Brief Articles

Lead Optimization Providing a Series of Flavone Derivatives as Potent Nonsteroidal Inhibitors of the Cytochrome P450 Aromatase Enzyme

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Following our SAR studies on aromatase inhibitors, new compounds were designed by appropriately modifying the structure of flavone **1** using our previously reported CoMFA model. While the introduction of substituents on the 2-phenyl ring alone did not cause improvement in potency, these modifications and the removal of the 7-methoxy group led to compounds showing inhibitory activity in the nanomolar range, comparable to the marketed drug fadrozole.

Introduction

One of the main approaches to control postmenopausal hormone-dependent breast cancer involves the reduction of plasma and tissue levels of estrogens via the inhibition of a key enzyme of their biosynthetic process, and research was soon focused on aromatase.[1] This multienzymatic complex, formed by cytochrome P450 XIX (CYP19) and NADPH-cytochrome P450 reductase, catalyzes the conversion of androgens to estrogens through the aromatization of the A ring of androgen substrates and has been considered a particularly attractive target for the treatment of hormone-dependent breast cancer. Since the early discovery of aromatase inhibitory activity in the known drug aminoglutethimide,[2] the extensive SAR studies performed in the past 30 years to search for potent, selective, and less toxic compounds have led to the development of a second and a third generation of nonsteroidal inhibitors, which have recently been reviewed.[3] Among different classes, the azoles still hold a prominent position as demonstrated by the recently marketed drugs, which are now considered a valid alternative to tamoxifene as a first line treatment for advanced breast cancer,[4] and recently new series of nonsteroidal aromatase inhibitors have been synthesized by groups working in the field.[5−8]

We have been interested for some years in the study of aromatase inhibitors, and on the basis of a CoMFA analysis,[9,10] new aromatase inhibitors were synthesized[11] in which the key features of the azole family of aromatase inhibitors (nitrogen-containing heterocycle and H-bond accepting group) were inserted into different scaffolds, such as chromone, xanthone, and flavone, which are known in the literature to bear aromatese inhibition properties.[12,13] Starting from these results, we performed additional SAR studies, obtaining several series of new aromatase inhibitors. In this paper we report some modifications performed on the earlier described flavone **1**[11] to improve the biological activity of this lead compound. **1** was aligned into the recently updated enantioselective CoMFA model[14] following the previously described alignment hypothesis.[10] Briefly, the phenyl group was aligned onto the *p*-cyanophenyl of *S*-fadrozole (Figure 1A), and as a consequence, the chromone nucleus was found to be fairly well aligned onto the *p*-cyanophenyl of *R*-fadrozole (Figure 1B). In Figure 1C, **1** is shown surrounded by the CoMFA contour maps of the model of ref 14. In light of this picture, we thought the activity of **1** ($IC_{50} = 0.55\ \mu M$) could be improved in two ways: (i) by inserting into the 2-phenyl ring of the flavone a suitable substituent mimicking the cyano group of *S*-fadrozole, eventually enabling the establishment of an H-bond interaction with the Ser478 of the biological counterpart[15] (**2a**−**c**, Table 1); (ii) by removing the 7-methoxy group of **1** (**3a**−**e**, Table 1). The biological activities of the resulting compounds were then predicted using our CoMFA model,[14] and it turned out that the designed structural modifications were predicted to improve the aromatase inhibitory potency with respect to the parent compound **1**, whose calculated pIC_{50} was 6.11 (Table 1).

Compounds **2a**−**c** (Scheme 1) were synthesized starting from 2,4-dihydroxypropiophenone, which was heated with the appropriately substituted benzoyl chloride and the corresponding sodium salt to give the 7-hydroxy-3-methylflavones **4a**,**b** after hydrolysis with sulfuric acid. The hydroxy group in position 7 was methylated with dimethylsulfate to give the intermediates **5a**,**b**, which were then brominated with *N*-bromosuccinimide to give **6a**,**b**, and the reaction with imidazole led to the desired compounds (**2a**,**b**). For the synthesis of **2c** a different route was used because of the instability of the nitrile in the presence of sulfuric acid. 2,4-Dihydroxypropiophenone was first methylated and then reacted with 4-cyanobenzoyl chloride and sodium 4-cyanobenzoate to give the corresponding 7-methoxyflavone **5c**, which was then treated as above to obtain the final compound. For the synthesis of **3a**−**e** (Scheme 1), starting from 2-hydroxypropiophenone and the appropriately substituted benzoyl chlorides and corresponding sodium salts, the flavones **8a**,**e** were obtained. These were then brominated (**9a**,**e**) and reacted with imidazole to give the final compounds.

The new compounds were tested for inhibition of aromatase, and to assess their selectivity toward related enzymes, their ability to inhibit another cytochrome P450 enzyme was also

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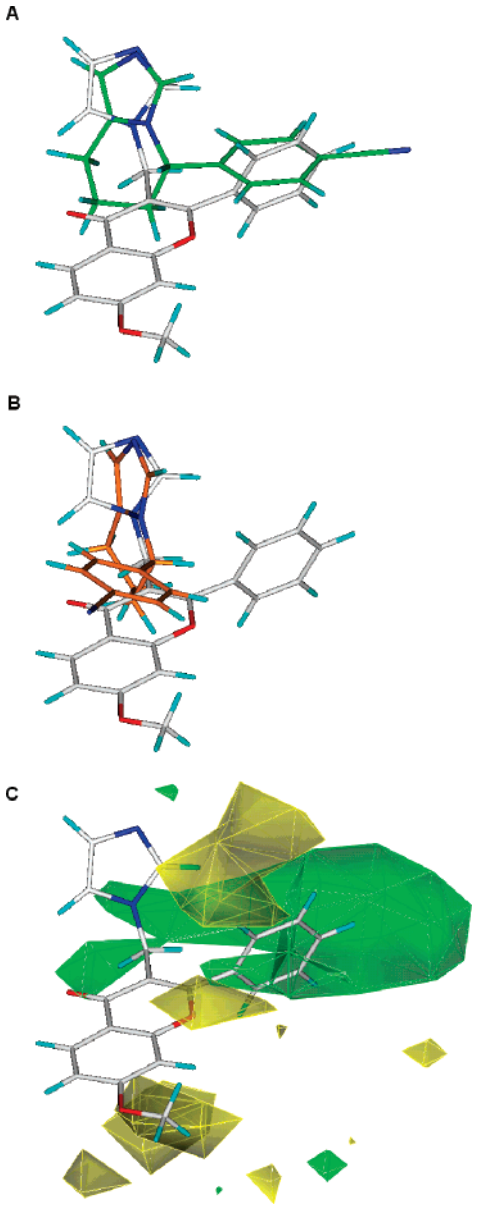


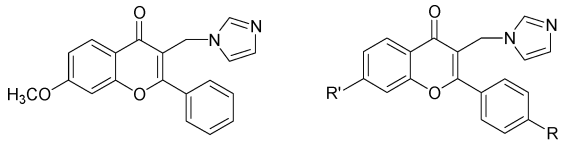
Figure 1. (A) Superimposition of **1** onto *S*-fadrozole following the previously reported alignment strategy. As a consequence, (B) the chromone nucleus is fairly well aligned onto the *p*-cyanophenyl of *R*-fadrozole. (C) The CoMFA contour maps are plotted in the 3D space surrounding the inhibitor **1**. The CoMFA contour map levels are those reported in Figure 1.

evaluated, namely, 17α-hydroxylase/C17,20-lyase (CYP17), catalyzing the key step of androgen biosynthesis.

Results and Discussion

All the new compounds showed inhibition of aromatase, and some of them proved to be active at the nanomolar level (Table 1). In particular, **3b** was slightly more active than the marketed drug fadrozole. Moreover, all the new compounds proved to be highly selective for aromatase with respect to CYP17 because no IC_{50} for the inhibition of this enzyme could be calculated. Considering the results collected in Table 1, the introduction of a NO_2 group on the phenyl ring in the 7-methoxy series (**2a**) seemed to have no significant effect on CYP19 inhibition. This could be due to an overall increase of the hydrophilic character, which counterbalanced the positive effect of a possible H-bond interaction between NO_2 and Ser478 of CYP19. Actually, high hydrophobicity of CYP19 inhibitors has been demonstrated to

Table 1. Structures and Biological Activities of the Target Compounds against CYP19 and CYP17

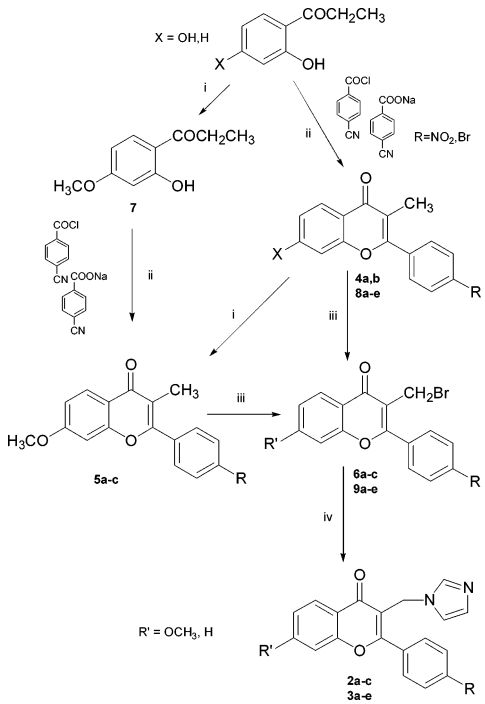


1 **2a-c, 3a-e**

	R	R′	CYP19[a] IC_{50}, μM[c]	CYP17[b] % inhibition at 2.5 μM	CYP19 pIC_{50obsd}	CYP19 pIC_{50pred}	Δ
1		OCH_3	0.55	44	6.26	6.11	0.15
2a	NO_2	OCH_3	0.47	9	6.33	6.78	−0.45
2b	Br	OCH_3	4.1	29	5.39	6.44	−1.05
2c	CN	OCH_3	1.8	12	5.74	6.56	−0.82
3a	H	H	0.071		7.15	6.22	0.93
3b	NO_2	H	0.045		7.35	7.20	0.15
3c	Br	H	0.44	27	6.36	6.62	−0.26
3d	CN	H	0.069		7.16	6.84	0.32
3e	OCH_3	H	0.080	26	7.10	6.90	0.20
fadrozole			0.052				

[a] Substrate is 500 nM 1β-[^{3}H]androstenedione. [b] Substrate is 25 μM progesterone. [c] The given values are mean values of at least three experiments. The deviations were within ±5%.

Scheme 1[a]



[a] Reagents and conditions: (i) K_2CO_3, $(CH_3)_2SO_4$, reflux 7 h; (ii) (1) 180−190 °C, 7 h; (2) H_2SO_4, reflux 15 min; (iii) NBS, CCl_4, reflux 5 h; (iv) imidazole, CH_3CN, reflux 6 h.

be a fundamental physicochemical requirement for good CYP19 inhibition.[11] Since our CoMFA model does not include log *P* or other descriptors to account for hydrophobic features, **2a**, which carried a strong H-bond acceptor group, was predicted to be more potent than it actually was (pIC_{50pred} = 6.78 vs pIC_{50obsd} = 6.33). In agreement with this observation was also the activity of **2c**. In previously reported series, the CN group was shown to be a worse H-bond acceptor compared to the NO_2 substituent, and this was confirmed here by the pIC_{50pred} of **2c**, which was lower than the pIC_{50pred} of **2a** (6.56 and 6.78, respectively). Still, **2c** was observed to be significantly less potent with an experimental pIC_{50obsd} of 5.74. A slightly different scenario could be observed for the highly hydrophobic Br

derivative **2b**, which is not able to establish an H-bond with the biological counterpart, thus likely missing an important interaction with the protein target. This may be responsible for both its low potency ($pIC_{50obsd} = 5.39$) and its CoMFA-based overestimated activity ($pIC_{50pred} = 6.44$).

More promising results were obtained with the removal of the methoxy group because **3a** showed activity in the nanomolar range, about an 8-fold increase with respect to the parent compound **1**. This seemed to confirm the design hypothesis based on our CoMFA model, the pIC_{50pred} of **3a** (6.22) however being much lower than its pIC_{50obsd} (7.15). This indeed proved that a decrease of steric hindrance in a sterically disallowed region increased the potency of inhibitors. Likely, our CoMFA model underestimated **3a** potency because the compound lacks the H-bond acceptor group, which is a fundamental feature for the statistical model.[14] This definitively arose by the analysis of the predicted activity of the most potent compound (**3b**), which underwent both structural modifications invoked by our design hypothesis, namely, the removal of the bulky methoxy substituent and the introduction of an excellent H-bond acceptor (NO_2) on the phenyl ring. The pIC_{50obsd} of **3b** was 7.35, in very good agreement with the pIC_{50pred} (7.20). Fairly good predictions were also obtained for the other compounds (**3c**−**e**, Table 1), the Br derivative being the least potent of the present series of molecules. To evaluate the overall predictive capability of our CoMFA model toward flavone-based molecules, we calculated the r^2_{pred} [16] to be 0.700 for the eight new compounds. Indeed, this value may be considered a satisfactory predictive value for a series of newly designed molecules.

In conclusion, the introduction of substituents in position 4′ on the phenyl ring of the flavone (**2a**−**c**) did not cause any improvement in potency. On the contrary, the removal of the methoxy group in position 7, together with the introduction of the same substituents on the phenyl ring, led to a series of compounds (**3a**−**e**) showing inhibitory activity in the nanomolar range, comparable to the marketed drug fadrozole. Moreover, the activities of the new compounds were predicted by the CoMFA model, and we can conclude that the overall predictive capability of the model toward flavone-based molecules, as quantitatively indicated by the r^2_{pred} value, is fairly good.

Experimental Section

Chemistry. General Methods. All melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ (unless otherwise indicated) on a Varian Gemini 300 spectrometer with Me_4Si as the internal standard. Mass spectra were recorded on a Waters ZQ 4000 spectrometer operating in the electrospray (ES) mode. Silica gel (Merck, 230−400 mesh) was used for purification with flash chromatography. Elemental analyses were within 0.4% of theoretical values. Compounds were named following IUPAC rules as applied by AUTONOM, PC software for nomenclature in organic chemistry from Beilstein-Institut and Springer.

7-Hydroxy-3-methyl-4′-nitroflavone (4a). A mixture of 2,4-dihydroxypropiophenone (1.8 g, 11 mmol), 4-nitrobenzoyl chloride (4.1 g, 22 mmol), and sodium 4-nitrobenzoate (6.3 g, 34.3 mmol) was heated to 180−190 °C for 7 h. Water was added, and the solid was filtered and refluxed for 15 min in 50% H_2SO_4, then poured into ice. The precipitate was filtered and resuspended in 2 N NaOH, filtered, and purified by column chromatography (petroleum ether/ethyl acetate 4:1) to give 2.3 g (70%) of **4a**, mp 278−280 °C. ^{1}H NMR (DMSO-d_6): δ 2.00 (s, 3H, CH_3), 6.80 (d, $J = 1.8$ Hz, 1H, arom), 6.90 (dd, $J = 1.8$ and 8.4 Hz, 1H, arom), 8.00 (d, $J = 8.4$ Hz, 1H, arom), 8.05 (d, $J = 8.4$ Hz, 2H, arom), 8.40 (d, $J = 8.4$ Hz, 2H, arom), 10.80 (s, 1H, OH).

7-Methoxy-3-methyl-4′-nitroflavone (5a). A mixture of **4a** (1.6 g, 5.4 mmol), dry K_2CO_3 (0.75 g, 5.4 mmol), and dimethyl sulfate (0.7 g, 5.4 mmol) in acetone was refluxed for 7 h. The mixture was hot-filtered and evaporated to dryness. Purification by column chromatography (petroleum ether/ethyl acetate 4:1) gave 1.10 g (65%) of **5a**, mp 202−204 °C. ^{1}H NMR: δ 2.20 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 6.85 (d, $J = 1.8$ Hz, 1H, arom), 7.00 (dd, $J = 1.8$ and 8.4 Hz, 1H, arom), 7.85 (d, $J = 8.4$ Hz, 2H, arom), 8.15 (d, $J = 8.4$ Hz, 1H, arom), 8.40 (d, $J = 8.4$ Hz, 2H, arom).

3-(Bromomethyl)-7-methoxy-4′-nitroflavone (6a). To a solution of **5a** (3 g, 9.8 mmol) in CCl_4 (150 mL) were added *N*-bromosuccinimide (1.85 g, 9.8 mmol) and a catalytic amount of benzoyl peroxide, and the mixture was refluxed for 5 h. The mixture was hot-filtered and evaporated to dryness to give 1.2 g of **6a** that was used without further purification. ^{1}H NMR: δ 3.90 (s, 3H, OCH_3), 4.40 (s, CH_2), 7.00−7.40 (m, 2H, arom), 7.50−8.45 (m, 5H, arom).

3-(Imidazolylmethyl)-7-methoxy-4′-nitroflavone (2a). A mixture of **6a** (1.2 g, 3 mmol) and imidazole (0.6 g, 9 mmol) in dry acetonitrile (40 mL) was refluxed under N_2 for 6 h and evaporated to dryness. The residue was purified by flash cromathography (ethyl acetate) to give 0.52 g (46%) of **2a**, mp 195 °C (dec). ^{1}H NMR: δ 3.90 (s, 3H, OCH_3), 5.00 (s, 2H, CH_2), 6.85−7.10 (m, 4H, arom), 7.40 (s, 1H, arom), 7.65 (d, $J = 8.4$ Hz, 2H, arom), 8.20 (d, $J = 8.4$ Hz, 1H, arom), 8.40 (d, $J = 8.4$ Hz, 2H, arom). MS: *m*/*z* 378 (M + 1). Anal. ($C_{20}H_{15}N_3O_5$) C, H, N.

Biology. All the compounds were tested according to previously reported methods.[11]

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Supporting Information Available: Experimental details for intermediates **4b**−**c**, **5b**−**c**, **6b**−**c**, **7**, **8a**−**e**, **9a**−**e** and final compounds **2b**−**c** and **3a**−**e**; computational chemistry methods; elemental analysis results of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) r^2_{pred} was calculated with the following equation: $r^2_{pred} = (SD - PRESS)/SD$, where SD is the sum of the squared deviations of each observed activity value for each molecule of the test set ($pIC50_{obsd}$ values of Table 1) from the mean of the observed activity values of the training set ($pIC50_{obsd}$ reported in ref 12) and PRESS is the sum of the squared deviations between predicted and observed values for the molecules of the test set ($pIC50_{pred}$ and $pIC50_{obsd}$ of Table 1, respectively).

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