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Synthesis of new potent and selective aromatase inhibitors based on long-chained diarylalkylimidazole and diarylalkyltriazole molecule skeletons

Arto Karjalainen[a,*], Arja Kalapudas[a,1], Marja Södervall[a,1], Olavi Pelkonen[b], Risto Lammintausta[a,2]

[a]*Department of Synthetic Chemistry, Orion Corporation, Orion Pharma, R&D, P.O. Box 65, Espoo, Finland*
[b]*Department of Pharmacology and Toxicology, University of Oulu, PL 5000, FIN-90401 Oulu, Finland*

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Abstract

A series of long-chained diarylalkylimidazoles and diarylalkyltriazoles were synthesized and evaluated for the inhibitory potency for aromatase (estrogen synthetase) activity in human placental microsomes. The relative specificity of inhibition was evaluated by measuring the inhibition of cholesterol side-chain cleavage enzyme (desmolase) in human placental mitochondria and the inhibition of 7-ethoxycoumarin *O*-deethylase (a typical drug-metabolizing enzyme activity) in rat liver microsomes. The structural requirements including substituent effects for the strongest potency and for the highest specificity were delineated. α,ω-Diarylalkyltriazoles and imidazoles were the most interesting molecules, in which the geometric and optical isomerism displayed remarkable selectivity for aromatase inhibition.

Keywords: Aromatase (inhibitors); Breast cancer; Desmolase; Cholesterol side chain cleavage

1. Introduction

Aromatase inhibitors (Bhatnagar et al., 1990a; Vanden Bossche et al., 1994) are potentially useful in treating some estrogen-dependent cancers, such as breast cancer, because they inhibit the enzyme, aromatase, that catalyses the last step in the pathway to the formation of hormonally active estrogens (estradiol and others) from androgenic precursors (such as androstenedione or testosterone). Aromatase inhibition is the prerequisite for the therapeutic effect and according to the current opinion estrogen synthesis must be inhibited in all tissues capable of the synthesis (Goss and Gwyn, 1994).

Aromatase (CYP19) belongs to a group of cytochrome P450 (CYP) enzymes, named so because the terminal oxygen activator in the enzyme complex is the heme protein, cytochrome P450 (Simpson et al., 1994). The P450 enzymes are important catalysts in many other steroid hormone-synthesizing pathways. For example, glucocorticoids and mineralocorticoids in the adrenal glands are synthesized through pathways in which particular P450 enzymes participate as rate-limiting steps. Of particular importance in all steroidogenic tissues is cholesterol side-chain cleavage step catalyzed by P450 SCC or desmolase (CYP11A1), because cholesterol is a precursor for all steroid hormones (Miller, 1988). If desmolase is inhibited, it leads to a decrease of production of all steroid hormones, including gluco- and mineralocorticoids, with all consequent side-effects and problems. The probability of inhibition of different P450 enzymes by any particular inhibitor is rather high, because different P450 enzymes and especially their active sites show considerable homology; their structures resemble each other to a considerable extent. This relative similarity of all P450 enzymes is consequently the principal source of potential problems with aromatase inhibitors. A molecular model of aromatase based on structures of bacterial P450s has been published (Graham-Lorence et al., 1995), but its stability to predict affinities and turnover numbers of potential substrates and inhibitors has not been surveyed.

Perhaps the most important step in which aromatase inhibitors may cause inadvertent, not-sought-for inhibition, is cholesterol side-chain cleavage. Naturally, the goal is to synthesize inhibitors that display as much specificity as

*Corresponding author. Tel.: +358-9-4292-963; fax: +358-9-4293-600.

E-mail address: arto.karjalainen@orion.fi (A. Karjalainen)

[1]Present address: Hormos Medical Ltd., University of Oulu, Department of Chemistry, PL 3000, FIN-90401 Oulu, Finland.

[2]Present address: Hormos Medical Ltd., BioCity, Turku, FIN-20520 Turku, Finland.

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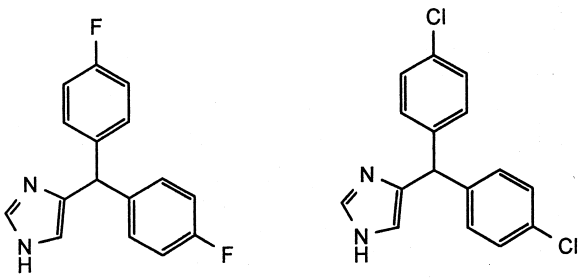


Fig. 1. Eli Lilly's substances displaying aromatase inhibition.

possible in terms of different P450 enzymes; i.e., selectivity is one of the most important goals in attempts to create new aromatase inhibitors. As an example, aminoglutethimide (Fig. 3) (Jones et al., 1990), the only widely used aromatase inhibitor thus far, displays equal inhibitory potencies towards aromatase and desmolase and actually produces harmful consequences based on the inhibition of all other steroid-synthesizing pathways (Santen et al., 1990). Our project for the development of potent and selective aromatase inhibitors started in 1986 after the observation that Eli Lilly (Hirsch and Taylor, 1984) had filed a patent application including 4,4′-difluoro- and 4,4′-dichloro-substituted 4(5)-(α,α-diphenylmethyl)-imidazoles covered by our patent (Karjalainen and Kurkela, 1981) (Fig. 1). Eli Lilly's research team had observed that these structures had yet another interesting pharmacological effect, in addition to the α-receptor effects we had observed with the derivatives: they were aromatase inhibitors. The intended indication for the use of these compounds was the treatment of breast cancer.

Another interesting and stimulating point was that previously we had also synthesized long-chained 4(5)-arylalkylimidazoles, for example 4(5)-[4-(2,6-dimethylphenyl)butyl]-1H-imidazole (**1**) (see Fig. 2) (Karjalainen and Kurkela, 1986), which we had found to have antimycotic and antibacterial properties. Because these substances and those of Eli Lilly (Fig. l) resembled structurally ordinary antimycotic imidazole derivatives, which inhibit P450 enzyme participating in ergosterol synthesis in yeast, we hypothesized that these long-chained compounds might inhibit aromatase as well.

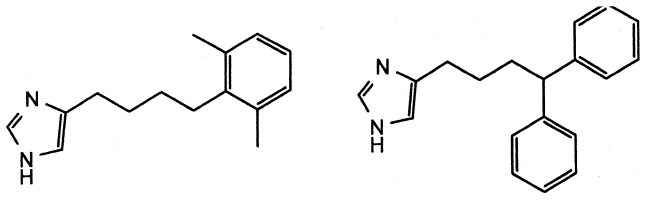


Fig. 2. Chemical structures of two early lead compounds.

The synthetic program was devised to be based on the structure–activity relationships, which encompassed the screening of our compounds by both aromatase and cholesterol side-chain cleavage assays and in the selected cases by 7-ethoxycoumarin *O*-deethylase assay representing a xenobiotic-metabolizing P450 enzyme.

The expectation that the long-chained 4(5)-arylalkylimidazoles might be aromatase inhibitors, proved correct. Compound (**1**) was a moderate inhibitor.

A new interesting lead was then provided by hybridizing the structural features of Eli Lilly's compounds and of compound (**1**). The diarylmethylene moiety connected into imidazole by a lengthened carbon chain led to rather potent and selective compounds (Karjalainen et al., 1992). For example, 4(5)-(4,4-diphenylbutyl)-1H-imidazole (**2**) (see Fig. 2) was a relatively potent aromatase inhibitor (IC_{50} 2 μM), but less potent in inhibiting desmolase (IC_{50} between 66 and 320 μM). We prepared skeletal modifications, in which the other aryl group was at various positions along the hydrocarbon bridge (Karjalainen et al., 1995a,b, 1996). In this study we found that in general these kind of structures had activity on aromatase inhibition and the substituents in the phenyl rings had influence on potency and selectivity. Also, the double bond in the carbon bridge of the α,ω-diarylalkyl derivatives brought additional beneficial effects especially in terms of selectivity.

An extensive screening program over these long-chained diarylalkyl substances was carried out later on with 2- and 1-substituted imidazoles and then logically with triazoles (Karjalainen et al., 1997), because the attachment of a short diarylmethylene group in the 1-position in imidazole and triazole had earlier been successful in producing potent and selective aromatase inhibitors as well. Examples of these are Ciba Geigy's CGS-18320B and Letrozole (CGS 20267) (Fig. 3) (Bhatnagar et al., 1990b).

Other known imidazole or triazole aromatase inhibitors are Ciba Geigy's Fadrozole (CGS 16949A) (Browne et al., 1991), Jansen's Vorozole (Vanden Bossche et al., 1994) and Zeneca's Anastrozole (Dukes et al., 1996) (Fig. 3).

It was found earlier, for example by Ciba-Geigy and by Zeneca, that the substituents such as F or CN were favourable as for the activity. Substitution of our long-chained diarylalkylimidazoles and diarylalkyltriazoles with the fluoro and cyano substituents gave similar results. One of the most interesting members of these series was the diastereomeric triazole 4-[3-(4-fluorophenyl)-2-hydroxy-1-[1,2,4]triazol-1-ylpropyl]benzonitrile, finrozole (MPV-2213 ad) (**3**) (see Fig. 4), in which the optical isomerism achieved by introducing a hydroxyl group into the alkyl chain brought conspicuous additional effect on selectivity.

In this report we give a summary of the syntheses and the results of the structure–activity screening of these long-chained 4(5)-diarylalkyl imidazoles (I and II in Fig. 4) and 1-diarylalkylimidazoles and triazoles (III in Fig. 4) leading to new groups of selective aromatase inhibitors, of which the most interesting representatives are the com-

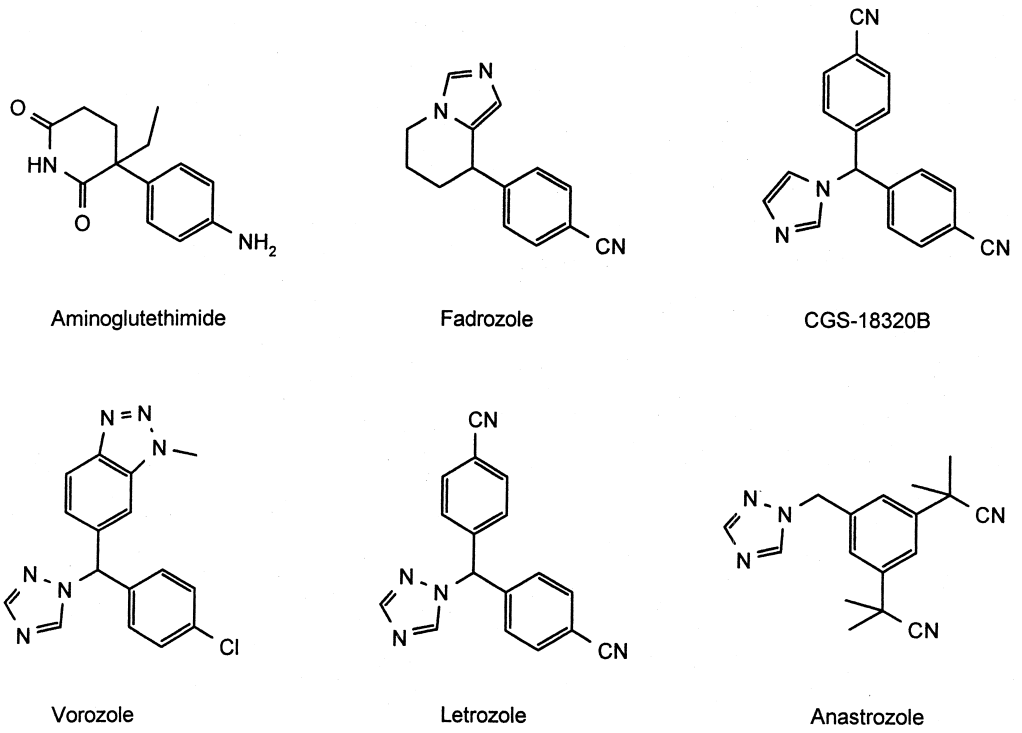


Fig. 3. Chemical structures of aminoglutethimide and known imidazole or triazole derivatives of aromatase inhibitors.

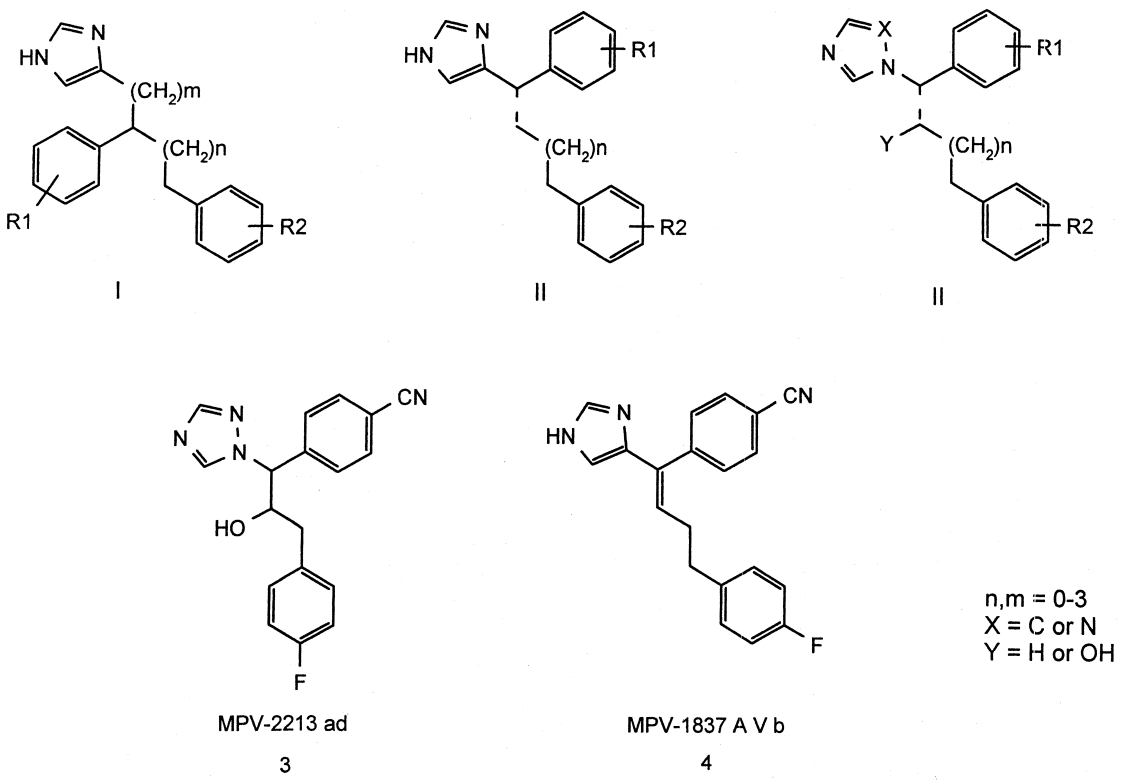


Fig. 4. Chemical structures of three main series of synthesised imidazole and triazole compounds and those of MPV-2213 ad and MPV-1837 A V b, which demonstrate a high degree of potency and selectivity with respect to aromatase inhibition.

pounds MPV-2213 ad (**3**) and 4-[4-(4-fluorophenyl)-1-(1H-imidazol-4-yl)-but-1-enyl]benzonitrile (MPV-1837 A V b) (**4**) (Fig. 4).

2. Chemistry

2.1. Syntheses

2.1.1. Synthesis of 4(5)-substituted imidazoles

In the course of our earlier projects, in which we developed new selective α_2-agonists (e.g., detomidine, medetomidine and dexmedetomidine) (Karjalainen, 1981; Karjalainen et al., 1982, 1989; Karjalainen and Kurkela, 1985) we synthesized a lot of variously substituted 4(5)-arylalkyl-imidazoles. In that connection we developed a new useful method to produce the 4(5)-substituted aryl alkyl imidazole skeleton starting from 4(5)-imidazole carbaldehyde by allowing it to react in the Grignard reaction with an arylmagnesiumbromide or with an arylalkylmagnesiumbromide in tetrahydrofuran at elevated temperature (Karjalainen, 1981). Because of the difficulties encountered in producing 4(5)-imidazole carbaldehyde in large quantities it was found more favourable to use the protected imidazole, 1-benzyl-5-imidazole carbaldehyde as the starting reagent (Parhi, 1985).

Also in this study 1-benzyl-5-imidazole carbaldehyde was a useful starting material in the synthesis of 4(5)-substituted diarylalkylimidazoles by various methods (Karjalainen et al., 1992, 1995a,b, 1996, 1997). In the following chapters we present the main lines of the used methodology. Our referred patents give detailed description of the syntheses with the analytical data and in the experimental part of this report we have included only some typical examples from these.

2.1.1.1. Synthesis of 4(5)-(ω,ω-diarylalkyl and -alkenyl)-1H-imidazoles

Scheme A presents the basic method, which we have used to produce of the 4(5)-(ω,ω-diarylalkyl)-1H-imidazole skeleton.

Here 1-benzyl-5-imidazole carbaldehyde reacted with diarylalkylmagnesium halides to afford the structures A I. The end products A III and A V were prepared from A I by a successive sequence of reactions comprising dehydration and catalytic hydrogenation. When synthesizing the unsaturated compounds A III, the *N*-benzyl group was eliminated by catalytic hydrogenation or by hydrogen transfer reaction in ethanol before the dehydration to give the intermediates A II. Alternatively the synthesis of A V directly from A I could be achieved by hydrogen transfer reaction with ammonium formate and Pd/C by heating in acetic acid (Ram and Spicer, 1987).

Diarylalkylhalides were either commercially available or they were prepared according to the general methods. The synthesis of the starting materials, used especially for the preparation of 4(5)-(ω,ω-diarylalkyl)-1H-imidazoles with a four-carbon chain bridge, was carried out as depicted in Scheme B.

In this method a benzophenone, by applying the Reformatsky reaction, was allowed to react with ethyl bromoacetate to yield the hydroxyl ester B I. The ethyl ester B II could be achieved from this by dehydration and hydrogenation. The reduction of the ester group to alcohol and the replacement of hydroxyl with bromine produced 3,3-diarylpropylbromide B III.

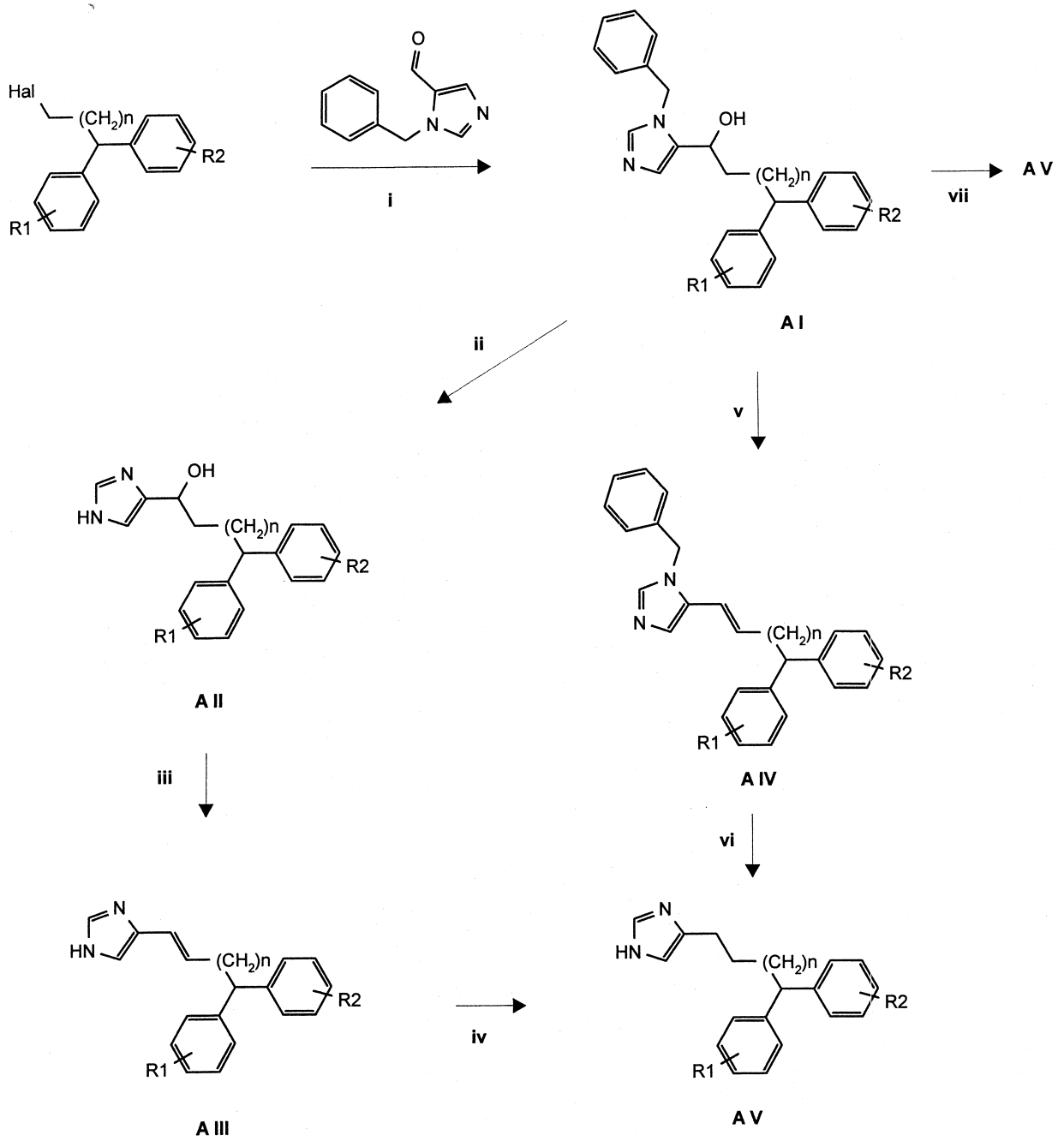
The 4(5)-(ω,ω-diarylalkyl)-1H-imidazoles, in which the substituents in the aromatic rings are the same, were synthesized easily according to the Scheme C. 1-Benzyl-5-imidazole carbaldehyde was allowed firstly to react with malonic acid in pyridine (Doebner modification of the Knoevenagel reaction) to 5-(1-benzylimidazole)-acrylic acid C I. The *N*-benzyl group of imidazole was removed and the double bond in the alkyl chain was reduced simultaneously by catalytic hydrogenation in acidic conditions and the propionic acid intermediate was esterified to achieve 4(5)-imidazole propionic acid ethyl ester C II. Alternatively the hydrogenation of C I was carried out in neutral conditions to preserve the protection and thus to produce 1-benzyl-5-imidazole propionic acid ethyl ester C V. The compounds C II or C V reacted further with aryl magnesium bromide to afford (3,3-diaryl-3-hydroxy-propyl)-imidazoles C III or C VI, respectively. The end products C VIII could be achieved by a sequence of reactions involving dehydration, debenzylation by hydrogenation over palladium as well as reduction of the double bond as described previously.

To produce symmetrically substituted diaryl derivatives with a 4-alkyl carbon chain bridge, the side chain extension with an appropriate carboxylic functionality in the end of the chain was needed. This was achieved through a series of conventional reactions as outlined in Scheme D.

These reactions comprised the reduction of the ester group of the intermediate C V to the alcohol with lithium aluminum hydride followed by halogenation. The substitution of the halogen was then accomplished through the reaction with sodium cyanide and the carboxylic ester functionality was introduced through the hydrolysis followed by esterification.

A special method used to prepare a group of differentially substituted 4(5)-3,3-diarylpropyl imidazoles is outlined in Scheme E.

According to this procedure the aldol condensation of 1-benzyl-5-imidazole carbaldehyde with substituted acetophenone gave the unsaturated ketone E I. The double bond was hydrogenated selectively under neutral conditions at room temperature to produce the ketone E II. The diarylalkylimidazole E III was obtained by the reaction of the ketone E II with an aryl magnesium bromide. 4(5)-3,3-Diarylpropylimidazole E IV could be prepared as described in the latter part of Scheme C.

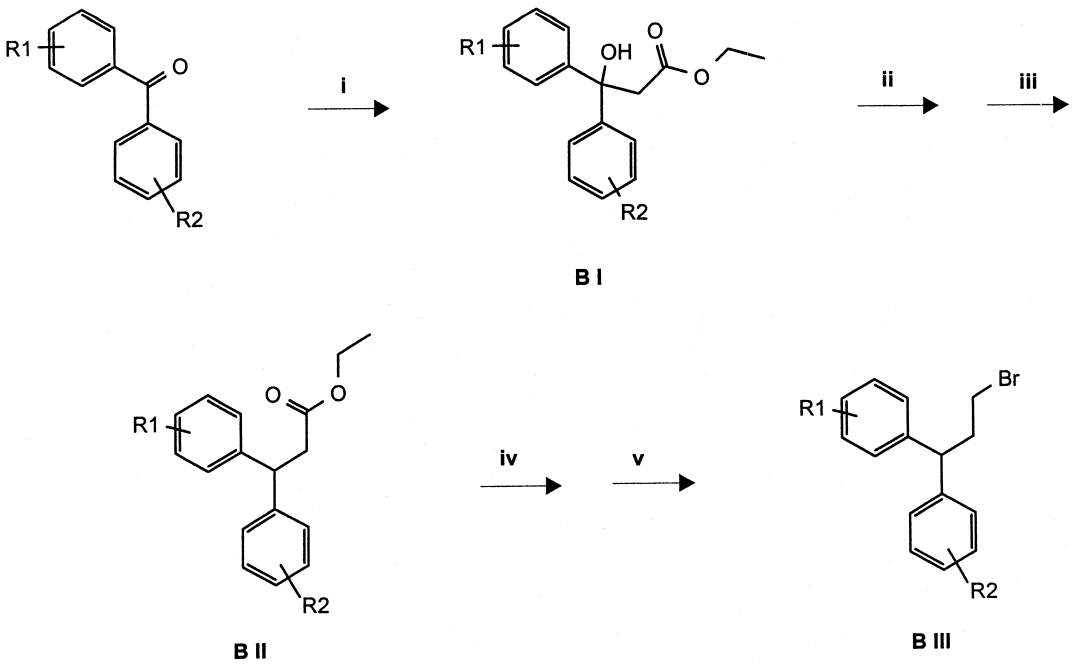


i: Mg, THF; ii: $HCOONH_4$, Pd/C, ethanol, refl. or H_2, Pd/C, ethyl acetate, RT; iii: K_2SO_4 or conc. HCl; iv: H_2, Pd/C, ethanol; v: H^+; vi: H_2, Pd/C, H^+, ethanol; vii: $HCOONH_4$, Pd/C, CH_3COOH, refl.

Scheme A.

2.1.1.2. Preparation of 4(5)-(α,ω-diarylalkyl- and alkenyl)-1H-imidazoles

The 4(5)-(α,ω-diarylalkyl- and -alkenyl)-1H-imidazoles were synthesized according to the general routes F–G. According to route F the reaction of 1-benzyl-5-imidazole carbaldehyde with appropriate arylalkylmagnesium bromide gave the alcohol F I. This was followed by the oxidation with manganese dioxide. The resulting ketone F II was converted to the α,ω-diarylalkylimidazole F III in the Grignard reaction followed by deprotection, dehydration and hydrogenation affording the end product F V. The hydrogenation of the alcohol F III with ammonium formate and palladium on carbon as catalyst gave a convenient straight entry to the end product F V. The geometric



Scheme B.

isomers of F IV were separated and the assignment of *Z* and *E* configurations were made by means of 1D NOE difference and NOESY NMR spectroscopic techniques (Kalapudas et al., 1993) (Scheme F).

As an alternative approach for the preparation of the intermediate F III (Scheme G), imidazolyl aryl ketone was allowed to react with arylalkylmagnesium bromide.

Yet another method for the preparation of 4(5)-α,ω-diarylalkenylimidazoles and α,ω-diarylalkylimidazoles utilized the McMurry reaction (McMurry, 1989), in which the reductive carbonyl coupling of the imidazolyl aryl ketone with an appropriate aldehyde in the presence of titanium(0) resulted directly in the formation of 4(5)-α,ω-diarylalkenylimidazole as outlined in Scheme H. 4(5)-(β,ω-Diarylalkenyl- and -alkyl)-1H-imidazoles could be prepared by the same method starting from 1-benzyl-5-imidazole carbaldehyde and an appropriate diaryl ketone.

2.1.1.3. Preparation of para-cyanoderivatives

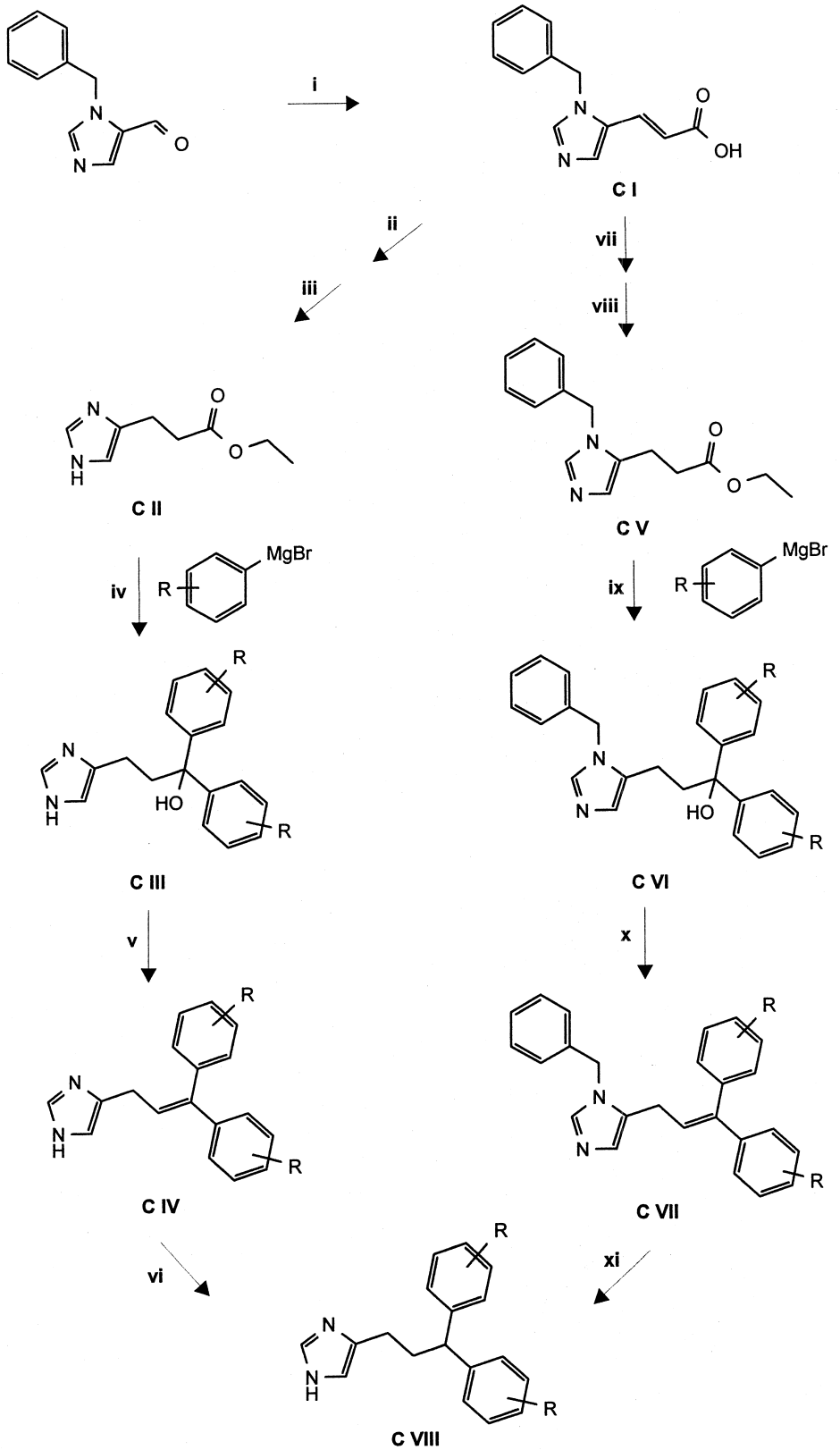
The methods used in the synthesis of 4(5)-(diarylalkyl- and -alkenyl)imidazoles include reaction conditions in which the cyano substituent is unstable. That is why the strategy for the preparation of the cyano-substituted end products was based on the methodology to produce the cyano group from an appropriate precursor group in a late stage of the reaction pathway. *t*-Butyl amino carbonyl, which is rather stable towards organometal reagents and hydrogenation was used as such a precursor. The *t*-butyl amino carbonyl moiety could be converted to the cyano group by treatment with thionyl chloride, phosphorous trichloride or phosphorous pentachloride.

2.1.2. Preparation of N-substituted (α,ω-diarylalkyl- and -alkenyl)imidazoles and triazoles

Saturated *N*-substituted α,ω-diarylalkylimidazoles and triazoles J II were prepared according to Scheme J (Karjalainen et al., 1997). In this procedure imidazole or triazole underwent a direct *N*-alkylation with an appropriately substituted benzyl halide to give J I. Treatment of compound J I with *n*-butyl lithium followed by alkylation with an appropriate aryl alkyl halide gave the end product J II.

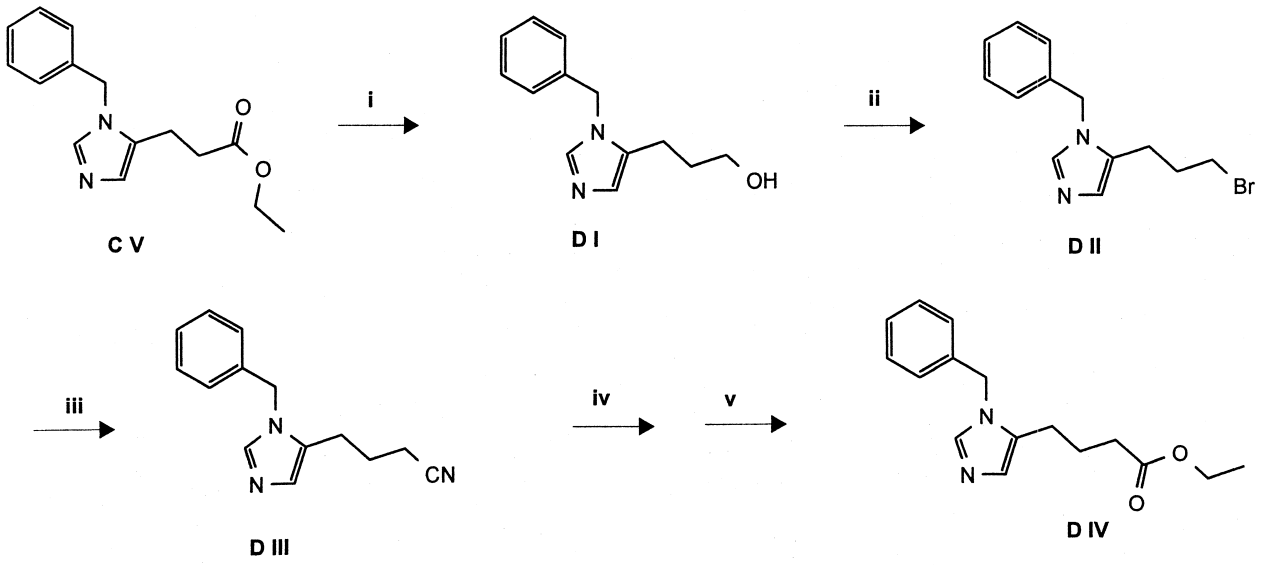
An alternative method used to synthesize the compound J II was the direct *N*-alkylation of imidazole or triazole with diarylalkyl halide. *N*-Substituted ω,ω-diarylalkyl imidazoles and triazoles could also be prepared with this method starting from appropriate diarylalkyl halides.

1-Substituted α,ω-diaryl-β-hydroxyalkyltriazoles and imidazoles K II and unsaturated *N*-α,ω-diaryltriazoles and imidazoles K III were synthesized starting from *N*-benzyl triazoles and imidazoles, which first were treated with *n*-butyl lithium to produce the carbanion of the benzylic carbon. Then the reaction with appropriately substituted arylalkyl aldehydes afforded KII and the following elimi-



i: malonic acid, pyridine, 100 °C; ii: H_2, Pd/C, 4N HCl, RT; iii: abs. ethanol, HCl-gas; iv: THF; v: K_2SO_4, 150-155 °C or conc. HCl, ethanol, refl.; vi: H_2, Pd/C, RT; vii: H2, Pd/C, ethanol, RT; viii; abs. ethanol, HCl-gas, refl.; ix: THF; x: conc. HCl, ethanol, refl.; xi: 2N HCl, ethanol, H_2, Pd/C, 80 °C

Scheme C.



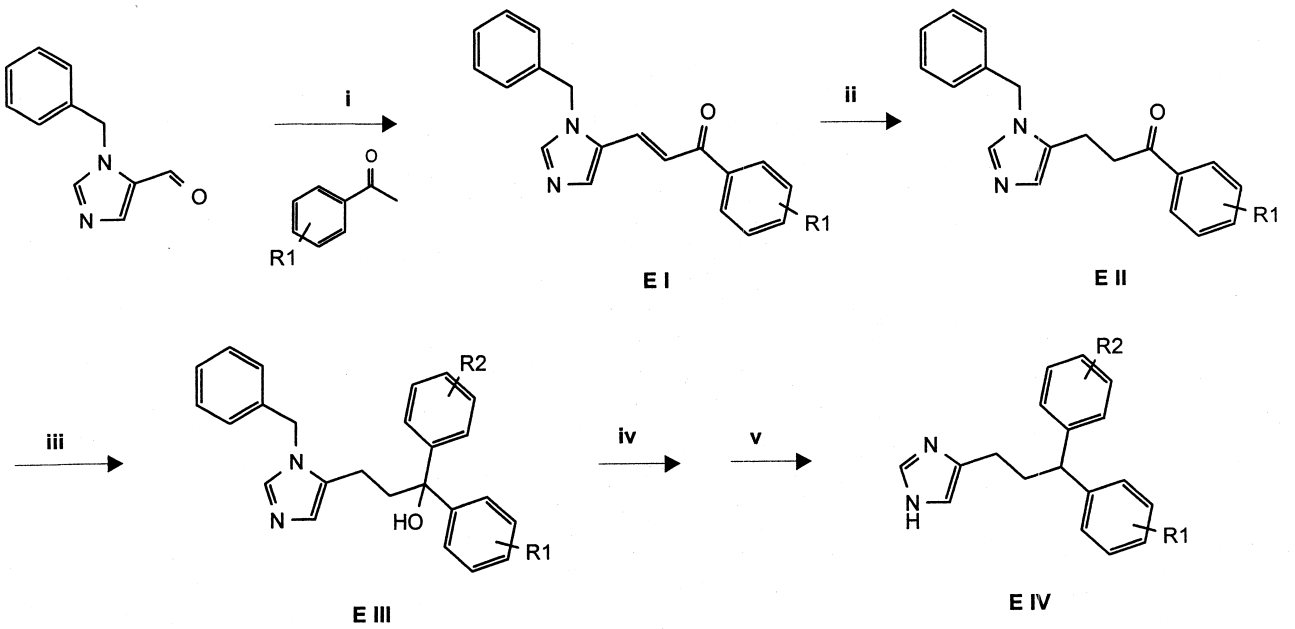
i: $LiAlH_4$, THF; ii: HBr; iii: NaCN; iv: NaOH; v: H^+, ethanol

Scheme D.

nation of water by the treatment with acid yielded K III. Alternatively the nucleophilic addition of the carbanion to an appropriately substituted arylalkyl esters gave K I, which was then reduced with sodiumborohydride to K II (Scheme K).

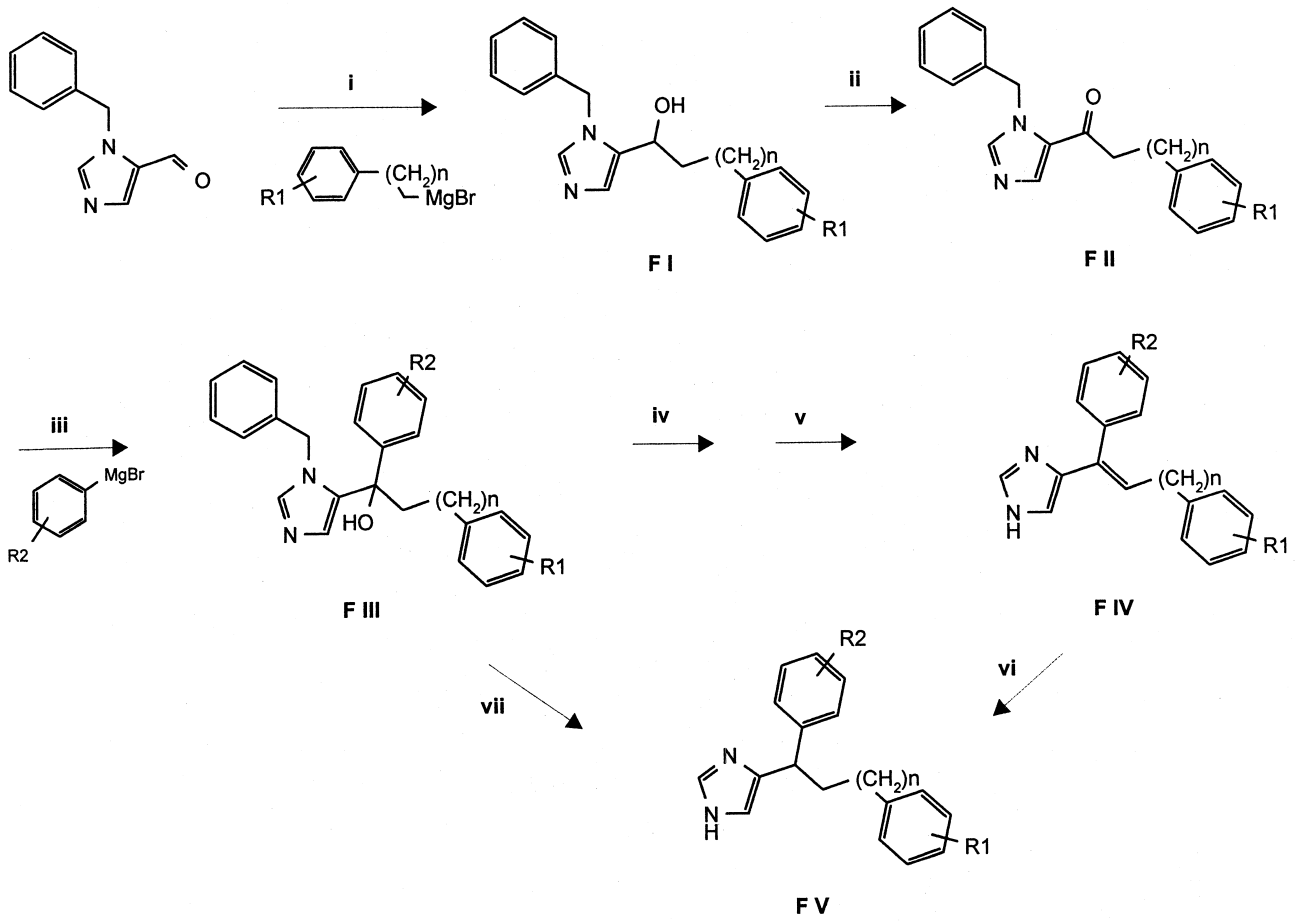
Unsaturated imidazole derivatives L II were prepared also by the treatment of a ketone L I with thionyl bisimidazole (Scheme L) (Ogata et al., 1987).

1-(1,3-Diaryl-3-hydroxypropyl)triazoles M III were prepared by heating 1,3-diarylpropenones M I and triazole in the presence of Triton B followed by reduction with sodiumborohydride (Scheme M).



i: OH^-, EtOH or MeOH; ii: H_2, Pd/C, ethanol; iii: $R_2PhMgBr$, THF; iv: HCl, ethanol; v: H_2, Pd/C, H^+, ethanol

Scheme E.



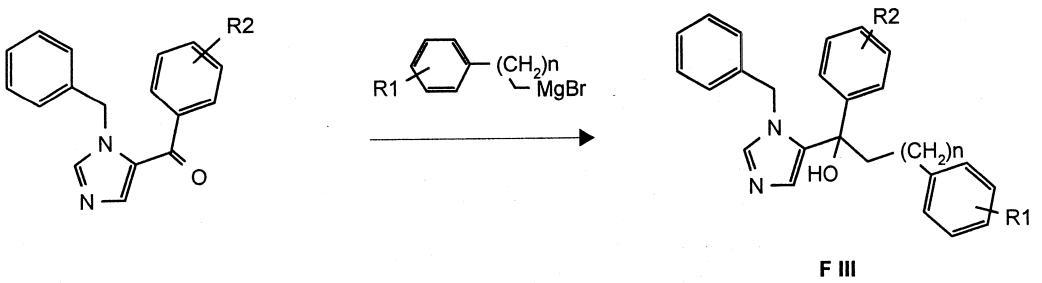
i: THF; ii: MnO_2, C_2Cl_4; iii: THF; iv: H_2, Pd/C, H^+, ethanol, RT; v: HCl, ethanol; vi: Pd/C, ethanol, RT; vii: $HCOONH_4$, Pd/C, CH_3COOH, refl.

Scheme F.

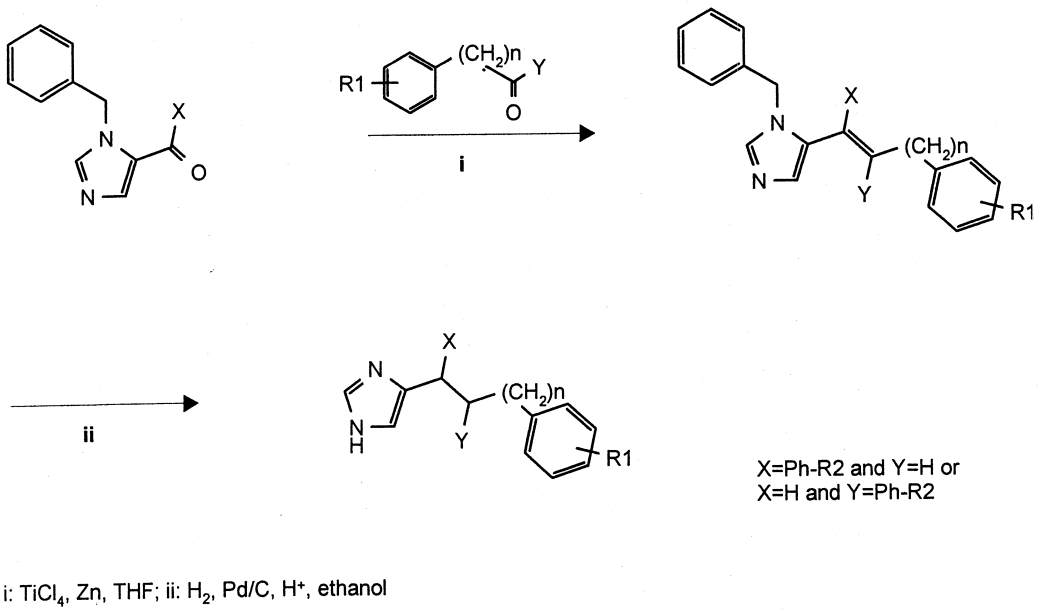
3. Experimental section

3.1. Chemistry

Melting points were determined using a Buchi 510 glass capillary melting point apparatus. Melting and boiling points are uncorrected. NMR spectra were recorded with a Bruker AC-P300 spectrometer using TMS as the internal reference in the indicated solvent at an ambient temperature. Chemical shifts were measured downfield from TMS. MS spectral data were obtained on a Kratos MS 80 RF Autoconsole instrument. The spectra were considered consistent with the assigned structures. The estimation of the isomeric purity was based on the ^1H NMR spectra. The separation of enantiomers and measurement of optical purity was done by chiral HPLC using Hewlett-Packard



Scheme G.



i: $TiCl_4$, Zn, THF; ii: H_2, Pd/C, H^+, ethanol

Scheme H.

HP 1090 apparatus. The silic acid used in the flash chromatography was silica gel 60, 230–400 mesh.

3.1.1. General methods for the preparation of diarylalkylimidazole and diarylalkyltriazole molecular skeleton

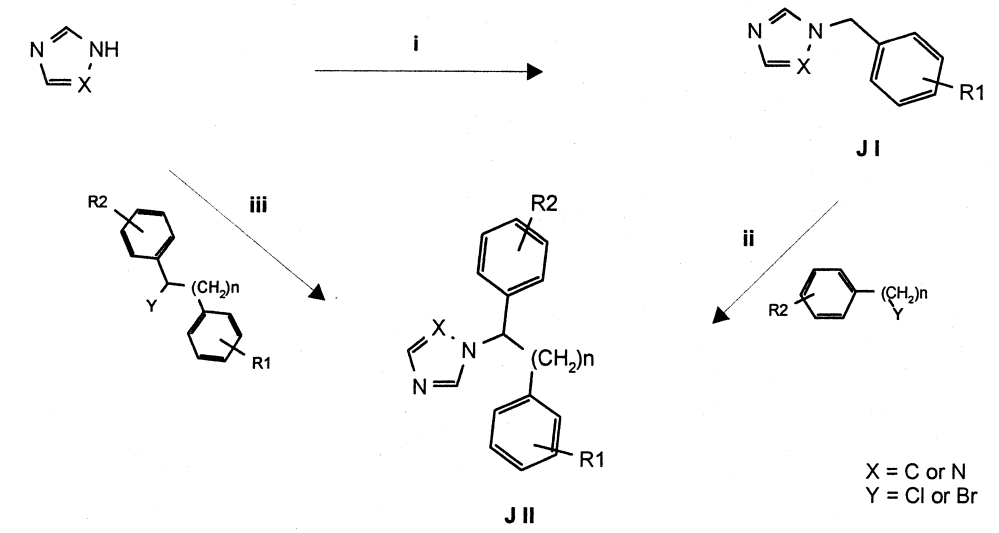
The detailed syntheses and the analytical parameters of the compounds of this study are presented in our patent publications, which are referred in the text above and in the following just a few selected examples of these are given to illustrate the principal methods used to prepare these structures as outlined in Schemes A–M.

3.2. Scheme A

3.2.1. 4-(4,4-Diphenylbutyl)-1H-imidazole ***(2)***

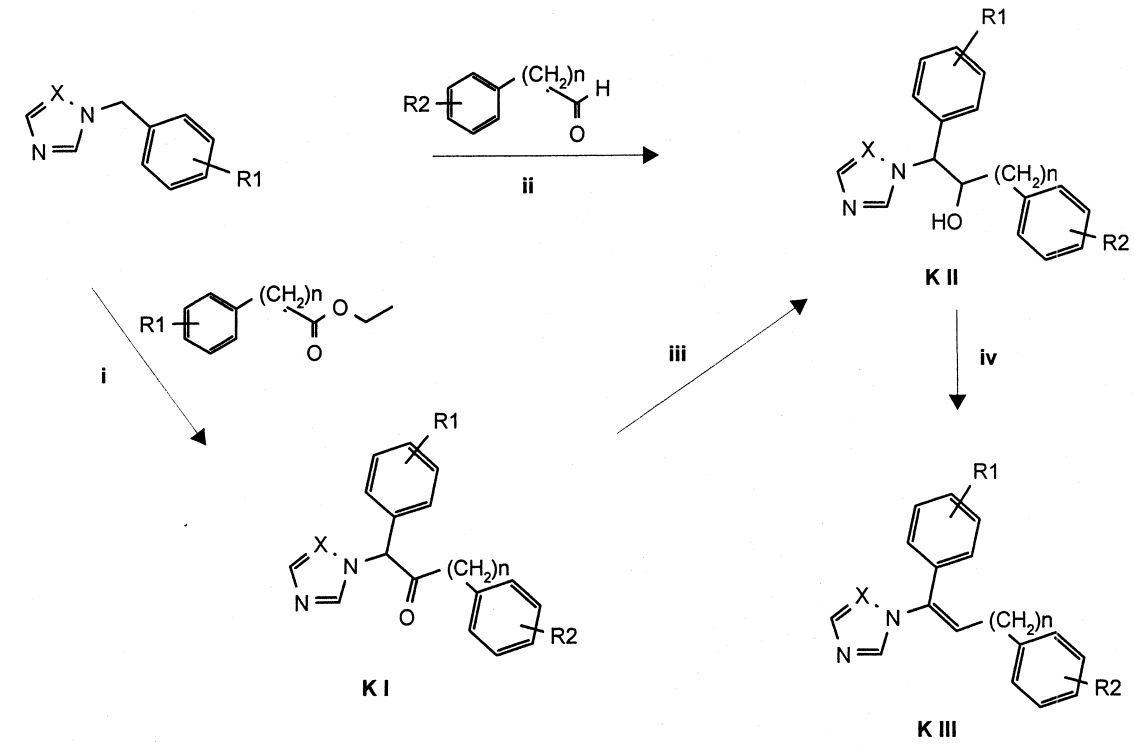
3.2.1.1. 1-Benzyl-5-(1-hydroxy-4,4-diphenylbutyl)-1H-imidazole

Two grams of magnesium turnings are covered with 60 ml of dry tetrahydrofuran. To the mixture is then added dropwise a solution of 1-bromo-3,3-diphenylpropane (22.9 g) in 20 ml of dry tetrahydrofuran at such a rate that a smooth reaction is maintained. After the addition is complete, the reaction mixture is refluxed for one addition-



i: R1-Ph-CH_2Br; ii: n-BuLi, THF; iii: NaH, DMF

Scheme J.



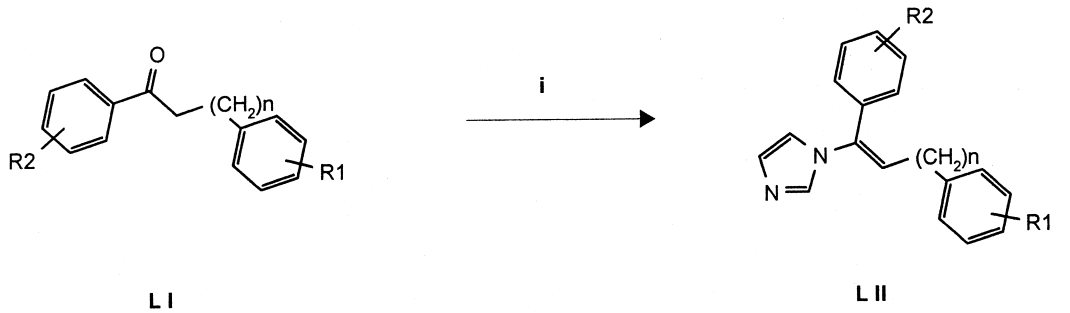
i: n-BuLi, THF, ii: n-BuLi, THF, iii: $NaBH_4$; iv: H^+, ethanol

Scheme K.

al hour and cooled to room temperature. The reaction mixture is then added dropwise to a solution of 1-benzyl-5-imidazolecarbaldehyde (7.35 g) in 80 ml of tetrahydrofuran at 60°C. After the addition is complete, the reaction mixture is refluxed for 2 h, cooled and poured into cold water. Tetrahydrofuran is evaporated and to the solution is added conc. hydrochloric acid. The solution is cooled and the precipitate which contains the product as hydrochloride salt is removed by filtration, washed with water and dried. Yield 14.1 g. M.p. 160–168°C: ^{1}H NMR (as HCl-salt, MeOH-d4) δ 1.50–2.30 (m, 4H), 3.82 (t, 1H), 4.65 (t, 1H), 5.43 (s, 2H), 7 05–7.50 (m, 16H), 8.62 (d, 1H).

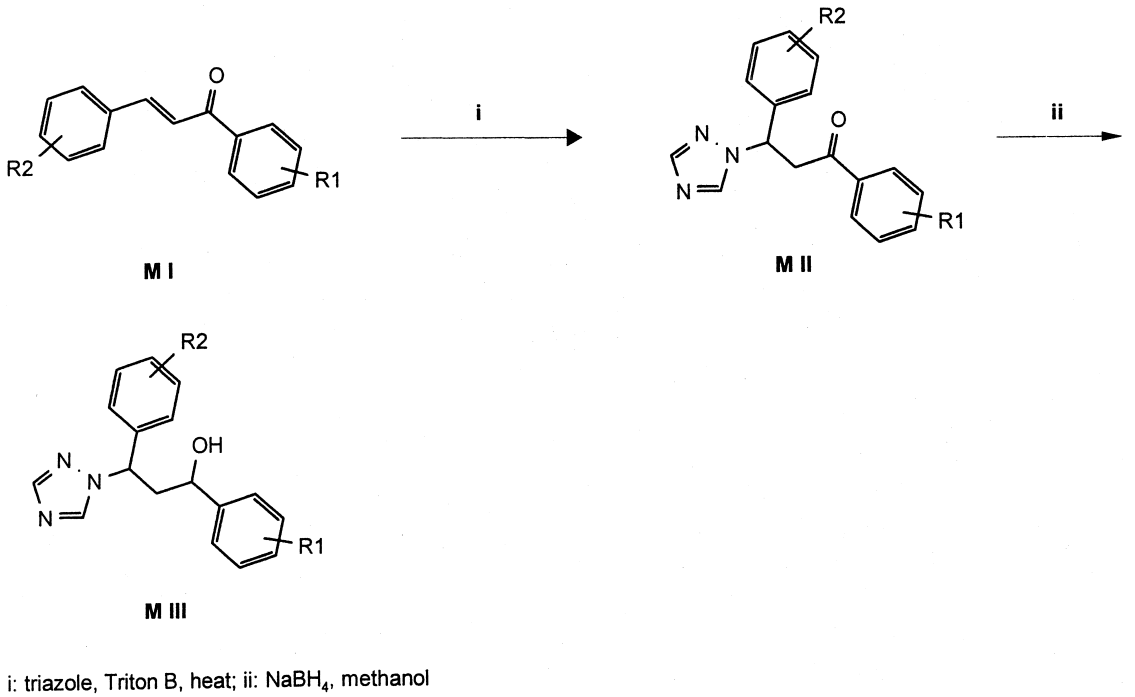
3.2.1.2. 1-Benzyl-5-(4,4-diphenyl-1-butenyl)-1H-imidazole

1-Benzyl-5-(1-hydroxy-4,4-diphenylbutyl)-1H-imidazole hydrochloride (5.0 g) and 30.0 g of anhydrous potassium hydrogen sulphate are heated at 150°C for 4 h. The mixture is cooled, 90 ml of ethanol is added to dissolve the product. The mixture is then filtered and the filtrate is evaporated to minor volume. Water is added and the mixture is made alkaline with sodium hydroxide. The product is extracted in methylene chloride, washed with water and evaporated to dryness. The product is then made to hydrochloride salt with dry hydrochloric acid in dry



i: imidazole, $SOCl_2$, THF

Scheme L.



Scheme M.

ethylacetate. Yield is 2.9 g. M.p. 204–206°C: ^{1}H NMR (as HCl-salt, MeOH-d_4) δ 2.88–3.05 (m, 2H), 4.08 (t, 1H), 5.29 (s, 2H), 6.22–6.32 (m, 2H), 7.00–7.50 (m, 16H), 8.87 (d, 1H).

3.2.1.3. 1-Benzyl-5-(4,4-diphenylbutyl)-1H-imidazole

1-Benzyl-5-(4,4-diphenyl-1-butenyl)-1H-imidazole hydrochloride (2.0 g) is dissolved in ethanol and a catalytic amount of Pd/C (10%) is added. The reaction mixture is agitated vigorously at room temperature in a hydrogen atmosphere until the uptake of hydrogen ceases. The mixture is filtered and the filtrate is evaporated to dryness. The residue which is the product is purified by flash chromatography eluting with the mixture of methylene chloride–methanol. Yield 1.3 g. M.p. of the hydrochloride salt is 200–202°C: ^{1}H NMR (as HCl-salt, MeOH-d_4) δ 1.30–1.70 (m, 2H), 1.85–2.20 (m, 2H), 2.61 (t, 2H), 3.83 (t, 1H), 5.35 (s, 2H), 7.05–7.50 (m, 16H), 8.89 (d, 1H).

3.2.1.4. 4-(4,4-Diphenylbutyl)-1H-imidazole

1-Benzyl-5-(4,4-diphenylbutyl)-1H-imidazole hydrochloride (0.6 g) is hydrogenated in the mixture of 20 ml of 2 N hydrochloric acid and 10 ml of ethanol at 80°C Pd/C (10%) as catalyst. When the uptake of the hydrogen ceases, the reaction mixture is cooled, filtered and evaporated to dryness. Water is added and the mixture is made alkaline with sodium hydroxide. The product is then extracted to methylene chloride which is washed with water, dried with sodium sulphate and evaporated to dryness. The residue is the product as base and it is made to its hydrochloride in ethyl acetate using dry hydrogen chloride. Yield 0.2 g. M.p. 204–206°C: ^{1}H NMR (as HCl-salt, MeOH-d_4) δ 1.40–1.90 (m, 2H), 1.90–2.30 (m, 2H), 2.75 (t, 2H), 3.95 (t, 1H), 7.00–7.40 (m, 11H), 8.72 (d, 1H); HRMS Calcd for $C_{19}H_{20}N_2$: (M+) 276.1622. Found: (M+) 276.1626.

3.3. Scheme F

*3.3.1. 4-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)-1-butenyl]-1H-imidazole, isomers a **(77)** and b **(4)***

3.3.1.1. 1-Benzyl-5-[4-(4-fluorophenyl)-1-hydroxybutyl]-1H-imidazole

Magnesium turnings (5.4 g) are covered with 50 ml of dry tetrahydrofuran. A solution of 1-bromo-3-(4-fluorophenyl)propane (48.7 g) in 200 ml of dry tetrahydrofuran is then added dropwise to the mixture at such a rate that a smooth reaction is maintained. After the addition is complete, the reaction mixture is refluxed for one additional hour and cooled to room temperature. To the reaction mixture is then added dropwise the solution of 1-benzyl-5-imidazolecarbaldehyde (20.9 g) in 200 ml of tetrahydrofuran. After the addition is complete, the reaction mixture is refluxed for 2 h, cooled and poured into cold saturated ammonium chloride solution. Tetrahydrofuran phase is removed and the water phase is extracted three times with ethyl acetate. Organic phases are combined, dried and evaporated to dryness. The residue is suspended with diethyl ether and filtered. Yield 86%; ^{1}H NMR (as base, $CDCl_3$) δ 1.5–1.75 (m, 2H), 1.76–1.84 (m, 2H), 2.53 (t,

2H), 4.49 (t, 1H), 5.20 and 5.26 (AB q, 2H), 6.91–7.1 (m, 7H), 7.30–7.35 (m, 3H), 7.48 (s, 1H).

3.3.1.2. 1-Benzyl-5-[4-(4-fluorophenyl)-1-oxobutyl]-1H-imidazole

The mixture of 36.1 g of 1-benzyl-5-(4-(4-fluorophenyl)-1-hydroxybutyl]-1H-imidazole and 53 g manganese dioxide in 550 ml of tetrachloroethylene is refluxed stirring for 4 h. The reaction mixture is filtered through siliceous earth and the filtrate is evaporated to dryness. The product is crystallized from acetone as hydrochloride salt: ^{1}H NMR (as base, $CDCl_3$) δ 1.9–2.0 (m, 2H), 2.58 (t, 2H), 2.76 (t, 2H), 5.52 (s, 2H), 6.9–7.32 (m, 9H), 7.63 (s, 1H), 7.75 (s, 1H).

3.3.1.3. 1-Benzyl-5-[4-(4-fluorophenyl)-1-hydroxy-1-(4-tert-butylaminocarbonylphenyl)butyl]-1H-imidazole

p-tert-Butylaminocarbonylphenyl bromide (9.9 g) is dissolved in 200 ml of dry tetrahydrofuran and cooled to −70°C. To the solution is added dropwise *n*-butyl lithium (5.3 g) in hexane and the mixture is stirred for 1 h. 1-Benzyl-5-[4-(4-fluorophenyl)-1-oxobutyl]-1H-imidazole (10.4 g) in 200 ml of tetrahydrofuran is added to the mixture at 70°C and the mixture is allowed to warm to room temperature and stirring is continued overnight. Saturated ammonium chloride is added to the mixture and the solution is extracted with ethyl acetate. Ethyl acetate fractions are combined and evaporated to dryness: ^{1}H NMR (as base, $CDCl_3$+MeOH-d_4) δ 1.15–1.35 (m, 1H), 1.47 (s, 9H), 1.6–1.8 (m, 1H), 2.15–2.25 (m, 2H), 2.52 (t, 2H), 4.74 and 4.80 (AB q, 2H), 6.80–7.31 (m, 13H), 7.57 (d, 2H).

3.3.1.4. 4-[4-(4-Fluorophenyl)-1-hydroxy-1-(4-tert-butyl aminocarbonylphenyl)butyl]-1H-imidazole

1-Benzyl-5-[4-(4-fluorophenyl)-1-hydroxy-1-(4-*tert*-butylaminocarbonylphenyl)butyl]-1H-imidazole hydrochloride (12.95 g) is dissolved into aqueous ethanol (400 ml) and 1.3 g of 10% Pd/C is added. Ammonium formate (8.17 g) is added to the boiling solution in small portions. The mixture is refluxed for 3 h. Then the reaction mixture is filtered through siliceous earth and the filtrate is evaporated to dryness. The residue is dissolved to methylene chloride, washed with 2 M sodium hydroxide and water, dried and evaporated to dryness. Yield 79%; ^{1}H NMR (as base, $CDCl_3$) δ 1.3-1.5 (m, 1H), 1.47 (s, 9H), 1.6–1.8 (m, 1H), 2.0–2.3 (m, 2H), 2.45–2.6 (m, 2H), 6.78 (s, 1H), 6.91 (t, 2H), 6.94–7.07 (m, 2H), 7.43 (d, 2H), 7.50 (s, 1H), 7.61 (d, 2H).

3.3.1.5. 4-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)-1-butenyl]-1H-imidazole, isomers a and b

4-[4-(4-Fluorophenyl)-1-hydroxy-1-(4-*tert*-butylaminocarbonylphenyl)butyl]-1H-imidazole (7.6 g) is dissolved into thionyl chloride (75 ml) and refluxed for 2 h. Thionyl chloride is evaporated and the residue is dissolved to ethyl acetate, washed with 2 M sodium hydroxide solution and water, dried and evaporated to dryness. The residue is crystallized from ethyl acetate as hydrogen chloride salt (isomer a). The mother liquid is washed with 2 M sodium hydroxide solution and water, dried and concentrated. Diethyl ether is added to the solution and the precipitated product is filtered (isomer b). Yields 53% isomer a and 17% isomer b; ^{1}H NMR (as HCl-salt, MeOH-d_4) isomer a δ 2.4 (q, 2H), 2.77 (t, 2H), 6.47 (t, 1H), 6.92–7.00 (m, 2H), 7.03 (s, 1H), 7.08–7.13 (m, 3H), 7.21–7.24 (m, 2H), 7.76–7.78 (m, 2H), 8.87 (d, 1H); isomer b δ 2.61 (q, 2H), 2.85 (t, 2H), 6.61 (t, 1H), 6.96–7.01 (m, 2H), 7.16–7.21 (m, 3H), 7.34 (d, 1H), 7.4 (d, 2H), 7.71 (d, 2H), 8.93 (d, 1H); HRMS Calcd for $C_{20}H_{16}FN_3$: (M+) 317.1325. Found: (M+) 317.1328.

3.4. Scheme H

3.4.1. 4-(2,3-Diphenylpropyl)-1H-imidazole ***(16)***

3.4.1.1. 1-Benzyl-5-(2,3-diphenyl-1-propenyl)-1H-imidazole

A Flask is charged with Zn (39.8 g, 0.162 mol) and 200 ml of tetrahydrofuran. $TiCl_4$ (57.3 g, 0.306 mol) is added dropwise to the mixture at 0–10°C. Then the mixture is refluxed for 1 h. Ten grams (0.051 mol) desoxybenzoin and 11.39 g (0.061 mol) 1-benzyl-5-imidazolyl aldehyde in 100 ml of THF is added to the mixture at room temperature. The mixture is heated to boiling and the refluxing is continued for 3 h. After cooling to room temperature the mixture is poured to 10% K_2CO_3 solution. Toluene is added and the mixture is filtered through siliceous earth. Toluene phase is separated and the water layer is extracted again with toluene. Toluene extracts are combined, washed with water, dried with $MgSO_4$ and evaporated to dryness. The crude product is purified by flash chromatography with methylene chloride and methanol (9.75:0.25) as eluent: MS *m*/*z* (%) 350 (M+) (8), 259 (4), 197 (57), 105 (100), 92 (6), 77 (58).

3.4.1.2. 4-(2,3-Diphenylpropyl)-1H-imidazole

1-Benzyl-5-(2,3-diphenyl-1-propenyl)-1H-imidazole (0.67 g, 1.9 mmol) is dissolved to ethanol–water solution (25:15). Pd/C (10%, 0.07 g) and 0.6 g (9.5 mmol) of ammonium formate in 15 ml of water is added to the mixture. After refluxing for 2 h the mixture is filtered and the filtrate is evaporated to dryness. The residue is dissolved into methylene chloride and washed many times with water. Methylene chloride is evaporated and the residue is dissolved into 2 M hydrogen chloride solution. The solution is extracted twice with diethyl ether. The water layer is made alkaline and extracted with methylene chloride. The methylene chloride phase is dried and evaporated to dryness. The product is then made to

hydrochloride salt with dry hydrogen chloride gas in diethyl ether. Yield 16%; MS *m/z* (%) 262 ($M^{+\cdot}$) (10), 197 (3), 181 (22), 171 (18), 82 (100); HRMS Calcd for $C_{18}H_{18}N_2$: (M+) 262.1466. Found: (M+) 262.1470.

3.5. Scheme J

3.5.1. 1-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)butyl]-1H-imidazole **(73)**

1-(4-Cyanobenzyl)-imidazole (1 g, 5.4 mmol) is dissolved into dry tetrahydrofuran (30 ml) and cooled to −70°C. *n*-BuLi in hexane (5.4 mmol) is added dropwise into the reaction mixture. After stirring for additional 30 min at −70°C, 3-(4-fluorophenyl)-propyl bromide (1.5 g, 6.9 mmol) in THF (10 ml) is added to the mixture and stirring is continued for 2 h. Then the mixture is allowed to warm to room temperature. Saturated aqueous ammonium chloride solution is added to the mixture, shaken and then the layers are separated. THF-phase is dried and evaporated to dryness. The residue is crystallized from isopropanol as hydrogen chloride salt. The filtrate is purified by flash chromatography: ^{1}H NMR (HCl-salt, MeOH-d_4) δ 1.5–1.63 (quintet, 2H), 2.3–2.5 (m, 2H), 2.7 (t, 2H), 5.75 (t, 1H), 6.94–7.00 (m, 2H), 7.14–7.19 (m, 2H), 7.62 (d, 2H), 7.63 (s, 1H), 7.78 (s, 1H), 7.8 (d, 2H), 9.6 (s, 1H); HRMS Calcd for $C_{20}H_{18}FN_3$: (M+) 319.1481. Found: (M+) 319.1485.

3.5.2. 1-[1-(4-Cyanophenyl)-3-(4-fluorophenyl)propyl]-1,2,4-triazole **(72)**

The mixture of 1-chloro-1-(4-cyano-phenyl)-3-(4-fluorophenyl)propane (4.18 g, 15 mmol) and 1,2,4-triazole sodium derivative (1.37 g, 15 mmol) in DMF (30 ml) is heated mildly for 4 h. DMF is evaporated. The residue is dissolved in ethyl acetate and washed with water. The organic layer is dried and the solvent is evaporated. The product is purified with flash chromatography (Silica gel 60 mesh 230–400, eluent: methylene chloride–methanol 99:1); ^{1}H NMR (HCl-salt, MeOH-d_4) δ 2.55–2.65 (m, 3H), 2.78–2.84 (m, 1H), 5.83 (dd, 1H), 7.00 (t, 2H), 1.17 (dd, 2H), 7.68 (d, 2H), 6.78 (d, 2H), 8.75 (s, 1H), 9.69 (s, 1H); HRMS Calcd for $C_{18}H_{15}FN_4$: (M+) 306.1278. Found: (M+1) 307.1359.

3.5.3. 1-[1-(4-Cyanophenyl)-3-(4-fluorophenyl)propyl]-1H-imidazole **(71)**

1-[1-(4-Cyanophenyl)-3-(4-fluorophenyl)propyl]-1H-imidazole **(71)** is prepared according to the same procedure using 1-chloro-1-(4-cyanophenyl)-3-(4-fluorophenyl)propane and 1H-imidazole sodium derivative as starting materials: ^{1}H NMR (HCl-salt, MeOH-d_4) δ 2.59–2.80 (m, 4H), 5.72 (m, 1H), 7.00 (t, 2H), 7.19 (dd, 2H), 7.63 (d, 2H), 7.67 (s, 1H), 7.81 (d, 2H), 7.84 (s, 1H), 9.23 (s, 1H); HRMS Calcd for $C_{19}H_{16}FN_3$: (M+) 305.1325. Found: (M+) 305.1352.

3.6. Scheme K

3.6.1. 1-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)-1-butenyl]-1,2,4-triazole, isomers a **(82)** and b **(83)**

3.6.1.1. 1-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)-2-hydroxybutyl]-1,2,4-triazole, diastereomers a+d and b+c

1-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)-2-hydroxybutyl]-1,2,4-triazole is prepared from 1-(4-cyanobenzyl)-1,2,4-triazole (10.8 mmol), *n*-BuLi (10.8 mmol) and 3-(4-fluorophenyl)propionaldehyde (13 mmol) according to Scheme J. The product is purified by flash chromatography: ^{1}H NMR (base, $CDCl_3$): diastereomer a+d δ 0.5–1.7 (m, 2H), 2.6–2.73 (m, 1H), 2.8–2.9 (m, 1H), 4.4–4.5 (m, 1H), 5.23 (d, 1H), 6.96 (t, 2H), 7.11 (dd, 2H), 7.48 (d, 2H), 7.66 (d, 2H), 8.05 (s, 1H), 8.08 (s, 1H); diastereomer b+c δ 1.5–1.7 (m, 2H), 2.63–2.73 (m, 1H), 2.8–2.9 (m, 1H), 4.3–4.4 (m, 1H), 5.26 (d, 1H), 6.95 (t, 2H), 7.05 (dd, 2H), 7.38 (d, 2H), 7.65 (d, 2H), 8.07 (s, 1H), 8.12 (s, 1H).

3.6.1.2. 1-[1-(4-Cyanophenyl)-4-(4-fluorophenyl)-1-butenyl]-1,2,4-triazole, isomers a and b

1-[1-(4-Cyano-phenyl)-4-(4-fluorophenyl)-2-hydroxybutyl]-1,2,4-triazole (0.42 g, 1.32 mmol) is dissolved into acetonitrile. Phosphorous pentachloride (0.27 g, 1.3 mmol) is added into the solution and the mixture is refluxed for 2 h. Acetonitrile is evaporated, the residue is dissolved with 2 M aqueous sodium hydroxide and extracted with methylene chloride. Methylene chloride is dried and the product is crystallized from ethyl acetate as hydrogen chloride salt: ^{1}H NMR (HCl-salt, MeOH-d_4) isomer a δ 2.42 (q, 2H), 2.85 (t, 2H), 6.85 (t, 1H), 7.0 (t, 2H), 7.16–7.21 (m, 2H), 7.37 (d, 2H), 7.74 (d, 2H), 8.82 (s, 1H), 9.38 (s, 1H); HRMS Calcd for $C_{19}H_{15}FN_4$: (M+) 318.1278. Found: (M+) 318.1281; isomer b δ 2.53 (q, 2H), 2.83 (t, 2H), 6.63 (t, 1H), 6.98 (t, 2H), 7.12–7.17 (m, 2H), 7.33 (d, 2H), 7.80 (d, 2H), 8.62 (s, 1H), 9.33 (s, 1H).

3.7. Scheme M

3.7.1. 1-[1-(4-Cyanophenyl)-3-(4-fluorophenyl)-3-hydroxypropyl]-1,2,4-triazole **(93)** and **(94)**

3.7.1.1. 3-(4-Cyanophenyl)-1-(4-fluorophenyl)prop-2-en-1-one

4-Cyanobenzaldehyde (0.1 mol) and 4-fluoroacetophenone (0.1 mol) are dissolved in methanol (150 ml) and solid sodium hydroxide is added to make the solution alkaline. The mixture is stirred in room temperature for 6 h. The product is filtered off and washed with

methanol: ^{1}H NMR ($CDCl_3$) δ 7.21 (t, 2H), 7.59 (d, 1H), 7.73 (s, 4H), 7.79 (d, 1H), 8.08 (dd, 2H).

3.7.1.2. 3-(4-Cyanophenyl)-1-(4-fluorophenyl)-3-(1-triazolyl)propanone

3-(4-Cyanophenyl)-1-(4-fluorophenyl)-prop-2-en-1-one (2.5 g, 10 mmol), 1,2,4-triazole (0.7 g, 10 mmol) and one drop of Triton B are heated to solution. The cooled mixture is diluted with ether and the product is filtered off. The product is used for the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 3.61 (dd, 1H), 4.37 (dd, 1H), 6.25 (dd, 1H), 7.15 (t, 2H), 7.55 (d, 2H), 7.68 (d, 2H), 7.95 (s, 1H), 7.99 (m, 2H), 8.23 (s, 1H).

3.7.1.3. 1-[1-(4-Cyanophenyl)-3-(4-fluorophenyl)-3-hydroxypropyl]-1,2,4-triazole

3-(4-Cyanophenyl)-1-(4-fluorophenyl)-3-(1-triazolyl)-propanone (25 mmol) is dissolved in methanol. Sodium borohydride (12.6) is added and the mixture is stirred in 30°C for 1 h. The mixture is rendered with 2 M hydrochloric acid and the solvent is evaporated. The residue is dissolved into ethyl acetate, washed with dilute sodium hydroxide and water, dried and the solvent is evaporated. The mixture is rendered with 2 M hydrochloric acid and the solvent is evaporated. The residue is dissolved into ethyl acetate, washed with dilute sodium hydroxide and water, dried and the solvent is evaporated. The product is purified by flash chromatography as a mixture of diastereomers (a+d:b+c, 2:1): ^{1}H NMR (base, $CDCl_3$) δ 2.27–2.37 and 2.54–2.63 (2m, together 1H), 2.76–2.88 (m, 1H), 4.26 and 4.41 (2dd, together 1H), 5.62 and 5.91 (2dd, together 1H), 7.03 and 7.04 (2t, together 2H), 7.22–7.31 (m, 2H), 7.50 and 7.55 (2d, together 2H), 7.65 and 7.69 (2d, together 2H), 7.94 and 8.04 (2s, together 1H), 8.05 and 8.22 (2s, together 1H).

The mixture of diastereomers is triturated with diethylether and filtered. The diastereomer a+d **(93)** is enriched in the insoluble material (>90%) and the diastereomer b+c **(94)** in the filtrate (>80%). Both diastereomers are further purified by recrystallization from toluene: ^{1}H NMR (HCl-salt, MeOH-d_4) diastereomer a+d δ 2.67 (ddd, 1H), 2.84 (dd, 1H), 4.54 (dd, 1H), 6.13 (dd, 1 H), 7.04 (t, 2H), 7.33 (dd, 2H), 7.77 (d, 2H), 7.81 (d, 2H), 8.79 (s, 1H), 9.86 (s, 1H); diastereomer b+c δ 2.43 (ddd, 1H), 2.94 (ddd, 1H), 4.33 (dd, 1H), 6.14 (dd, 1H), 7.05 (t, 2H), 7.34 (dd, 2H), 7.66 (d, 2H), 7.75 (d, 2H), 8.69 (s, 1H), 9.62 (s, 1H); HRMS Calcd for $C_{18}H_{15}FN_4O$: (M+) 322.1227. Found: (M+1) 323.1292.

3.7.2. Reference compounds

Fadrozole (Browne, 1985; Browne et al., 1991), letrozole (Bowman et al., 1990), CGS 18320B (Bowman et al., 1990) and the compound **(23)** (Hirsch and Taylor, 1984) were prepared according to the literature.

3.8. Biochemical assays

3.8.1. Chemical sources

All chemicals were from commercial sources and were of the purest grade available.

3.8.2. Enzyme sources

Human placentas were obtained after normal delivery at term. Tissue was rinsed with ice-cold buffer, coagulated blood and connective tissue were excised from trophoblastic tissue and the selected area was first cut into small pieces with scissors and then homogenized in a Waring Blendor for 20 s. The resulting homogenate was further homogenized with a Potter-Elvehjem homogenizer with four strokes each lasting about 10 s. Mitochondrial and microsomal pellets (10 000×g and 100 000×g pellets, respectively, were isolated by differential centrifugation (Pelkonen and Pasanen, 1981). Pellets were resuspended in phosphate buffer, pH 7.4, into the final protein concentration of about 20 mg/ml.

For the ECOD assay, liver microsomes from untreated Sprague–Dawley rats were prepared by the standard ultracentrifugation method.

3.8.3. Aromatase assay

Aromatase activity was measured by the method of Pasanen (1985). It involved evaporation of an acetone solution containing the substrate (10 μM androstenedione, containing 100 000 dpm of [^{3}H]androstenedione) and 0.5 mg Tween 80 to dryness under a nitrogen stream. The residue was then dissolved in 1 ml of an incubation mixture containing 10 mM $MgCl_2$, 2 mM NADPH, 25 mM phosphate buffer, pH 7.4, and 1 mg microsomal protein containing aromatase enzyme. After incubation for 40 min at +37°C the enzyme reaction was stopped by adding 100 μl ice-cold 33.3% TCA. After centrifugation for 2 min the supernatant (1 ml) was taken into a 1-ml plastic injection syringe and forced through a water-equilibrated Sep-Pak® into a counting vial. Ten ml of scintillation liquid were added and samples were counted in an LKB Rack Betascintillation counter. The tritiated water formed during the aromatase reaction was quantitated by subtracting the control value from the sample value.

3.8.4. Assay for cholesterol side-chain cleavage enzyme (desmolase)

Cholesterol side-chain cleavage activity was measured by the method of Pasanen and Pelkonen (1984). The substrate was prepared according to Hanukoglu and Jefcoate (1980). The mixture containing the following substances, 5 μM cholesterol (including 100 000 dpm [^{3}H]cholesterol), 5 μM cyanoketone, 10 mM $MgCl_2$, 2 mM NADPH, 25 mM phosphate buffer, pH 7.4, and 1 mg mitochondrial protein, was incubated for 30 min at +37°C and the reaction was stopped by adding 900 μl methanol. ^{14}C-Labelled pregnenolone was added to each incubation

vial as an internal standard and the methanol-precipitated protein was centrifuged. The clear supernatant was loaded onto a pre-equilibrated Sep-Pak® column (75% methanol) and the pregnenolone formed in the reaction was eluted into the counting vials with 80% methanol. Ten ml of Scintillation liquid were added and samples were counted in an LKB Rack Beta scintillation counter with a double label program. The pregnenolone formed during the incubation was quantitated by subtracting the control value from the sample value.

3.8.5. 7-Ethoxycoumarin O-deethylase assay (ECOD)

The ability of the compounds to inhibit the enzyme ECOD was investigated by the in vitro assay method according to Aitio (1978). The incubation mixture including cofactors (200 mM KCl, 10 mM MgCl2, 2.5 mM NADP, 6 mM glucose-6-phosphate and 4 U glucose-6-phosphate dehydrogenase) and 0.5 mg microsomal protein from rat liver in 0.1 M sodium–potassium phosphate buffer, pH 7.4 (total volume 0.49 ml), was preincubated at +37°C for 2 min and the reaction was started by adding 10 μl of 5 mM 7-ethoxycoumarin (final concentration 0.1 mM). After 10-min incubation, the reaction was stopped by adding 0.5 ml 6% trichloroacetate and the mixture was spun at 3000 rpm for 10 min. From the clear supernatant, 0.5 ml was taken to a fluorometer cuvette containing 2 ml 1.6 M glycine–NaOH buffer, pH 10.4, just before measuring the fluorescence with the Hitachi spectrophotofluorometer with settings excitation 390 nm and emission 440 nm. Authentic 7-hydroxycoumarin was used for the calibration and for the construction of the standard curve.

3.8.6. Inhibition experiments

In inhibition experiments, the studied substance in a final concentration from 0.1 μM to 1 mM was added into incubation mixture in a volume of 5–10 μl, usually as an ethanol or DMSO solution. The same volume of the solute was added into control incubation tubes (especially DMSO is slightly inhibitory itself). The IC_{50} values (concentration causing a 50% inhibition) were determined graphically on a semilogarithmic paper. Enzymes from different individual placentas gave approximately similar IC_{50} values (data not illustrated).

4. Results and discussion

4.1. Screening of basic skeletons

The observation that MPV-400 A III (**1**) (Fig. 1) was a relatively potent aromatase inhibitor (IC_{50}15 μM) started our rational modification of the long-chained 4(5)-arylalkyl imidazole and 4(5)-diarylalkyl imidazole skeletons (**2**) and (**5–22**) (Table 1).

The effect of the length of the carbon chain on the aromatase inhibition was tested first with a series of monoarylalkyl imidazoles, in which the number of the bridge carbons varied from one to five (compounds **(5–9)** in Table 1). It was observed that the optimal length of the carbon bridge, optimizing the effect on the aromatase inhibition, is three or four carbons (see compounds **(7–8)** for aromatase: IC_{50} 35 and 12 μM, respectively).

While screening diarylalkyl imidazoles, an important finding was made: an additional aryl group increased aromatase activity considerably for example in compounds (**9**) and (**5**) as compared to corresponding mono aryl structures (**13**) and (**10**), respectively. The lengthening of the bridge carbon chain to comprise three or four carbons will produce the most potent aromatase inhibitors (compare compounds (**2, 10–14**)) in this series too. It was of some interest that 4(5)-(diphenylmethyl)imidazole (**10**) (Table 1), the basic skeleton of one of the reference compounds, 4(5)-[bis(4-fluorophenyl)methyl]-1H-imidazoles (**23**) (in Table 7) was about 10-fold less potent than the diarylalkyl imidazole (**2**) containing four bridge carbons.

Interestingly, the long-chained 4(5)-diarylalkyl imidazoles (e.g. compound (**2**)) showed good selectivity for aromatase over desmolase as well, which was the crucial matter as to the continuation of the synthetic program.

Diarylalkyl derivatives, in which the position of one of the aryl rings was varied in the bridge, gave also a good effect on aromatase inhibition. For example, 4(5)-(1,3-diphenylpropyl)-1H-imidazole (**15**), had a IC_{50} value of 4.5 μM for aromatase.

The substitution of the 1-position of imidazole with the diarylalkyl moiety preserved some activity (see compound (**22**); IC_{50} for aromatase 16 μM), but the substitution of the 2-position of imidazole reduced activity radically (for example 2-(4,4-diphenylbutyl)-2H-imidazole: IC_{50} over 1000 μM).

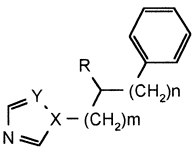
4.2. Screening the effect of substituents

With respect to substituent effects, especially two series of compounds were screened: ω,ω-diarylalkyl 4(5)-imidazoles with three or four carbons in the bridge (compounds (**24–52**) Table 2).

As a rule in these skeletons the substituents had surprisingly little effect with respect to the aromatase inhibition and selectivity. There are, however, exceptions. In the three-carbon bridge series the *para*-cyano or the *para*-cyano combined with the *para*-fluoro substitution increased the potency of the skeleton considerably, as compared the compound **(12)** (IC_{50}15 μM) (Table 1) with the compounds **(26)** (IC_{50} 0.5 μM) and **(38)** (IC_{50} 0.5 μM) (Table 2). However, also in this case, substituents had no significant effect on the selectivity.

The effect of the fluoro and cyano substituents in the α,ω- and β,ω-diarylalkyl framework could be observed to be much more favourable than in the ω,ω-framework. With the cyano group at the *para*-position of the α-phenyl and hydrogen or fluorine at the *para*-position of the ω-phenyl,

Table 1
Effects on aromatase (A) and desmolase (D) inhibition for a series of the basic carbon skeleton modifications in the mono- and diarylalkylimidazoles and triazoles



Compound	R	*m*	*n*	X	Y	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	D/A	Method of synthesis	References
5	H	0	0	C	N	400.0	ND			Karjalainen (1981)
6	H	1	0	C	N	300.0	ND			Karjalainen (1981)
7	H	2	0	C	N	35.0	ND		A	
8	H	3	0	C	N	12.0	105	9	A	
9	H	4	0	C	N	50.0	ND		A	
10	Ph	0	0	C	N	25.0	ND			Karjalainen (1981)
11	Ph	1	0	C	N	49.0	9.0	<1	H	
12	Ph	2	0	C	N	15.0	ND		C	Karjalainen et al. (1992)
2	Ph	3	0	C	N	2.0	320.0	160	A	Karjalainen et al. (1995b)
13	Ph	4	0	C	N	1.7	68.0	40	A	Karjalainen et al. (1995b)
14	Ph	5	0	C	N	14.5	61.0	4	A	Karjalainen et al. (1995b)
15	Ph	0	2	C	N	4.5	7.5	2	F	Karjalainen et al. (1995b)
16	Ph	1	1	C	N	45.0	18.0	<1	H	
17	Ph	1	2	C	N	5.5	12.0	2	H	Karjalainen et al. (1995a)
18	Ph	2	1	C	N	7.5	19.0	3	E	Karjalainen et al. (1992)
19	Ph	0	4	C	N	25.0	ND		A	Karjalainen et al. (1995b)
20	Ph	3	1	C	N	4.7	27.0	6	B	Karjalainen et al. (1995b)
21	Ph	2	0	N	C	20.0	28.0	1	J	
22	Ph	3	0	N	C	16.0	52.5	3	J	

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

both the potency and the selectivity of these structures were increased as seen in the connection of the compound **(60)** (Table 3). Also on the β,ω-diaryl skeleton, the tested fluorine and cyano were beneficial (compare compound **(17)** in Table 1 with compound **(66)** in Table 3). Surprisingly, however, the selectivity was not so impressive with this framework.

We connected α,ω-diaryl framework with substituents also to the 1-position of imidazole and triazole, and both the potency and the selectivity increased now considerably (see compounds **(71–74)** in Table 4).

4.3. Geometric isomerism

Introduction of a double bond into the appropriately substituted α,ω-diarylalkyl framework linked to imidazole or triazole and separation of *E*- and *Z*-isomers offered an interesting possibility to affect selectivity. The isomer possessing favourable spatial configuration proved to have many-fold increased selectivity when compared to the other isomer. Especially this increase in selectivity was very pronounced with the four-carbon bridged α,ω-diarylalkene framework, connected to 4(5)-imidazole moiety, as seen by comparing the effects of the geometric isomers of the compound 4-[4-(4-fluorophenyl)-1-(1H-imidazol-4-yl)but-1-enyl]benzonitrile, **(4)** and **(77)** (Table 5). Also with 1-triazoles and with 1-imidazoles this effect is rather conspicuous as compared the isomers of 4-[4-(4-fluorophenyl)-1-[1,2,4]triazol-1-ylbut-1-enyl]benzonitrile (**(82)** and **(83)**) and 4-[4-(4-fluorophenyl)-1-imidazol-1-ylbut-1-enyl]benzonitrile (**(84)** and **(85)**) (Table 5).

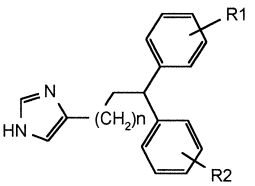
The effect of relative positions of fluoro and cyano groups was also studied and observed to be important as for the activity and selectivity. It was somewhat surprising to find that dicyano substitution did not give this selectivity difference (see compounds **(78)** and **(79)** in Table 5).

With 1-substituted imidazoles the geometric isomerism gave similar selectivity difference between the geometric isomers (compare compounds **(84)** and **(85)** in Table 5). Interestingly, however, the introduction of a double bond in this case did not seem to bring any improvement in the selectivity compared to the selectivity of the corresponding saturated structure 4-[4-(4-fluorophenyl)-1-imidazol-1-ylbutyl]benzonitrile **(73)** (Table 4).

4.4. Triazoles and optical isomerism

When our best skeletal framework was attached to 1-position of triazole ring (compounds **(69), (70), (72), (74), (80–83)** in Tables 4 and 5), it was found that all the important structure–activity characteristics for activity and

Table 2
Effects on aromatase (A) and desmolase (D) inhibition for various substituent modifications in the basic ω,ω-diarylalkylimidazoles



Compound	R1	R2	*n*	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	D/A	Method of synthesis	References
24	H	*p*-Me	1	7.1	50.0	7	E	Karjalainen (1992)
25	H	*p*-F	1	10.1	440.0	44	E	Karjalainen (1992)
26	H	*p*-CN	1	0.48	14.0	29	E	Karjalainen et al. (1996)
27	*o*-Me	*o*-Me	1	33.0	8.2	<1	C	Karjalainen (1992)
28	*m*-Me	*m*-Me	1	20.0	29.0	14	C	Karjalainen (1992)
29	*p*-Me	*p*-Me	1	14.0	48.0	3	C	Karjalainen (1992)
30	*o*-Me	*o*-Me	1	8.6	32.0	4	C	Karjalainen (1992)
31	*o*-Me	*o*-Me	1	3.2	36.0	11	C	Karjalainen (1992)
32	*p*-OMe	*p*-OMe	1	8.6	26.0	3	C	Karjalainen (1992)
33	*m*-F	*m*-F	1	19.0	20.5	1	C	Karjalainen (1992)
34	*p*-F	*p*-F	1	5.0	30.0	6	E	Karjalainen (1992)
35	*p*-CF_3	*p*-F	1	5.2	110.0	21	E	
36	*p*-CF_3	*p*-CF_3	1	32.0	ND		C	
37	*p*-CF_3	*p*-CN	1	2.4	61.0	25	E	Karjalainen et al. (1996)
38	*p*-CN	*p*-F	1	0.5	21.0	42	E	Karjalainen et al. (1996)
39	*p*-CN	*p*-OMe	1	0.85	33.0	39	E	Karjalainen et al. (1996)
40	*p*-CN	*p*-CN	1	0.86	36.0	19	C	Karjalainen et al. (1996)
41	H	*o*-Me	2	3.4	29.0	8	A	Karjalainen et al. (1995b)
42	H	*m*-Me	2	2.5	26.0	10	A	Karjalainen et al. (1995b)
43	H	*o*-F	2	16.0	38.0	2	A	Karjalainen et al. (1995b)
44	H	*p*-F	2	2.8	80.0	29	A	Karjalainen et al. (1995b)
45	H	*p*-Et	2	8.5	65.0	8	A	Karjalainen et al. (1995b)
46	*m*-Me	*m*-Me	2	28.0	48.0	2	A	Karjalainen et al. (1995b)
47	*p*-Me	*p*-Me	2	3.5	110.0	31	A	Karjalainen et al. (1995b)
48	*p*-CF_3	*p*-CF_3	2	120.0	340.0	3	A	
49	*p*-NH_2	*p*-NH_2	2	26.0	210.0	8	C	Karjalainen et al. (1995b)
50	*p*-NO_2	*p*-NO_2	2	20.0	37.0	2	C	Karjalainen et al. (1995b)
51	*p*-F	*p*-F	2	3.3	175.0	53	A	Karjalainen et al. (1995b)
52	*p*-CN	*p*-CN	2	1.6	47.0	29	B	Karjalainen et al. (1996)

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

selectivity found by screening 4(5)-imidazoles, were valid with 1-triazoles as well.

In the course of this research we observed that in vivo activity was better with triazoles than with imidazoles and so our focus in the structure–activity screening, when making further modifications in the skeleton, was shifted to 1-triazoles.

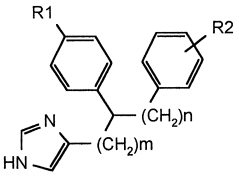
We synthesized 1-substituted α,ω-diarylalkyl triazoles with a hydroxyl group in the β-carbon of the bridge, creating thus an additional chiral centre giving a new aspect as to achieving selectivity (see compounds (**3**) and (**86–94**) in Table 6). The separated diastereomers displayed substantial additional selectivity differences. The length of the bridge carbon chain containing three carbons and appropriate substitution pattern were also with these modifications the crucial matters with respect to the favourable effects.

4.5. Comparison with reference compounds

The comparison of the potency and selectivity of our best structures, e.g., compounds **(3)**, **(4)** and **(89)**, with the selected known inhibitors was made and is presented in Table 7. As reference compounds were used letrozole, fadrozole, CGS 18320B and 4-[bis(4-fluorophenyl)-methyl]-1H-imidazole **(23)**, which was included in Eli Lilly's patent application. The reference compounds were prepared according to the literature and studied in conjunction in our own compounds.

The potency of compounds **(3)**, **(4)** and **(89)** to inhibit

Table 3
Effects on aromatase (A) and desmolase (D) inhibition for various substituent modifications in the basic diarylalkylimidazoles



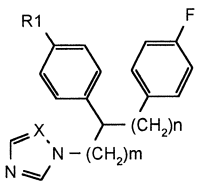
Compound	R1	R2	*m*	*n*	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	D/A	Method of synthesis	References
53	F	H	0	4	210.0	19.0	9	F	Karjalainen et al. (1995b)
54	F	*o*-Me	0	4	3.5	16.0	5	F	Karjalainen et al. (1995b)
55	F	*m*-Me	0	4	3.8	57.0	15	F	Karjalainen et al. (1995b)
56	F	*p*-Me	0	4	8.5	170.0	20	F	Karjalainen et al. (1995b)
57	F	H	0	3	0.8	31.0	41	F,G	Karjalainen et al. (1995b)
58	CN	H	0	3	0.21	12.0	57	F	Karjalainen et al. (1996)
59	F	*p*-F	0	3	0.63	29.0	46	H,G	
60	CN	*p*-F	0	3	0.23	20.0	87	F	Karjalainen et al. (1996)
61	F	H	0	2	0.7	22.0	31	H,G	Karjalainen et al. (1995b)
62	F	*p*-F	0	2	2.2	22.0	10	G	
63	F	H	1	1	4.4	3.4	1	H	
64	F	H	1	2	2.2	28.0	13	H	Karjalainen et al. (1995a)
65	F	*p*-F	1	2	1.3	20.0	15	H	Karjalainen et al. (1995a)
66	CN	*p*-F	1	2	0.5	6.6	13	H	

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

aromatase proved to be superior to aminoglutethimide and they were at least equally potent as the other reference compounds. The best representatives of our structures gave a very high selectivity ratio towards desmolase, with compounds **(3)** and **(4)** selectivity ratio being over 3850 and 5268, respectively.

The compound, 4-[(4-cyanophenyl)(1H-imidazol-4-yl)methyl]benzonitrile **(95)**, a structural relative of letrozole, was synthesized (according to the method C) for comparison of selectivity and activity between 1-substituted and 4(5)-substituted diarylalkyl imidazoles. Rather low aromatase inhibition potency and poor selectivity of

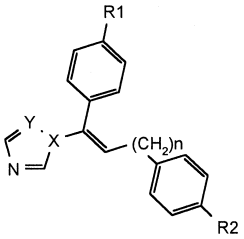
Table 4
Effects on aromatase (A) and desmolase (D) inhibition for substituent modifications in some α,ω- and ω,ω-diarylalkylimidazoles and triazoles



Compound	R1	*m*	*n*	X	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	D/A	Method of synthesis	References
67	H	2	0	C	5.0	21.0	4	J	
68	F	2	0	C	2.2	33.0	15	J	
69	F	2	0	N	36.0	36.0	1	J	
70	F	0	2	N	4.0	11.0	3	J	
71	CN	0	2	C	0.05	11.8	246	J	
72	CN	0	2	N	0.19	20.0	105	J	
73	CN	0	3	C	0.04	17.0	236	J	Karjalainen et al. (1997)
74	CN	0	3	N	0.12	27.0	225	J	

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

Table 5
Effects on aromatase (A) and desmolase (D) inhibition for *Z,E*-isomerism in some α,ω-diarylalkylimidazoles and triazoles



Compound	Isomer	R1	R2	*n*	X	Y	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	D/A	Method of synthesis	References
75	a	CN	F	1	C	N	0.19	36.0	189	F	Karjalainen et al. (1996)
76	b	CN	F	1	C	N	0.33	3.4	10	F	Karjalainen et al. (1996)
77	a	CN	F	2	C	N	0.21	33.0	157	F	Karjalainen et al. (1996)
4	b	CN	F	2	C	N	0.19	>1000	5268	F	Karjalainen et al. (1996)
78	a	CN	CN	2	C	N	1.1	6.3	6	F	Karjalainen et al. (1996)
79	b	CN	CN	2	C	N	0.5	5.4	11	F	Karjalainen et al. (1996)
80	a	CN	F	1	N	N	0.12	5.7	48	K	Karjalainen et al. (1997)
81	b	CN	F	1	N	N	0.05	165.0	3300	K	Karjalainen et al. (1997)
82	a	CN	F	2	N	N	0.65	16.0	25	K	Karjalainen et al. (1997)
83	b	CN	F	2	N	N	0.14	300.0	2140	K	Karjalainen et al. (1997)
84	a	CN	F	2	N	C	0.34	4.0	18	K	Karjalainen et al. (1997)
85	b	CN	F	2	N	C	0.06	13.5	225	K	Karjalainen et al. (1997)

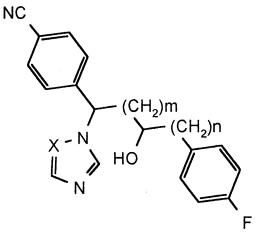
[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

(95) pointed out that with 4(5)-substituted imidazoles the lengthened carbon backbone is the essential structural feature for the high potency and the great selectivity.

4.6. Effects on drug-metabolizing cytochrome P450

In the light of potential drug interactions, it is important

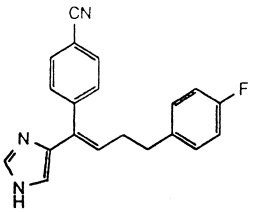
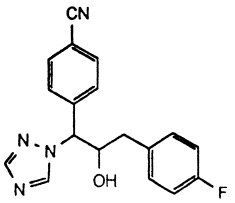
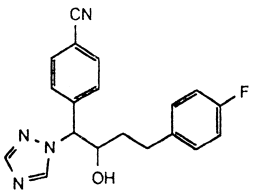
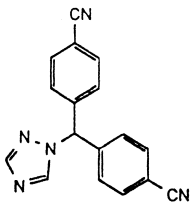
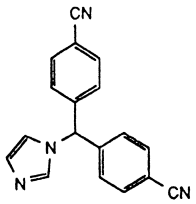
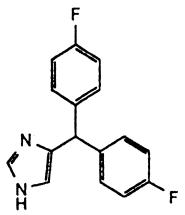
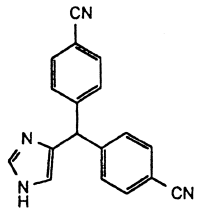
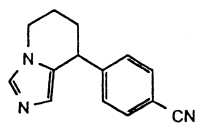
Table 6
Effects on aromatase (A) and desmolase (D) inhibition for optical isomerism in α,ω-diarylalkylimidazoles and triazoles



Compound	Isomer	*m*	*n*	X	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	D/A	Method of synthesis	References
86	a+d	0	0	N	42.0	>1000	24	K	
87	b+c	0	0	N	17.0	215.0	13	K	
3	a+d	0	1	N	0.22	>1000	3850	K	Karjalainen et al. (1997)
88	b+c	0	1	N	0.5	305	610	K	Karjalainen et al. (1997)
89	a+d	0	2	N	0.95	>1000	1054	K	Karjalainen et al. (1997)
90	b+c	0	2	N	3.9	350	90	K	Karjalainen et al. (1997)
91	a+d	0	2	C	0.18	49.0	272	K	Karjalainen et al. (1997)
92	b+c	0	2	C	0.5	65	130	K	Karjalainen et al. (1997)
93	a+d	1	0	N	0.37	315	851	M	Karjalainen et al. (1997)
94	b+c	1	0	N	0.7	420	600	M	Karjalainen et al. (1997)

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

Table 7
Comparative effects on aromatase (A), desmolase (D) and ECOD inhibition for some known and some of our novel α,ω-diarylalkylimidazoles and triazoles

Compound	Structure	A: IC_{50} (μM)[a]	D: IC_{50} (μM)[a]	ECOD: IC_{50} (μM)[a]
4		0.19	>1000	235.0
3		0.18	>1000	1000
89		0.95	>1000	38.0
Letrozole		0.20	185.0	102.0
CGS 18320B		0.06	15.5	16.5
23 (Eli Lilly)		0.3	3.3	
95		1.00	3.0	44.0
Fadrozole		0.35	34.0	34.0

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition.

to screen affinities of chemicals to drug-metabolizing enzymes, especially to cytochrome P450 enzymes. In this study, rat liver microsomal 7-ethoxycoumarin *O*-deethylase (ECOD) activity, representative for many different P450 enzymes, was employed. The general conclusion is that most imidazole derivatives are rather potent inhibitors of ECOD, whereas triazoles exhibit much less inhibition. However, taking into consideration that many inhibitors display rather restricted CYP selectivity (see Pelkonen et al., 1998), this conclusion has to be regarded as very preliminary.

Especially the most potent and selective compounds **(3)** and **(4)** were virtually devoid of any affinity to rat liver P450 enzymes (see Table 7), and later similar findings were observed with human liver microsomes (unpublished results). Interestingly also compound **(4)**, an imidazole derivative, was very selective with respect to ECOD. The geometric isomerism plays an important role in this selectivity too: the *Z*-isomer **(4)** is about nine times more selective than the *E*-isomer **(77)** (Table 5). The same kind of comparison between the diastereomers **(3)** and **(88)** (Table 6) reveals that the optical isomerism has a strong effect on selectivity in the case of ECOD as well, although both diastereomers are very selective.

5. Conclusions

Some interim conclusions could be drawn on the basis of results described above: a good aromatase inhibition is achieved with the structures including a framework of diarylalkyl moiety comprising a lengthened carbon chain linked to the 1- or 4(5)-positions of imidazole or 1-position of triazole. An especially favourable framework seems to be the α,ω-diarylalkyl with three or four carbon atoms in the bridge, which connects the framework to the heterocycle. The best substituents, as for the aromatase activity, are the cyano group in the α-phenyl ring and fluorine atom in the ω-phenyl ring. Although the substituents bring some selectivity, particularly geometric and optical isomerism give rise to the high selectivity.

The outcome of this synthetic program was a large group of potent and selective aromatase inhibitors, of which the most selective representatives are the structures MPV-2213 C II a+d **(3)** and MPV-1837 A V b **(4)**. The MPV-2213 C II a+d **(3)** also demonstrated the desired effect, i.e., the inhibition in estrogen synthesis in experimental animals (unpublished results) and in human volunteers in vivo (Ahokoski et al., 1998).

Acknowledgements

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