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New Aromatase Inhibitors. Synthesis and Biological Activity of Aryl-Substituted Pyrrolizine and Indolizine Derivatives

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Abstract—We report herein the design and the synthesis of some aryl-substituted pyrrolizine and indolizine derivatives, on the basis of a hypothetical pharmacophore structure designed to fit the catalytic site of the human cytochrome P450 aromatase. The in vitro biological evaluation of these compounds allowed us to point out two new potent non-steroidal aromatase inhibitors, MR 20494 and MR 20492, with IC_{50} values in the range of 0.1 μM.

Introduction

Inhibitors of the cytochrome P450 aromatase are therapeutic agents for the treatment of estrogen dependent diseases such as breast cancer,[1] and constitute today an alternative to antihormone treatment.[2] More recently, antisense oligodeoxynucleotides were also developed to inhibit human aromatase gene expression.[3] Moreover, several nonsteroidal inhibitors of aromatase have hitherto been reported. Most of them belong to the azole-type and are commercially available or currently under clinical evaluation (fadrozole, vorozole, letrozole, and anastrozole).[4–6] However, and particularly in relation to results obtained in vivo and with side effects due to these compounds,[7,8] the synthesis of more powerful and more specific aromatase inhibitors still remains a challenge.

With this view, some benzocycloalkene-type inhibitors have been designed and synthesized,[9–12] and among them, we have recently described the synthesis and the biological evaluation of new 3-amino-2-arylmethyl indenones.[13] We showed that derivative **1** (MR 20814, Scheme 1) inhibits efficiently in vitro human aromatase activity with an IC_{50} of 3.5 ± 1.2 μM and an apparent K_i/K_m of 6.7 ± 0.6, while it was considerably less active towards equine aromatase ($IC_{50} > 10$ μM and $K_i/K_m = 100.0 \pm 6.4$), used as a useful comparative molecular model to understand the mammalian active site of this enzyme.[13]

This previous 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 human enzyme. As shown by comparative molecular modeling between human and equine enzymes, this interaction led to dispose of the amino group of **1** at the entry of an extra-hydrophobic pocket, already described by Laughton et al.[14] In the case of equine aromatase, we proposed that the primary structure and the conformation of the enzyme cannot allow this position.[13]

In order to confirm this hypothesis and to improve the activity of some related compounds, we undertook the pharmacomodulation of **1**. In particular, we carried out the substitution of its amino group by an hydrophobic moiety likely to occupy, in an optimal way, the extra-hydrophobic pocket within the active site of the enzyme. This work allowed us to report, in a preliminary paper,[15] the synthesis and the biological activity of compound **2** (MR 20496, Scheme 1), in which the amino group of **1** was replaced by a phenyl ring, while its pyridin moiety was frozen in a *Z* geometry. Derivative **2** inhibits strongly human aromatase in vitro ($IC_{50} = 0.472 \pm 0.092$ μM).

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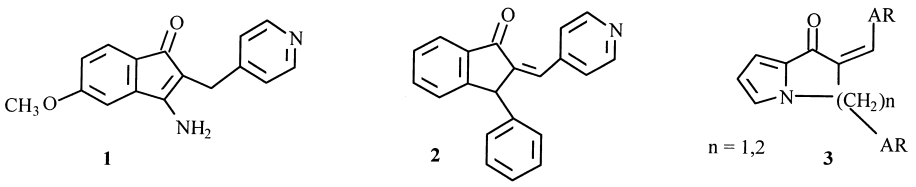


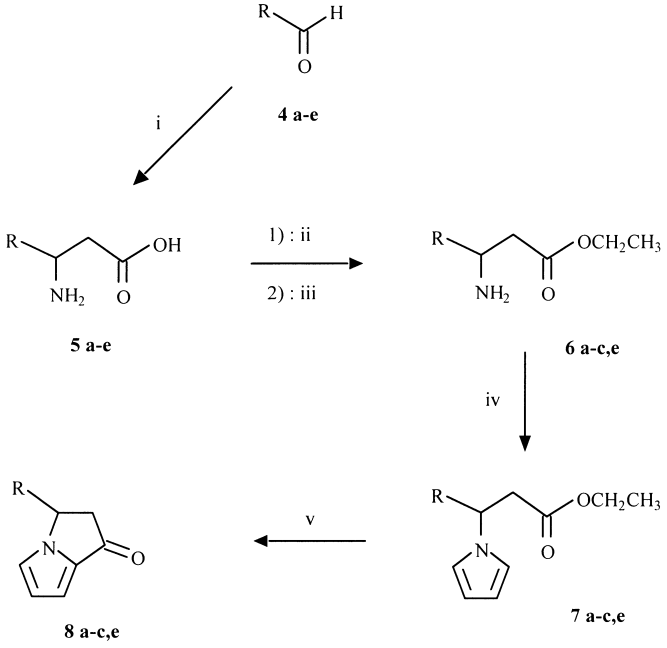
Scheme 1.

These results altogether led us to design a hypothetical pharmacophore structure, responsible for the aromatase inhibition, and we report herein the synthesis and the biological evaluation of some new aryl-substituted pyrrolizine and indolizine derivatives **3**, fitting this model.

Chemistry

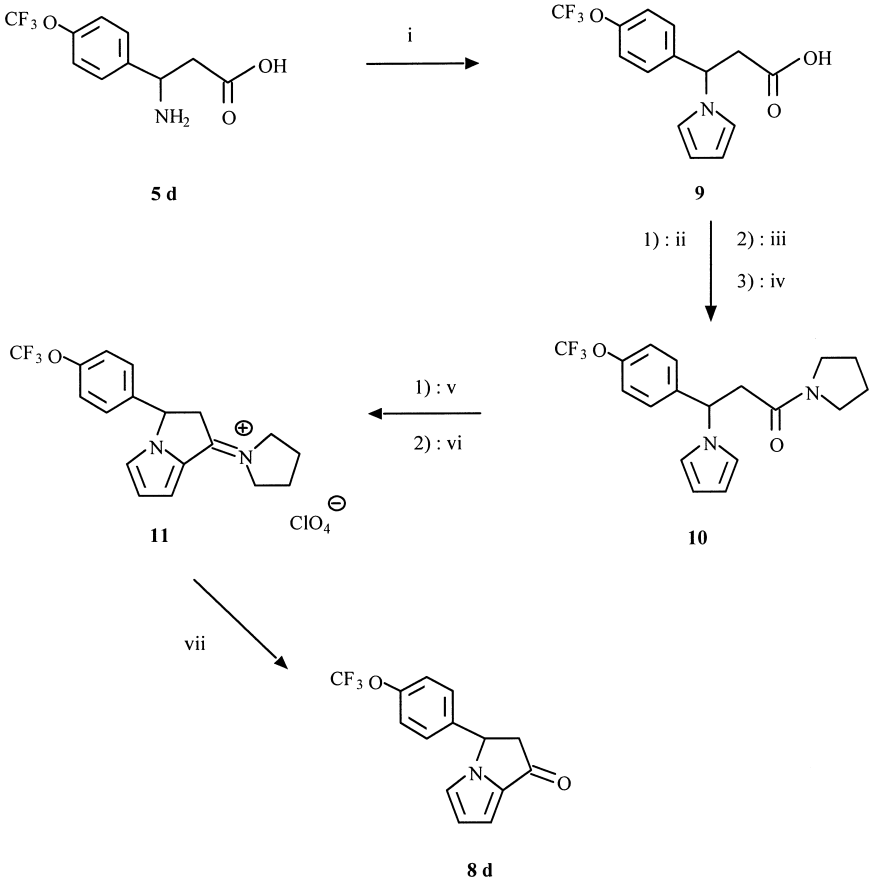
We previously reported the access to 3-phenyl-2,3-dihydro-1*H*-pyrrolizin-1-one **8a**, starting from 3-amino-3-phenylpropionic acid **5a**.[16] The synthetic pathway we used involved successively a Clauson–Kaas reaction and subsequently a Vilsmeier intramolecular cyclisation leading to **8a** after an alkaline hydrolysis of the Vilsmeier salt.

Recent works showed that the pyrrole ring could be acylated by an ester function under the action of boron tribromide in dichloromethane.[17] This efficient ring closure method led us to reinvestigate the synthesis of the pyrrolizinone series and particularly to develop it starting from the β-amino acids **5a–e**. We previously described[18,19] **5a**, **5b** and **5e**, that we obtained, starting from benzaldehydes **4a** and **4b** or from thiophene-3-carboxaldehyde **4e**, according to the Rodionow–Johnson reaction.[20,21] Application of the same treatment, involving malonic acid and ammonium acetate, to 4-cyano- **4c** or 4-trifluoromethoxybenzaldehyde **4d** led to the new aminophenylpropionic acids **5c** and **5d** (Scheme 2). Esterification of the carboxylic acid group of **5a–c,e** was carried out, without protection of the amino group, through a treatment with thionyl chloride in ethanol, followed by displacement of the intermediary salt with ammonia in ether.[22] The expected ethyl aminopropionates **6a–c,e** were then involved in a Clauson–Kaas reaction,[23,24] using 2,5-dimethoxytetrahydrofuran in acetic acid, and yielded the ethyl arylpyrrolylpropionates



R					
	a	b	c	d	e

Scheme 2. (i) $CH_2(CO_2H)_2$, $AcONH_4$, EtOH or MeCN; (ii) $SOCl_2$, EtOH; (iii) NH_3 gas, EtO_2; (iv) 2,5-diOMeTHF, AcOH; (v) BBr_3, CH_2Cl_2.



Scheme 3. (i) 2,5-diOMeTHF, AcOH; (ii) TEA, $(CH_3)_2CO$; (iii) $ClCO_2Et$, $(CH_3)_2CO$; (iv) pyrrolidine, $(CH_3)_2CO$; (v) $POCl_3$, toluene; (vi) $HClO_4$, H_2O; (vii) NaOH, H_2O.

7a–c,e. Cyclisation of the latter into the pyrrolizinones **8a–c,e** finally took place using boron tribromide in methylene chloride at room temperature.

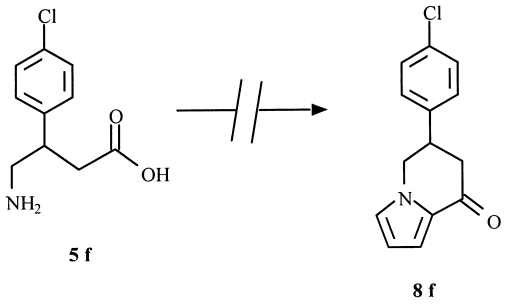
However, we could not apply this short sequence to 3-amino-3-(4-trifluoromethoxyphenyl)-propionic acid **5d**, due to the sensitivity of the alkoxy groups to the action of boron tribromide. For this reason, the trifluoromethoxypyrrolizinone **8d** was prepared, in a similar manner as for the initial synthesis of **8a**, via a Vilsmeier cyclisation, using phosphorus oxychloride as reagent, and a subsequent hydrolysis (Scheme 3).

On the other hand, using the same pathway as for **8d**, we also reported the synthesis of 6-(4-chlorophenyl)-5,6,7,8-tetrahydroindolizin-8-one **8f**,[25] starting from the corresponding γ-amino acid, baclofen **5f**, a commercially available antispastic drug (Scheme 4). The vinyl homologue of the latter, vigabatrin **5g**, lent itself to the previous sequence using boron tribromide and led to 5-vinyl-5,6,7,8-tetrahydroindolizin-8-one **8g**, in a similar manner as for **8a–c,e** (Scheme 5).

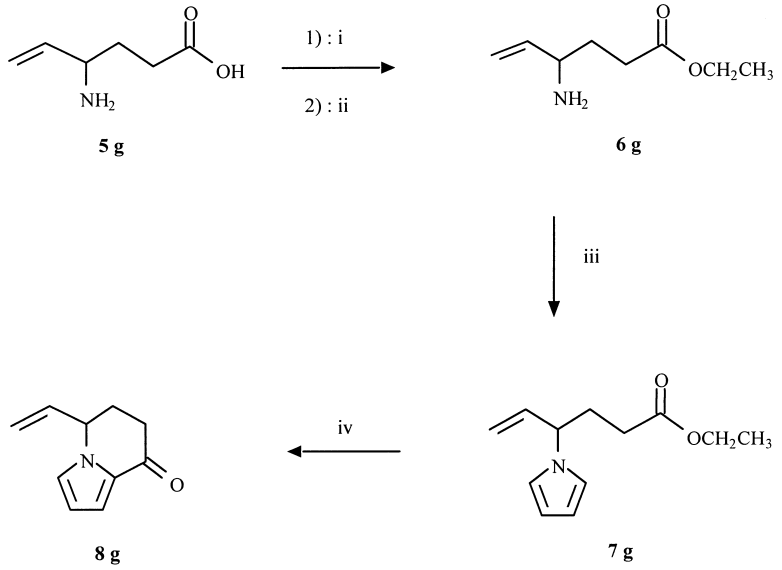
All the synthesised pyrrolizinones **8a–e** and indolizinones **8f,g** were finally involved in an aldolisation reaction, using various arylaldehydes such as benzaldehyde, 2,4-dimethoxybenzaldehyde, pyridine-2-, -3- or -4-carboxaldehydes (Schemes 6 and 7). The reactions were carried out, either in an ethanolic solution of sodium hydroxide or in a heterogeneous system (dichloromethane/water) using tetrabutylammonium hydrogen sulfate as catalyst. In all cases, the reaction led selectively to the *Z* forms of **12–29**, as deduced from their ^1H NMR spectra (data not shown).

Biology

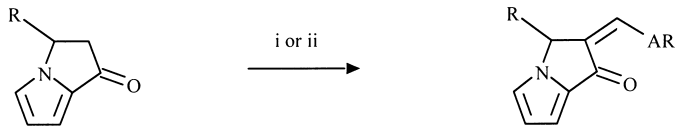
This study showed that compounds **12–20**, **25** and **26** did not inhibit at 10 μM human aromatase in placental microsomes. According to the results presented in Table 1, compounds **24** (MR 16088) and **27** (MR 20493) were

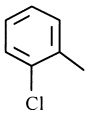
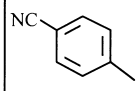
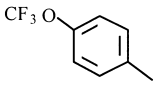
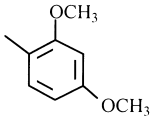


Scheme 4.

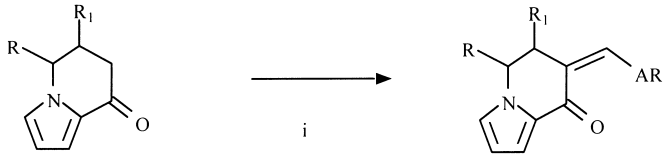


Scheme 5. (i) $SOCl_2$, EtOH; (ii) NH_3 gas, EtO_2; (iii) 2,5-diOMeTHF, AcOH; (iv) BBr_3, CH_2Cl_2.



AR \ R					
	**12** (MR 20431)	**13** (MR 20444)	-	-	**14** (MR 20427)
	**15** (MR 20446)	**16** (MR 20443)	-	-	**17** (MR 20428)
	**18** (MR 20447)	-	**19** (MR 16092)	**20** (MR 16090)	-
	**21** (MR 20445)	-	-	**22** (MR 16089)	-
	**23** (MR 20491)	-	-	**24** (MR 16088)	-

Scheme 6. (i) AR-CHO, NaOH, EtOH; (ii) AR-CHO, $nBu_4NH^+{}^-HSO_4$, CH_2Cl_2, H_2O.



8 f,g 25-29 (Z)

	AR			
R	R_1			
	H	**25** (MR 20475)	**26** (MR 20479)	**27** (MR 20493)
H	Cl	-	**28** (MR 20494)	**29** (MR 20492)

Scheme 7. (i) AR-CHO, $nBu_4NH^+ \ ^-HSO_4$, CH_2Cl_2, H_2O.

Table 1. (A) Inhibition of human aromatase in placental microsomes by pyrrolizine derivatives. (B) Inhibition of human aromatase in placental microsomes by indolizine derivatives[a]

	Compounds	IC_{50} (μM)	K_i/K_m
A	4-OHA	0.45 ± 0.06	1.9 ± 0.8
	MR 20445 (21)	3.0 ± 0.6	12.7 ± 0.1
	MR 20491 (23)	1.6 ± 0.4	2.1 ± 0.2
	MR 16089 (22)	0.65 ± 0.07	—
	MR 16088 (24)	9.4	—
B	Fadrozole	0.06 ± 0.03	0.1 ± 0.0
	MR 20493 (27)	7.4 ± 1.6	7.3 ± 0.9
	MR 20494 (28)	0.11 ± 0.06	0.5 ± 0.3
	MR 20492 (29)	0.15 ± 0.01	1.0 ± 0.3

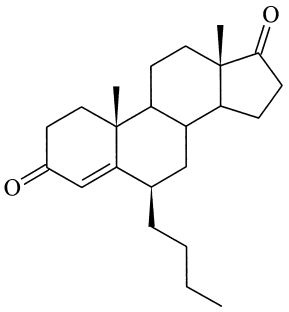
IC_{50} of compounds **12**–**20**, **25**–**26** were > 10 μM.
K_m (Δ_4) = 10.7 ± 3.3 nM

[a]Aromatase activity was evaluated by measuring ^3H_2O released from 200 nM [1β,2β-^{3}H]androstenedione at 37 °C for 15 min, in the presence of inhibitors. 4-Hydroxyandrostenedione (4-OHA) was used as control. The inhibition of aromatase in human placental (195 μg) microsomes was performed in the presence of 60 μM NADPH, H^+. The blank was realised without adding NADPH, H^+. The results are expressed as % to a standard control which was incubated with NADPH, H^+ and without inhibitor, and are the mean of triplicate experiments ± SD. (—): not determined. For legend, see above. Fadrozole was used as control.

weak inhibitors in these conditions, while **21** (MR 20445), **23** (MR 20491) and **22** (MR 16089) were as potent as 4-OHA. Finally, compounds **28** (MR 20494) and **29** (MR 20492) were very potent inhibitors of human aromatase, slightly less than Fadrozole.

Discussion

For the pyrrolizinone series, the compounds bearing a *meta* or *para* pyridinylmethylene substituent are actives. The main interaction of these ligands with the receptor is the coordination bond between the nitrogen of pyridine group and the heme iron.[13] This bond imposes the orientation of the ligand and therefore the position of the hydrophobic group inside the active site. These variations of positions of the ligands inside the active site could explain the variation of affinity. The comparison of the three dimensional structures of these compounds and steroid aromatase inhibitors, like the compound **30**[26] with hydrophobic group, showed a close position of the hydrophobic groups between the two structures when the pyridin group for these series and C19 methyl group of steroid inhibitor have the same orientation (Fig. 1). These results confirm the presence of the extrahydrophobic surface, explaining the activities of these series. By comparing **21** to **22** and **23** to **24**, two opposite effects are observed, in terms of activity, with the 4-trifluoromethoxyphenyl group. This group is very hydrophobic with a theoretical logP[27] of 3.2 (compared to a value of 2.8 for *para* chloro phenyl or 2.1 for 4-methoxyphenyl group). The improvement of activity of **22** compared to **21** could be explained by this factor. For **24** compared to **23**, a steric hindrance could explain this result. Indeed, the better affinity of **23** compared to **22** must be due to a closer hydrophobic contact between the ligand and residues of the receptor, and this reinforces the idea of the steric hindrance with a 4-trifluoromethoxyphenyl group.



30

For the tetrahydroindolizinone series, these compounds have a better flexibility with an envelope conformation allowing us to place the hydrophobic group in two different equatorial positions (see Fig. 2 for one conformation). This flexibility is surely the main factor explaining the improvement of the activities. It is interesting to see the same activities for the *meta* or *para* pyridinylmethylene group. From these data, by molecular modeling, we can design new structures which will be a combination of the three dimensional characteristics of these two ligands. This work is actually in progress.

Experimental Protocols

Chemistry

Melting points were determinated on a Kofler block and are uncorrected. IR spectra were recorded on a Mattson 1000 FTIR spectrophotometer and only noteworthy absorptions (reciprocal centimeters) are listed. ^{1}H NMR spectra were recorded at 400 MHz with tetramethylsilane as an internal standard using a JEOL JNM-LA 400 spectrometer. Splitting patterns have been designated as follows: s = singlet; b s = broad singlet; d = doublet; t = triplet; q = quartet; qt = quintuplet; dd = double doublet; m = multiplet. Analytical TLC was carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV$_{254}$) with visualisation by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values.

3-Amino-3-(4-cyanophenyl)propionic acid 5c. To a solution of 4-cyanobenzaldehyde **4c** (13.1 g, 0.1 mol) in acetonitrile (200 mL) were added malonic acid (10.7 g, 0.1 mol) and ammonium acetate (15.4 g, 0.2 mol). The reaction mixture was refluxed for 48 h. The precipitate was filtered, washed with water and dried to give **5c** as a colorless solid (26%) which was used without further purification; mp > 260 °C; IR (KBr) 3100–2450 ($^+NH_3$ CO_2^-), 2230 (CN), 1630 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.82 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'} = J_{H\text{-}5'\ H\text{-}6'} = 7.5$ Hz, H-3′ and H-5′), 7.60 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'} = 7.5$ Hz, H-2′ and H-6′), 4.33 (m, 1H, H-3), 3.5 (b s, 3H, $^+NH_3$), 2.49 (m, 1H, H-2a), 2.37 (m, 1H, H-2b); anal. $C_{10}H_{10}N_2O_2$ (C, H, N).

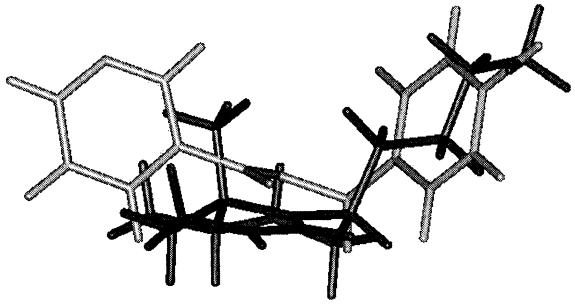


Figure 1. Superimposition of compounds **21** and **30**.

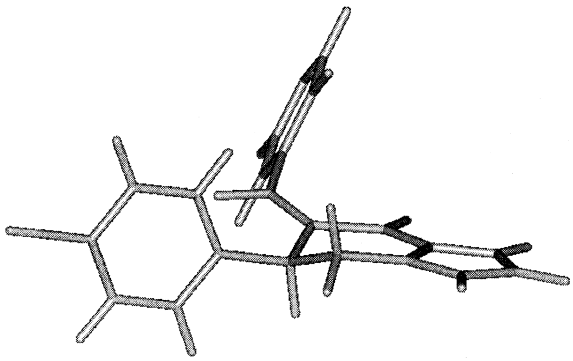


Figure 2. One equatorial conformation of compound **30**.

3-Amino-3-(4-trifluoromethoxyphenyl)propionic acid 5d. To a solution of 4-trifluoromethoxybenzaldehyde **4d** (19.0 g, 0.1 mol) in ethanol (200 mL) were added malonic acid (10.7 g, 0.1 mol) and ammonium acetate (15.4 g, 0.2 mol). The reaction mixture was refluxed for 6 h. The precipitate was filtered, washed with hot ethanol and dried to give **5d** as a colorless solid (58%) which was used without further purification; mp > 260 °C; IR (KBr) 3100–2450 ($^+NH_3$ CO_2^-), 1630 (CO), 1260, 1210, 1165 (CF_3); ^{1}H NMR (DMSO-d_6) δ: 7.51 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'} = J_{H\text{-}5'\ H\text{-}6'} = 8.5$ Hz, H-3′ and H-5′), 7.27 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'} = 8.5$ Hz, H-2′ and H-6′), 4.26 (t, 1H, $J = 8$ Hz, H-3), 3.6 (b s, 3H, $^+NH_3$), 2.5 (m, 2H, H-2a and H-2b); anal. $C_{10}H_{10}NO_3F_2$ (C, H, N).

Ethyl 3-amino-3-arylpropionates and ethyl 4-amino-4-vinylbutyrate 6a–c,e or g. General method: to a stirred suspension of 3-amino-3-arylpropionic acid **5a–c** or **e** or 4-amino-4-vinylbutyric acid (0.050 mol) **5g** in ethanol (100 mL) was added dropwise at room temperature thionyl chloride (0.055 mol). Stirring was maintained for 20 min and the solution was then evaporated to dryness under reduced pressure. The solid residue was triturated in ether (50 mL), filtered and dissolved in a mixture of water (30 mL) and ether (300 mL). The reaction mixture was then bubbled for 2 min at room temperature with an ammonia gas flow. The organic layer was separated, dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to give **6a–c,e** or **g** as an oil which was used without further purification.

Ethyl 3-amino-3-phenylpropionate 6a. Yellow oil (53%); IR (KBr) 3200 (NH_2), 1740 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.2 (m, 5H, Ph), 4.15 (m, 1H, H-3), 3.95 (q, 2H, $J = 7.2$ Hz, CH_2), 2.54 (m, 2H, H-2); 1.8 (b s, 2H, NH_2),

1.05 (t, 3H, $J=7.2$ Hz, CH_3); anal. $C_{11}H_{15}NO_2$ (C, H, N).

Ethyl 3-amino-3-(2-chlorophenyl)propionate 6b. Yellow oil (71%); IR (KBr) 3200 (NH_2), 1750 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.62 (d, 1H, $J_{H\text{-}3'\ H\text{-}4'}=7.7$ Hz, H-3′), 7.3 (m, 3H, H-6′, H-5′ and H-4′), 4.62 (dd, 1H, $J_{H\text{-}3\ H\text{-}2b}=8.3$ Hz, $J_{H\text{-}3\ H\text{-}2a}=4.8$ Hz, H-3), 4.03 (q, 2H, $J=7$ Hz, CH_2), 2.56 (dd, 1H, $J_{H\text{-}2a\ H2b}=15$ Hz, $J_{H\text{-}2a\ H\text{-}3}=4.8$ Hz, H-2a), 2.46 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}=15$ Hz, $J_{H\text{-}2b\ H\text{-}3}=8.3$ Hz, H-2b), 1.7 (b s, 2H, NH_2), 1.02 (t, 3H, $J=7$ Hz, CH_3); anal. $C_{11}H_{14}NO_2Cl$ (C, H, N).

Ethyl 3-amino-3-(4-cyanophenyl)propionate 6c. Yellow oil (80%); IR (KBr) 3380, 3310 (NH_2), 2230 (CN), 1730 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.56 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'}=J_{H\text{-}5'\ H\text{-}6'}=8.5$ Hz, H-3′ and H-5′), 7.43 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'}=J_{H\text{-}6'\ H\text{-}5'}=8.5$ Hz, H-2′ and H-6′), 4.41 (t, 1H, $J=7$ Hz, H-3), 4.06 (q, 2H, $J=7$ Hz, CH_2), 2.56 (d, 2H, $J=7$ Hz, H-2a and H-2b), 1.7 (b s, 2H, NH_2), 1.16 (t, 3H, $J=7$ Hz, CH_3); anal. $C_{12}H_{14}N_2O_2$ (C, H, N).

Ethyl 3-amino-3-thien-3-ylpropionate 6e. Orange oil (44%); IR (KBr) 3200 (NH_2), 1750 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.19 (d, 1H, $J_{H\text{-}5'\ H\text{-}4'}=4.8$ Hz, H-5′), 7.07 (s, 1H, H-2′), 6.97 (d, 1H, $J_{H\text{-}4'\ H\text{-}5'}=4.8$ Hz, H-4′), 4.40 (dd, 1H, $J_{H\text{-}3\ H\text{-}2b}=8.8$ Hz, $J_{H3\ H\text{-}2a}=4.8$ Hz, H-3), 4.04 (q, 2H, $J=72$ Hz, CH_2), 2.60 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}=15.7$ Hz, $J_{H\text{-}2a\ H\text{-}3}=4.8$ Hz, H-2a), 2.53 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}=15.7$ Hz, $J_{H\text{-}2b\ H\text{-}3}=8.8$ Hz, H-2b), 1.8 (b s, 2H, NH_2), 1.14 (t, 3H, $J=7.2$ Hz, CH_3); anal. $C_9H_{13}NO_2S$ (C, H, N).

Ethyl 4-amino-4-vinylbutyrate 6g. Colorless oil (93%); IR (KBr) 3200 (NH_2), 1750 (CO); ^{1}H NMR (DMSO-d_6) δ: 5.72 (m, 1H, H-vinyl), 5.04 (d, 1H, $J=10$ Hz, H-vinyl), 5.04 (d, 1H, $J=17$ Hz, H-vinyl), 4.03 (q, 2H, $J=7$ Hz, CH_2), 3.15 (m, 1H, H-4), 2.28 (t, 2H, $J=7.5$ Hz, H-2a and H-2b), 1.6 (m, 4H, H-3a, H-3b and NH_2), 1.16 (t, 3H, $J=7$ Hz, CH_3); anal. $C_8H_{15}NO_2$ (C, H, N).

Ethyl 3-aryl-3-(pyrrol-1-yl)propionates and ethyl 4-(pyrrol-1-yl)-4-vinylbutyrate 7a–c,e or g. General method: to a solution of ethyl 3-amino-3-arylpropionates **6a–c** or **e** or ethyl 4-amino-4-vinyl butyrate **6g** (0.045 mol) in acetic acid (100 mL) was added 2,5-dimethoxytetrahydrofuran (5.9 mL, 0.045 mol). The reaction mixture was refluxed for 1 h and then evaporated to dryness. The residue was taken up in ether (100 mL) and filtered. The filtrate was washed with water, dried over sodium sulfate and evaporated under reduced pressure to give **7a–c,e** or **g** as an oil.

Ethyl 3-phenyl-3-(pyrrol-1-yl)propionate 7a. Orange oil (86%); IR (KBr) 1750 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.3 (m, 5H, Ph), 6.94 (m 2H, H-α pyrrol), 6.03 (m, 2H, H-β pyrrol), 5.66 (t, 1H, $J_{H\text{-}3\ H\text{-}2}=6.8$ Hz, H-3), 4.01 (q, 2H, $J=7.1$ Hz, CH_2), 2.32 (m, 2H, H-2), 1.07 (t, 3H, $J=7.1$ Hz, CH_3); anal. $C_{15}H_{17}NO_2$ (C, H, N).

Ethyl 3-(2-chlorophenyl)-3-(pyrrol-1-yl)propionate 7b. Orange oil (78%); IR (KBr) 1750 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.3 (m, 4H, Ph), 6.90 (m, 2H, H-α pyrrol), 6.02 (m, 3H, H-β pyrrol and H-3), 4.03 (q, 2H, $J=7$ Hz, CH_2), 3.38 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}=16$ Hz, $J_{H\text{-}2a\ H\text{-}3}=9$ Hz, H-2a), 3.28 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}=16$ Hz, $J_{H\text{-}2b\ H\text{-}3}=6.3$ Hz, H-2b), 1.07 (t, 3H, $J=7.1$ Hz, CH_3); anal. $C_{15}H_{16}NO_2Cl$ (C, H, N).

Ethyl 3-(4-cyanophenyl)-3-pyrrol-1-ylpropionate 7c. Orange oil (80%); IR (KBr) 2230 (CN), 1725 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.61 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'}=J_{H\text{-}5'\ H\text{-}6'}=8.5$ Hz, H-3′ and H-5′), 7.22 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'}=J_{H\text{-}6'\ H\text{-}5'}=8.5$ Hz, H-2′ and H-6′), 6.72 (m, 2H, H-α pyrrol), 6.19 (m, 2H, H-β pyrrol), 5.71 (dd, 1H, $J_{H\text{-}3\ H\text{-}2a}=9$ Hz, $J_{H\text{-}3\ H\text{-}2b}=6.3$ Hz, H-3), 4.10 (q, 2H, $J=7$ Hz, CH_2), 3.23 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}=16$ Hz, $J_{H\text{-}2a\ H\text{-}3}=9$ Hz, H-2a), 3.13 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}=16$ Hz, $J_{H\text{-}2b\ H\text{-}3}=6.3$ Hz, H-2a), 1.17 (t, 3H, $J=7$ Hz, CH_3); anal. $C_{16}H_{16}N_2O_2$ (C, H, N).

Ethyl 3-(pyrrol-1-yl)-3-(thien-3-yl)propionate 7e. Orange oil (59%); IR (KBr) 1740 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.22 (d, 1H, $J_{H\text{-}5'\ H4'}=4.8$ Hz, H-5′), 7.02 (s, 1H, H-2′), 6.85 (d, 1H, $J_{H\text{-}4'\ H5'}=4.8$ Hz, H-4′), 6.69 (m, 2H, H-α pyrrol), 6.12 (m, 2H, H-β pyrrol), 5.67 (t, 1H, $J_{H\text{-}3\ H\text{-}2}=7$ Hz, 1H, H-3), 4.08 (q, 2H, $J=7$ Hz, CH_2), 3.12 (d, 2H, $J_{H\text{-}2\ H\text{-}3}=7.7$ Hz, H-2a and H-2b), 1.14 (t, 3H, $J=7$ Hz, CH_3); anal. $C_{13}H_{15}NO_2S$ (C, H, N).

Ethyl 4-(pyrrol-1-yl)-4-vinylbutyrate 7g. Orange oil (78%); IR (KBr): 1740 (CO); ^{1}H NMR (DMSO-d_6) δ: 6.74 (t, 2H, H-α pyrrol), 6.19 (m, 3H, H-β pyrrol and H-vinyl), 5.10 (d, 1H, $J=10$ Hz, H-vinyl), 5.05 (d, 1H, $J=17$ Hz, H-vinyl), 4.55 (m, 1H, H-4), 4.03 (q, 2H, $J=7$ Hz, CH_2), 2.1 (m, 4H, H-2a, H-2b, H-3a and H-3b), 1.15 (t, 3H, $J=7$ Hz, CH_3); anal. $C_{12}H_{17}NO_2$ (C, H, N).

3-Aryl-2,3-dihydro-1H-pyrrolizin-1-ones and 5-vinyl-5,6,7,8-tetrahydroindolizin-8-one 8a–c,e or g. General method: to a stirred solution of ethyl 3-aryl-3-(pyrrol-1-yl)propionates **7a–c** or **e** or ethyl 4-(pyrrol-1-yl)-4-vinylbutyrate **7g** (0.019 mol) in methylene chloride (100 mL) was added dropwise at 0 °C a solution of boron tribromide in methylene chloride (1 M, 110 mL). The reaction mixture was stirred for 30 min at room temperature and hydrolysed with sodium hydrogen carbonate at 0 °C. The organic layer was washed with water (2×100 mL), dried over calcium chloride and evaporated to dryness. The solid residue was purified by a silica-gel column with methylene chloride as eluent to give **8a–c,e** or **g** as crystals.

3-Phenyl-2,3-dihydro-1*H*-pyrrolizin-1-one 8a. Colorless crystals (50%); mp 98 °C (literature).

3-(2-Chlorophenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one 8b. Colorless crystals (59%); mp 112 °C; IR (KBr) 1685 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.50 (dd, 1H, $J_{H\text{-}3'\ H\text{-}4'}=7.7$ Hz, $J_{H\text{-}3'\ H\text{-}5'}=1.4$ Hz, H-3′), 7.35 (dt, 1H, $J_{H\text{-}4'\ H\text{-}3'}=7.7$ Hz, $J_{H\text{-}4'\ H\text{-}5'}=7.7$ Hz, $J_{H\text{-}4'\ H\text{-}6'}=1.7$ Hz, H-4′), 7.28 (dt, 1H, $J_{H\text{-}5'\ H\text{-}4'}=7.7$ Hz, $J_{H\text{-}5'\ H\text{-}6'}=7.7$ Hz, $J_{H\text{-}5'\ H\text{-}3'}=1.7$ Hz, H-5′), 7.16 (dd, 1H, $J_{H\text{-}5\ H\text{-}6}=2.2$ Hz, $J_{H\text{-}5\ H\text{-}7}=1.1$ Hz, H-5), 6.72 (dd, 1H, $J_{H\text{-}7\ H\text{-}6}=3.9$ Hz, $J_{H\text{-}7\ H\text{-}5}=1.1$ Hz, H-7), 6.68 (dd, 1H, $J_{H\text{-}6'\ H\text{-}5'}=7.7$ Hz, $J_{H\text{-}6'\ H\text{-}4'}=1.4$ Hz, H-6′), 6.56 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}=3.9$ Hz, $J_{H\text{-}6\ H\text{-}5}=$

2.2 Hz, H-6), 6.04 (dd, 1H, $J_{H\text{-}3\ H\text{-}2a}$ = 8 Hz, $J_{H\text{-}3\ H\text{-}2b}$ = 3.1 Hz, H-3), 3.65 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}$ = 18 Hz, $J_{H\text{-}2a\ H\text{-}3}$ = 8 Hz, H-2a), 2.73 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}$ = 18 Hz, $J_{H\text{-}2b\ H\text{-}3}$ = 3.1 Hz, H-2b); anal. $C_{13}H_{10}NOCl$ (C, H, N).

3-(4-Cyanophenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one 8c. Yellow crystals (80%); mp 104 °C (ether); IR (KBr) 2230 (CN), 1695 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.61 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'} = J_{H\text{-}5'\ H\text{-}6'}$ = 8.5 Hz, H-3′ and H-5′), 7.12 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'}$ = 8.5 Hz, H-2′ and H-6′), 6.78 (m, 2H, H-5 and H-7), 6.53 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.8 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.5 Hz, H-6), 5.54 (dd, 1H, $J_{H\text{-}3\ H\text{-}2a}$ = 9 Hz, $J_{H\text{-}3\ H\text{-}2b}$ = 3 Hz, H-3), 3.53 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}$ = 18 Hz, $J_{H\text{-}2a\ H\text{-}3}$ = 9 Hz, H-2a), 2.81 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}$ = 18 Hz, $J_{H\text{-}2b\ H\text{-}3}$ = 3 Hz, H-2b); anal. $C_{14}H_{10}N_2O$ (C, H, N).

3-(4-Trifluoromethoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one 8d. A suspension of *N*(3-(4-trifluoromethoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-yl)pyrrolidinium perchlorate **11** (10.8 g, 0.025 mol) in an aqueous solution of sodium hydroxide (1 N, 50 mL) was heated at 60 °C for 30 min. The cooled reaction mixture was then extracted with chloroform (2×100 mL). The organic layers were collected, washed with water (100 mL), dried over magnesium chloride and evaporated to dryness under reduced pressure to give a green oil which was dissolved in hot ethanol (20 mL). The solution was charcoaled, filtered and cooled to give, by dilution with water (10 mL), **8d** as a colorless solid (37%); mp 84 °C (propan-2-ol); IR (KBr) 1695 (CO), 1265, 1220, 1165 (CF_3); ^{1}H NMR ($CDCl_3$) δ: 7.26 (s, 1H, H-5), 7.23 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'} = J_{H\text{-}5'\ H\text{-}6'}$ = 8.5 Hz, H-3′ and H-5′), 7.13 (s, 1H, H-7), 6.84 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'}$ = 8.5 Hz, H-2′ and H-6′), 6.57 (m, 1H, H-6), 5.57 (dd, 1H, $J_{H\text{-}3\ H\text{-}2a}$ = 9 Hz, $J_{H\text{-}3\ H\text{-}2b}$ = 3 Hz, H-3), 3.56 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}$ = 18 Hz, $J_{H\text{-}2a\ H\text{-}3}$ = 9 Hz, H-2a), 2.92 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}$ = 18 Hz, $J_{H\text{-}2b\ H\text{-}3}$ = 3 Hz, H-2b); anal. $C_{14}H_{10}NO_2F_3$ (C, H, N).

3-(Thien-3-yl)-2,3-dihydro-1*H*-pyrrolizin-1-one 8e. Colorless crystals (45%); mp 110 °C; IR (KBr) 1680 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.50 (m, 2H, H-2′ and H-5′), 7.13 (d, 1H, $J_{H\text{-}4'\ H\text{-}5'}$ = 4.8 Hz, H-4′), 6.99 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 2.2 Hz, H-5), 6.64 (d,1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.8 Hz, H-7), 6.49 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.8 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.2 Hz, H-6), 5.82 (dd, 1H, $J_{H\text{-}3\ H\text{-}2a}$ = 7.8 Hz, $J_{H\text{-}3\ H\text{-}2b}$ = 3.6 Hz, H-3), 3.50 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}$ = 18 Hz, $J_{H\text{-}2a\ H\text{-}3}$ = 7.8 Hz, H-2a), 2.95 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}$ = 18 Hz, $J_{H\text{-}2b\ H\text{-}3}$ = 3.6 Hz, H-2b); anal. $C_{11}H_9NOS$ (C, H, N).

5-Vinyl-5,6,7,8-tetrahydroindolizin-8-one 8g. Colorless crystals (49%); mp 66 °C; IR (KBr) 1680 (CO); ^{1}H NMR ($CDCl_3$) δ: 6.91 (dd, 1H, $J_{H\text{-}1\ H\text{-}2}$ = 3.9 Hz, $J_{H\text{-}1\ H\text{-}3}$ = 1.5 Hz, H-1), 6.76 (dd, 1H, $J_{H\text{-}3\ H\text{-}2}$ = 2.5 Hz, $J_{H\text{-}3\ H\text{-}1}$ = 1.5 Hz, H-3), 6.16 (dd, 1H, $J_{H\text{-}2\ H\text{-}1}$ = 3.9 Hz, $J_{H\text{-}2\ H\text{-}3}$ = 2.5 Hz, H-2), 5.83 (m, 1H, H-vinyl), 5.22 (dd, 1H, J = 10.2 Hz, J = 1 Hz, H-vinyl), 4.90 (dd, 1H, J = 17 Hz, J = 1 Hz, H-vinyl), 4.62 (m, 1H, H-5), 2.50 (m, 1H, H-7a), 2.38 (m, 1H, H-7b), 2.26 (m, 1H, H-6a), 2.06 (m, 1H, H-6b); anal. $C_{10}H_{11}NO$ (C, H, N).

3-(4-Trifluoromethoxyphenyl)-3-pyrrol-1-ylpropionic acid 9. To a solution of 3-amino-3-(4-trifluoromethoxyphenyl)propionic acid **5d** (10 g, 0.04 mol) in acetic acid (150 mL) was added 2,5-dimethoxytetrahydrofuran (7.8 mL, 0.04 mol). The reaction mixture was refluxed for 1 h and then evaporated to dryness. The residue was taken up in ether (200 mL) and the suspension was filtered. The filtrate was washed with water (2×100 mL), then dried over sodium sulfate and the solvent was removed under reduced pressure to give **9** as a colorless oil (95%); IR (KBr) 3300–2500 (OH), 1715 (CO), 1260, 1215, 1165 (CF_3); ^{1}H NMR (DMSO-d_6) δ: 10.5 (b s, 1H, OH), 7.42 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'} = J_{H\text{-}5'\ H\text{-}6'}$ = 8.5 Hz, H-3′ and H-5′), 7.29 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'}$ = 8.5 Hz, H-2′ and H-6′), 6.95 (m, 2H, H-α pyrrol), 5.99 (m, 2H, H-β pyrrol), 5.66 (t, 1H, J = 7 Hz, H-3), 3.25 (m, 2H, H-2a and H-2b); anal. $C_{14}H_{12}NO_3F_3$ (C, H, N).

***N*-(3-(4-Trifluoromethoxyphenyl)-3-pyrrol-1-ylpropionyl)-pyrrolidine 10.** To a stirred solution of 3-(4-trifluoromethoxyphenyl)-3-pyrrol-1-ylpropionic acid **9** (18.3 g, 0.06 mol) in acetone (150 mL) at 0 °C was added dropwise triethylamine (9.3 mL, 0.066 mol). After 30 min, ethyl chloroformate (5.9 mL, 0.06 mol) was added dropwise at 0 °C. After 30 min, pyrrolidine (5.1 mL, 0.06 mol) was added in the same conditions. The reaction mixture was refluxed for 1 h and then evaporated to dryness. The oily residue was taken up in ether (250 mL) and filtered. The filtrate was washed with water (2×100 mL), dried over calcium chloride and the solvent was removed under reduced pressure to give **10** as a colorless solid (40%); mp 85 °C (ether/petroleum ether); IR (KBr) 1624 (CO), 1280, 1195, 1160 (CF_3); ^{1}H NMR ($CDCl_3$) δ: 7.2 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 6.72 (m, 2H, H-α pyrrol), 6.16 (m, 2H, H-β pyrrol), 4.10 (m, 1H, H-3), 3.20 (m, 5H, H-α pyrrolidine and H-2a), 2.14 (m, 1H, H-b), 1.10 (m, 4H, H-β pyrrolidine); anal. $C_{18}H_{19}N_2O_2F_3$ (C, H, N).

***N*-(3-(4-Trifluoromethoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-yl)pyrrolidinium perchlorate 11.** Phosphoryl chloride (4.4 mL, 0.048 mol) was added to a solution of *N*-(3-(4-trifluoromethoxyphenyl)-3-pyrrol-1-ylpropionyl)pyrrolidine **10** (8 g, 0.024 mol) in toluene (100 mL). The reaction mixture was refluxed for 1 h and then evaporated to dryness. The oily residue was washed with petroleum ether (2×100 mL) and poured into water (100 mL). The mixture was refluxed again for another 1 h and filtered. The filtrate was cooled and adjusted to pH = 9 with sodium hydrogen carbonate. Perchloric acid was finally added to acidify the solution to pH = 2. The precipitate was filtered, washed with water (50 mL) and dried to give **11** as a green solid (95%); mp 100 °C (dec) (ethanol); IR (KBr) 1670 (CN), 1260, 1220, 1170 (CF_3), 1090 (ClO_4); ^{1}H NMR ($CDCl_3$) δ: 7.30 (d, 2H, $J_{H\text{-}3'\ H\text{-}2'} = J_{H\text{-}5'\ H\text{-}6'}$ = 8.5 Hz, H-3′ and H-5′), 7.22 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'}$ = 8.5 Hz, H-2′ and H-6′), 7.11 (m, 1H, H-5), 6.98 (m, 1H, H-7), 6.74 (m, 1H, H-6), 5.75 (m, 1H, H-3), 4.34 (dd, 1H, $J_{H\text{-}2a\ H\text{-}2b}$ = 18 Hz, $J_{H\text{-}2a\ H\text{-}3}$ = 9 Hz, H-2a), 3.90 (m, 4H, H-α pyrrolidinium), 3.52 (dd, 1H, $J_{H\text{-}2b\ H\text{-}2a}$ = 18 Hz, $J_{H\text{-}2b\ H\text{-}3}$ = 3 Hz, H-2b), 2.22 (m, 4H, H-β pyrrolidinium); anal. $C_{18}H_{18}N_2O_5ClF_3$ (C, H, N).

(*Z*) 2-Alkylidene-3-aryl-2,3-dihydro-1*H*-pyrrolizin-1-ones and (*Z*) 7-alkylidene-5-vinyl-5,6,7,8-tetra-hydroindolizin-

8-ones 12–27. General method A: an aqueous sodium hydroxide solution (6 N, 2 mL) was added to a solution of 3-aryl-2,3-dihydro-1*H*-pyrrolizin-1-ones **8a**–**e** or 5-vinyl-5,6,7,8-tetrahydroindolizin-8-one **8g** (0.002 mol) in ethanol (5 mL). Arylcarboxaldehyde (0.0024 mol) was then added to the reaction mixture wich was stirred at room temperature for 12 h. The precipitate was filtered, washed with water (2×20 mL) and ether (2×20 mL). A silica-gel column was used, with cyclohexane:ethyl acetate (70:30) as eluent, to give the *Z*-form of **12**–**18**, **21**, **25** or **26**.

General method B: to a solution of 3-aryl-2,3-dihydro-1*H*-pyrrolizin-1-ones **8a**–**e** or 5-vinyl-5,6,7,8-tetrahydroindolizin-8-one **8g** (0.002 mol) and arylcarboxaldehyde (0.0022 mol) in methylene chloride (20 mL) were added a solution of sodium hydroxide (6 N, 0.006 mol) and tetrabutylammonium hydrogen sulfate (catalytic amount). The reaction mixture was stirred at room temperature for 2 h. After addition of methylene chloride (40 mL), the organic layer was separated, washed with water, dried over calcium chloride and evaporated to dryness. A silica-gel column was used, with cyclohexane:ethyl acetate (70:30) as eluent, to give the *Z*-form of **19**, **20**, **22**–**24** or **27**.

(*Z*) 2-Benzylidene-3-phenyl-2,3-dihydro-1*H*-pyrrolizin-1-one 12. Yellow crystals (method A, 90%); mp 182 °C; IR (KBr) 1650 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.53 (s, 1H, H-vinyl), 7.49 (m, 2H, Ph), 7.3 (m, 8H, Ph), 7.14 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 2.1 Hz, H-5), 6.85 (s, 1H, H-3), 6.81 (d, 1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.7 Hz, H-7), 6.49 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.7 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.1 Hz, H-6); anal. $C_{20}H_{15}NO$ (C, H, N).

(*Z*) 2-Benzylidene-3-(2-chlorophenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one 13. Yellow crystals (method A, 85%); mp 128 °C; IR (KBr) 1690 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.62 (s, 1H, H-vinyl), 7.2 (m, 10H, Ph and H-5), 6.84 (s, 1H, H-3), 6.81 (d, 1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.9 Hz, H-7), 6.38 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.9 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.2 Hz, H-6); anal. $C_{20}H_{14}$ NOCl (C, H, N).

(*Z*) 2-Benzylidene-3-(thien-3-yl)-2,3-dihydro-1*H*-pyrrolizin-1-one 14. Yellow crystals (method A, 77%); mp 148 °C; IR (KBr) 1670 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.65 (s, 1H, H-vinyl), 7.50 (m, 3H, Ph), 7.40 (d, 1H, $J_{H\text{-}5'\ H\text{-}4'}$ = 4.8 Hz, H-5′), 7.31 (m, 3H, Ph et H-2′), 7.25 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 2.3 Hz, H-5), 6.95 (s, 1H, H-3), 6.90 (d, 1H, $J_{H\text{-}4'\ H\text{-}5'}$ = 4.8 Hz, H-4′), 6.80 (d, 1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.4 Hz, H-7), 6.50 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.4 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.3 Hz, H-6); anal. $C_{18}H_{13}NOS$ (C, H, N).

(*Z*) 2-(2,4-Dimethoxyphenyl)methylene-3-phenyl-2,3-dihydro-1*H*-pyrrolizin-1-one 15. Yellow crystals (method A, 87%); mp 131 °C; IR (KBr) 1650 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.80 (s, 1H, H-vinyl), 7.35 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'}$ = 8.8 Hz, H-6′), 7.2 (m, 5H, Ph), 7.08 (d, 1H, $J_{H\text{-}5\ H\text{-}6'}$ = 2.4 Hz, H-5), 6.7 (m, 2H, H-3 and H-7), 6.50 (d, 1H, $J_{H\text{-}3'\ H\text{-}5'}$ = 2 Hz, H-3′), 6.45 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.6 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.4 Hz, H-6), 6.38 (dd, 1H, $J_{H\text{-}5'\ H\text{-}6'}$ = 8.8 Hz, $J_{H\text{-}5'\ H\text{-}3'}$ = 2 Hz, H-5′), 3.82 (s, 3H, CH_3), 3.74 (s, 3H, CH_3); anal. $C_{22}H_{19}NO_3$ (C, H, N).

(*Z*) 3-(2-Chlorophenyl)-2-(2,4-dimethoxyphenyl)methylene-2,3-dihydro-1*H*-pyrrolizin-1-one 16. Yellow crystals (method A, 94%); mp 183 °C; IR (KBr) 1680 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.80 (s, 1H, H-vinyl), 7.50 (d, 1H, $J_{H\text{-}3''\ H\text{-}4''}$ = 7.7 Hz, H-3″), 7.3 (m, 4H, Ph and 5), 6.7 (m, 2H, H-7 and H-3), 6.71 (d, 1H, $J_{H\text{-}6''\ H\text{-}5''}$ = 7.7 Hz, H-6″), 6.45 (m, 2H, H-6 and H-3′), 6.35 (d, 1H, $J_{H\text{-}5'\ H\text{-}6'}$ = 8.8 Hz, 1H, H-5′), 3.82 (s, 3H, CH_3), 3.72 (s, 3H, CH_3); anal. $C_{22}H_{18}NO_3Cl$ (C, H, N).

(*Z*) 2-(2,4-Dimethoxyphenyl)methylene-3-(thien-3-yl)-2,3-dihydro-1*H*-pyrrolizin-1-one 17. Yellow crystals (method A, 44%); mp 112 °C; IR (KBr) 1670 (CO); ^{1}H NMR (DMSO-d_6) δ: 7.77 (s, 1H, H-vinyl), 7.56 (d, 1H, $J_{H\text{-}5'\ H\text{-}4'}$ = 4.8 Hz, H-5′), 7.4 (m, 2H, H-6″ and H-2′), 7.18 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 2.3 Hz, H-5), 6.84 (d, 1H, $J_{H\text{-}4'\ H\text{-}5'}$ = 4.8 Hz, H-4′), 6.80 (s, 1H, H-3), 6.72 (d, 1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.4 Hz, H-7), 6.53 (s, 1H, H-3″), 6.46 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.4 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.3 Hz, H-6), 6.33 (d, 1H, $J_{H\text{-}5''\ H\text{-}6''}$ = 8.5 Hz, H-5′), 3.85 (s, 3H, CH_3), 3.78 (s, 3H, CH_3); anal. $C_{20}H_{17}NO_3S$ (C, H, N).

(*Z*) 3-Phenyl-2-(pyridin-2-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 18. Yellow crystals (method A, 68%); mp 196 °C; IR (KBr) 1690 (CO); ^{1}H NMR (DMSO-d_6) δ: 8.55 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'}$ = 5 Hz, H-6′), 7.7 (m, 2H, H-4′ and H-3′), 7.46 (s, 1H, H-vinyl), 7.2 (m, 7H, Ph, H-5′ and H-5), 6.97 (d, 1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.9 Hz, H-7), 6.84 (dd, 1H, $J_{H\text{-}6\ H\text{-}7}$ = 3.9 Hz, $J_{H\text{-}6\ H\text{-}5}$ = 2.2 Hz, H-6), 6.50 (s, 1H, H-3); anal. $C_{19}H_{14}N_2O$ (C, H, N).

(*Z*) 3-(4-Cyanophenyl)-2-(pyridin-2-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 19. Yellow crystals (method B, 85%); mp 102 °C (ethanol/water); IR (KBr) 2230 (CN), 1695 (CO); ^{1}H NMR ($CDCl_3$) δ: 8.51 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'}$ = 5 Hz, H-6′), 7.65 (m, 1H, H-5′), 7.54 (s, 1H, H-vinyl), 7.52 (d, 2H, $J_{H\text{-}3''\ H\text{-}2''}$ = $J_{H\text{-}5''\ H\text{-}6''}$ = 8.5 Hz, H-3″ and H-5″), 7.43 (d, 2H, $J_{H\text{-}2''\ H\text{-}3''}$ = $J_{H\text{-}6''\ H\text{-}5''}$ = 8.5 Hz, H-2″ and H-6″), 7.41 (d, 1H, $J_{H\text{-}3'\ H\text{-}4'}$ = 5 Hz, H-3′), 7.16 (m, 1H, H-4′), 6.96 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 3.5 Hz, H-5), 6.93 (m, 1H, H-7), 6.86 (m, 1H, H-6), 6.53 (s, 1H, H-3); anal. $C_{19}H_{13}N_3O$ (C, H, N).

(*Z*) 3-(4-Trifluoromethoxyphenyl)-2-(pyridin-2-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 20. Yellow crystals (method B 70%); mp 152 °C (ether); IR (KBr) 1685 (CO), 1265, 1215, 1165 (CF_3); ^{1}H NMR ($CDCl_3$) δ: 8.54 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'}$ = 5 Hz, H-6′), 7.63 (m, 1H, H-5′), 7.52 (s, 1H, H-vinyl), 7.39 (d, 1H, $J_{H\text{-}3'\ H\text{-}4'}$ = 5 Hz, H-3′), 7.32 (d, 2H, $J_{H\text{-}3''\ H\text{-}2''}$ = $J_{H\text{-}5''\ H\text{-}6''}$ = 8.5 Hz, H-3″ and H-5″), 7.14 (m, 1H, H-4′), 7.04 (d, 2H, $J_{H\text{-}2''\ H\text{-}3''}$ = $J_{H\text{-}6''\ H\text{-}5''}$ = 8.5 Hz, H-2″ and H-6″), 6.95 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 3.5 Hz, H-5), 6.92 (m, 1H, H-7), 6.89 (m, 1H, H-6), 6.53 (s, 1H, H-3); anal. $C_{20}H_{13}N_2O_2F_3$ (C, H, N).

(*Z*) 3-Phenyl-2-(pyridin-3-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 21. Yellow crystals (method A, 45%); mp 166 °C; IR (KBr) 1700 (CO); ^{1}H NMR ($CDCl_3$) δ: 8.6 (s, 1H, H-2′), 8.44 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'}$ = 4 Hz, H-6′), 7.64 (s, 1H, H-vinyl), 7.54 (d, 1H, $J_{H\text{-}4'\ H\text{-}5}$ = 7.7 Hz, H-4′), 7.4 (m, 5H, Ph), 7.14 (dd, 1H, $J_{H\text{-}5'\ H\text{-}4'}$ = 7.7 Hz, $J_{H\text{-}5'\ H\text{-}6'}$ = 4 Hz, H-5′), 6.93 (d, 1H, $J_{H\text{-}7\ H\text{-}6}$ = 3.9 Hz, H-7), 6.90 (d, 1H, $J_{H\text{-}5\ H\text{-}6}$ = 2.2 Hz, H-5), 6.50 (dd, 1H,

$J_{H\text{-}6\ H\text{-}7} = 3.9$ Hz, $J_{H\text{-}6\ H\text{-}5} = 2.2$ Hz, H-6), 6.32 (s, 1H, H-3); anal. $C_{19}H_{14}N_2O$ (C, H, N).

(*Z*) 3-(4-Trifluoromethoxyphenyl)-2-(pyridin-3-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 22. Yellow crystals (method B, 65%); mp 136 °C (ether); IR (KBr) 1690 (CO), 1265, 1215, 1165 (CF_3); ^{1}H NMR ($CDCl_3$) δ: 8.61 (s, 1H, H-2′), 8.47 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'} = 4$ Hz, H-6′), 7.66 (s, 1H, H-vinyl), 7.51 (d, 1H, $J_{H\text{-}4'\ H\text{-}5'} = 8$ Hz, H-4′), 7.27 (d, 2H, $J_{H\text{-}3''\ H\text{-}2''} = J_{H\text{-}5''\ H\text{-}6''} = 8.5$ Hz, H-3″ and H-5″), 7.18 (m, 1H, H-5′), 7.11 (d, 2H, $J_{H\text{-}2''\ H\text{-}3''} = J_{H\text{-}6''\ H\text{-}5''} =$ 8.5 Hz, H-2″ and H-6″), 6.96 (d, 1H, $J_{H\text{-}5\ H\text{-}6} = 2.2$ Hz, H-5), 6.91 (m, 1H, H-7), 6.54 (m, 1H, H-6), 6.37 (s, 1H, H-3); anal. $C_{20}H_{13}N_2O_2F_3$ (C, H, N).

(*Z*) 3-Phenyl-2-(pyridin-4-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 23. Yellow crystals (method B, 33%); mp 148 °C; IR (KBr) 1685 (CO); ^{1}H NMR ($CDCl_3$) δ: 8.48 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'} = 4.9$ Hz, H-2′ and H-6′), 7.6 (m, 3H, H-3′, H-5′ and H-vinyl), 7.2 (m, 5H, Ph), 6.95 (d, 1H, $J_{H\text{-}7\ H\text{-}6} = 3.9$ Hz, H-7), 6.90 (d, 1H, $J_{H\text{-}5\ H\text{-}6} =$ 2.2 Hz, H-5), 6.31 (dd, 1H, $J_{H\text{-}6\ H\text{-}7} = 3.9$ Hz, $J_{H\text{-}6\ H\text{-}5} =$ 2.2 Hz, H-6), 6.16 (s, 1H, H-3); anal. $C_{19}H_{14}N_2O$ (C, H, N).

(*Z*) 3-(4-Trifluoromethoxyphenyl)-2-(pyridin-4-ylmethylene)-2,3-dihydro-1*H*-pyrrolizin-1-one 24. Yellow crystals (method B, 55%); mp 92 °C (ether/petroleum ether); IR (KBr) 1685 (CO), 1270, 1215, 1165 (CF_3); ^{1}H NMR ($CDCl_3$) δ: 8.52 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'} = 5$ Hz, H-2′ and H-6′), 7.62 (s, 1H, H-vinyl), 7.26 (d, 2H, $J_{H\text{-}3''\ H\text{-}2''} =$ $J_{H\text{-}5''\ H\text{-}6''} = 8.5$ Hz, H-3″ and H-5″), 7.15 (m, 4H, H-3′, H-5′, H-2″ and H-6), 6.98 (d, 1H, $J_{H\text{-}5\ H\text{-}6} =$ Hz, H-5), 6.91 (m, 1H, H-7), 6.56 (m, 1H, H-6), 6.33 (s, 1H, H-3); anal. $C_{20}H_{13}N_2O_2F_3$ (C, H, N).

(*Z*) 7-Benzylidene-5-vinyl-5,6,7,8-tetrahydroindolizin-8-one 25. Yellow crystals (method A, 35%); mp 86 °C; IR (KBr) 1680 (CO); ^{1}H NMR ($CDCl_3$) δ: 7.88 (s, 1H, H-vinyl), 7.3 (m, 5H, Ph), 7.18 (d, 1H, $J_{H\text{-}1\ H\text{-}2} = 3.6$ Hz, H-1), 6.88 (d, 1H, $J_{H\text{-}3\ H\text{-}2} = 2.4$ Hz, H-3), 6.32 (dd, 1H, $J_{H\text{-}2\ H\text{-}1} = 3.6$ Hz, $J_{H\text{-}2\ H\text{-}3} = 2.4$ Hz, H-2), 5.88 (m, 1H, H-vinyl), 5.25 (d, 1H, $J = 10$ Hz, H-vinyl), 5.03 (d, 1H, $J = 17$ Hz, H-vinyl), 4.70 (m, 1H, H-5), 3.32 (dd, 1H, $J_{H\text{-}6a\ H\text{-}6b} = 14.9$ Hz, $J_{H\text{-}6a\ H\text{-}5} = 4.4$ Hz, H-6a), 3.17 (dd, 1H, $J_{H\text{-}6b\ H\text{-}6a} = 14.9$ Hz, $J_{H\text{-}6b\ H\text{-}5} = 6.8$ Hz, H-6b); anal. $C_{17}H_{15}NO$ (C, H, N).

(*Z*) 7-(Pyridin-3-ylmethylene)-5-vinyl-5,6,7,8-tetrahydroindolizin-8-one 26. Yellow oil (method A, 28%); IR (KBr) 1670 (CO); ^{1}H NMR ($CDCl_3$) δ: 8.53 (s, 1H, H-2′), 8.45 (d, 1H, $J_{H\text{-}6'\ H\text{-}5'} = 4.8$ Hz, H-6′), 7.69 (s, 1H, H-vinyl), 7.58 (d, 1H, $J_{H\text{-}4'\ H\text{-}5'} = 7.9$ Hz, H-4′), 7.25 (dd, 1H, $J_{H\text{-}5'\ H\text{-}4'} = 7.9$ Hz, $J_{H\text{-}5'\ H\text{-}6'} = 4.8$ Hz, H-5′), 7.06 (d, 1H, $J_{H\text{-}1\ H\text{-}2} = 4$ Hz, H-1), 6.81 (d, 1H, $J_{H\text{-}3\ H\text{-}2} = 2.5$ Hz, H-3), 6.22 (dd, 1H, $J_{H\text{-}2\ H\text{-}1} = 4$ Hz, $J_{H\text{-}2\ H\text{-}3} = 2.5$ Hz, H-2), 5.78 (m, 1H, H-vinyl), 5.15 (d, 1H, $J = 10$ Hz, H-vinyl), 4.92 (d, 1H, $J = 17$ Hz, H-vinyl), 4.65 (m, 1H, H-5), 3.18 (dd, 1H, $J_{H\text{-}6a\ H\text{-}6b} = 14.9$ Hz, $J_{H\text{-}6a\ H\text{-}5} = 4.7$ Hz, H-6a), 3.05 (dd, 1H, $J_{H\text{-}6b\ H\text{-}6a} = 14.9$ Hz, $J_{H\text{-}6b\ H\text{-}5} =$ 6.3 Hz, H-6b); anal. $C_{16}H_{14}N_2O$ (C, H, N).

(*Z*) 7-(Pyridin-4-ylmethylene)-5-vinyl-5,6,7,8-tetrahydroindolizin-8-one 27. Yellow crystals (method B, 57%); mp 70 °C; IR (KBr) 1660 (CO); ^{1}H NMR ($CDCl_3$) δ: 8.60 (d, 2H, $J_{H\text{-}2'\ H\text{-}3'} = J_{H\text{-}6'\ H\text{-}5'} = 5.1$ Hz, H-2′ and H-6′), 7.70 (s, 1H, H-vinyl), 7.2 (m, 3H, H-3′, H-5′ and H-1), 6.85 (d, 1H, $J_{H\text{-}3\ H\text{-}2} = 2.5$ Hz, H-3), 6.29 (dd, 1H, $J_{H\text{-}2\ H\text{-}1} = 4$ Hz, $J_{H\text{-}2\ H\text{-}3} = 2.5$ Hz, H-2), 5.80 (m, 1H, H-vinyl), 5.22 (d, 1H, $J = 10$ Hz, H-vinyl), 4.97 (d, 1H, $J = 17$ Hz, H-vinyl), 4.68 (m, 1H, H-5), 3.20 (dd, 1H, $J_{H\text{-}6a\ H\text{-}6b} = 15$ Hz, $J_{H\text{-}6a\ H\text{-}5} = 4.4$ Hz, H-6a), 3.07 (dd, 1H, $J_{H\text{-}6b\ H\text{-}6a} = 15$ Hz, $J_{H\text{-}6b\ H\text{-}5} = 6.4$ Hz, H-6b); anal. $C_{16}H_{14}N_2O$ (C, H, N).

Pharmacology

Preparation of microsomes. Human placental and equine testicular microsomes were prepared as previously described.[13,28] Briefly, we have taken in each instance the available tissue presenting the highest specific aromatase activity. Fresh tissue washed with 0.50 M KCl was first homogenised in 50 mM phosphate buffer pH 7.5 containing 0.25 M sucrose, 1 mM DTT, and 4 μM androstenedione in order to preserve the enzyme active site, and then centrifuged at 20,000 *g*. The supernatant was further ultracentrifuged at 100,000 *g* and the final pellet was dissolved in the same buffer containing 20% glycerol, 1 mM DTT, 0.2 mM EDTA-4 Na, 4 μM androstenedione, and stored at −80 °C until use. Protein concentration was evaluated according to Bradford[29] using bovine serum albumin as standard, and Coomassie brilliant blue as dye-reagent.

Inhibition studies with microsomes. Aromatase activity was evaluated by measuring ^3H_2O released from 200 nM [1β,2β-^{3}H]androstenedione (specific activity: 1554 GBq/mmol) at 37 °C for 15 min according to Auvray et al.,[13] in the presence of various inhibitors from 0 to 15 μM. Reactions with microsomes (195 and 20 μg of human and equine microsomal proteins respectively for determination of IC_{50} values) were initiated by adding 60 μM NADPH, H^+ to a final volume of 0.5 mL and stopped by adding 1 mL of chloroform. Steroids were then extracted by incubation with a charcoal/dextran solution (7%/1.5%) and the radioactivity of the aqueous phase was measured as previously described.[28] Control incubations were realised by incubating microsomes, substrate and inhibitors without NADPH, H^+ in the same conditions. Results are the mean of triplicate experiments ± SD and are expressed as pmol estrogen formed/min·mg microsomal proteins.

Kinetic studies. Concentration ranges were 0–600 nM for inhibitors and 6–100 nM for substrate. Aromatase activity was evaluated at the linear portion of the Michaelis–Menten plot by incubating 5 μg (human) or 4 μg (equine) of microsomal proteins with substrate and various inhibitors at 37 °C for 12 min with human aromatase or 8 min with the equine enzyme. K_m and K_i values were determined graphically by using respectively Lineweaver–Burk and Dixon representations.

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