scaffold (compounds **22g**−**i**). A reason for the higher activity of the xanthone-based compounds with respect to the chromone-based ones might be the higher lipophilicity of the former derivatives, which could facilitate the access and/or the binding to the aromatase active site. We calculated the log *P* values[30] of compounds **22a**−**f**: −0.45, −0.14, 0.98, 1.14, 1.45, and 2.57, respectively. From molecular biology studies,[31] it is known that the aromatase active site contains several hydrophobic residues, and consequently, the hydrophobic effect could play a critical role in the stabilization of the enzyme−inhibitor complex. The importance of hydrophobicity in the molecular recognition was earlier pointed out by Hansch[32] and recently brought to the attention of drug designers by Davis and Teague.[33]

Of course, lipophilicity cannot be the only driving force leading to a strong binding to aromatase, as shown by the good activity of the chromone derivative **22j** (despite its log $P = 0.11$) and by the relatively lower potency of the "inverted" xanthone derivatives **22g**−**i**. For the latter, which show hydrophobic and electronic properties similar to those of compounds **22d**−**f**, the only reasonable hypothesis to explain the lower activity is the possibility of some conformational restraint that might prevent the molecules from assuming a conformation that locates the pharmacophoric functions in optimal spatial orientation for binding to the enzyme.

The fairly good inhibitory activity of compounds **22j**,**l**,**n** shows that the presence of a strong H-bonding function (like CN or NO_2) is not strictly necessary to obtain aromatase inhibition. Our idea (see Inhibitor Design) was that the carbonyl group present in these molecules could serve as an H-bond acceptor either in the same orientation of the fadrozole CN or in an alternative one. This hypothesis seems to be confirmed. However, it has to be noted that any conjecture about the actual inhibitor binding mode remains speculative until the structure of some ligand−enzyme complex is resolved by an experimental method (X-ray crystallography, NMR). Interestingly, for these three compounds, the order of potency is reversed with respect to lipophilicity; in fact, the calculated log *P* values for compounds **22j**,**l**,**n** are 0.11, 1.71, and 2.13, respectively.

As stated in the Introduction, to assess the selectivity of the new aromatase inhibitors, we extended the biological investigation of the compounds to another P450 enzyme, namely P450 17. The data reported in Table 2 show that indeed the most potent aromatase inhibitors **22d**−**f** are selective, as they do not inhibit P450 17. On the other hand, it is quite remarkable that the "inverted" xanthone derivatives **22g**−**i** are good inhibitors of 17α-hydroxylase, even so they are less selective than compounds **22d**−**f**. Interestingly, slight structural modifications decrease P450arom inhibition strongly and concurrently increase P450 17 inhibition markedly. Similar evidences have been obtained by us with 3- and 4-pyridyl-substituted tetrahydrocyclopropanaphthalenes,[19b] imidazolyl(methyl)-substituted tetrahydronaphthalenes,[19c] and imidazolylmethyl-substituted biphenyls.[27] Considering the relevance of the target and the high P450 17 inhibitory activity, and taking into account also that compound **22i** is rather selective with respect to aromatase, it might be a lead for the development of a drug candidate for prostatic diseases.

Finally, a comment about the use of the CoMFA models as an aid in the design of the new aromatase inhibitors is deserved. As stated above, we used theoretical models and classical SAR concepts in conjunction to design the structural modification of the chromone and xanthone nuclei. Perhaps, it would have been too ambitious to expect accurate quantitative predictions from the CoMFA models applied to molecules structurally different enough from those of the training set. Actually, the predicted activity data for aromatase inhibition differ from about 0.2 up to more than 2.5 log units from the experimentally observed values. The reasons may be numerous, but they probably reside in the inaccurate description of the molecular properties by the steric and electrostatic CoMFA fields and in the arbitrariness of the molecules' alignment. Nonetheless, the statistical model proved to be of help in supporting some decision about structural modifications (substituent positions) and in verifying the consistency of some molecules with the design hypothesis. The conclusion might be that an appropriate balance of quantitative and qualitative thinking can still be considered the best approach for the molecular design applied to pharmacological targets.

Conclusions

In conclusion, we have shown that by appropriately modifying the (di)benzopyran-4-one nucleus, it is possible to obtain potent and selective aromatase inhibitors. Compounds endowed with such an activity can be considered for the development as anticancer drugs, provided that they are selective in their action against

the target. The most interesting derivatives presented here (compounds **22d**,**e**) show a human aromatase inhibitory activity superior to that of the known drug fadrozole and are selective with respect to another human P450 enzyme, namely P450 17. On the other hand, based on the same xanthone nucleus, we obtained compounds **22g**−**i** that show fairly high potency as P450 17 inhibitors, the latter enzyme being a target for the development of drugs for the treatment of prostatic diseases.

Further experiments on the selectivity toward other P450 enzymes and on the in vivo activity must be performed to answer the question whether the xanthones described in this paper are novel drug candidates for the treatment of steroidal hormone-dependent diseases.

Experimental Section

Chemistry. General Methods. All melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. ^{1}H NMR were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$ solutions, with Me_4Si as the internal standard. Mass spectra were recorded on a VG 7070 E spectrometer. Elemental analyses were within 0.4% of theoretical value unless otherwise indicated.

8-Methyl-5-nitro-4*H*-benzopyran-4-one (2). A solution of 8-methyl-4*H*-benzopyran-4-one (**1**; 3.2 g, 0.02 mol) in H_2SO_4 (500 mL) was treated with fuming HNO_3 (1.26 g, 0.02 mol) keeping the temperature between 0 and 5 °C. The reaction mixture was stirred 1 h at room temperature, then poured into ice. The separated solid was collected by filtration, and compound **2** (2.8 g, 70%) was obtained: mp 172−174 °C (toluene); ^{1}H NMR δ 2.55 (s, 3H), 6.4 (d, J = 6.1 Hz, 1H, H-2), 7.3 (d, J = 6.3 Hz, 1H), 7.6 (d, J = 6.3 Hz, 1H), 7.95 (d, J = 6.1 Hz, 1H, H-3).

5-Amino-8-methyl-4*H*-benzopyran-4-one (3). A solution of **2** (9 g, 0.043 mol) in THF was hydrogenated at room temperature using $Pd/CaCO_3$ as catalyst. After filtration, the solvent was removed under reduced pressure and the residue was crystallized from toluene to yield compound **3** (6.15 g, 80%): mp 141−143 °C; ^{1}H NMR δ 2.3 (s, 3H), 6.15 (d, J = 4.64 Hz, 1H, H-2), 6.3 (broad, 2H, NH_2), 6.35 (d, J = 7.8 Hz, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.7 (d, J = 4.64 Hz, 1H, H-3).

8-Methyl-5-cyano-4*H*-benzopyran-4-one (4). To a solution of **3** (1.75 g, 0.01 mol) in H_2SO_4, a solution of $NaNO_2$ (0.69 g, 0.01 mol) in water was added dropwise, keeping the temperature below 5 °C. The reaction mixture was stirred 1 h at room temperature, and then it was added to a solution of 1.5 g of CuCN and 2 g of NaCN in H_2O at 50−60 °C. The mixture was heated at 100 °C for 20 min, stirred overnight at room temperature and filtered. The residue, on crystallizing from toluene, gave 0.94 g (50%) of **4**: mp 193−195 °C; ^{1}H NMR δ 2.55 (s, 3H), 6.45 (d, J = 6.0 Hz,1H, H-2), 7.55−7.7 (m, 2H), 7.9 (d, J = 6.0 Hz, 1H, H-3).

5-Bromo-8-methyl-4*H*-benzopyran-4-one (5). Compound **3** (1.75 g, 0.01 mol) was treated in HCl 1:1 with a solution of $NaNO_2$ (0.69 g, 0.01 mol) in water as previously described. The solution obtained was added to a boiling solution of CuBr (1.7 g) in water. The mixture was stirred overnight at room temperature and then extracted with CH_2Cl_2, washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate 7/3) to give 1.2 g of **5** (50%): mp 85−87 °C; ^{1}H NMR δ 2.45 (s, 3H), 6.35 (d, J = 6.0 Hz, 1H, H-2), 7.25−7.4 (m, 2H), 7.85 (d, J = 6.0 Hz, 1H, H-3).

4-Methyl-1-nitro-9*H*-9-xanthenone (6). A mixture of 15.3 g (0.1 mol) of 2-methyl-5-nitrophenol, 12.3 g (0.1 mol) of *o*-chlorobenzoic acid, 20 g of K_2CO_3, 0.5 g of Cu and 0.5 g of CuI in 300 mL of nitrobenzene was heated in an oil bath at 170−180 °C for 8 h. The solvent was steam distilled, and the residue was filtered and acidified with HCl. The separated solid was filtered, washed with water and suspended in a saturated solution of $NaHCO_3$. The suspension obtained was filtered and acidified. The solid was collected by filtration, washed with water and dried, obtaining 17 g (60%) of 2-(2-methyl-5-nitrophenoxy)benzoic acid: mp 145−147 °C (ligroin); ^{1}H NMR δ 2.45 (s, 3H), 6.9−8.2 (m, 7H, arom). 16.4 g (0.06 mol) of the previous compound was added portionwise to 240 mL of H_2SO_4 concentrated, keeping the temperature below 50 °C. The reaction mixture was then heated at 110 °C for 40 min, cooled and poured into ice. After filtration, the product was dried and crystallized from toluene, affording 12 g (80%) of **6**: mp 255−257 °C; ^{1}H NMR δ 2.55 (s, 3H), 7.4−8.1 (m, 6H, arom).

1-Amino-4-methyl-9*H*-9-xanthenone (7). Using the same procedure described for compound **3**, compound **7** (6.1 g, 90%) was obtained from compound **6** (7.65 g, 0.03 mol): mp 146−148 °C (ligroin); ^{1}H NMR δ 2.35 (s, 3H), 6.4 (d, J = 7.3 Hz,-1H), 6.55 (broad, 2H, NH_2), 7.2−8.25 (m, 5H, arom).

4-Methyl-9-oxo-9*H*-1-xanthenecarbonitrile (8). Using the same procedure described for compound **4**, compound **8** (1.2 g, 50%) was obtained from **7** (2.25 g, 0.01 mol): mp 220−222 °C dec (toluene); ^{1}H NMR δ 2.6 (s, 3H), 7.3−8.3 (m, 6H, arom).

1-Bromo-4-methyl-9*H*-9-xanthenone (9). Using the same procedure described for compound **5**, compound **9** (1.73 g, 60%) was obtained from **7** (2.25 g, 0.01 mol): mp 147−148 °C (ligroin); ^{1}H NMR δ 2.5 (s, 3H), 7.2−8.3 (m, 6H, arom).

1-Methyl-4-nitro-9*H*-9-xanthenone (11). A solution of 1-methyl-9*H*-9-xanthenone (**10**; 6.0 g, 0.028 mol) in H_2SO_4 (700 mL) was treated with fuming HNO_3 (1.78 g, 0.028 mol), keeping the temperature between 0 and 5 °C. The reaction mixture was stirred 1 h at room temperature and poured into ice. The separated solid was collected by filtration and purified by flash chromatography (eluent: hexane/ethyl acetate, 9/1). 5.0 g (70%) of **11** was obtained: mp 199−200 °C (toluene); ^{1}H NMR δ 3.05 (s, 3H), 7.4−8.3 (m, 6H, arom).

4-Amino-1-methyl-9*H*-9-xanthenone (12). Using the same procedure described for compound **4**, compound **12** (3.0 g, 90%) was obtained from **11** (3.8 g, 0.015 mol): mp 137−140 °C (ligroin); ^{1}H NMR δ 2.8 (s, 3H), 3.6 (broad, 2H, NH_2), 7.2−8.25 (m, 5H, arom).

1-Methyl-9-oxo-9*H*-4-xanthenecarbonitrile (13). Using the same procedure described for compound **4**, compound **13** (1.2 g, 50%) was obtained from **12** (2.25 g, 0.01 mol): mp 158−160 °C (toluene); ^{1}H NMR δ 3.1 (s, 3H), 7.3−8.3 (m, 6H, arom).

4-Bromo-1-methyl-9*H*-9-xanthenone (14). Using the same procedure described for compound **5**, compound **14** (1.73 g, 60%) was obtained from **12** (2.25 g, 0.01 mol): mp 159−161 °C (ligroin); ^{1}H NMR δ 3.0 (s, 3H), 7.2−8.2 (m, 6H, arom).

8-(Bromomethyl)-5-nitro-4*H*-benzopyran-4-one (15b). A mixture of 8-methyl-5-nitro-4*H*-benzopyran-4-one (**2**; 2.8 g, 0.0137 mol), *N*-bromosuccinimide (2.44 g, 0.0137 mol) in the presence of a catalytic amount of benzoyl peroxide in 100 mL of CCl_4 was refluxed 4 h and then hot filtered. The solvent was evaporated to dryness and the residue crystallized from ligroin, to give 2.7 g of the desired compound (70%): mp 151−153 °C; ^{1}H NMR δ 4.65 (s, 2H), 6.4 (d, J = 4.8 Hz,1H, H-2), 7.35 (d, J = 7.2 Hz, 1H), 7.8 (d, J = 7.2 Hz, 1H), 7.95 (d, J = 4.8 Hz, 1H, H-3).

8-(Bromomethyl)-5-cyano-4*H*-benzopyran-4-one (15a). Using the previous procedure, compound **15b** (0.92 g, 70%) was obtained from **4** (0.94 g, 0.005 mol): mp 202−204 °C (ligroin); ^{1}H NMR δ 4.8 (s, 2H), 6.45 (d, J = 7.6 Hz, 1H, H-2), 7.7−7.85 (m, 2H), 7.95 (d, J = 7.6 Hz, 1H, H-3).

5-Bromo-8-(bromomethyl)-4*H*-benzopyran-4-one (15c). Using the previous procedure, compound **15c** (1.1 g, 70%) was obtained from **5** (1.2 g, 0.005 mol): mp 115−117 °C (ligroin); ^{1}H NMR δ 4.65 (s, 2H), 6.35 (d, J = 6.0 Hz,1H, H-2), 7.35 (d, J = 8.0 Hz, 1H), 7.6 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 6.0 Hz, 1H, H-3).

4-(Bromomethyl)-1-nitro-9*H*-9-xanthenone (15e). Using the previous procedure, compound **15e** (1.7 g, 65%) was obtained from **6** (2.0 g, 0.008 mol): mp 83−85 °C (toluene); ^{1}H NMR δ 5.5 (s, 2H), 7.4−8.2 (m, 6H, arom).

4-(Bromomethyl)-9-oxo-9*H*-1-xanthenecarbonitrile (15d). Using the previous procedure, 0.96 g (60%) of oily compound was obtained from **8** (1.2 g, 0.005 mol), and it was used in the subsequent step without further purification.

1-Bromo-4-(bromomethyl)-9*H*-9-xanthenone (15f). Using the previous procedure, 0.73 g (60%) of oily compound was obtained from **9** (0.96 g, 0.003 mol), and it was used in the subsequent step without further purification.

1-(Bromomethyl)-4-nitro-9*H*-9-xanthenone (15h). Using the previous procedure, 0.4 g (60%) of oily compound was obtained from **11** (0.5 g, 0.002 mol), and it was used in the subsequent step without further purification.

1-(Bromomethyl)-9-oxo-9*H*-4-xanthenecarbonitrile (15g). Using the previous procedure, 0.28 g (60%) of oily compound was obtained from **13** (0.47 g, 0.002 mol), and it was used in the subsequent step without further purification.

4-Bromo-1-(bromomethyl)-9*H*-9-xanthenone (15i). Using the previous procedure, 0.44 g (60%) of oily compound was obtained from **14** (0.57 g, 0.002 mol), and it was used in the subsequent step without further purification.

7-(Bromomethyl)-4*H*-benzopyran-4-one (18). Using the previous procedure and starting from 1.6 g (0.01 mol) of 7-methyl-4*H*-benzopyran-4-one (**16**), 1.43 g (60%) of the desired compound was obtained: mp 89−93 °C (ligroin); ^{1}H NMR δ 4.5 (s, 2H), 6.35 (d, J = 5.2 Hz,1H, H-2), 7.3−7.5 (m, 2H), 7.8 (d, J = 5.2 Hz, 1H, H-3), 8.2 (d, J = 6.3 Hz, 1H).

3-(Bromomethyl)-9*H*-9-xanthenone (19). Using the previous procedure and starting from 2.1 g (0.01 mol) of 3-methyl-9*H*-9-xanthenone (**17**), 1.73 g (60%) of the desired compound was obtained: mp 104−107 °C (ligroin); ^{1}H NMR δ 5.5 (s, 2H), 6.85−8.35 (m, 7H, arom).

3-(Bromomethyl)-7-methoxy-2-phenyl-4*H*-benzopyran-4-one (21). Using the previous procedure and starting from 2.6 g (0.01 mol) of 3-methyl-7-methoxy-2-phenyl-4*H*-benzopyran-4-one (**20**), 1.89 g (55%) of the desired compound was obtained: mp 115−117 °C (ligroin); ^{1}H NMR δ 3.9 (s, 3H), 5.5 (s, 2H), 6.85−8.5 (m, 8H, arom).

General Method for Preparation of Imidazol-1-yl Derivatives 22a−j,l,n. A mixture of the selected bromomethyl derivative (0.005 mol) and imidazole (0.015 mol) in 50 mL of acetonitrile was refluxed for 7 h under nitrogen. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (eluent: toluene/acetone 4/1).

^{1}H NMR and MS for Compounds 22a−j,l,n ($CDCl_3$). **22a**: ^{1}H NMR δ 5.45 (s, 2H), 6.5 (d, J = 6.52 Hz, 1H, H-2), 6.95−7.75 (m, 5H, arom + CH imidazole), 7.9 (d, J = 6.52 Hz, 1H, H-3); MS m/z (relative abundance) 251 (M^+, 46.7), 184 (100), 158 (29.3), 32 (16.8). **22b**: ^{1}H NMR δ 5.45 (s, 2H), 6.45 (d, J = 6.25 Hz, 1H, H-2), 6.95−7.65 (m, 5H, arom + CH imidazole), 7.95 (d, J = 6.25 Hz, 1H, H-3); MS m/z (relative abundance) 271 (M^+, 10.1), 174 (27.4), 68 (100), 41 (48.2). **22c**: ^{1}H NMR δ 5.35 (s, 2H), 6.4 (d, J = 5.55 Hz, 1H, H-2), 6.95−7.65 (m, 5H, arom + CH imidazole), 7.8 (d, J = 5.55 Hz, 1H, H-3); MS m/z (relative abundance) 305 (M^+, 10.2), 260 (36.5), 193 (100), 167 (28.9). **22d**: ^{1}H NMR δ 5.4 (s, 2H), 7.0−8.3 (m, 9H, arom + C−H imidazole); MS m/z (relative abundance) 301 (M^+, 9.2), 235 (100), 68 (97.9), 41 (61.5). **22e**: ^{1}H NMR δ 5.45 (s, 2H), 7.0−8.3 (m, 9H, arom + C−H imidazole); MS m/z (relative abundance) 321 (M^+, 44.8), 237 (40.9), 224 (100), 68 (92.9). **22f**: ^{1}H NMR δ 5.55 (s, 2H), 7.1−8.3 (m, 9H, arom + C−H imidazole); MS m/z (relative abundance) 354 (M^+, 7.8), 276 (31.8), 243 (100), 209 (96.5). **22g**: ^{1}H NMR δ (DMSO-d_6) 6.0 (s, 2H), 6.8−8.3 (m, 9H, arom + C−H imidazole); MS m/z (relative abundance) 301 (M^+, 7.9), 145 (100), 67 (16.6), 40 (35.6). **22h**: ^{1}H NMR δ 6.1 (s, 2H), 7.0−8.3 (m, 9H, arom + C−H imidazole); MS m/z (relative abundance) 321 (M^+, 7.2), 68 (100), 44 (95.5), 41 (53). **22i**: ^{1}H NMR δ 6.2 (s, 2H), 7.0−8.3 (m, 9H, arom + C−H imidazole); MS m/z (relative abundance) 354 (M^+, 6.2), 275 (35.6), 242 (100), 179 (28.9). **22j**: ^{1}H NMR δ 5.25 (s, 2H), 6.3 (d, J = 4.89 Hz, 1H, H-2), 6.9−8.05 (m, 6H, arom + CH imidazole), 8.2 (d, J = 4.89 Hz, 1H, H-3); MS m/z (relative abundance) 226 (M^+, 96), 159 (100), 131 (42.4), 51 (24.1). **22l**: ^{1}H NMR δ 5.35 (s, 2H), 6.9−8.3 (m, 10H, arom + C−H imidazole); MS m/z (relative abundance) 276 (M^+, 10), 261 (80), 152 (66), 76 (100). **22n**: ^{1}H NMR δ 3.9 (s, 3H), 5.05 (s, 2H), 6.8−8.2 (m, 11H, arom + C−H imidazole); MS m/z (relative abundance) 332 (M^+, 62.7), 266 (17.5), 265 (100), 115 (69.9).

General Method for Preparation of 1,3,4-Triazol-1-yl Derivatives 22k,m,o. A mixture of the selected bromomethyl derivative (0.005 mol) and 1,3,4-triazole (0.015 mol) in 50 mL of acetonitrile was refluxed for 7 h under nitrogen. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (eluent: toluene/acetone 4/1).

^{1}H NMR and MS for Compounds 22k,m,o ($CDCl_3$). 2k: ^{1}H NMR δ 5.5 (s, 2H), 6.35 (d, J = 5.33 Hz, 1H, H-2), 7.3−8.25 (m, 6H, arom + CH triazole + H-3); MS m/z (relative abundance) 227 (M^+, 100), 200 (48), 159 (47), 131 (44). **22m**: ^{1}H NMR δ 5.45 (s, 2H), 7.0−8.3 (m, 9H, arom + C−H triazole); MS m/z (relative abundance) 277 (M^+, 100), 250 (14.6), 209 (42), 181 (36). **22o**: ^{1}H NMR δ 3.9 (s, 3H), 5.25 (s, 2H), 6.8−8.5 (m, 10H, arom + C−H triazole); MS m/z (relative abundance) 333 (M^+, 17.2), 278 (51.1), 264 (100), 115 (31.7).

Biological Methods. Enzyme Preparations. The enzymes were prepared according to described methods: human placental aromatase,[34] human testicular 17α-hydroxylase as described for the rat enzyme.[19a]

Enzyme Assays. The enzyme assays were performed as described: aromatase,[34] 17α-hydroxylase,[27] kinetic study of **22e**.[26]

Molecular Modeling. The construction of molecular models and the interpolation with the previously developed CoMFA models were performed by means of the SYBYL software.[35] Details about conformational analysis, structure optimization, and CoMFA alignment are reported in ref 19b.

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