Aromatase Inhibitors: Synthesis, Biological Activity, and Structure of 1,2-Imidazolylmethylcyclopentanol Derivatives

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Two series of 1,2-disubstituted imidazolylmethylcyclopentanol derivatives (5a—d, 10a—d) were prepared by using easily available methyl 2-oxocyclopentanecarboxylate as the starting material. Evaluation of the aromatase inhibitory activities *in vitro* was performed. Their activities were compared with those of a steroidal aromatase inhibitor, Formestane, and a non-steroidal inhibitor, Fadrozole. Among these compounds, the aromatase inhibitory activities of 5d, 10a, 10b, 10c, 11a, 15a, and 15b were more potent than Formestane. One compound, 1-(4-chlorobenzyl)-*cis*-2-(1*H*-imidazol-1-ylmethyl)cyclopentanol (10a) was in particular identified as a potent aromatase inhibitor *in vitro*, exhibiting an IC_{50} value of 4×10^{-8} M. The enantiomers of 10a were separated, and their absolute configurations were determined by X-ray crystallography.

Key words aromatase inhibitor; 1,2-disubstituted imidazolylmethylcyclopentanol; X-ray crystallography

Aromatase catalyzes the final stage of steroid hormone biosynthesis, namely the conversion from androgen to estrogen. Thus inhibition of this enzyme should lead to a reduced supply of estrogens. Consequently, the therapeutic potential of aromatase inhibitors is thought to be the endocrine treatment of estrogen-dependent breast cancer in post-menopausal woman.[1-4] Various types of aromatase inhibitors have been developed, and several of them are undergoing clinical studies.[5-7] Although tremendous efforts have been put into the development of aromatase inhibitors, only Formestane was launched in the U.K. and South Africa for the treatment of breast cancer in 1993.

In order to seek new inhibitors of aromatase which were more potent and selective than Formestane, a research program was initiated in our laboratory. We have developed several azole derivatives, which possess a 1,2-disubstituted cyclopentanol skeleton for agrochemical fungicides.[8] The target of the action site of azole fungicides is 14-demethylase (CYP51),[9] one of the cytochrome P450 super family enzymes, which is a critical enzyme for ergosterol biosynthesis. Ergosterol is known to be an essential component of the fungal cell membrane.[10] Azole fungicides serve as ergosterol biosynthesis inhibitors such as Ketoconazole.[11] Meanwhile, aromatase (CYP19)[9] is also a member of the cytochrome P-450 super family enzymes. Therefore, we considered the similar reactivity of 14-demethylase and aromatase based on the presence of a heme molecule which should be coordinated by compounds containing a nitrogen atom. Then our interest focused on the posturation that these azole fungicides might have potential as aromatase inhibitors. Thus, we evaluated the aromatase inhibitory activities of these compounds and compared them with Formestane[12] as a steroidal type inhibitor and Fadrozole[13] as a non-steroidal type inhibitor (Fig. 1).

We report here the synthesis and aromatase inhibitory activities of novel 1,2-disubstituted cyclopentanol derivatives and related compounds.

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Results and Discussion

Charts 1—3 depicts the synthetic routes and structures of the compounds discussed in this paper. 1,2-Disubstituted imidazolylmethylcyclopentanols (**5a—d**, **10a—d**) were prepared from methyl 2-oxocyclopentanecarboxylate (**1**) as the starting material. The reaction of **1** with an appropriate substituted benzyl bromide in *N*,*N*-dimethylformamide (DMF) afforded 2-benzylated compounds (**2**) in 88% yield,[14] which were treated by 12.5% aqueous sulfuric acid to give decarboxylated compounds (**3**) in 87% yield.[15] Methylenation of **3** with trimethylsulfoxonium iodide and sodium hydride in dimethyl sulfoxide (DMSO)[16] gave approximately a 10 : 1 diastereomeric mixture of epoxides (**4**) in 83% yield, which were ring-opened by sodium imidazolate in DMF at 70 °C to give 1,2-disubstituted imidazolylmethylcyclopentanols (**5**) in yield of 93%. The reversed arrangement of the imidazolylmethyl and phenyl groups on the cyclopentane ring was achieved by the procedure of Chart 2. After ketalization of **1** with ethylene glycol, **6** was treated with lithium aluminum hydride to give alcohol **7** in 89% yield, which was mesylated and imidazolylated to give **8** in 70% yield. Complete removal of the carbonyl protecting group of **8** was done by acidic hydrolysis to give the ketone 9.[17] Addition of appropriate Grignard reagents to the ketone 9 gave a 1 : 1 diasteromeric mixture of substituted cyclopentanols, **10** and **11** in 90% yield. The stereochemistry of *cis*-**10** and *trans*-**11** were determined by ^{1}H-NMR difference nuclear Overhauser effect (NOE) experiment on compounds **10** and **11**. This indicated the proximity between imidazolyl methyl proton

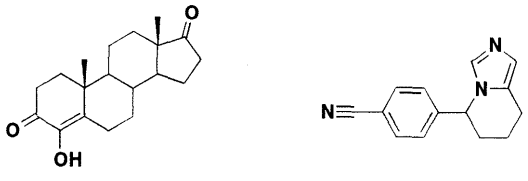


Fig. 1. The Structure of Formestane (Left) and Fadrozole (Right)

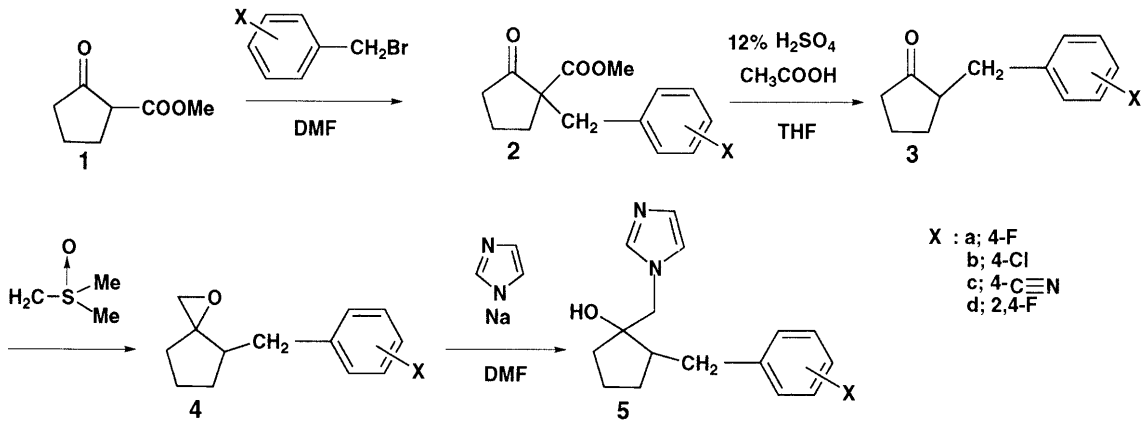
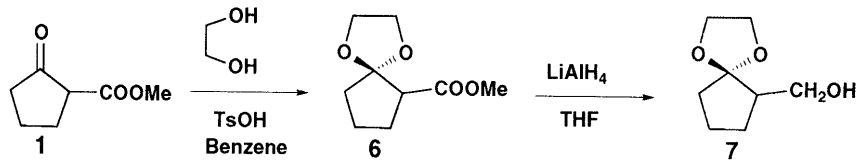


Chart 1. Synthetic Route of **5a**, **5b**, **5c**, and **5d**



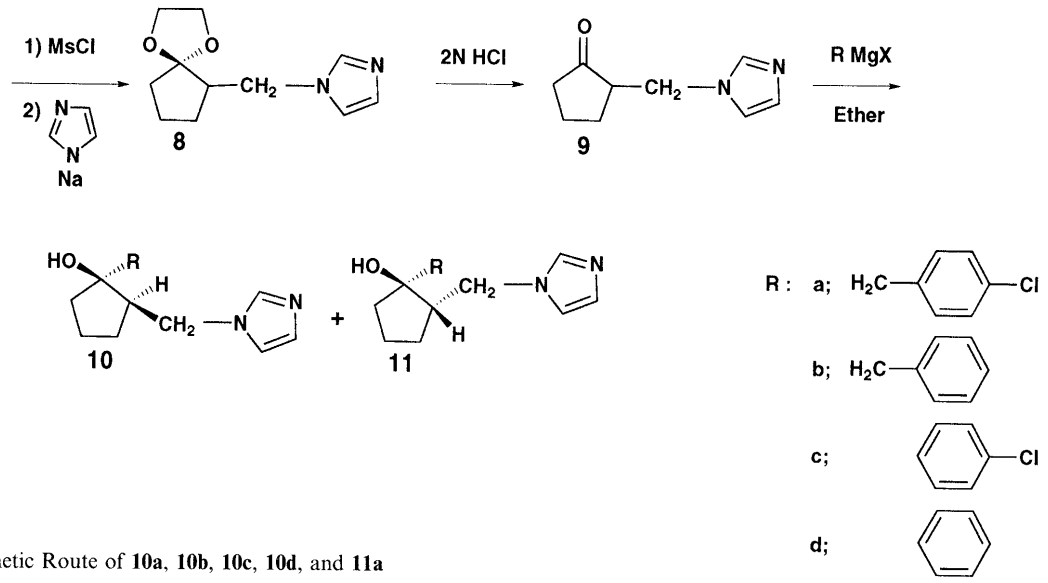


Chart 2. Synthetic Route of **10a**, **10b**, **10c**, **10d**, and **11a**

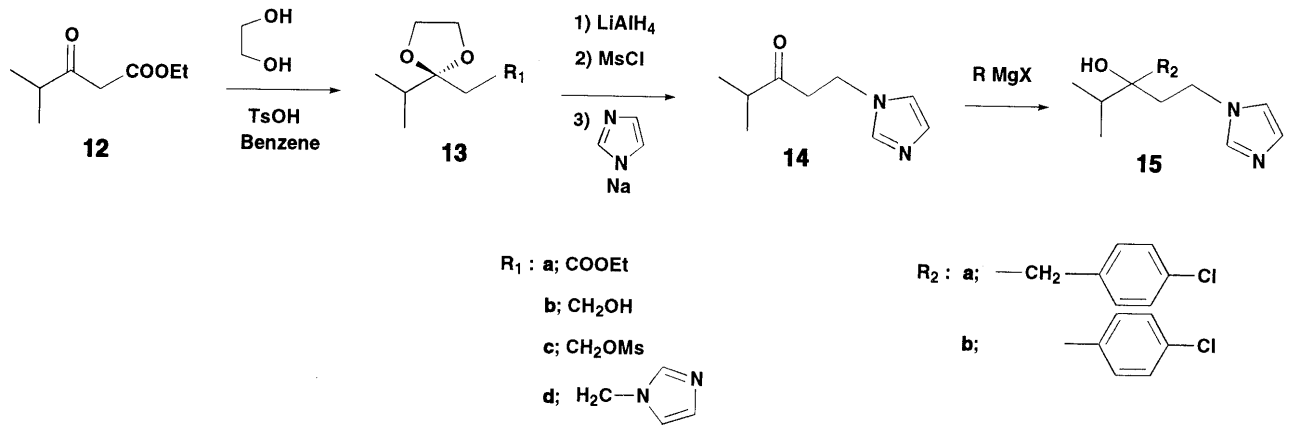


Chart 3. Synthetic Route of **15a** and **15b**

and benzyl proton in **11**, from which *trans* configulation of **11** was thought and finally confirmed by X-ray diffraction analysis.

Ring-opened analogs of **10** were synthesized according to the procedure of Chart 3. Following ketalization of **12** with ethylene glycol (95%), **13** was reduced by lithium

Table 1. Aromatase Inhibitory Activity of the Compounds

	IC_{50} (M)
5a	4×10^{-6}
5b	6×10^{-6}
5c	6×10^{-6}
5d	3×10^{-7}
(+/−) **10a**	4×10^{-8}
(+) **10a**	4×10^{-7}
(−) **10a**	2.5×10^{-8}
10b	5×10^{-7}
10c	3×10^{-7}
10d	3.5×10^{-6}
11a	2×10^{-7}
15a	1×10^{-7}
15b	1×10^{-6}
Formestane	3.5×10^{-6}
Fadrozole	2×10^{-8}
Androstenedione	1×10^{-6}

All results are mean of triplicated determinations and expressed 50% inhibitory concentration (M).

alminium hydride (91%) and converted to mesylate **13c** (92%). After **13c** was imidazolylated, the ketal was removed to give the ketaric **14** in 90% yield. Addition of appropriate Grinard regents to **14** gave adducts **15a** and **15b**. The structural assignments of the synthesized compounds were based on ^{1}H-NMR, IR and mass spectral data.

Table 1 summarizes the IC_{50} values of the compounds which showed activities in the order of 10^{-6} to 10^{-8} M in the human placental microsome system. In an attempt to characterize whether or not 1,2-disubstituted imidazolylmethylcyclopentanol derivatives have aromatase inhibitory activities, the investigation was started with compounds of Chart 1 type. Test compounds exhibited dose dependent aromatase inhibitory activity *in vitro*. **5a**, **5b** and **5c** possessed moderate aromatase inhibitory activities, and they were as potent as Formestane. Hence, the modification of the 4-position of the phenyl group of compounds of Chart 1 type did not affect the aromatase inhibitory activity. In contrast, by the introduction of fluorine atoms at *ortho* and *para* positions of the phenyl group, activity of the compounds (**5d**) was increased by the factor of more than 10 times fold, compared with the mother compound (**5b**). The reversed arrangement of the imidazolylmethyl and phenyl groups on the cyclopentane ring (compounds of Chart 2 type) increased the activities dramatically. All of them were more potent than the parental structure, **5b**. Among these compounds, the activity of (+/−)**10a** was 100-fold higher than those of the parental compound and Formestane, and the activity was comparable to that of Fadrozole (IC_{50}: 2.0×10^{-8} M). The geometrical isomer (**11a**), the modification of the phenyl group (**10b**, **10c**, **10d**), or the modification of the cyclopentane ring (compounds of Chart 3 type (**15a**, **15b**)) all resulted in diminished activities from that of **10a**. Therefore, the orientation and distance between the imidazolylmethyl and phenyl moieties were suggested to play an important role in the expression of aromatase inhibitory activity. The lipophillic portion near the imidazole moiety appears to be especially important. To obtain further understanding of (+/−)**10a** activity,

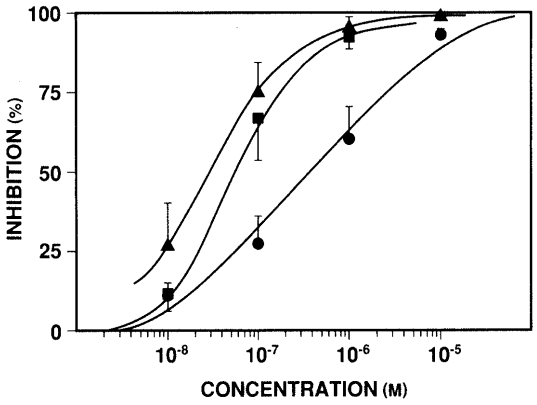


Fig. 2. Aromatase Inhibitory Activity of **10a** Enantiomers

■, (+/−) **10a**; ▲, (−) **10a**; ●, (+) **10a**; all results are expressed mean +/− standard devisiation of triplicate studies.

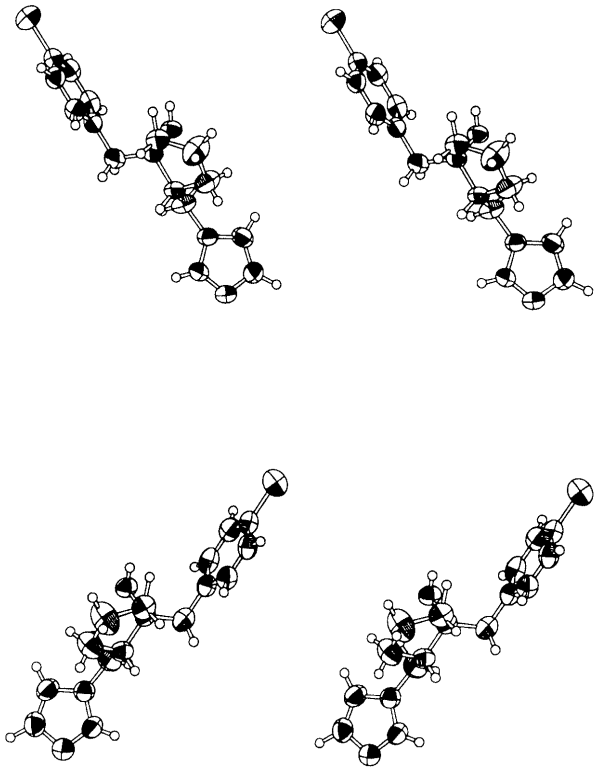


Fig. 3. ORTEP Structure of (−) **10a** (Upper) and (+) **10a** (Lower)

the absolute configurations of (+)**10a** and (−)**10b** were investigated. Optical resolution of (+/−)**10a** was performed by HPLC using a preparative chiral column (Chiralcel OD), and two enantiomers were then obtained. The activity of the (+)-enantiomer ((+)**10a**) was less than those of the racemate ((+/−)**10a**) and the (−)-enantiomer ((−)**10a**) (Fig. 2). Based on the X-ray crystallography analysis, the absolute configuration of the (−)-enantiomer ((−)**10a**) was assigned to the 1*S*,2*R*-cyclopentanol skeleton, and the ORTEP structures of (−)**10a** and (+)**10a** are displayed in Fig. 3. The separation of (+/−)**10a** into its enantiomers (−)**10a** and (+)**10a** demonstrated that the (−)-enantiomers with the 1*S*,2*R* absolute configuration was responsible for the higher aromatase inhibitory activity of (+/−)**10a**. It might be thought that compound (−)**10a** should be fitted with an active site of aromatase from human placenta better than

(+)-enantiomer.

Conclusions

Two series of 1,2-disubstituted imidazolylmethylcyclopentanols and related ring-opened compounds, whose structures are different from reported aromatase inhibitor, were shown to have noteworthy activity toward human placental aromatase. Among 1,2-disubstituted imidazolylmethylcyclopentanols, **5d**, (−)**10a**, (+)**10a**, **10b**, **10c**, **11a**, **15a** and **15b** were more potent than Formestane and above all, (−)**10a** exhibited the most potent activity, being equivalent to that of Fadrozole. Most potent compound has the structure with 1*S*,2*R*-cyclopentanol skeleton. These results implied that the orientation and distance between the imidazolylmethyl and phenyl moieties on the cyclopentane ring played an important role in the expression of the aromatase inhibitory activity *in vitro* of these derivatives.

Experimental

The melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. All temperatures are expressed in degrees centigrade. The ^{1}H-NMR spectra were recorded on a JEOL GX500 (500 MHz) spectrometer, and chemical shifts were given in δ with Me_4Si as an internal standard. The IR spectra were recorded on a JASCO-A202 IR spectrophotometer. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer in the EI mode. Optical rotations were determined on a JASCO E-180 polarimeter in MeOH. Chromatography columns were prepared with silica gel (70—230 mesh, E. Merck). Elemental analyses were carried out with a Yanagimoto MT-3 automatic elemental analyzer. X-ray diffraction analysis was done on a Rigaku AFC7R diffractometer with graphite monochromated CuK_α radiation and a 12 kW rotating anode generator using the $\omega-2\theta$ scan technique. The intensities of 1726 independent reflections with $\theta<75$ were measured, of which 1484 were classified as observed with $I>3\sigma$ (I).

2-Carbomethoxy-2-(4-fluorobenzyl)cyclopentanone (2a) To a stirred suspension of *n*-hexane washed sodium hydride (0.94 g, 39 mmol, 1.3 eq) in DMF (15 ml) was added dropwise a solution of methyl 2-oxocyclopentanecarboxylate (**1**) (4.26 g, 30 mmol, 1.0 eq) in DMF (15 ml) at 0 °C. After stirring at 25 °C for 0.5 h, 4-fluorobenzyl bromide (6.80 g, 36 mmol, 1.2 eq) was added at 0 °C and the mixture was stirred overnight. The reaction mixture was quenched by the addition of crushed ice and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography using *n*-hexane–ethyl acetate (4 : 1) to afford **2a** (6.61 g, 88.3%) as a solid. Recrystallization from ethyl acetate–*n*-hexane gave colorless prisms; mp 58—60 °C. ^{1}H-NMR ($CDCl_3$): δ 1.64 (1H, m), 1.92 (2H, m), 2.03 (1H, m), 2.39 (2H, m), 3.08 (1H, d, J=13.8 Hz), 3.18 (1H, d, J=13.8 Hz), 3.72 (3H, s), 6.94 (2H, m), 7.09 (2H, m). IR (KBr): 1750 (s), 1722 (s) cm^{-1}. MS *m/z*: 250 (M^+).

2-Carbomethoxy-2-(4-chlorobenzyl)cyclopentanone (2b) Following a procedure similar to that described for **2a** using 4-chlorobenzyl bromide (6.28 g, 39 mmol, 1.2 eq) instead of 4-fluorobenzyl bromide, **2b** (5.55 g) was obtained as a colorless oil in 69.5% yield. ^{1}H-NMR ($CDCl_3$): δ 1.65 (1H, m), 1.91 (2H, m), 2.05 (1H, m), 2.38 (2H, m), 3.07 (1H, d, J=14.0 Hz), 3.17 (1H, d, J=14.0 Hz), 3.72 (3H, s), 7.06 (2H, m, J=8.3 Hz), 7.23 (2H, m, J=8.3 Hz). IR (neat): 1775 (s), 1730 (s) cm^{-1}. MS *m/z*: 266 (M^+).

2-Carbomethoxy-2-(4-cyanobenzyl)cyclopentanone (2c) Methyl 2-oxocyclopentanecarboxylate (**1**), (2.79 g, 19.6 mmol) was alkylated as described above to give **2c** (4.94 g) as a crystalline residue in 98% yield. Recrystallization from ethyl acetate–*n*-hexane gave colorless crystals; mp 104—106 °C. ^{1}H-NMR ($CDCl_3$): δ 1.70 (1H, m), 1.86 (1H, m), 1.96 (1H, m), 2.08 (1H, m), 2.43 (2H, m), 3.12 (1H, d, J=13.8 Hz), 3.27 (1H, d, J=13.8 Hz), 3.72 (3H, s), 7.26 (2H, m, J=8.0 Hz), 7.56 (2H, m, J=8.0 Hz). IR (KBr): 2255 (s), 1746 (m), 1725 (s) cm^{-1}. MS *m/z*: 257 (M^+).

2-Carbomethoxy-2-(2,4-difluorobenzyl)cyclopentanone (2d) Methyl 2-oxocyclopentanecarboxylate (**1**) (2.84 g, 20 mmol, eq) was alkylated as described above to give **2d** (5.14 g, 95.8%) as a crystalline mass, which was recrystallized from ethyl acetate–*n*-hexane to provide an analytical sample as colorless prisms; mp 55—58 °C. ^{1}H-NMR ($CDCl_3$): δ 1.77 (1H, m), 1.91 (2H, m), 2.07 (1H, m), 2.43 (2H, m), 3.02 (1H, d, J=14.2 Hz), 3.01 (1H, d, J=14.2 Hz), 3.72 (3H, s), 6.77 (2H, m), 7.15 (1H, m). IR (KBr): 1720 (s) cm^{-1}. MS *m/z*: 268 (M^+).

2-(4-Fluorobenzyl)cyclopentanone (3a) A mixture of **2a** (14.5 g, 57.8 mmol, 1.0 eq), glacial acetic acid (100 ml) and 12.5% aqueous sulfuric acid (50 ml) was heated at reflux under argon for 4 h. The mixture was poured over crushed ice (50 g) and extracted with diethyl ether. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to dryness to give the crude product (12.7 g), which was purified by silica gel column chromatography using *n*-hexane–ethyl acetate (6 : 1) to give **3a** (9.7 g) as a pale yellow oil in 86.9% yield. ^{1}H-NMR ($CDCl_3$): δ 1.54 (1H, m), 1.74 (1H, m), 1.95 (1H, m), 2.08 (2H, m), 2.33 (2H, m), 2.55 (1H, dd, J_1=13.8 Hz, J_2=9.2 Hz), 3.09 (1H, dd, J_1=13.8 Hz, J_2=4.6 Hz), 6.95 (2H, m), 7.11 (2H, m). IR (neat): 1741 (s) cm^{-1}.

2-(4-Chlorobenzyl)cyclopentanone (3b) **2b** (5.6 g, 20.8 mmol) was decarboxylated as described above to give **3b** (3.63 g) as a colorless oil in 83.6% yield. ^{1}H-NMR ($CDCl_3$): δ 1.53 (1H, m), 1.74 (1H, m), 1.96 (1H, m), 2.10 (2H, m), 2.33 (2H, m), 2.53 (1H, dd, J_1=13.8 Hz, J_2=9.1 Hz), 3.09 (1H, dd, J_1=13.8 Hz, J_2=4.4 Hz), 7.10 (2H, d, J=8.3 Hz), 7.24 (2H, d, J=8.3 Hz). IR (neat): 1740 (s) cm^{-1}.

2-(4-Cyanobenzyl)cyclopentanone (3c) **2c** (4.94 g, 19.2 mmol) was decarboxylated as described above to give **3c** (2.83 g) as a pale yellow oil in 73.9% yield. ^{1}H-NMR ($CDCl_3$): δ 1.50 (1H, m), 1.76 (1H, m), 1.97 (1H, m), 2.09 (2H, m), 2.36 (2H, m), 2.62 (1H, dd, J_1=13.8 Hz, J_2=9.2 Hz), 3.17 (1H, dd, J_1=13.8 Hz, J_2=4.4 Hz), 7.28 (2H, d, J=8.5 Hz), 7.57 (1H, d, J=8.3 Hz). IR (neat): 1740 (s) cm^{-1}.

2-(2,4-Difluorobenzyl)cyclopentanone (3d) **2d** (5.14 g, 21.6 mmol) was decarboxylated as described above to give **3d** (3.57 g) as a pale yellow oil in 78.7% yield. ^{1}H-NMR ($CDCl_3$): δ 1.53 (1H, m), 1.75 (1H, m), 1.97 (1H, m), 2.07 (2H, m), 2.35 (2H, m), 2.56 (1H, dd, J_1=14.2 Hz, J_2=9.2 Hz), 3.12 (1H, dd, J_1=14.2 Hz, J_2=4.6 Hz), 6.86 (2H, m), 7.14 (1H, m). IR (neat): 1738 (s) cm^{-1}.

4-(4-Fluorobenzyl)-1-oxaspiro[2.4]heptane (4a) To a stirred solution of trimethylsulfoxonium iodide (6.63 g, 30.1 mmol, 1.3 eq) in DMF (35 ml) was added *n*-hexane washed sodium hydride (0.67 g, 27.8 mmol, 1.2 eq) at 0 °C. After the solution was stirred at 25 °C for 0.5 h, **3a** (4.45 g, 23.2 mmol, 1.0 eq) was added. The reaction mixture was stirred at room temperature for 3.0 h, quenched with ice cold water (30 ml), and extracted with diethyl ether (100 ml × 2). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness to give crude **4a** (4.01 g, 83.9 %), which was used for the next reaction without any purification. ^{1}H-NMR ($CDCl_3$): δ 1.50 (2H, m), 1.80 (3H, m), 2.04 (1H, m), 2.22 (1H, m), 2.38 (1H, dd, J_1=13.8 Hz, J_2=9.9 Hz), 2.54 (1H, dd, J_1=13.8 Hz, J_2=5.0 Hz), 2.68 (1H, d, J=4.6 Hz), 2.81 (1H, d, J=4.6 Hz), 6.95 (2H, m), 7.11 (2H, m). IR (neat): 1518 (s) cm^{-1}.

4-(4-Chlorobenzyl)-1-oxaspiro[2.4]heptane (4b) Following a procedure similar to that described for **4a**, using **3b** (3.63 g, 17.4 mmol, 1.0 eq) instead of **3a**, **4b** (2.62 g) was obtained as a pale yellow oil in 67.7 % yield. ^{1}H-NMR ($CDCl_3$): δ 1.50 (2H, m), 1.80 (3H, m), 2.05 (1H, m), 2.23 (1H, m), 2.37 (1H, dd, J_1=13.8 Hz, J_2=9.9 Hz), 2.54 (1H, dd, J_1=13.8 Hz, J_2=5.0 Hz), 2.68 (1H, d, J=4.6 Hz), 2.82 (1H, d, J=4.6 Hz), 7.09 (2H, m, J=8.3 Hz), 7.22 (2H, m, J=8.3 Hz). IR (neat): 1498 (s) cm^{-1}.

4-(4-Cyanobenzyl)-1-oxaspiro[2.4]heptane (4c) Following a procedure similar to that described for **4a**, using 2-(4-cyanobenzyl) cyclopentanone (**3c**) (1.40 g, 7.3 mmol, 1.0 eq) instead of **3a**, **4c** (1.28 g) was obtained as a pale yellow oil in 85.3% yield.

4-(2,4-Difluorobenzyl)-1-oxaspiro[2.4]heptane (4d) Following a procedure similar to that described for **4a**, **3d** (3.56 g, 17 mmol, 1.0 eq) instead of **3a**, **4d** (3.35 g) was obtained as a pale yellow oil in 87.9 % yield.

2-(4-Fluorobenzyl)-1-(1*H*-imidazol-1-ylmethyl)cyclopentanol (5a) A mixture of **4a** (0.67 g, 3,3 mmol, 1.0 eq), sodium imidazolate (0.38 g, 4.2 mmol, 1.3 eq) and DMF (10 ml) was heated to 70 °C under argon overnight. The mixture was poured into ice cold water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo* to give crude material (0.93 g). Recrystallization from ethyl acetate–*n*-hexane gave colorless prisms **5a** (0.80 g, 93%); mp 138—142 °C. ^{1}H-NMR ($CDCl_3$): δ 1.57 (3H, m), 1.78 (3H, m), 1.91 (1H, m), 2.56 (1H, dd, J_1=13.8 Hz, J_2=9.2 Hz), 2.71 (1H, dd, J_1=13.8 Hz, J_2=5.5 Hz), 3.89 (1H, d, J=14.2 Hz), 3.98 (1H, d, J=14.2 Hz), 6.97 (3H, m), 7.01 (1H, s), 7.13 (2H, m), 7.49 (1H, s). IR

(KBr): 3200 (s), 1600 (m), 1520 (s) cm^{-1}. MS *m/z*: 274 (M^+). *Anal.* ($C_{16}H_{19}FN_2O$) C, H, N: Calcd 70.1, 7.0, 10.2. Found 70.0, 6.9, 10.6.

2-(4-Chlorobenzyl)-1-(1*H*-imidazol-1-ylmethyl)cyclopentanol (5b) A mixture of **4b** (1.31 g, 5.9 mmol, 1.0 eq), sodium imidazolate (0.69 g, 7.7 mmol, 1.3 eq) and DMF (10 ml) was heated at 70 °C for 4 h under argon. The usual workup and purification by silica gel column chromatography afforded **5b** (0.71 g, 41.5%); mp 112—114 °C. ^{1}H-NMR ($CDCl_3$): δ 1.55 (3H, m), 1.78 (3H, m), 1.90 (1H, m), 2.55 (1H, dd, $J_1=13.5$ Hz, $J_2=9.9$ Hz), 2.71 (1H, dd, $J_1=13.5$ Hz, $J_2=5.2$ Hz), 3.85 (1H, d, $J=14.2$ Hz), 4.03 (1H, d, $J=14.2$ Hz), 6.98 (1H, m), 7.06 (1H, s), 7.11 (2H, d, $J=8.3$ Hz), 7.25 (2H, d, $J=8.3$ Hz), 7.66 (1H, s). IR (KBr): 1518 (s)cm^{-1}. MS *m/z*: 290 (M^+). *Anal.* ($C_{16}H_{19}ClN_2O$) C, H, N: Calcd 66.1, 6.6, 9.6. Found 65.8, 6.4, 9.5.

2-(4-Cyanobenzyl)-1-(1*H*-imidazol-1-ylmethyl)cyclopentanol (5c) According to a procedure similar to that described for the preparation of **5a** and **5b**, **4c** (1.24 g, 5.8 mmol, 1.0 eq) was heated with sodium imidazolate (0.68 g, 7.6 mmol, 1.3 eq) at 70 °C overnight. The usual workup and purification by silica gel column chromatography afforded **5c** (0.41 g, 25%); mp 112—114 °C. ^{1}H-NMR ($CDCl_3$): δ 1.57 (3H, m), 1.70 (2H, m), 1.80 (1H, m), 1.88 (1H, m), 2.63 (1H, dd, $J_1=13.8$ Hz, $J_2=10.3$ Hz), 2.77 (1H, dd, $J_1=13.8$ Hz, $J_2=4.6$ Hz), 3.86 (1H, d, $J=14.2$ Hz), 4.00 (1H, d, $J=14.2$ Hz), 6.96 (1H, s), 7.00 (1H, s), 7.28 (2H, d, $J=8.3$ Hz), 7.50 (1H, s), 7.57 (2H, d, $J=8.3$ Hz). IR (KBr): 3525 (m), 2225 (s), 1624 (w), 1501 (s) cm^{-1}. MS *m/z*: 281 (M^+). *Anal.* ($C_{17}H_{19}N_3O$) C, H, N: Calcd 72.6, 6.8, 14.9. Found 72.8, 6.7, 14.6.

2-(2,4-Difluorobenzyl)-1-(1*H*-imidazol-1-ylmethyl)cyclopentanol (5d) According to a procedure similar to that described for the preparation of **5a** and **5b**, **4d** (1.67 g, 7.5 mmol, 1.0 eq) was heated at 70 °C overnight. The usual workup and recrystallization from ethyl acetate–*n*-hexane afforded **5d** (0.61 g, 28.0%). mp 134—136 °C. ^{1}H-NMR ($CDCl_3$): δ 1.58 (3H, m), 1.71 (1H, m), 1.79 (2H, m), 1.92 (1H, m), 2.58 (1H, dd, $J_1=13.7$ Hz, $J_2=10.0$ Hz), 2.78 (1H, dd, $J_1=13.7$ Hz, $J_2=4.6$ Hz), 3.86 (1H, d, $J=14.2$ Hz), 4.04 (1H, d, $J=14.2$ Hz), 6.88 (2H, m), 6.97 (1H, s), 7.00 (1H, s), 7.15 (1H, m), 7.52 (1H, s). IR (KBr): 3200 (s), 1618 (m), 1601 (s), 1510 (s) cm^{-1}. MS *m/z*: 292 (M^+). *Anal.* ($C_{16}H_{18}F_2N_2O$) C, H, N: Calcd 65.7, 6.2, 9.6. Found 65.6, 6.2, 9.7.

Methyl 2,2-Ethylenedioxycyclopentanecarboxylate (6) A mixture of methyl-2-oxocyclopentane carboxylate (**1**) (63.9 g, 0.45 mmol, 1.0 eq), ethylene glycol (280 g, 4.5 mmol, 10.0 eq), *p*-toluenesulfonic acid·H_2O (0.86 g, 4.5 mmol, 0.01 eq) and benzene was heated to reflux. The water azeotrope was collected by a Dean–Stark trap. The benzene layer was separated and dried over Na_2SO_4. After concentration of the solvent, the residue was purified by vacuum distillation (72 °C, 3 mmHg) to give **6** (78.3 g, 93.5%) as a clear oil. ^{1}H-NMR ($CDCl_3$): δ 1.65 (1H, m), 1.82 (2H, m), 2.11 (3H, m), 2.92 (1H, t, $J=7.33$ Hz), 3.70 (3H, s), 3.90 (4H, m). IR (neat): 1730 (s) cm^{-1}.

1,1-Ethylenedioxy-2-hydroxymethylcyclopentane (7) To a stirred suspension of lithium aluminum hydride (0.78 g, 20.5 mmol, 1.2 eq) in dry tetrahydrofuran (THF) (20 ml) was added **6** (3.18 g, 17.1 mmol, 1.0 eq) at 0 °C. The mixture was stirred at room temperature overnight, quenched by addition to ice water, and filtered through celite 535. The filtrate was washed with brine, dried over Na_2SO_4, and concentrated to give crude **7** (2.43 g, 89.9%). ^{1}H-NMR ($CDCl_3$): δ 1.54—1.77 (5H, m), 1.79—1.87 (1H, m), 2.12—2.17 (1H, m), 3.61—3.71 (2H, m), 3.90—3.99 (4H, m). IR (neat): 3500 cm^{-1}.

2-(1*H*-Imidazol-1-ylmethyl)cyclopentane Ethylene Ketal (8) Methanesulfonyl chloride (3.43 g, 30 mmol, 1.2 eq) was added dropwise to a stirred solution of **7** (3.95 g, 25 mmol, 1.0 eq) and triethylamine (3.28 g, 32.5 mmol, 1.3 eq) in CH_2Cl_2 at −15 °C. The mixture was stirred at −15 °C for 0.5 h and washed with brine. The organic layer was separated and dried over Na_2SO_4. After the vaporization of the solvent, 2-methanesulfonyloxymethyl cyclopentane ethylene ketal was obtained. A mixture of the 2-methanesulfonyl compound (4.7 g, 19.9 mmol, 1.0 eq), sodium imidazolate (1.75 g, 19.5 mmol, 0.98 eq) and DMF (23.7 ml) was heated to 90 °C under argon for 1.0 h. The reaction mixture was poured into ice water and extracted with dichloromethane. The organic layer was washed with distilled water, dried over Na_2SO_4 and concentrated to give crude 8, which was purified by silica gel column chromatography using ethyl acetate to give **8** (3.66 g, 70%). ^{1}H-NMR ($CDCl_3$): δ 1.05—2.05 (6H, m), 2.05—2.75 (1H, m), 3.18 (1H, dd, $J_1=14.0$ Hz, $J_2=6.2$ Hz), 3.83 (4H, d, $J=1.4$ Hz), 4.15 (1H, dd, $J_1=14.0$ Hz, $J_2=6.2$ Hz), 6.94 (1H, d), 7.04 (1H, s), 7.48 (1H, br s). IR (neat): 1510 cm^{-1}.

2-(1*H*-Imidazol-1-ylmethyl)cyclopentanone (9) A mixture of **8** (3.56 g, 17.1 mmol, 1.0 eq) and 2 N HCl (17.8 ml) was heated to 60 °C under argon for 4.0 h and then cooled to room temperature. The mixture was neutralized with 1 N potassium hydroxide and extracted with dichloromethane. The organic layer was washed with distilled water, dried over Na_2SO_4, and concentrated to give crude **9**, which was purified by silica gel column chromatography using chloroform–methanol (15 : 1) to give **9** (2.54 g, 90.5%) as an oil. ^{1}H-NMR ($CDCl_3$): δ 1.07—2.73 (7H, m), 4.05 (1H, s), 4.15 (1H, s), 6.78 (1H, d, $J=1.6$ Hz), 6.92 (1H, s), 7.33 (1H, br). IR (neat): 1740 cm^{-1}.

1-(4-Chlorobenzyl)-*cis*-2-(1*H*-imidazol-1-ylmethyl)cyclopentanol (10a) and (11a) To a stirred suspension of magnesium (1.48 g, 60.9 mmol, 2.0 eq) in dry ether (15 ml) was added dropwise a solution of 4-chlorobenzyl bromide (12.5 g, 60.9 mmol, 2.0 eq) in ether (40 ml) over 25 min. The ether solution became cloudy and was refluxed. After 2 h, a solution of **9** (5.0 g, 30.5 mmol, 1.0 eq) in dry THF (50 ml) was added dropwise over 10 min. The reaction mixture was stirred at room temperature under argon for 2 h, quenched with 1 N aqueous HCl solution, and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to dryness to afford crude material (8.83 g), which was recrystallized from ethyl acetate–*n*-hexane to give a 1 : 1 diastereomeric mixture of cyclopentanol (5.19 g, 58.7%) **10a** and **11a** (1 : 1). The diastereomers (2.0 g) were separated by flash column chromatography (LiChroprep, Si 60, 270 g) using *n*-hexane–acetone (1 : 1) to give **10a** (1.01 g) and **11a** (0.70 g); **10a**; colorless crystals; mp 150—151.5 °C. ^{1}H-NMR ($CDCl_3$): δ 1.47 (1H, m), 1.58 (2H, m), 1.80 (3H, m), 2.11 (1H, m), 2.62 (2H, s), 3.92 (1H, dd, $J_1=13.8$ Hz, $J_2=8.7$ Hz), 4.16 (1H, dd, $J_1=13.8$ Hz, $J_2=6.2$ Hz), 6.93 (1H, s), 7.04 (1H, s), 7.12 (2H, d, $J=8.2$ Hz), 7.28 (2H, d, $J=8.2$ Hz), 7.50 (1H, s). IR (KBr): 1525 (s) cm^{-1}. MS *m/z*: 290 (M^+). *Anal.* ($C_{16}H_{19}ClN_2O$) C, H, N: Calcd 66.1, 6.6, 9.6. Found 66.1, 6.5, 9.5; **11a**; colorless crystals; mp 155—157 °C. ^{1}H-NMR ($CDCl_3$): δ 1.42 (2H, m), 1.73 (3H, m), 1.93 (1H, m), 2.37 (1H, m), 2.67 (1H, d, $J=13.3$ Hz), 2.82 (1H, d, $J=13.3$ Hz), 3.73 (1H, dd, $J_1=13.5$ Hz, $J_2=11.2$ Hz), 4.30 (1H, dd, $J_1=13.5$ Hz, $J_2=4.4$ Hz), 6.93 (1H, s), 7.05 (1H, s), 6.99 (2H, d, $J=8.3$ Hz), 7.30 (2H, d, $J=8.3$ Hz), 7.46 (1H, s). IR (KBr): 1502 (s) cm^{-1}. MS *m/z*: 290 (M^+). *Anal.* ($C_{16}H_{19}ClN_2O$) C, H, N: Calcd 66.1, 6.6, 9.6. Found 66.1, 6.4, 9.5.

Optical Resolution of 10a A 400 mg portion of racemic mixture **10a** was eluted from a preparative optical active column (Chiralcel OD 20 × 250 nm 10 mm, Daicel Chemical Industries, Tokyo, Japan) using isopropanol–*n*-hexane (3 : 7). The first enantiomer eluted (−)**10a** which weighed 170 mg. Recrystallization from methanol–water gave colorless prisms (−)**10a** (82.3 mg); mp 172—173 °C; $[\alpha]_D^{25}$: −31.3° ($c=0.57$, methanol). A second elution gave (+)**10a** which weighed 170 mg. Recrystallization from methanol–water afforded colorless prisms (+)**10a** (76.8 mg); mp 171—173 °C; $[\alpha]_D^{25}$: 32.2° ($c=0.57$, methanol).

X-Ray Analysis of (−) 10a A crystal of dimensions 0.25 × 0.25 × 0.25 mm was selected for analysis. Crystal data: $C_{16}H_{19}ClN_2O$; FW = 290.81; colorless; prismatic; orthorhombic; space group: $P2_12_12_1$ (#19), $a=8.958(1)$ Å, $b=29.310(2)$ Å, $c=5.823(2)$ Å, $V=1528.9(4)$ Å^3, $z=4$, $D_{calc}=1.21$ g/cm^3, $\mu(CuK_\alpha)=21.59$ cm^{-1}. The cell constants and the orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 20 carefully centered reflections. The structure was solved by direct methods (SAP191).[18] All hydrogen atoms were refined isotropically. The structure was refined by full-matrix least-squares refinement with anisotropic thermal parameters to a final *R* value of 0.035.

1-Benzyl-*cis*-2-(1*H*-imidazol-1-ylmethyl)cyclopentanol (10b) Following a procedure similar to that described above, the reaction of **9** with benzyl magnesium bromide afforded **10b** (98.7 mg); mp 94—98 °C. ^{1}H-NMR ($CDCl_3$): δ 1.42—1.63 (4H, m), 1.73—1.82 (3H, m), 1.83—1.92 (1H, m), 2.09—2.15 (1H, m), 2.69 (2H, s), 3.90 (1H, dd, $J_1=13.74$ Hz, $J_2=8.70$ Hz), 4.12 (1H, dd, $J_1=13.74$ Hz, $J_2=5.95$ Hz), 6.92 (1H, s), 7.04 (1H, s), 7.19—7.20 (2H, m), 7.28—7.35 (3H, m), 7.49 (1H, s). IR (KBr): 1520 (s) cm^{-1}. *Anal.* ($C_{16}H_{20}N_2O$) C, H, N: Calcd 74.5, 7.9, 10.9. Found 74.3, 7.6, 10.8.

1-(4-Chlorophenyl)-*cis*-2-(1*H*-imidazol-1-ylmethyl)cyclopentanol (10c) Following a procedure similar to that described above, the reaction of **9** (0.82 g, 5 mmol, 1.0 eq) with 4-chlorophenyl magnesium bromide afforded **10c** (486 mg, 42.3%); mp 128—131 °C. ^{1}H-NMR ($CDCl_3$): δ 1.68—2.00 (5H, m), 2.23—2.29 (1H, m), 2.33—2.39 (1H, m), 3.86—3.94 (2H, m), 6.69 (1H, s), 6.90 (1H, s), 7.26 (1H, s), 7.31 (1H, d, $J=8.7$ Hz), 7.38 (1H, d, $J=8.7$ Hz). IR (KBr): 1522 (s) cm^{-1}. MS *m/z*: 276 (M^+). *Anal.* ($C_{15}H_{17}ClN_2O$) C, H, N: Calcd 65.1, 6.2, 10.1. Found 65.3, 6.2, 10.2.

1-Phenyl-*cis*-2-(1*H*-imidazol-1-ylmethyl)cyclopentanol (10d) To a stirred solution of 1.8 M phenyllithium solution (1.83 ml, 3.3 mmol, 1.1 eq) in dry THF was added dropwise a solution of **9** (0.49 g, 3 mmol, 1.0 eq) in THF (2 ml) at −78°C. After stirring for 2 h, the mixture was quenched with saturated ammonium chloride solution (3 ml). The reaction mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give crude material **10d** (649 mg) which was purified with silica gel column chromatography using ethyl acetate to give **10d** (260 mg, 35.6%) as a colorless solid; mp 92—95 °C. ^{1}H-NMR ($CDCl_3$): δ 1.77—2.00 (5H, m), 2.26—2.32 (1H, m), 2.39—2.41 (1H, m), 3.92 (2H, m), 6.70 (1H, s), 6.89 (1H, s), 7.29 (1H, s), 7.27 (1H, m), 7.35 (2H, t, J=7.33 Hz), 7.45 (2H, d, J=7.33 Hz). IR (KBr): 1520 (s), 1501 (s) cm^{-1}. MS m/z: 242 (M^+). *Anal.* ($C_{15}H_{18}N_2O$) C, H, N: Calcd 74.4, 7.5, 11.6. Found 74.1, 7.3, 11.5.

Ethyl 3,3-Ethylenedioxy-4-methylpentanoate (13a) A mixture of ethyl 4-methyl-3-oxopentanoate (**12**) (5.43 g, 34.3 mmol, 1.0 eq), ethylene glycol (7.45 g, 120.1 mmol, 3.5 eq) and *p*-toluenesulfonic acid (137.2 mg, 4 mg/mmol) in benzene was heated to reflux for 6 h and the water was collected through a Dean–Stark trap. The reaction mixture was then cooled, neutralized with triethylamine, washed with distilled water and brine, dried over Na_2SO_4, and concentrated to give the crude ketal (**13a**) (6.84 g). This was purified by silica gel column chromatography (100 g, Merck) using *n*-hexane–ethyl acetate (6 : 1). By this procedure, pure **13a** (6.6 g, 95.1%) was obtained. ^{1}H-NMR ($CDCl_3$): δ 0.96 (3H, s), 0.97 (3H, s), 1.27 (3H, m), 2.12 (1H, m), 2.67 (2H, s), 3.96 (2H, m), 4.02 (2H, m), 4.14 (2H, m). IR (neat): 1704 (s) cm^{-1}.

3,3-Ethylenedioxy-4-methyl-1-pentane-1-ol (13b) To a stirred suspension of lithium aluminum hydride (2.3 g, 57.9 mmol, 1.5 eq) in dry THF (50 ml) was added dropwise **13a** (7.8 g, 38.6 mmol, 1.0 eq) at 0 °C under argon. This mixture was stirred for 18 h and poured into ice water. The mixture was filtered through celite 535 and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give crude **13b** (6.30 g), which was purified by silica gel column chromatography (130 g, Merck) using *n*-hexane–ethyl acetate (2 : 1) to give pure **13b** (5.64 g, 91.2%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 0.94 (3H, s), 0.95 (H, s), 1.95 (3H, m), 3.75 (2H, t), 4.00 (4H, m). IR (neat): 3450 cm^{-1}.

3,3-Ethylenedioxy-4-methylpentyl Methanesulfonate (13c) Methanesulfonyl chloride (4.4 g, 38.4 mmol, 1.1 eq) was added dropwise to a stirred solution of **13b** (5.6 g, 34.9 mmol, 1.0 eq) in pyridine (28 ml) at 0 °C. This mixture was stirred at 0 °C for 3 h and treated with 1 N HCl (14 ml) to pH 5. The resulting mixture was extracted with ethyl acetate (140 ml × 2). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give crude material (9.5 g), which was purified by silica gel column chromatography (200 g, Merck) using *n*-hexane–ethylacetate (1 : 1) to give **13c** (7.65 g, 92.0%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 0.94 (3H, s), 0.95 (3H, s), 1.88 (1H, m), 2.13 (2H, t), 3.01 (3H, s), 3.95 (4H, s), 4.33 (2H, t).

3,3-Ethylenedioxy-1-(1*H*-imidazol-1-yl)-4-methylpentane (13d) A mixture of **13c** (7.6 g, 31.7 mmol, 1.0 eq), sodium imidazolate (4.3 g, 47.8 mmol, 1.5 eq) in DMF (46 ml) was heated at 80 °C for 2 h. The reaction mixture was poured into ice cold water (200 ml) and then extracted with ethyl acetate (200 ml × 3), washed with brine and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* afforded crude **13d** (4.96 g, 73.9%), which was purified by silica gel column chromatography (10 g, Merck) using *n*-hexane–ethyl acetate (1 : 1) to give pure **13d** as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 0.94 (3H, s), 0.95 (3H, s), 1.89 (1H, m), 2.13 (2H, t), 3.99 (6H, m), 6.92 (1H, s), 7.05 (1H, s), 7.48 (1H, s).

1-(1*H*-Imidazolyl)-4-methylpentane-3-one (14) A mixture of sulfonic acid (23.3 ml), THF (47 ml), distilled water (23.3 ml) and **13d** (4.9 g, 23.3 mmol) was stirred at room temperature overnight. After neutralization with saturate sodium bicarbonate, the mixture was extracted with ethyl acetate (150 ml × 4). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give **14** (3.49 g, 90.1%) as an oil. ^{1}H-NMR ($CDCl_3$): δ 1.05 (3H, s), 1.06 (3H, s), 2.53 (1H, m), 2.90 (2H, t), 4.25 (2H, t), 6.89 (1H, s), 7.02 (1H, s), 7.47 (1H, s). IR (KBr): 3400 (s), 1715 (s), 1518 (s) cm^{-1}.

3-(4-Chlorobenzyl)-1-(1*H*-imidazolyl)-4-methylpentane-3-ol (15a) To a stirred suspension of magnesium (156 mg, 6 mmol, 2.0 eq) in dry ether (2 ml) was added dropwise 4-chlorobenzylchloride (1.24 g, 6.0 mmol, 2.0 eq) at room temperature. The reaction mixture was stirred for 30 min at room temperature, 15 min at reflux and cooled to 0 °C. To this mixture was added **14** (500 mg, 3 mmol, 1.0 eq). After 30 min, the reaction mixture was allowed to warm to room temperature and stirred overnight. This mixture was quenched by addition of water (5 ml) and extracted with ethyl acetate (20 ml × 2). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give crude **15a** (990 mg), which was purified by silica gel column chromatography (15 g, Merck) using *n*-hexane–ethyl acetate–methanol (1 : 1 : 0.3) to give pure **15a** (557 mg, 71.6%); mp 161.5—162 °C. ^{1}H-NMR ($CDCl_3$): δ 0.98 (3H, d), 1.04 (3H, d), 1.78 (2H, m), 2.00 (1H, m), 2.66 (1H, d), 2.86 (1H, d), 3.98 (1H, m), 4.05 (1H, m), 6.85 (1H, s), 7.03 (1H, s), 7.15 (2H, d), 7.30 (2H, d), 7.44 (1H, s). IR (KBr): 3240 (s), 1510 (s), 1495 (s) cm^{-1}. MS m/z: 292 (M^+). *Anal.* ($C_{16}H_{21}ClN_2O$) N: Calcd 9.6. Found 9.4.

3-(4-Chlorophenyl)-1-(1*H*-imidazolyl)-4-methylpentane-3-ol (15b) To a stirred suspension of bromochlorobenzene (201.8 mg, 1.05 mmol, 1.2 eq) in dry THF was added *n*-butyllithium solution (0.53 ml, 0.97 mmol, 1.1 eq) in *n*-hexane at −78 °C. This mixture was stirred at −78 °C for 1.0 h. To this mixture **14** (146 mg, 0.88 mmol, 1.0 eq) was added dropwise at −78 °C. This mixture was stirred at −78 °C for 2 h, quenched with ammonium chloride solution (2 ml) at −78 °C, and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to dryness to give crude **15b** (204.3 mg) which was purified by silica gel column chromatography (15 g, Merck) using *n*-hexane–ethyl acetate–methanol (5 : 10 : 3) to give **15b** (165.6 mg, 78.3%) as a crystal mass. ^{1}H-NMR ($CDCl_3$): δ 0.69 (3H, d, J=7.87 Hz), 0.99 (3H, d, J=7.87 Hz), 1.63 (1H, br, OH), 2.04 (1H, q, J=7.89 Hz), 2.23 (1H, m), 2.33 (1H, m), 3.50 (1H, m), 3.95 (1H, d), 6.79 (1H, s), 7.00 (1H, s), 7.26 (1H, s), 7.32 (2H, d, J=8.71 Hz), 7.36 (2H, d, J=8.71 Hz). IR (KBr): 3200, 2975, 1520, 1500 cm^{-1}. MS m/z: 242 (M^+). *Anal.* ($C_{15}H_{19}ClN_2O$) N: Calcd. 10.0. Found 10.2.

Formestane, Androstenedione and Fadrozole Formestane (androst-4-ene-3,17-dione, 4-hydroxy) and androstenedione (androst-4-ene-3,17-dione) were purchased from Sigma (St. Louis MO). Fadrozole was synthesized according to the method of Browne *et al.*[19] in our laboratory.

Aromatase Inhibitory Activity Test compounds were dissolved in dimethylsulfoxide (DMSO) (Kishida Kagaku, Tokyo, Japan) and further dilution was made with 10% DMSO (v/v) in 67 mM phosphate buffer (pH 7.2). Final DMSO concentrations during the experiments were equal or less than 0.1% (v/v).

The experiments were conducted according to the method of Covey *et al.*[20] with a slight modification. Briefly, human placental microsomes (0.1 mg protein) were incubated in the presence of 67 mM phosphate buffer (pH 7.2) with 1×10^{-6} M, 2 kBq/ml [19-^{14}C]androst-4-ene-3,17-dione (Du Pont, Wilmington, DE) and 2×10^{-3} M NADPH (Sigma, St. Louis, MO) in a total reaction volume of 0.5 ml. The incubation mixture also contained test compounds and an NADPH regenerating system (4×10^{-3} M glucose-6-phosphate (Sigma, St. Louis, MO) and 4 units/ml glucose-6-phosphate dehydrogenase (Oriental Yeast, Tokyo, Japan)). Then the reaction was allowed to proceed for 30 min at 37 °C and was terminated by the addition of 5 ml of ice cold chloroform. The mixture was agitated in a vortex mixer for 45 s and centrifuged at 700 ***g*** for 5 min. After centrifugation, 0.1 ml of the aqueous phase was transferred to a scintillation vial, and the radioactivity was quantified by liquid scintillation spectrometry. Thus the aromatase activities of the mixtures were radiometrically determined.

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