Aromatase Inhibition by an 11,13-Dihydroderivative of a Sesquiterpene Lactone

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ABSTRACT

Compounds that inhibit aromatase activity are used for the treatment of breast cancer. A group of sesquiterpene lactones inhibit aromatase activity and also exert cytotoxicity through their reactive α-methylene-γ-lactone group. To synthesize sesquiterpene lactones with greater specificity for aromatase inhibition and lower cytotoxicity, we chemically reduced the α-methylene-γ-lactone group in the active aromatase inhibitor 10-epi-8-deoxycumambrin B (compound 1), to obtain the new compound 11αH,13-dihydro-10-epi-8-deoxycumambrin B (compound 2). Reduction of the α-methylene-γ-lactone group abrogated the cytotoxic activity of compound 1 against the JEG-3, HeLa, and COS-7 cell lines. Compound 2 had higher aromatase inhibitory activity than compound 1 (IC50 = 2 ± 0.5 μM versus 7 ± 0.5 μM, Ks app. = 1.5 μM versus 4.0 μM) and was a more potent type II ligand to the heme iron present in the cytochrome P450arom active site. Compound 2 inhibited aromatase activity in JEG-3 cells in a comparable manner to the inhibitor aminoglutethimide (AG) used clinically for the treatment of breast cancer. Additionally, compound 2 inhibited androstenedione-induced uterine hypertrophy in sexually immature mice (41% of uterine weight suppression for compound 2 versus 51% for AG). We conclude that the anti-aromatase activity of sesquiterpene lactones does not depend on the presence of the highly reactive α-methylene-γ-lactone group, whereas their cytotoxicity does. These findings may facilitate the development of safer agents for breast cancer therapy.

Estrogens play an important role in both normal endocrine processes and in certain hormone-dependent diseases such as breast cancer. The biosynthesis of estrogens is catalyzed by the enzyme complex aromatase. The use of aromatase inhibitors (e.g., aminoglutethimide) for breast cancer treatment is a promising therapeutic approach (Brodie et al., 1999; Njar and Brodie, 1999).

Sesquiterpene lactones (SQLs) are a large and structurally diverse group of plant metabolites. More than 3000 structures have been reported from the family of Asteraceae plants (Heinrich et al., 1998). Several biological activities exerted by SQL have been reported, including anti-tumor (Robles et al., 1995; Beekman et al., 1997), anti-inflammatory (Hall et al., 1980), anti-migraine (Groenewegen et al., 1986), gastric cytoprotective (Giordano et al., 1992), and neurotoxic effects (Cheng et al., 1992). Recently, it was also shown that SQL are inhibitors of smooth muscle contractility (Hay et al., 1994), cyclooxygenase and proinflammatory cytokines induction (Hwang et al., 1996), and nuclear factor-κB activation (Hehner et al., 1998; Lyas et al., 1998). These activities are mediated by the α-methylene-γ-lactone function, and when present in the molecule, also by the α,β-unsaturated cyclopentenone ring. These chemical groups can be considered as powerful alkylating agents by a Michael-type addition of a suitable nucleophile, e.g., nucleophilic attack of cysteine sulfhydryl groups, on the α,β-unsaturated carbonyl group. However, this alkylating activity is non-specific, leading to inhibition of a large number of enzymes or factors involved in key biological processes (Heinrich et al., 1998).

We recently reported that a group of SQLs isolated from various Asteraceae species in northwestern Argentina competitively inhibits the aromatase activity of human placental microsomes (Blanco et al., 1997). All the SQLs that we studied had cytotoxicity and possessed the α-methylene-γ-lactone function. We postulated that this group would not be directly involved in the aromatase inhibitory activity. In this study, we chemically reduced the C-11, C-13 double-bond group.

ABBREVIATIONS: SQL, sesquiterpene lactone; compound 1, 10-epi-8-deoxycumambrin B; compound 2, 11αH,13-dihydro-10-epi-8-deoxycumambrin B; EIMS, electron impact mass spectroscopy; COSY, homonuclear correlation spectroscopy; HETCOR, heteronuclear correlation spectroscopy; P450, cytochrome P450; MTS, tetrazolium compound; DMSO, dimethyl sulfoxide; Ks app., spectral binding constant.
present in the structure of the most potent inhibitor in our series, the SQL 10-epi-8-deoxycumambrin B (compound 1), to produce the dihydroderivative 11βH,13-dihydro-10-epi-8-deoxycumambrin B (compound 2). We then compared the ability of the two compounds to inhibit aromatase activity in human placental microsomes and to exert cytotoxicity in the cell lines JEG-3, HeLa, and COS-7. We tested the aromatase inhibitory activity of compound 2 on JEG-3 cells and in a murine model.

**Experimental Procedures**

**Materials.** The SQL compound 1 has been isolated and characterized as previously reported (Sosa et al., 1989).

Radiolabeled [1,2,6,7-3H]testosterone (80 Ci/mmol) and [3,4,5-3H]cholesterol (51 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). Their purity was checked by thin-layer chromatography according to the manufacturer’s instructions. All other nonradioactive steroids, NADPH, NADP⁺, dt-diithiothreitol, EDTA, and other reagents were purchased from Sigma (St. Louis, MO). Aminoglutethimide was purchased from CIBA-GEIGY (Basel, Switzerland).

**Animals.** Female sexually immature (24 ± 2 days) Rockefeller W1 mice (15–17 g) were obtained from our departmental animal facility. The animals were housed in an air-conditioned room at 21°C (12 h light/dark cycle), and provided with food and water ad libitum. Mice were acclimatized for 5 days in the experimental animal house before the experiments.

**Biological Preparations.** Human placental microsomes and human placental mitochondrias were obtained as reported by us (Genti-Raimondi et al., 1993) and as described by Tuckey (1992), respectively.

**Protein concentration** was determined according to the Bradford procedure using bovine serum albumin as standard (Bradford, 1976).

**Cell Cultures.** JEG-3 choriocarcinoma cell line was purchased from the American Type Culture Collection (Manassas, VA) at passage number 127. COS-7 and HeLa cell lines were obtained from our departmental cell line collection.

JEG-3 cells were grown in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal calf serum, glutamine (2 mM), streptomycin (0.1 mg/ml), and penicillin (100 U/ml) at 37°C in a humidified 95% air/5% CO₂ atmosphere in 175 cm² plastic culture flasks. Medium was changed twice weekly. HeLa and COS-7 cells were grown essentially under the same conditions, with the culture medium supplemented with 5% fetal calf serum.

**Preparation and Characterization of Compound 2.** To a solution of compound 1 (353 mg, 1.42 mmol) in dry MeOH (70 ml), NaBH₄ was added (246 mg, 6.51 mmol) with stirring at room temperature. After 50 min, the mixture was acidified with diluted HCl (diluted with H₂O) and extracted with CHCl₃ (30 ml). The solvent (340 mg) was purified by flash column chromatography on silica gel (benzene-acetone 94:6), yielding 304 mg (86%) of compound 2 (Fig. 1).

**Spectral Studies.** Spectroscopic studies were carried out as described by Kellis and Vickers (1984) and as communicated previously (Blanco et al., 1997).

**Cytotoxicity Testing.** The cytotoxic activity of the compounds on the cell lines was evaluated by two experimental approaches. 1) The percentage of viable cells was estimated with trypan blue dye exclusion using microsomal observations of the gross morphological changes produced during the incubations with different concentrations of the compounds. 2) The number of viable cells was estimated using the CellTiter 96 AQueous NonRadioactive Cell Proliferation Assay kit (Promega Corporation, Madison, WI) according to the manufacturer’s protocol. This kit is composed of a solution of a tetrazolium compound (MTS) and an electron-coupling reagent (phenazine methosulfate). MTS is bioreduced by cells into a formazan that is soluble in tissue culture medium. The absorbance of the formazan at 490 nm can be measured directly and it is proportional to the number of living cells. The conversion of MTS into the aqueous soluble formazan is accomplished by dehydrogenase enzymes found in metabolically active cells (Promega Technical Bulletin, No. 169). Depending on cell growth rate (doubling time), cell size, and duration of the experiment, we selected the optimal cell number so that the control cultures would remain in exponential growth phase during the experiment. Cells were plated on 96-well plates; the SQLs were added to cultures from stock solutions (100% dimethyl sulfoxide; DMSO), and in all cases the final concentration of dimethyl sulfoxide did not exceed 0.2% (v/v).

**Assay of Aromatase Activity in Human Placental Microsomes.** The inhibitory aromatase activities of compounds 1 and 2 were determined as described previously (Blanco et al., 1997).

**Assay of Cholesterol Side Chain Cleavage Enzyme Activity in Human Placental Mitochondria.** The effects of compound 2 and aminoglutethimide on the cholesterol side chain cleavage activity were determined as described previously (Blanco et al., 1997).

**Assay of Aromatase Activity in Human Choriocarcinoma JEG-3 Cell Line.** Aromatase activity was determined by measuring the amount of [3H]estradiol plus [3H]testosterone. Aliquots of a cell suspension were incubated at 37°C in the presence of substrate and the inhibitor or its solvent (dimethyl sulfoxide). The total volume of the incubation mixture was always 1.0 ml. Varying concentrations of compound 2 or aminoglutethimide were added from 100% DMSO stock solutions. After addition to the incubation mixtures, final DMSO concentrations were always equal to or less than 0.2%. At the end of the incubation, the steroids were extracted, identified, and quantified as previously described (Genti-Raimondi et al., 1993).

**Fig. 1.** Reduction of compound 1 to compound 2 with sodium borohydride.

1. 10-epi-8-deoxycumambrin B
2. 11βH, 13-dihydro-10-epi-8-deoxycumambrin B
140 nM unlabeled testosterone and 10 nM \(^{3}H\)testosterone. A cell suspension of \(2 \times 10^{5}\) cells/ml and an incubation time of 4 h were chosen. Under these conditions, conversion of substrate was always less than 10% in the controls.

To perform the kinetic characterization of compound 2, aromatase activity was measured with a mixture of unlabeled testosterone and \(^{3}H\)testosterone varying the concentration from 50 to 300 nM. Non-inhibited aromatase activity was measured in the presence of the solvent (DMSO), and inhibited activity was obtained in the presence of fixed concentrations of compound 2.

**Assay of Aromatase Inhibitory Activity in Immature Female Mice.** The aromatase inhibitory activity in vivo was determined using our standard assay based on the inhibition of androstenedione-induced uterine hypertrophy in sexually immature mice. Originally, the assay was described using immature female rats (Bhatnagar et al., 1990). We have validated the assay on sexually immature female Rockefeller W1 mice with reproducible results. Androstenedione was dissolved in olive oil for injection (s.c.), aminogluthethimide and compound 2 were dissolved in water and administered orally using a plastic tube connected to a syringe. The animals were randomly divided in four experimental groups before treatment. Group 1: negative control (olive oil s.c.), group 2: positive control (androstenedione 50 mg/kg/day s.c.), group 3: aminoglutethimide treatment (androstenedione 50 mg/kg/day s.c. + aminogluthethimide 70 mg/kg/day p.o.), and group 4: compound 2 treatment (androstenedione 50 mg/kg/day s.c. + compound 2, 70 mg/kg/day p.o.). Treatments were administered once a day in parallel for all four groups for four consecutive days. On day 4, animals were sacrificed by cervical dislocation 8 h after the last treatment. The uterus was dissected and weighted in a Mettler AT 261 Delta range balance (±0.1 mg), and body weights were determined before sacrifice using a Mettler PM 4600 Delta range balance (±10 mg). Results were expressed as a ratio: \(\text{uterine weight (mg)/body weight (g)}\) \(\times 100\).

**Molecular Modeling.** The structures of the inhibitors and substrates were minimized using AM1 calculations with Molecular Orbital Package (MOPAC).

**Data Analysis.** The concentration of the compounds required to reduce control activity by 50% (IC\(_{50}\)) was calculated by nonlinear regression analysis using Sigma Plot version 3.1 (1995, Jandel Corporation, Chicago, IL). Results are expressed as the mean ± S.D.

Values of \(K_{m}\) and \(V_{max}\) were estimated graphically from plots of 1/velocity versus 1/substrate using linear regression analysis, which in all cases resulted in straight-line plots, with linear regression correlation coefficients very close to unity. The replots from the slope of each reciprocal plot versus the corresponding inhibitor concentrations were generated, and the \(K_{i}\) value for the compound was determined. Values represent the mean of at least three experiments performed in duplicate.

The reported spectral binding constant (\(K_{spp}\)) was calculated using standard graphical analysis (Kellis and Vickery, 1984). A plot was generated by representing 1/absorbance 419 – 390 nm versus 1/compound concentration. Values represent the mean of three different absorption spectra performed under the same experimental conditions.

Cell growth inhibition was calculated using the formula: Growth inhibition (%) = \(1 - \left(\frac{\text{absorbance of treated cells} - \text{absorbance of culture medium}}{\text{absorbance of untreated cells} - \text{absorbance of culture medium}}\right)\) \(\times 100\) (Francois et al., 1996).

The results of the in vivo assay were compared using the Mann-Whitney \(U\) test. A \(P\) value less than 0.05 was taken as significant.

**Results**

**Spectroscopic Characterization of Compound 2.** Spectroscopy confirmed the structural identity of compound 2. The EIMS spectrum of 2 revealed a molecular ion at \(m/z\) 250, indicating a molecular weight of two more mass units than 1.

The \(^{1}H\) NMR spectrum of 2 did not show signals corresponding to the exocyclic double-bond protons H-13a and H-13b. In comparison with the spectrum of compound 1 (Sosa et al., 1989), it showed an extra methyl signal at \(\delta\) 1.22 corresponding to the CH\(_3\) group attached to C-11. The spectrum also showed a new signal at \(\delta\) 2.23 (dq, 11.3 and 7 Hz) corresponding to the proton attached to C-11. The coupling constant value of 11.3 Hz between H-11 and H-7 indicated that these two protons maintain a trans-diaxial relationship. As H-7 is always in \(\alpha\) orientation, H-11 adopts the opposite orientation (\(\beta\)), clearly indicating that the methyl group attached to C-11 (H\(_{3}\)-13) is in \(\alpha\)-orientation.

The disappearance of the C-11, C-13 double bond was also observed in the \(^{13}C\) NMR spectrum. Instead of the signals at \(\delta\) 140.28 (C-11) and \(\delta\) 118.65 (C-13) observed in the spectrum of compound 1, the spectrum of compound 2 shows one more CH\(_3\) signal at \(\delta\) 12.85 (C-13) and a signal of a CH group at \(\delta\) 41.91 corresponding to C-11. The full assignment of the NMR signals was aided by the 2D experiments COSY and HETCOR.

**Aromatase Inhibitory Activity of Compound 2 in Human Placental Microsomes.** To determine whether compound 2 inhibited the aromatase activity in human placental microsomes, we performed dose-response experiments. For comparative purposes, we carried out simultaneous assays with compound 1. The compounds inhibited the aromatase activity of human placental microsomes with an IC\(_{50}\) = 2.0 ± 0.5 \(\mu M\) (compound 2) and IC\(_{50}\) = 7.0 ± 0.5 \(\mu M\) (compound 1), respectively. Figure 2A shows the time course of the aromatization of 50 pmol of testosterone in 1.0 ml (1 \(\times 10^{5}\) cpm/ml) by human placental microsomes in the presence and absence of compound 2. Under these conditions, 10 \(\mu M\) compound 2 inhibits the aromatization by approximately 80% (Fig. 2A, inset). The reaction rate in the presence of the inhibitor remains linear for 10 min. The lack of time dependence of inhibition indicates that no significant conversion of the inhibitor, to more or to less active forms, occurs during the incubation. The dihydroderivative (compound 2) was then tested for this ability to cause time-dependent inactivation. When human placental microsomes were preincubated in the presence of 10 \(\mu M\) compound 2, and in the absence of substrate, variation of the pre-equilibration time did not affect the fractional inhibition or time course in the presence and absence of NADPH cofactor (data not shown). Figure 2B shows a Lineweaver-Burk plot of the inhibition of human placental aromatase by compound 2. Inhibition was competitive with respect to the substrate testosterone (i.e., apparent increase in \(K_{m}\) values with no significant changes in \(V_{max}\) values in the presence of inhibitor and therefore a decrease of the \(V_{max}/K_{m}\) ratio). A replot of the slopes of the lines (shown in the inset) yielded a \(K_{i}\) = 1.5 \(\mu M\). In the absence of inhibitor, the average \(K_{m}\) value for testosterone was 31 nM.

The kinetic data suggest that compound 2 may inhibit the aromatization by competing with testosterone for the substrate-binding site of the enzyme. This possibility was further investigated by monitoring the effects of the compound on the spectral absorption properties of the enzyme. When partially purified aromatase was first equilibrated with testosterone, the addition of increasing concentrations of compound 2 (12.5–150 \(\mu M\)) produced a similar type II difference spectrum to compound 1 (Blanco et al., 1997), with a Soret peak at 419 nm. This phenomenon was dose-dependent.
Graphical analysis of the titration showed only one kind of binding site that exhibits a $K_{\text{app}} = 16 \mu M$ (data not shown). Interestingly, there is a correlation between the better inhibitory constant of compound 2 ($K_i = 1.5 \mu M$) and the spectral binding constant ($K_{\text{app}} = 16 \mu M$) in comparison with the kinetic and spectral data obtained for compound 1 ($K_i = 4 \mu M$, $K_{\text{app}} = 29 \mu M$) under similar experimental conditions (Blanco et al., 1997).

Some aromatase inhibitors inhibit in a dose-dependent manner the activity of other cytochrome P450-mediated steroid hydroxylation. The cholesterol side chain cleavage enzyme catalyzes the three hydroxylations required for the conversion of cholesterol to pregnenolone. This is the first step in the steroid biosynthetic pathway. This enzyme is affected by aminogluthethimide (Cash et al., 1967; Bhatnagar et al., 1990). When we compared the effects of aminogluthethimide and compound 2 (100 and 200 nM) on cholesterol side chain cleavage activity in human placental mitochondria, no significant inhibitory activity was observed for compound 2 (data not shown).

### Effect of Compounds 1 and 2 on Cellular Viability

To compare the effect of the α-methylene-γ-lactone group of the SQL structures on cellular viability, we incubated JEG-3 cells with 100 μM compounds 1 or 2 for 24 h. The cells showed morphological changes after 1 h of incubation with compound 1, whereas incubation with 100 μM compound 2 did not show significant effect on the cells, even when the exposure was prolonged for 48 h (data not shown). These observations were in agreement with results of trypan blue staining of the treated cells versus the nontreated controls. In order to confirm these morphological analyses, cell cultures of the cell lines JEG-3, HeLa, and COS-7 were exposed to different concentrations (range 0.1–100 μM) of test compounds 1 and 2 for 24 h to later assess cell viability through a colorimetric assay (Fig. 3). The SQL carrying the α-methylene-γ-lactone function (compound 1) was cytotoxic, whereas compound 2 was not cytotoxic under the same experimental conditions. Similar profiles of growth inhibition were obtained with HeLa and COS-7 cell lines.

### Aromatase Inhibitory Activity of Compound 2 in JEG-3 Cells

The noncytotoxic dihydroderivative compound 2 was evaluated as a potential aromatase inhibitor using cultures of JEG-3 cells. Figure 4 shows dose-response curves obtained for compound 2 and aminogluthethimide. Under the same experimental conditions, IC$_{50}$ values obtained were
very similar for both compounds; IC\textsubscript{50} = 10 ± 5 μM for compound 2 and IC\textsubscript{50} = 15 ± 5 μM for aminogluthethimide, respectively. Figure 5 shows a Lineweaver-Burk plot obtained for compound 2 using testosterone as substrate. Compound 2 acts as a competitive inhibitor of the aromatase activity. An inhibition constant \( K_i = 36 \) μM was extrapolated (see inset). In the absence of inhibitor, the average \( K_m \) value for testosterone was 128 nM.

**Aromatase Inhibitory Activity of Compound 2 in Vivo**. To determine whether compound 2 inhibited aromatase activity in an in vivo model we performed experiments on sexually immature female mice. The ovarian aromatase activity converts androstenedione into estrogens, which stimulates the increment of the uterine weight in the immature animal. In the presence of an aromatase inhibitor, the androstenedione-induced uterine hypertrophy is abolished (Bhatnagar et al., 1990; Hartmann et al., 1994).

Compound 2 (70 mg/kg/day p.o.) inhibited androstenedione-induced uterine hypertrophy in sexually immature mice (Fig. 6). The inhibitory effect was similar to aminogluthethimide using the same administration route and equimolar dose. There was a significant difference between the group of animals treated with androstenedione alone (positive control) and the group treated with androstenedione and compound 2 concomitantly (\( P = 0.01 \)). Also, there was a significant difference between the group treated with androstenedione alone (positive control) and the group of animals treated with androstenedione and aminogluthethimide concomitantly (\( P = 0.006 \)). There was not a significant difference between treatment groups 3 and 4 (\( P = 0.06 \)), respectively. The average percentage of inhibition for compound 2 was 41% (\( n = 9 \)), and the average percentage of inhibition for aminogluthethimide was 54% (\( n = 9 \)).

**Discussion**

In this study, we found that the chemical reduction of the C-11, C-13 exocyclic double bond of compound 1 does not affect the capacity of the compound to inhibit aromatase activity, but it eliminates the cytotoxic activity of the molecule. We characterized the aromatase inhibitory activity of the novel semisynthetic derivative compound 2. The results showed that this compound is a competitive inhibitor of the aromatase activity of human placental microsomes with a similar potency to aminogluthethimide. The UV-Vis difference spectroscopy data support the kinetic evidence, and we can conclude that compound 2 competes with the steroidal substrate for the aromatase active site. In addition, compound 2 is an aromatase inhibitor more specific than aminogluthethimide, since it does not affect the cholesterol side chain cleavage activity (P450\textsubscript{sec}) of human placental mitochondria.

The cytochrome P450 aromatase is a membrane-bound
protein that has resisted structure-function analysis by means of X-ray crystallographic methods because of its resistance to solubilization, and hence, to crystallization. For these reasons, several three-dimensional models have been proposed. These models were based on other cytochrome P450s whose structures have been resolved. One of the most complete and detailed three-dimensional models of aromatase P450 was proposed by Graham-Lorence et al. (1995). This model was used in our previous work to suggest the possible interactions between the P450 aromatase active site and the SQ. Based on this model, the three-dimensional computer-generated structures of the inhibitor (compound 2) and substrate (testosterone), and our present data, we suggest that: 1) the carbonyl group at C-12 of compound 2 would interact with the K473 (lysine) residue in the aromatase active site analogously to the substrate testosterone; 2) the C-10 hydroxyl group in β orientation would coordinate with the heme iron present in the aromatase active site; and 3) the reduction of the α-methylene group produces a more apolar region in the inhibitor that could be better positioned in the extra hydrophobic pocket predicted for the aromatase active site (Fig. 7). This pocket is located below the α-face of the steroidal substrate corresponding to the C-4, C-6, and C-7 positions of its skeleton and can accommodate bulky substituents (Laughton et al., 1993; Graham-Lorence et al., 1995; Liu et al., 1995; Kao et al., 1996). This could explain the minor increase in the inhibitory potency of compound 2 (Ki = 1.5 μM) in comparison with compound 1 (Ki = 4.0 μM) and the correlation with the spectral binding constants obtained for both inhibitors (Ks app, 1 = 29 μM, Ks app, 2 = 16 μM). The loss of the cytotoxic activity observed for compound 2 due to the reduction of the α-methylene exocyclic group dictated that we test its potential aromatase inhibitory activity in a cellular model. According to the results obtained with the JEG-3 choriocarcinoma cell line, the aromatase inhibitory activity of compound 2 is significant and similar to the drug aminoglutethimide, the latter being in agreement with that reported by Krekels et al. (1991). Compound 2 inhibited aromatase activity in immature female mice stimulated with androstenedione. These finding are potentially interesting, but need further confirmation in other cellular and animal models suitable for the study of aromatase inhibitors (Dukes, 1997).

SQs constitute a large group of terpenoids with many biological activities mediated by α,β-unsaturated carbonyl structures, such as an α-methylene-γ-lactone or an α,β-unsubstituted cyclopentenone. We have demonstrated that the α-methylene-γ-lactone group is not necessarily required for aromatase inhibition and that aromatase inhibition can be maintained without causing cytotoxicity. The elimination of this highly reactive and nonspecific chemical moiety from the original compound would probably improve the general pharmacological profile of this novel aromatase inhibitor. These findings open new avenues for future modifications designed to enhance the activity of these active natural compounds.

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References


Fig. 7. Three-dimensional models of compound 2 (empty spheres—L = sesquiterpene lactone) and testosterone (filled spheres—S = steroid). Models are superimposed by overlapping by hand the carbonyl group at C-12 of the inhibitor and the carbonyl group at C-3 of the steroid, placed in a schematic aromatase enzyme pocket (not in scale) and showing all the possible interactions.


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