Imidazolylmethylbenzophenones as Highly Potent Aromatase Inhibitors†

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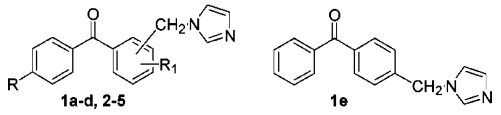
Abstract: Suppression of tumor and plasma estrogen levels by inhibition of aromatase is one of the most effective treatments for post-menopausal breast cancer patients. Starting from an easy, synthetically accessible, benzophenone scaffold, a new class of potent aromatase inhibitors was synthesized, endowed with high selectivity with respect to 17α-hydroxylase/17,20-lyase (CYP17). Compounds **1b** and **1d** proved to be among the most potent inhibitors described so far.

Estrogens are known to be implicated in breast cancer occurrence and development, and different strategies have been devised to control or block the progression of hormone-dependent breast cancer. The action of estrogens on their receptor can be blocked by the use of selective estrogen receptor modulators (SERMs[a]), like tamoxifen. These compounds have been widely used as first line therapy in post-menopausal women with advanced breast cancer, but they are known to possess proestrogenic effects, particularly on the endometrium and vascular system, and this partial agonism has been associated with the possible development of tumors.[1] A different approach comes from the reduction of hormone levels by inhibition of cytochrome P450 aromatase (CYP19),[2] a key enzyme involved in the synthesis of estrogens, promoting the aromatization of the A ring of androgen precursors. Third generation aromatase inhibitors (AIs), such as letrozole, anastrozole, and exemestane, are now considered a valid alternative to tamoxifene as first line treatment of advanced breast cancer.[3] The search for potent and selective AIs still remains an attractive subject, and some interesting compounds have recently been developed by different groups working in the field.[4−7] In addition, in a previous paper, we described some xanthenone-based molecules as potent inhibitors.[8]

In this letter, we report on a new class of potent inhibitors of aromatase, characterized by a rather flexible imidazolylmethylbenzophenone scaffold, which offers the advantage of a large synthetic accessibility. Moreover, these compounds could allow us to establish whether the conformationally constrained scaffold of the previously synthesized series of molecules is an essential element for optimal interaction with the enzyme and to determine the best positioning on this scaffold of the imidazole nitrogen that coordinates the iron atom of the heme group. To this aim, the position of the imidazole ring on the benzophenone moiety was varied, substituents were introduced on the phenyl ring carrying the imidazole, because the same pattern of substitution was also present in the previous series, and an additional benzene was placed on the other phenyl ring to explore the spatial requirements of that part of the molecule. The structures of the new compounds are reported in Chart 1.

Chart 1. Structures of the Studied Compounds



The synthesis was performed, according to Scheme 1, via Friedel−Craft acylation of benzene or biphenyl with the appropriately substituted benzoyl chloride to give **6a**−**d**, **7**, **9**, and **10**, followed by bromination of the methyl group with NBS and subsequent substitution with imidazole to provide compounds **1a**−**d** and **2**−**4**. 2-Methyl-5-nitrobenzoic acid **8**, which was not commercially available, was prepared via nitration of *o*-toluic acid. For the synthesis of compound **5**, 2-methyl-5-nitrobenzophenone **9** was reduced with H_2 and $Pd/CaCO_3$ to give 2-methyl-5-aminobenzophenone **11**, and the amino group was substituted by a bromine atom to give **12** via the formation of the diazonium salt followed by addition of CuBr. This intermediate was then reacted as reported above to provide the final compound.

The new compounds were tested for inhibition of aromatase using human placental microsomes incubated with 1β[^{3}H]-androstenedione and measuring the tritiated water formed during the aromatization of the substrate, as previously described.[9] The inhibitory activity of the compounds toward 17α-hydroxylase/17,20-lyase (CYP17), another cytochrome P450 involved in the synthesis of androgens, was also assessed to evaluate their selectivity toward a related enzyme. For the CYP17 inhibition tests, human CYP17 expressed in *E. coli* and P450 reductase were used.[10] Another benzophenone derivative (**1e**),[11] previously reported in the literature as part of a large series of azole compounds tested for inhibition of aromatase, was also included in the evaluation and tested in our assays.

The results reported in Table 1 showed that all tested compounds were highly potent AIs, some of them exhibited activity in the low nanomolar range and they were selective toward aromatase with respect to CYP17. Interestingly, despite an increase in conformational flexibility, this series of molecules turned out to be more potent than the conformationally constrained series of compounds previously reported.[8] It can promptly be observed that the position of the imidazole side chain is of crucial importance for the activity, because moving it from position 2 (compound **1a**) to 3 (**1b**) on the benzophenone nucleus led to a 100-fold increase in potency. Compound **1e**, in which the imidazolylmethyl chain is in position 4, whose previously reported IC_{50} value was 10 nM,[11] proved to be significantly less active when tested in our assay (IC_{50} = 252 nM, both assays were run using human placental microsomes), showing activity comparable to the *ortho*-derivative **1a** (for further details see Supporting Information). The introduction of a nitro group in both **1b** and **1a**, leading to compounds **2** and **3** respectively, was of no significant influence for the most active one **1b**, but highly beneficial for **1a**, because it increased its potency of more than one log unit. Looking at this result in view of the possible binding mode of these molecules at the

† Dedicated to Prof. Piero Valenti on the occasion of his retirement.

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[a] Abbreviations: SERMs, selective estrogen receptor modulators; CYP19, aromatase; AIs, aromatase inhibitors; CYP17, 17α-hydroxylase/17,20-lyase.

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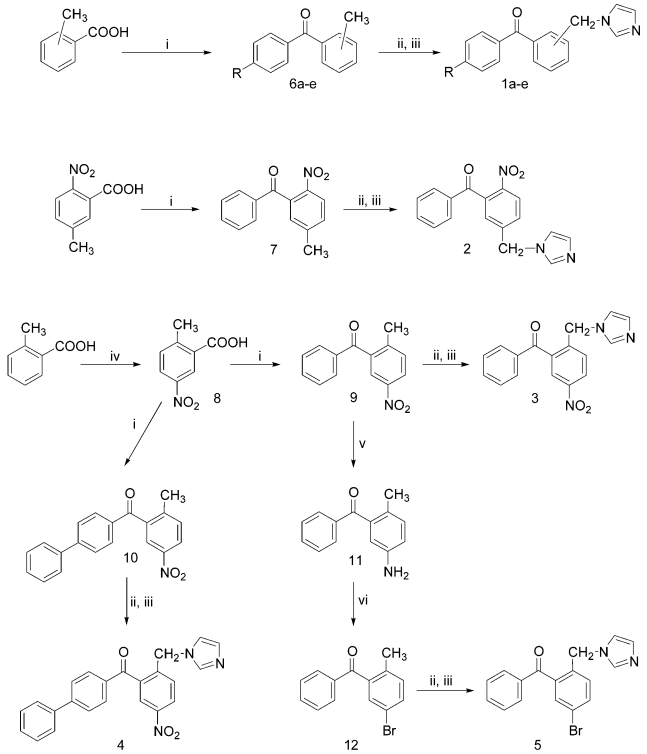
Scheme 1. Syntheses of the Studied Compounds[a]



[a] Reagents and conditions: (i) $SOCl_2$, reflux, then $AlCl_3$ or trifluoromethansulfonic acid, benzene or biphenyl, rt; (ii) NBS, benzoyl peroxide, CCl_4, reflux; (iii) imidazole, CH_3CN, N_2, reflux; (iv) KNO_3, H_2SO_4, 0 °C; (v) THF, H_2, $Pd/CaCO_3$, rt; (vi) HBr, $NaNO_2$, 0 °C, then hot CuBr solution, rt.

Table 1. Biological Activities of the New Compounds toward CYP19 and CYP17

compound	R	R_1	CH_2imid position	CYP19[a] IC_{50}[c] nM	CYP17[b] % inhib. at 2.5 μM
1a			2	560	NA[d]
1b			3	7.3	NA
1c	Ph		2	260	24
1d	Ph		3	5.3	NA
1e			4	252	NA
2		6-NO_2	3	31	NA
3		5-NO_2	2	25.9	NA
4	Ph	5-NO_2	2	17.7	17
5		5-Br	2	400	15
fadrozole				52	NA

[a] Human aromatase, placental microsomes, and substrate 1β[^{3}H]androstenedione, 500 nM. [b] Human CYP17 expressed in *E. coli*, substrate progesterone, 25 μM. [c] The given values are mean values of at least three experiments. The deviations were within ±5%. [d] NA = no activity detected.

enzyme active site (see below) and assuming that the coordination of the heme iron by the imidazole moiety would be the primary interaction, it could be reasoned that, while the ketone in compound **1b** could be located at the optimal distance from the imidazole moiety to properly interact via H-bond with the enzyme, this is not possible for **1a**. If this is the case, the nitro group is only critical for compound **3**, as its position relative to the imidazole could be the appropriate one to give the molecule the possibility to establish a correct H-bond interaction with the enzyme. A further evidence to support this hypothesis came from the introduction on the structure of **1a** of a bromine atom which, unlike the nitro group, is devoid of H-bond potential, leading to compound **5**: the potency did not improve with the introduction of this substituent. Activity seemed to be slightly

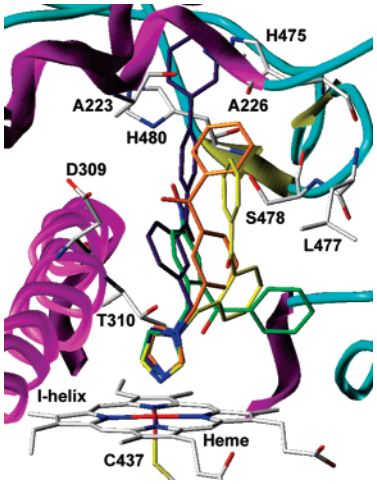


Figure 1. Docking complexes between aromatase and **1a** (yellow), **1b** (orange), **1d** (violet), and **3** (green) as outcome of docking simulations carried out with GOLD and cluster analysis performed with AClAP. The heme group and the I-helix are explicitly indicated for convenience. Moreover, some aromatase active site amino acids are also reported.

influenced by the presence of an additional phenyl ring in position 4′. For compounds **1a**, **1b**, and **3**, the introduction of this additional substituent (compounds **1c**, **1d**, and **4**, respectively) led to an increase in potency, which, though small, still showed that a bulky group in that position could be beneficial. In particular, compound **1d** proved to be among the most potent AIs synthesized so far (IC_{50} = 5.3 nM).

To further investigate the SAR of the present series of derivatives and to additionally validate the previously postulated "H-bonding hypothesis",[8] docking experiments were also performed using the recently developed homology-built aromatase model (PDB code 1TQA).[12] In Figure 1, the putative binding modes of compounds **1a** (yellow), **1b** (orange), **1d** (violet), and **3** (green) as outcome of docking simulations and cluster analysis[13,14] are reported. Docking simulations were carried out constraining ligands to properly coordinate the heme iron atom, while leaving the rest of the molecules free to move (see Supporting Information). This also allowed a proper orientation of the noncoordinating nitrogen of the imidazole ring that could, therefore, establish a H-bond with Thr310 (average distance between the heteroatoms of ~3.1 Å). From Figure 1 it clearly arises that the *meta*- (**1b**) and the *ortho*- (**1a**) derivatives interacted with the target in quite a different way. In details, the carbonyl oxygen atom of **1b** was positioned at ~3−4 Å from the carboxyl group of Asp309 of the I-helix and ~5−6 Å from the hydroxyl group of Ser478 of the very flexible C-terminal loop. Either residue could reasonably interact by means of a H-bond with the **1b** carbonyl group, thus accounting for the higher activity of this compound relative to **1a**, in which the same moiety is not properly oriented to establish similar interactions. In particular, **1a** carbonyl oxygen is far too distant (more than 7 Å) to interact with either Asp309 or Ser478. A comment is required on Asp309 that in this study was treated as ionized. While in this form it obviously cannot interact by means of H-bond, it is widely accepted that during the aromatization Asp309 can be protonated and, therefore, able to favorably interact with our compound.[15] Concerning Ser478, both ligand-[16,17] and target-based[18−20] computational studies point to this residue as a fundamental amino acid for the interaction with nonsteroidal AIs belonging to different classes. Also, mutagenesis experiments show that this residue is one of the amino acids located in the aromatase active site and involved

in the interaction with ligands.[21] The proposed binding mode (Figure 1) well confirmed the difference in potency between the *meta*- (**1b**, $IC_{50} = 7.3$ nM) and the *ortho*-derivative (**1a**, $IC_{50} = 560$ nM).

In Figure 1, the putative binding mode of **3**, carrying a NO_2 group in *para*-position with respect to the methylimidazole chain, is also reported. Docking simulations clearly pointed out the possibility for this additional group to establish H-bond interactions with either Ser478 or Asp309, which are equidistant from the $-NO_2$ oxygen (distance between heteroatoms $\sim$ 4.0 Å), thus accounting for the significantly increased potency ($IC_{50} = 25.9$ nM) with respect to the parent compound **1a**. Furthermore, to investigate the effects of the introduction of bulky substituents on the benzophenone scaffold, docking simulations were also performed with **1d**, namely, the *meta*-derivative with an additional phenyl ring in position 4′. A pose representative of a statistically populated cluster is reported in Figure 1. This binding mode showed that aromatase can accommodate sterically hindered ligands in this region of its active site, as **1d** could establish a few further interactions, in particular, either $\pi-\pi$ with His475 and His480 or hydrophobic with Ala223 and Ala226. Actually, **1d** proved to be the most potent AI that we reported so far, with an IC_{50} value of 5.3 nM.

In conclusion, a new class of highly potent AIs was synthesized, endowed with high selectivity with respect to CYP17, which combine remarkable activity (in the low nanomolar range) with higher accessibility via straightforward synthetic procedures when compared to the conformationally constrained xanthone derivatives.[8] Compound **1d** turned out to be our most potent AI, comparable to currently marketed drugs. Docking simulations in combination with cluster analysis were also carried out, providing a fairly good explanation for the binding mode of the present series of new inhibitors at the aromatase active site.

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Supporting Information Available: Full experimental procedures of both synthesis and computational studies and elemental analyses of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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