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Modulation of Cytochromes P450 with Xanthone-Based Molecules: From Aromatase to Aldosterone Synthase and Steroid 11β-Hydroxylase Inhibition

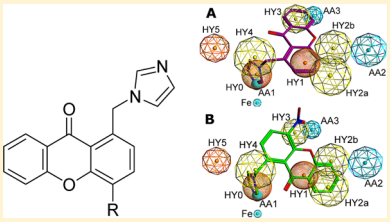
Silvia Gobbi,*[,†] Qingzhong Hu,[‡] Matthias Negri,[‡] Christina Zimmer,[‡] Federica Belluti,[†] Angela Rampa,[†] Rolf W. Hartmann,[‡] and Alessandra Bisi*[,†]

[†]Department of Pharmacy and Biotechnologies, University of Bologna, Via Belmeloro, 6, I-40126 Bologna, Italy

[‡]Pharmaceutical and Medicinal Chemistry, Saarland University, P.O. Box 151150, and Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), D-66041 Saarbrücken, Germany

Supporting Information

ABSTRACT: Imidazolylmethylflavones previously reported by us as aromatase inhibitors proved to be able to interact with aldosterone synthase (CYP11B2), a cytochrome P450 enzyme involved in the biosynthesis of the mineralcorticoid hormone aldosterone, and were used to obtain a pharmacophore model for this enzyme. Here, in the search for potential ligands for CYP11B2 and the related CYP11B1, a virtual screening of a small compounds library of our earlier synthesized aromatase inhibitors was performed and, according to the results and the corresponding biological data, led to the design and synthesis of a series of xanthones derivatives carrying an imidazolylmethyl substituent in position 1 and different substituents in position 4. Some very potent inhibitors were obtained; in particular, the 4-chlorine derivative was active in the low nanomolar or subnanomolar range on CYP11B2 and CYP11B1, respectively, proving that xanthone can be considered as an excellent scaffold, whose activity can be directed to different targets when appropriately functionalized.



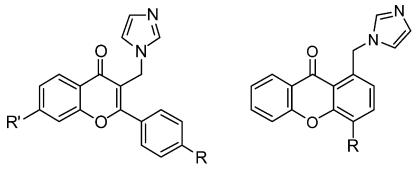
INTRODUCTION

The search for inhibitors of cytochrome P450 (CYP) enzymes catalyzing the biosynthesis of steroid hormones has been intensely pursued during the past several decades, since abnormally increased levels of these hormones, sharing closely related synthetic pathways, have proved to be involved in the development of several hormone-related diseases. Inhibition of the aromatase enzymatic complex (CYP19), which promotes the aromatization of the A ring of androgen substrates leading to estrogen hormones, is one of the approaches currently exploited for the treatment of estrogen-dependent breast cancer, aiming to reduce the estrogen concentration in blood.[1] Closely related 17α-hydroxylase/C17,20-lyase (CYP17), a key enzyme of androgen biosynthesis, is considered to be an interesting target for the treatment of benign prostatic hyperplasia and prostate cancer.[2]

Two other steroidogenic CYP enzymes, namely, 11β-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2), isoforms sharing a high degree of sequence homology (more than 90%), are involved in the biosyntheses of cortisol and aldosterone, respectively. In particular, the final steps of aldosterone biosynthesis are performed by these two enzymes that catalyze the 11-hydroxylation of 11-deoxycorticosterone to corticosterone, which is then further hydroxylated to 18-hydroxycorticosterone (18OH-B). In man, only the CYP11B2 isoform can perform the final oxidation of C18 to produce aldosterone, while CYP11B1 is mainly responsible for glucocorticoid formation.[3] These enzymes have raised researchers' attention as potential therapeutic targets,[2] since overproduction of cortisol can cause diseases such as hypercortisolism and Cushing's syndrome,[3] while hyperaldosteronism could be responsible for hypertension, and aldosterone has been more recently found to play a role in other cardiac diseases such as congestive heart failure or myocardial fibrosis.[4]

Recently, some imidazolylmethylflavones previously described by our group as aromatase inhibitors (Chart 1)[5,6] have been shown to be able to interact with aldosterone synthase when tested in a compound library screening.[7] They were then used as part of a training set in order to generate an extended pharmacophore model for this enzyme, in which the earlier described pharmacophoric points[8] were confirmed and a novel large hydrophobic area was identified.[7] After absorbing

Chart 1. Reference Structures



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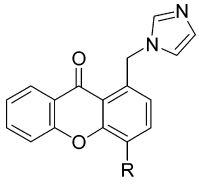
more CYP11B2 inhibitors that have been reported in the latest patents into the training set, the pharmacophore model was further improved to be a more powerful tool aiding the identification of new CYP11B2 inhibitors.[9] Inspired by the interesting finding that imidazolylmethylflavones inhibit CYP11B2, a small compound library of earlier synthesized aromatase inhibitors was virtually screened with this novel pharmacophore model as query using MOE. The pharmacophore search returned four compounds, characterized by the structurally related xanthone scaffold, that could be inhibitors of CYP11B2. In particular, these molecules (Chart 1 and Table 1, **1a–d**)[10,11] have a common feature, the imidazolylmethyl substituent in position 1, for coordinating the heme iron of these CYP enzymes, and different substituents (or no substituent) in position 4. The compounds were then experimentally tested for inhibition of CYP11B2 and CYP11B1 and were shown to be potent inhibitors of these enzymes, with the unsubstituted molecule **1a** showing lower activity with respect to the substituted ones (Table 1). In contrast, compound **1c**, substituted by a 4-Br, showed the highest potency, suggesting that apolar substituents in position 4 might lead to potent inhibition. To explore this option, a series of related xanthones derivatives have now been designed and synthesized, carrying groups with different polarity and size in position 4, in order to explore the chemical space, consider their role in the interaction with the enzyme, and investigate the requirements of this hydrophobic area. The new compounds (**1e–p**), collected in Table 1, were tested for their inhibition of CYP11B2 and CYP11B1. Due to the close structural resemblance to compounds originally designed for aromatase inhibition and in order to examine their potential selectivity, the new compounds were then further evaluated for inhibition of the other steroidogenic enzymes CYP19 and CYP17.

Table 1. Structures and Biological Profiles of the New Compounds



	R	CYP11B1[a] IC_{50} [nM][d]	CYP11B2[b] IC_{50} [nM][d]	SF[c] B1/B2	SF[c] B2/B1
1a[e]	H	99.1	114.3	0.9	
1b[f]	NO_2	22.3	31.1	0.7	
1c[f]	Br	6.7	8.5	0.8	
1d[f]	CN	13.4	19.5	0.7	
1e	Cl	0.5	3.0	0.2	6.0
1f	I	19.2	23.5	0.8	
1g	Ph	156.3	103.9	1.5	
1h	OCH_3	80.1	41.1	1.9	
1i	OCH_2CH_3	25.4	14.8	1.7	
1j	$OCH(CH_3)_2$	92.5	19.1	4.8	
1k	$OCH_2CH_2CH(CH_3)_2$	24.4	25.2	1.0	
1l	$OCH(CH_3)CH_2CH_3$	13.1	15.3	0.9	
1m		5.5	24.1	0.2	4.4
1n		268.4	99.7	2.7	
1o	OH	415.8	235.3	1.8	
1p	NH_2	785.3	676.5	1.2	
Fadrozole		6.3	0.8	7.9	

[a]Hamster fibroblasts expressing human CYP11B1; substrate deoxycorticosterone, 100 nM. [b]Hamster fibroblasts expressing human CYP11B2; substrate deoxycorticosterone, 100 nM. [c]SF = selectivity factor. [d]Mean value of at least three experiments; relative standard deviation less than 25%. [e]See ref 11. [f]See ref 10.

CHEMISTRY

The key intermediates for the synthesis of the studied compounds, 4-substituted-1-methylxanthones **4e–o**, were prepared starting from 4-amino-1-methylxanthone[10] **2** by reacting it with $NaNO_2$ and coupling the obtained diazonium salt with CuCl or NaI or H_2SO_4 to give **4e**, **4f**, and **3**, respectively, or via formation of arenediazonium tetrafluoroborate and coupling with potassium trifluoro(phenyl)borate to give **4g**.[12] Intermediate **3** was then alkylated with dimethyl sulfate or selected alkyl halides to obtain **4h–o** (Scheme 1). 1-Methylxanthones (**4e–o**) were then brominated with NBS, and by reaction with imidazole, compounds **1e–n** were obtained, as depicted in Scheme 2 (**6** was deprotected by refluxing it in acetic acid to give **1o**). Finally, **1p** was obtained by hydrogenation of the previously described 1-imidazolylmethyl-4-nitroxanthen-9-one[10] **1b** (Scheme 3).

RESULTS AND DISCUSSION

Following the results obtained with imidazolylmethylflavones that were selected among earlier synthesized aromatase inhibitors, a pharmacophore search was performed on an in-house compound library of previously synthesized CYP19 inhibitors with the recently described 3D-pharmacophore model of CYP11B2.[9] This pharmacophore model consisted of six hydrophobic/aromatic features (HY0–HY5) and two acceptor atom features (AA1 and AA2) (Figure 1). Among them, HY0 and AA1 together were attributed to the heterocycle that coordinated the heme iron with its sp^2 hybrid N, while HY1 represented the aromatic core. These pharmacophores were deemed essential for the CYP11B2 inhibition. Since the pharmacophore model was developed on the basis of various structurally diversified CYP11B2 inhibitors, which made the model more powerful in identifying potential CYP11B2 inhibitors, the possession of the rest of the pharmacophores may increase the affinity or improve the selectivity. However, it is not necessary to occupy as many pharmacophores as possible for the sake of ligand efficiency. This model was employed as the pharmacophore query to filter the 3D conformation databases generated from the small CYP19 inhibitor library by comparing the pharmacophore features of the query with the ligand annotation points of each conformation. If all the attributions, distances and constrains satisfied and the rigid-body superposition passed, a hit was identified. Only one hit for each compound was conserved according to the ranking of

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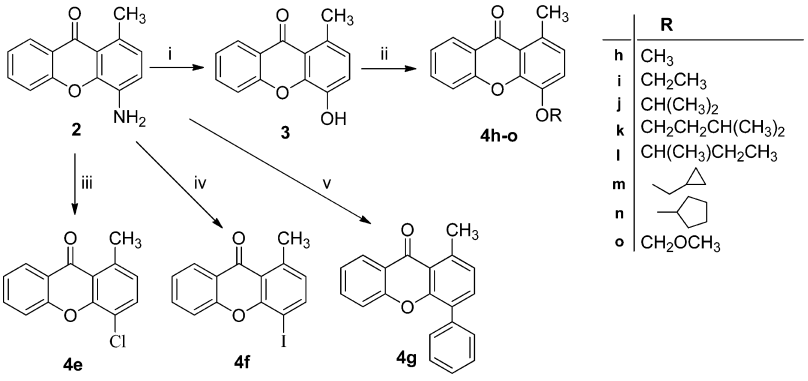
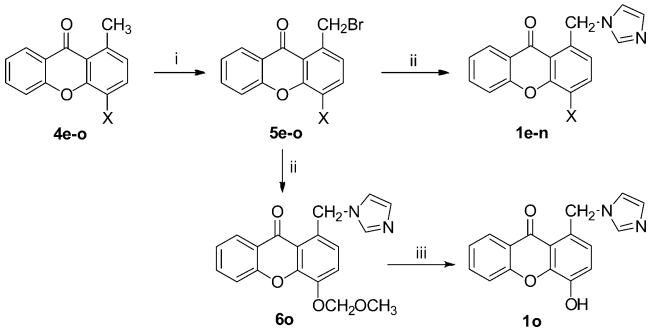
Scheme 1[a]



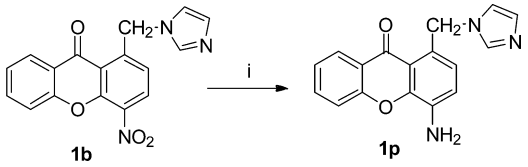
[a]Reagents and conditions: (i) H_2SO_4, $NaNO_2$, −5 °C to rt, then H_2SO_4, 80 °C; (ii) dimethyl sulfate, K_2CO_3, acetone or NaH, DMSO, alkyl halide; (iii) HCl, $NaNO_2$, −5 °C to rt, then CuCl, 80 °C; (iv) H_2SO_4, $NaNO_2$, −5 °C to rt, then NaI, 80 °C; (v) $NaBF_4$, HCl, H_2O, $NaNO_2$, −5 °C, then $Pd(CH_3COO)_2$, potassium trifluoro(phenyl)borate, dry dioxane, rt.

Scheme 2[a]



[a]Reagents and conditions: (i) NBS, CCl_4, benzoyl peroxide, *hν*, reflux; (ii) N_2, imidazole, reflux; (iii) CH_3COOH, H_2SO_4, reflux.

Scheme 3[a]



[a]Reagents and conditions: (i) H_2, Pd/C, THF, rt.

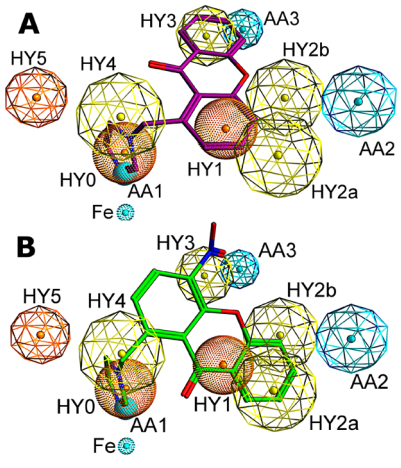


Figure 1. The fitting pattern of xanthone compounds **1a** (violet, A) and **1b** (green, B) in the pharmacophore model.

rmsd. Since the prediction ability of the model has been fully validated before,[9] no further refining of the query was performed. The virtual screening revealed that some molecules (**1a**−**d**),[10,11] characterized by a xanthone scaffold, could fit into the model, indicating potential inhibition of CYP11B2. Although only differing for the groups in position 4 of the xanthone core, these four compounds exhibited two different poses in the pharmacophore model. Both poses occupied HY0 and AA1 with the imidazolyl moiety, whereas the positions of the xanthone cores were different (Figure 1). Compounds **1a**, **1c** and **1d** showed a similar occupational pattern, with ring A of the xanthone nucleus covering the essential feature HY1 and ring C fitting the recently identified feature HY3 (pose 1, Figure 1a; compound **1a** as representative, violet). However, compound **1b** employed ring B and C to match features HY1 and HY2b, respectively (pose 2, Figure 1b, green). Strikingly, this second pose was only observed for compound **1b**, with the polar NO_2 group in position 4 filling HY3. The compounds were then tested and found to be potent inhibitors of both CYP11B1 and CYP11B2 enzymes (Table 1). The presence of a substituent in position 4 in the xanthone scaffold proved to be favorable for interaction, the unsubstituted **1a** having lower activity with respect to the substituted **1b**−**d**. In particular, a slight improvement in potency could be seen going from **1b**, substituted with a nitro group in position 4, to **1d**, featuring a cyano moiety, to **1c**, carrying a bromine, which showed activity in the low nanomolar range. However, no significant selectivity was observed between the two enzymes, and the compounds were slightly better inhibitors of CYP11B1 with respect to CYP11B2. Some selectivity could indeed be noted when these results were compared with those obtained for inhibition of CYP19 and CYP17[10,11] (Table 2), mainly for substituted compounds. In particular, **1b** showed a 30−40-fold selectivity for CYP11B1 and CYP11B2 with respect to CYP19, and for **1c** and **1d**, the selectivity ratios against CYP19 were 120−140 and 120−180, respectively, while selectivity toward CYP17 was generally lower.

Since xanthones with common pose 1 occupied HY3 with their C-ring, similar to the pattern of previously reported CYP11B2 inhibitors,[7] we were encouraged by this similarity to further modify this structure for more potent CYP11B2 inhibition. When tested, compound **1c** (pose 1) showed the highest potency, suggesting that apolar substituents in position 4 that could fit the hydrophobic regions HY2a or HY2b of the

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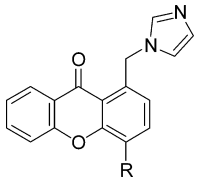
Table 2. Inhibition of Human CYP19 and CYP17 for the New Compounds 1e−p



	R	CYP19[a] IC_{50} nM[c]	CYP17[b] IC_{50} nM[c]
1a[d]	H	150[d]	880[d]
1b[e]	NO_2	970[e]	130[e]
1c[e]	Br	940[e]	42[e]
1d[e]	CN	2300[e]	220[e]
1e	Cl	480	260
1f	I	460	1346
1g	Ph	300	1886
1h	OCH_3	1100	610
1i	OCH_2CH_3	1973	>5000
1j	$OCH(CH_3)_2$	400	1250
1k	$OCH_2CH_2CH(CH_3)_2$	837	>5000
1l	$OCH(CH_3)CH_2CH_3$	575	>5000
1m		565	>5000
1n		260	>5000
1o	OH	170	493
1p	NH_2	6393	1737
Fadrozole		41	>5000
Abiraterone		>5000	72

[a]Human placental CYP19; substrate androstenedione, 500 nM. [b]*Escherichia coli* expressing human CYP17; substrate progesterone, 25 μM. [c]Mean value of at least three experiments; relative standard deviation less than 25%. [d]See ref 11. [e]See ref 10.

pharmacophore model might lead to potent inhibition. New compounds (**1e−p**) were then designed and synthesized in order to investigate the effect of different substitutions on this part of the molecule and to try to address the selectivity issue. The results of the testing on CYP11B2 and CYP11B1 are presented in Table 1 and show remarkable activity for most of the new compounds. Considering the substitution with halogen atoms (compounds **1c**, **1e**, and **1f**), it should be noted that it was proved to be highly favorable, leading to very potent compounds with activity in the low nanomolar range. In particular, inhibition seemed to slightly increase going from the bulky iodine (compound **1f**) to the chlorine containing **1e**, which showed an IC_{50} of 3.0 nM on CYP11B2. Moreover, activity on CYP11B1 showed the same tendency for these derivatives, with **1e** (IC_{50} = 0.5 nM) as the best inhibitor of this enzyme in the whole series. While this compound showed a 6-fold selectivity for CYP11B1 over CYP11B2, **1b** and **1f** were not able to discriminate between the two enzymes.

Notably, an increase in steric hindrance in position 4 on the xanthone nucleus seemed not to be easily accepted by these enzymes, especially CYP11B1, where the insertion of a phenyl group (compound **1g**) led to a decrease in activity. On the other hand, the introduction of alkoxy substituents, both linear or branched, seemed to enhance activity, particularly toward CYP11B2, with the only exception of **1n** carrying the large pentyloxy group, which again showed similar or lower potency with respect to the unsubstituted **1a** for CYP11B2 and CYP11B1, respectively. In particular, activity toward aldosterone synthase increased with respect to **1a** with the introduction of a methoxy group (compound **1h**, IC_{50} = 41.1 nM) and again with an ethoxy one (**1i**, IC_{50} = 14.8 nM), but further lengthening and branching of the chain did not lead to any significant improvement. Somewhat different behavior was noted for inhibition of CYP11B1; the introduction of the small methoxy substituent (**1h**) was not significant for activity, which was increased with an ethoxy substituent (**1i**, IC_{50} = 25.4 nM) but decreased by branching this short chain, as in **1j** (IC_{50} = 92.5 nM), and the introduction of a longer branched alkoxy group did not significantly improve potency, with the only exception of **1m**, carrying a cyclopropylmethoxy group (IC_{50} = 5.5 nM). Regarding selectivity of these alkoxy derivatives, it could be noted that shorter chains seemed to better differentiate between CYP11B2 and CYP11B1; for example, **1j** with an isopropoxy substituent was almost 5-fold more potent on aldosterone synthase. In contrast, compound **1m** showed a 4-fold selectivity for 11β-hydroxylase.

Finally, the introduction in position 4 of the xanthone nucleus of hydrophilic moieties such as OH and NH_2 (compounds **1o** and **1p**, respectively) led to a significant decrease in activity for both CYP11B2 and CYP11B1.

Taking into account the close similarity with our previously reported aromatase inhibitors, to further investigate the selectivity of the new synthesized compounds, the potential of compounds **1e−p** to inhibit CYP19 and CYP17 was also evaluated, and results are collected in Table 2. The compounds generally proved to be poor inhibitors of CYP17. In contrast to the lower selectivity of the synthesized compounds toward CYP11B2 when compared to fadrozole (SF = 7.9, Table 1), they exhibited higher selectivity than the reference compound for CYP11B2 and CYP11B1 with respect to CYP19, although their inhibition of CYP19 are within micro- or submicromolar range. Above all, compounds **1e** and **1i** showed 2−3 orders of magnitude higher activity on the target enzymes with respect to CYP19 and CYP17. For a quantitative outline of the selectivity of the new compounds for the different CYPs evaluated, a table reporting selectivity factors (SF) can be found in the Supporting Information (Table 1 SI).

■ CONCLUSIONS

In the search for potential ligands for CYP11B2 and CYP11B1, a series of molecules characterized by a xanthone scaffold, structurally related to previously reported aromatase inhibitors, has been designed and synthesized, on the basis of a recently described pharmacophore model of CYP11B2.[9] These compounds carry an imidazolylmethyl substituent in position 1 and different substituents in position 4. Among them, some very potent inhibitors were obtained, in particular, the 4-chlorine derivative **1e** proved to be active in the low nanomolar or subnanomolar range on CYP11B2 and CYP11B1,

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respectively. Generally speaking, the introduction of hydrophobic substituents of appropriate size seemed to enhance affinity for both enzymes, as expected from the docking simulations studies. It should be noted that the selectivity issue regarding these highly homologous enzymes still remains to be solved, although some of the new compounds could discriminate between the two: in particular, compound **1j**, carrying an isopropoxy group, showed a 5-fold selectivity for CYP11B2, and **1e** and **1m**, bearing chlorine and cyclopropylmethyl groups, respectively, were found to be the most selective for CYP11B1 (6- and 4-fold, respectively). On the other hand, a remarkable degree of selectivity was observed toward CYP19 and CYP17, indicating that a modulation of different steroidogenic cytochromes P450 can be obtained with an appropriate pattern of substitution on the xanthone nucleus. These results confirm that the xanthone can be considered as an excellent scaffold, whose activity can be directed to different targets when appropriately functionalized.

EXPERIMENTAL SECTION

Chemistry. General Methods. All melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$ solutions unless otherwise indicated, with Me_4Si as the internal standard. Mass spectra were recorded on a V.G. 7070 E spectrometer or on a Waters ZQ 4000 apparatus operating in electrospray (ES) mode. Silica gel (Merck, 230−400 mesh) was used for purification with flash chromatography. Chemical purities of the tested compounds were determined by elemental analysis (C, H, N) and confirmed ≥95% purity (see the Supporting Information). Compounds were named following IUPAC rules as applied by AUTONOM, a PC software for systematic names in organic chemistry (Beilstein-Institut and Springer).

*4-Hydroxy-1-methylxanthen-9-one (**3**).* 4-Amino-1-methyl-9-xanthenone[10] (**2**) (3 g, 13.5 mmol) was dissolved in H_2SO_4 (40 mL), and $NaNO_2$ (0.93 g, 13.5 mmol) in water was added dropwise keeping the temperature below −5 °C. The mixture was stirred for 30 min, allowed to go to room temperature (rt) for another 30 min, and poured into warm H_2SO_4 1:2 (300 mL) with stirring. The mixture was heated to 80 °C until the evolution of N_2 ceased and poured into ice and the precipitate was filtered to obtain **3** (0.9 g, 31%). Mp: 243−245 °C. ^{1}H NMR (acetone-d_6): δ 2.81 (s, 3H, CH_3), 7.31−7.32 (m, 1H, arom), 7.38−7.42 (m, 2H, arom), 7.49−7.52 (m, 1H, arom), 7.76−7.79 (m, 1H, arom), 8.21−8.23 (m, 1H, arom), 9.72 (s, 1H, OH).

*4-Chloro-1-methylxanthen-9-one (**4e**).* **2**[10] (1 g, 4.44 mmol) was dissolved in HCl 1:2 (12 mL), and $NaNO_2$ (0.31 g, 4.44 mmol) in water was added dropwise keeping the temperature below −5 °C. The mixture was stirred for 30 min, allowed to go to rt for further 30 min, and added to a cold solution of CuCl (0.53 g, 5.33 mmol) in HCl (3 mL). The mixture was heated to 80 °C until the evolution of N_2 ceased and poured into ice, and the precipitate was filtered to obtain **4e** (0.45 g, 42%, ligroin). Mp: 151−153 °C. ^{1}H NMR: δ 3.05 (s, 3H, CH_3), 7.30−7.48 (m, 3H, arom), 7.65−7.76 (m, 2H, arom), 8.25−8.32 (m, 1H, arom).

*4-Iodo-1-methylxanthen-9-one (**4f**).* **2**[10] (1 g, 4.44 mmol) was dissolved in H_2SO_4 (13 mL), and $NaNO_2$ (0.31 g, 4.44 mmol) in water was added dropwise keeping the temperature below −5 °C. The mixture was stirred for 30 min and allowed to go to rt for 30 min. A solution of NaI (0.66 g, 4.44 mmol) in water was added, and the mixture was warmed to 80 °C for 1 h and then poured into ice. The precipitate was filtered, dissolved in DCM, washed with $Na_2S_2O_3$ solution, and evaporated to dryness to obtain **4f** (0.4 g, 27%, ligroin). Mp: 188−190 °C. ^{1}H NMR: δ 3.11 (s, 3H, CH_3), 7.08−7.15 (m, 1H, arom), 7.31−7.45 (m, 2H, arom), 7.63−7.75 (m, 1H, arom), 8.06−8.12 (m, 1H, arom), 8.24−8.31 (m, 1H, arom).

*1-Methyl-4-phenylxanthen-9-one (**4g**).* 4-Amino-1-methyl-9-xanthenone[10] (**2**) (1 g, 4.44 mmol) was dissolved in a mixture of $NaBF_4$ (0.6 g, 5.7 mmol), HCl (4 mL), and H_2O (8 mL), and then $NaNO_2$ (0.31 g, 4.44 mmol) in water was added dropwise keeping the temperature below −5 °C. The mixture was stirred for 1 h, and the precipitate formed was filtered and dried to obtain a crude solid (1.25 g, 88%) (mp: 180−181 °C), which was added, under nitrogen, to a mixture of $Pd(CH_3COO)_2$ (44 mg, 0.2 mmol) and potassium trifluoro(phenyl)borate (0.86 g, 4.69 mmol) (see below for preparation). Dry dioxane (15 mL) was added and the mixture was stirred at rt for 5 h, diluted with diethyl ether, washed with brine, and evaporated to dryness. The crude was purified by flash chromatography (petroleum ether/ethyl acetate 9:1) to obtain **4g** (0.3 g, 26%). Mp: 95−97 °C. ^{1}H NMR: δ 2.85 (s, 3H, CH_3), 7.35−7.55 (m, 8H, arom), 7.59−7.65 (m, 1H, arom), 7.70−7.80 (m, 1H, arom), 8.34−8.40 (m, 1H, arom).

Potassium trifluoro(phenyl)borate was obtained from phenylboronic acid (0.9 g, 7.2 mmol) in CH_3OH by adding dropwise an aqueous solution of KHF_2 (1.86 g, 23.7 mmol) and stirring for 10 min; the solvent was evaporated and the residue was extracted with acetone and evaporated to obtain a crude solid (0.95 g, 72%). Mp: 295−298 °C.

*4-Methoxy-1-methylxanthen-9-one (**4h**).* To a solution of **3** (1 g, 4.42 mmol) in acetone (100 mL) were added K_2CO_3 (1.2 g, 8.69 mmol) and dimethyl sulfate (0.88 g, 7.93 mmol). The mixture was refluxed for 7 h, hot-filtered, and evaporated to dryness to obtain **4h** (1.07 g, 90%). Mp: 169−170 °C (lit.[13] mp: 168−169 °C). ^{1}H NMR: δ 2.85 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.32−7.45 (m, 4H, arom), 7.63−7.75 (m, 1H, arom), 8.25−8.36 (m, 1H, arom).

General Procedure for the Alkylation of 3. NaH (60%, 7.5 mmol) was suspended in DMSO (30−40 mL) and heated to 70 °C for 1 h under nitrogen. **3** (5 mmol) was added and the mixture was stirred for another 3 h, and then the selected alkyl halide (5 mmol) was added and the reaction was kept at the same temperature for 8 h. The mixture was then poured into ice, extracted with DCM, washed with water, dried over Na_2SO_4 and evaporated to dryness to obtain compounds **4i−n** (see the Supporting Information).

*4-Methoxymethoxy-1-methylxanthen-9-one (**4o**).* **3** (1.4 g, 6.19 mmol) in dry THF was added to a suspension of 60% NaH (0.18 g, 7.40 mmol) in dry THF at 0 °C under nitrogen. MOM-Cl (0.54 mL, 7.40 mmol) was added dropwise and the resulting solution was stirred at 0 °C for 1 h and then at rt overnight. The mixture was then poured into ice and filtered and the solid washed with water. The crude was purified by flash chromatography (toluene/acetone 9.75:0.25), to obtain **4o** (55%). Mp: 96−98 °C. ^{1}H NMR: δ 2.93 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 5.26 (s, 2H, OCH_2), 7.30−7.51 (m, 4H, arom), 7.63−7.76 (m, 1H, arom), 8.24−8.36 (m, 1H, arom).

General Procedure for the Bromination of 4e−o. To a solution of the selected methyl derivative (2 mmol) in CCl_4 (10−20 mL) were added *N*-bromosuccinimide (2 mmol) and a catalytic amount of benzoyl peroxide, and the mixture was refluxed for 5−7 h. The mixture was hot-filtered and evaporated to dryness to give a crude product (compounds **5e−o**; see the Supporting Information) that was used without further purification.

General Procedure for the Synthesis of Imidazolylmethyl Compounds 1e−n, 6o. A mixture of the selected bromomethyl derivative (1 mmol) and imidazole (3 mmol) in acetonitrile (40−50 mL) was refluxed for 7 h under nitrogen. The solvent was removed under reduced pressure and the residue was purified by flash chromatography.

*1-Imidazolylmethyl-4-chloroxanthen-9-one (**1e**).* Eluent: petroleum ether/ethyl acetate 1:4. Yield: 30%. Mp: 223−224 °C. ^{1}H NMR: δ 6.19 (s, 2H, CH_2), 6.96 (s, 1H, imi), 7.15 (s, 1H, imi), 7.38−7.55 (m, 3H, arom), 7.71−7.82 (m, 3H, arom + imi), 8.32−8.38 (m, 1H, arom). ^{13}C NMR: δ 43.99, 117.49, 119.52, 120.60, 120.98, 122.19, 124.58, 127.07, 128.51, 131.81, 134.59, 135.33, 135.73, 138.14, 154.94, 156.34, 177.58. ES-MS: 311 (M + 1). Anal. ($C_{17}H_{11}ClN_2O_2$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-iodoxanthen-9-one (**1f**).* Eluent: toluene/acetone 1:1. Yield: 26%. Mp: 105−107 °C (ethyl acetate/petroleum ether). ^{1}H NMR: δ 6.18 (s, 2H, CH_2), 6.99 (s, 1H, imi), 7.10 (s, 1H, imi), 7.38−7.51 (m, 3H, arom), 7.70−7.80 (m, 3H, arom + imi),

8.25−8.32 (m, 1H, arom). ^{13}C NMR: δ 43.12, 82.14, 119.16, 121.12, 122.11, 122.43, 124.08, 126.92, 128.82, 131.13, 134.01, 135.87, 137.99, 142.48, 156.65, 168.81, 176.11. ES-MS: 403 (M + 1). Anal. ($C_{17}H_{11}IN_2O_2$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-phenylxanthen-9-one (**1g**).* Eluent: petroleum ether/ethyl acetate 1:4. Yield: 21%. Mp: 178−180 °C (toluene). ^{1}H NMR: δ 5.95 (s, 2H, CH_2), 6.75 (s, 1H, imi), 6.90 (s, 1H, imi), 7.15−7.80 (m, 11H, arom + imi), 8.22−8.33 (m, 1H, arom). ^{13}C NMR: δ 44.70, 117.42, 119.09, 119.20, 119.56, 122.54, 124.19, 126.97, 128.06, 128.17, 128.32, 128.71 (2 C), 129.43 (2 C), 134.11, 134.93, 136.68, 139.22, 140.72, 155.04, 157.12, 178.25. ES-MS: 353 (M + 1). Anal. ($C_{23}H_{16}N_2O_2$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-methoxyxanthen-9-one (**1h**).* Eluent: toluene/acetone 4:1. Yield: 40%. Mp: 198−201 °C. ^{1}H NMR: δ 3.98 (s, 3H, OCH_3), 6.01 (s, 2H, CH_2), 6.92 (s, 1H, imi), 7.20 (s, 1H, imi) 7.33−7.78 (m, 5H, arom), 7.81 (s, 1H, imi), 8.32−8.35 (m, 1H, arom). ^{13}C NMR: δ 40.12, 52.14, 116.98, 119.35, 119.99, 120.37, 120.97, 122.11, 123.72, 124.92, 126.58, 128.12, 134.28, 138.01, 151.29, 152.10, 155.34, 178.24. ES-MS: 307 (M + 1). Anal. ($C_{18}H_{14}N_2O_3$) C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-ethoxyxanthen-9-one (**1i**).* Eluent: toluene/acetone 3:2. Yield: 12%. Mp: 170−173 °C . ^{1}H NMR: δ 1.47 (t, *J* = 4.10 Hz, 3H, CH_3), 4.12−4.24 (q, *J* = 4.10 Hz, 2H, OCH_2), 6.03 (s, 2H, CH_2), 6.96 (s, 1H, imi), 7.15−7.72 (m, 6H, arom + imi), 7.86 (s, 1H, imi), 8.21−8.38 (m, 1H, arom). ^{13}C NMR: δ 16.91, 40.77, 68.34, 117.24, 119.01, 119.87, 120.52, 120.88, 122.56, 123.28, 124.73, 126.15, 128.79, 134.41, 137.69, 151.38, 152.35, 155.03, 178.44. ES-MS: 321 (M + 1). Anal. ($C_{19}H_{16}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-isopropoxyxanthen-9-one (**1j**).* Eluent: toluene/acetone 4:1. Yield: 50%. Mp: 147−149 °C. ^{1}H NMR: δ 1.41 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 4.65−4.75 (m, 1H, OCH), 6.08 (s, 2H, CH_2), 6.97 (s, 1H, imi), 7.22 (s, 1H, imi), 7.34−7.71 (m, 5H, arom.), 7.82 (s, 1H, imi), 8.33−8.39 (m, 1H, arom). ^{13}C NMR: δ 22.10 (2 C), 40.38, 71.50, 117.40, 119.80, 119.91, 120.01, 120.90, 122.01, 123.80, 125.05, 126.86, 128.06, 134.75, 138.32, 151.48, 152.39, 155.21, 178.72. ES-MS: 335 (M + 1). Anal. ($C_{20}H_{18}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-(3-methylbutoxy)xanthen-9-one (**1k**).* Eluent: toluene/acetone 3:2. Yield: 12%. Mp: 146−148 °C. ^{1}H NMR: δ 0.98 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.75−1.82 (m, 2H, CH_2), 2.58−2.70 (m, 1H, CH), 4.15 (t, *J* = 4.20 Hz, 2H, OCH_2), 6.01 (s, 2H, CH_2), 6.95 (s, 1H, imi), 7.20 (s, 1H, imi), 7.31−7.70 (m, 5H, arom), 7.87 (s, 1H, imi), 8.28−8.36 (m, 1H, arom). ^{13}C NMR: δ 22.98 (2 C), 24.01, 38.20, 40.82, 63.12, 116.91, 118.87, 119.73, 120.32, 120.85, 122.37, 123.51, 125.38, 126.78, 128.61, 134.28, 138.17, 151.93, 152.88, 155.03, 178.54. ES-MS: 335 (M + 1). Anal. ($C_{22}H_{22}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-s-butoxyxanthen-9-one (**1l**).* Eluent: toluene/acetone 3:2. Yield: 32%. Mp: 150−153 °C. ^{1}H NMR: δ 1.03 (t, *J* = 4.40 Hz, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.72−1.85 (m, 2H, CH_2), 4.31−4.45 (m, 1H, OCH), 6.01 (s, 2H, CH_2), 6.92 (s, 1H, imi), 7.20 (s, 1H, imi), 7.35−7.65 (m, 5H, arom), 7.81 (s, 2H, imi), 8.34−8.41 (m, 1H, arom). ^{13}C NMR: δ 10.01, 20.25, 31.11, 40.51, 73.16, 117.17, 119.15, 119.74, 120.27, 120.98, 122.28, 123.52, 125.38, 126.73, 128.29, 134.57, 138.46, 151.67, 152.29, 155.17, 178.21. ES-MS: 349 (M + 1). Anal. ($C_{21}H_{20}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-cyclopropylmethoxyxanthen-9-one (**1m**).* Eluent: toluene/acetone 3:2. Yield: 14%. Mp: 276−179 °C. ^{1}H NMR: δ 0.39−0.45 (m, 2H, CH_2), 0.68−0.80 (m, 2H, CH_2), 1.37−1.44 (m, 1H, CH), 3.91 (d, *J* = 4.30 Hz, 2H, OCH_2), 6.12 (s, 2H, CH_2), 6.95 (s, 1H, imi), 7.25−7.51 (m, 4H, arom), 7.62−7.78 (m, 1H, arom), 7.94 (s, 1H, imi), 8.21−8.32 (m, 1H, arom). ^{13}C NMR: δ 3.01 (2 C), 10.92, 40.13, 78.38, 117.22, 119.38, 119.56, 120.38, 121.02, 122.37, 123.11, 125.27, 126.36, 128.15, 134.48, 138.26, 151.74, 152.32, 155.28, 178.53. ES-MS: 347 (M + 1). Anal. ($C_{21}H_{18}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-cyclopentyloxyxanthen-9-one (**1n**).* Eluent: toluene/acetone 4:1. Yield: 45%. Mp: 200−201 °C. ^{1}H NMR: δ 1.61−1.80 (m, 4H, 2 × CH_2), 1.86−2.03 (m, 4H, 2 tme CH_2), 4.85−4.97 (m, 1H, OCH), 6.03 (s, 2H, CH_2), 6.96 (s, 1H, imi), 7.21 (s, 1H, imi), 7.32−7.57 (m, 4H, arom), 7.65−7.78 (m, 1H, arom), 7.81 (s, 1H, imi), 8.25−8.35 (m, 1H, arom). ^{13}C NMR: δ 24.03 (2 C), 32.99 (2 C), 40.54, 80.76, 117.42, 119.80, 119.97, 120.51, 121.98, 123.80, 124.29, 124.40, 126.87, 127.65, 134.79, 138.06, 151.35, 152.60, 155.25, 178.76. ES-MS: 361 (M + 1). Anal. ($C_{22}H_{20}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-methoxymethoxyxanthen-9-one (**6o**).* Eluent: toluene/acetone 4:1. Yield: 75%. Mp: 148−150 °C. ^{1}H NMR: δ 3.62 (s, 3H, OCH_3), 5.46 (s, 2H, OCH_2), 6.19 (s, 2H, CH_2), 6.94 (s, 1H, imi), 7.23 (s, 1H, imi), 7.31−7.80 (m, 5H, arom), 7.85 (s, 1H, imi), 8.31−8.43 (m, 1H, arom).

*1-Imidazolylmethyl-4-hydroxyxanthen-9-one (**1o**).* A solution of **6o** (0.3 g, 0.9 mmol) in CH_3COOH 1:1 (10 mL) with a few drops of H_2SO_4 was refluxed for 15 min and poured into ice. The mixture was neutralized with K_2CO_3 and the precipitate formed was filtered and purified by flash chromatography (toluene/acetone 3:2) to obtain **1o** (0.21 g, 80%). Mp: 203−206 °C. ^{1}H NMR (DMSO): 5.81 (s, 2H, CH_2), 6.78 (s, 1H, imi), 7.18 (s, 1H, imi), 7.23−7.55 (m, 5H, arom + imi), 7.63−7.89 (m, 1H, arom), 8.18−8.26 (m, 1H, arom). ^{13}C NMR (DMSO): δ 40.61, 118.71, 120.32, 120.84, 121.11, 121.30, 122.35, 125.13, 125.22, 127.21, 128.12, 136.36, 138.25, 151.50, 153.85, 155.80, 179.10. ES-MS: 293 (M + 1). Anal. ($C_{17}H_{12}N_2O_3$): C, H, N (see the Supporting Information).

*1-Imidazolylmethyl-4-aminoxanthen-9-one (**1p**).* 1-Imidazolylmethyl-4-nitroxanthen-9-one[10] (**1b**) (0.45 g, 1.4 mmol) was dissolved in THF and hydrogenated at room temperature using a catalytic amount of Pd/C. After filtration, the solvent was removed under reduced pressure and the residue was crystallized from toluene to obtain **1p** (0.27 g, 70%). Mp: 218−220 °C (dec). ^{1}H NMR: δ 5.90 (s, 2H, CH_2), 7.01−7.15 (m, 2H, arom), 7.20 (s, 1H, imi), 7.35−7.65 (m, 3H, arom + imi), 7.68 (s, 1H, imi), −7.73−7.85 (m, 1H, arom), 8.25−8.34 (m, 1H arom). ^{13}C NMR: δ 40.27, 118.34, 120.15, 120.58, 121.52, 121.71, 122.62, 125.28, 125.36, 127.38, 128.42, 136.03, 138.47, 149.58, 151.89, 155.56, 178.76. ES-MS: 292 (M + 1). Anal. ($C_{17}H_{13}N_3O_2$): C, H, N (see the Supporting Information).

Biological Methods. *Inhibition of CYP11B1 and CYP11B2.* V79MZh cells expressing human or rat CYP11B1 or CYP11B2 were incubated with [1,2-^{3}H]-11-deoxycorticosterone (100 nM) as the substrate and the inhibitor at different concentrations.[14] The assay was performed as previously described.[15]

CYP17 Preparation and Assay. Human CYP17 was expressed in *E. coli*[16] (coexpressing human CYP17 and NADPH-P450 reductase), and the assay was performed using the method previously described with progesterone (25 μM) as the substrate and NADPH as the cofactor.[17,18]

CYP19 Preparation and Assay. Human CYP19 was obtained from microsomal preparations of human placenta[19] and the assay was performed using the ^3H_2O method as previously described with [1β-^{3}H]androstenedione (500 nM) as the substrate.[20]

Pharmacophore Search. The previously reported pharmacophore model[9] was employed as the pharmacophore query. Compounds were built and energy minimized in the MMFF94s force field with MOE 2008. 3D-conformational database was subsequently constructed with a strain limit of 4 kcal/mol using the Conformation Import module in MOE. Only one matched conformer was conserved for each compound according to the ranking of rmsd. No further refining of the query was performed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental and spectroscopic details for intermediate compounds **4i−n** and **5e−o**, elemental analyses of target compounds **1e−p**, and Table 1 SI. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: silvia.gobbi@unibo.it; alessandra.bisi@unibo.it. Phone/Fax: +39 051 2099732/34.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

CYP, cytochrome P450; CYP19, aromatase; CYP17, 17α-hydroxylase/17,20-lyase; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; 18OH-B, 18-hydroxycorticosterone.

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