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DESIGN AND SYNTHESIS OF A NEW TYPE OF NON STEROIDAL HUMAN AROMATASE INHIBITORS

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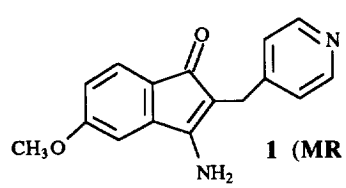
Abstract . The structure-activity relationship study of one of recently described aromatase inhibitors, compound **1** (MR20814), allowed us to design some related derivatives as potential new inhibitors. Among those we synthesized, chlorophenylpyridylmethylenetetrahydroindolizinone **5** (MR20492) exhibited *in vitro* a ten-fold higher inhibition of the enzyme ($IC_{50} = 0.2 \pm 0.0$ µM and $Ki = 10.3 \pm 3.3$ nM).

In the search of new leads for inhibition of the cytochrome P-450 aromatase, responsible for the estrogen biosynthesis, we have recently described the synthesis and biological evaluation of new 3-amino-2-arylmethylindenones.[1] Among those, derivative **1** strongly inhibits *in vitro* the human aromatase activity with an IC_{50} of 3.5 ± 1.2 µM and an apparent Ki of 86.2 ± 7.8 nM. This study also showed, on the basis of the characteristics of the UV difference spectrum (type II), an interaction between the pyridin nitrogen atom of **1** and the heme iron atom of the cytochrome.

The 3D molecular modelling study of the interaction between **1** and the active site of the human aromatase showed that the amino group of **1** could occupy the entry of the extrahydrophobic pocket, described by Laughton et al.[2], inside this active site. The synthesis of more powerful and more specific aromatase inhibitors still remains a challenge.[3] Thus, we undertook the pharmacomodulation of this new lead. In particular, we carried out the substitution of its amino group by an hydrophobic moiety likely to occupy, in an optimal way, the extrahydrophobic pocket within the active site of the enzyme.



1 (MR 20814)

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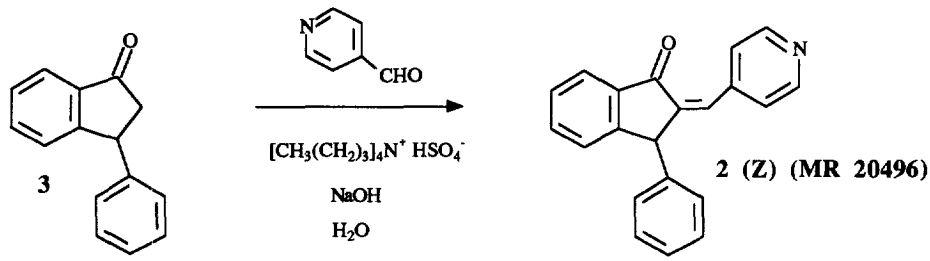
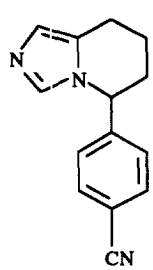
Results and discussion

Among the synthesized compounds, derivative **2** (MR 20496) appeared to inhibit strongly aromatase *in vitro* (IC_{50} = 0.8 ± 0.1 μM and Ki = 10.3 ± 4.8 nM; Km of androstenedione was 10.7 ± 3.3 nM). Its synthesis called upon the following sequence (Scheme 1) in which 3-phenylindan-1-one **3**[4] was submitted to the action of pyridine-4-carboxaldehyde in the presence of sodium hydroxide and tetrabutylammonium hydrogen sulfate. The Z geometry of **2** was deduced from its ^{1}H-NMR spectrum, which exhibit a vinyl-H singlet at 7.50 ppm in accordance with our previous work.[1] This restrained configuration could bring the pyridin nitrogen closer to the heme iron.

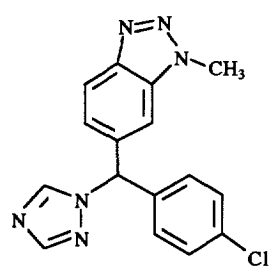


Scheme 1

The presence of a phenyl substituent in the structure of **2**, led us to connect this new indane structure with azole-type aromatase inhibitors, fadrozole and vorozole. In this azole family, it is known that the presence of a para-substituted phenyl group, occupying the extrahydrophobic pocket of the active site of aromatase, reinforces the interaction between the azole nitrogen atom of the inhibitors and the heme iron atom of the enzyme.[5]



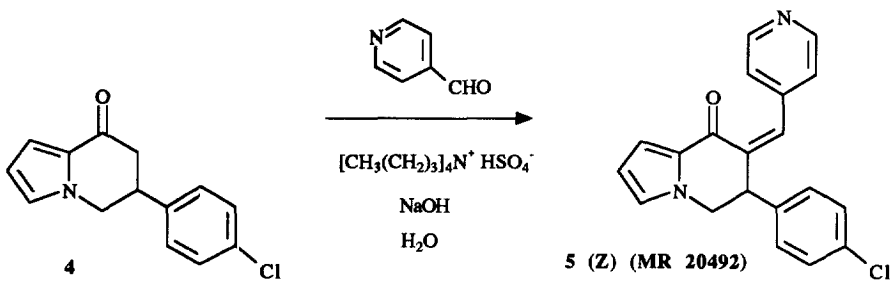
fadrozole



vorozole

Taking into account these considerations, and in order to obtain a molecule with a size comparable to vorozole, we diverted the structure of **2** towards those of their higher aza-analogues, the aryltetrahydroindolizinones, obtained from the previously described compound **4** [6] in a similar manner as for **2** (Scheme 2). Among these compounds, the (Z) 4-pyridylmethylene derivative **5** (MR 20492) exerted *in vitro* a potent aromatase inhibition (IC_{50} = 0.2 ± 0.0 μM and Ki = 10.3 ± 3.3 nM), ten-fold greater than with **1** and comparable to fadrozole (IC_{50} = 0.2 ± 0.1 μM and Ki = 0.6 ± 0.4 nM).

Compound **5**, issued from a structural compromise between azole- and benzocycloalkene-type inhibitors[7] can be considered as a new lead in the aromatase inhibitors area. Further studies, concerning its *in vivo* metabolism for its possible use in therapy of estrogen-dependent diseases, are currently under investigation.



Scheme 2

Synthesis : Preparation of 2 and 5 (general method)

To a solution of 3-phenylindan-1-one **3** (0.84 g, 4 mmol) or 6-(4-chlorophenyl)-5,6,7,8-tetrahydroindolizin-8-one **4** (1 g, 4 mmol) and pyridine-4-carboxaldehyde (0.47 g, 4.4 mmol) in methylene chloride (40 ml), was added a solution of sodium hydroxide (0.48 g, 12 mmol) and tetrabutylammonium hydrogen sulfate (catalytic amount) in water (3 ml). The reaction mixture was stirred at room temperature for 2 hours. After addition of methylene chloride (80 ml), the organic layer was separated, washed with water, dried over calcium chloride and evaporated to dryness. A silica-gel column was used to purify the residue with cyclohexane/ethyl acetate (70/30) as eluent.

(Z) 3-phenyl-2-(pyridin-4-ylmethylen)indan-1-one 2

Yellow crystals (29%); mp 147°C; IR (KBr) 1725 cm^{-1} (CO); ^1H NMR (400 MHz, $CDCl_3$) 8.44 (d, J = 5.0 Hz, 2H, H2' and H6'), 7.50 (m, 5H, Hα, H4, H7, H3' and H5'), 7.39 (m, 2H, H2" and H6"), 7.34 (t, J = 7.2 Hz, 1H, H5), 7.25 (t, J = 7.2 Hz, 1H, H6), 7.07 (m, 4H, H3", H4", H5" and H3); analysis calculated for $C_{21}H_{15}NO$: C, 84.82; H, 5.08; N, 4.71; found : C, 84.54; H, 5.14; N, 4.56.

(Z) 6-(4-chloro-phenyl)-7-(pyridin-4-ylmethylene)-5,6,7,8-tetrahydroindolizin-8-one 5

Yellow crystals (65%); mp 238°C; IR (KBr) 1665 cm^{-1} (CO); ^1H NMR (400 MHz, $CDCl_3$) 8.50 (d, J = 5.6 Hz, 2H, H2' and H6'), 7.94 (s, 1H, Hα), 7.26 (d, J = 8.0 Hz, 2H, H3" and H5"), 7.17 (m, 1H, H3), 7.10 (m, 4H, H3', H5', H2" and H6"), 6.72 (m, 1H, H1), 6.25 (m, 1H, H2), 4.59 (m, 1H, H6), 4.45 (dd, J = 12 and 3.5 Hz, 1H, H5a), 4.31 (dd, J = 12 and 2.5 Hz, H5b); analysis calculated for $C_{20}H_{15}N_2OCl$: C, 71.74; H, 4.51; N, 8.36; found : C, 71.59; H, 4.52; N, 8.19.

Biological Methods : Preparation of Microsomes

Human placental microsomes were prepared as described previously. [8] Protein concentration was evaluated according to Bradford [9] using bovine serum albumin as standard, and Coomassie brilliant blue as dye-reagent.

Biological Methods : Inhibition Studies

Aromatase activity was evaluated by measuring ^3H_2O released from [1ß,2ß-^{3}H]-androstenedione (concentrations of 8-100 nM for kinetic studies and 200 nM for IC_{50} determinations).[1, 10] Steroids were then extracted with a charcoal/dextran solution (7%/1.5%) and the radioactivity of the aqueous phase was measured as previously described. Control incubation was realized without cofactor in the same conditions. The results are the mean of triplicate experiments +/- SD and are expressed as pmol estrogen formed/min.mg microsomal protein.

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