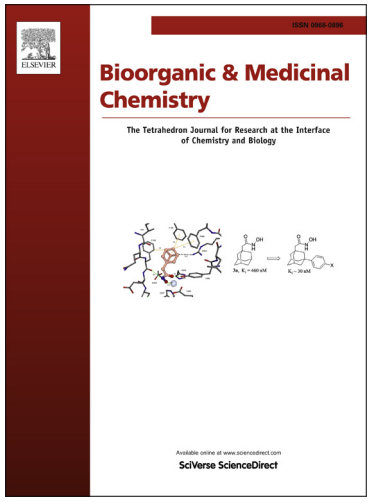

Discovery of 2-methyl-1-{1-[(5-methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}propan-2-ol: A novel, potent and selective type 5 17β-hydroxysteroid dehydrogenase inhibitor

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Discovery of 2-methyl-1-{1-[(5-methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}propan-2-ol: A novel, potent and selective type 5 17β-hydroxysteroid dehydrogenase inhibitor

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Abstract

Type 5 17β-hydroxysteroid dehydrogenase (17β-HSD5), also known as aldo-keto reductase 1C3 (AKR1C3), is a member of the aldo-keto reductase superfamily of enzymes and is expressed in the human prostate. One of the main functions of 17β-HSD5 is to catalyze the conversion of the weak androgen, androstenedione, to the potent androgen, testosterone. The concentration of intraprostatic 5α-dihydrotestosterone (DHT) in patients following chemical or surgical castration has been reported to remain as high as 39% of that of healthy men, with 17β-HSD5 shown to be involved in this androgen synthesis. Inhibition of 17β-HSD5 therefore represents a promising target for the treatment of castration-resistant prostate cancer (CRPC). To investigate this, we conducted high-throughput screening (HTS) and identified compound **2,** which displayed a structure distinct from known 17β-HSD5 inhibitors. To optimize the inhibitory activity of compound **2**, we first introduced a primary alcohol group. We then converted the primary alcohol group to a tertiary alcohol, which further enhanced the inhibitory activity, improved metabolic stability, and led to the identification of compound **17**. Oral administration of compound **17** to castrated nude mice bearing the CWR22R xenograft resulted in the suppression of androstenedione (AD)-induced intratumoral testosterone production. Compound **17** also demonstrated good isoform selectivity, minimal inhibitory activity against either CYP or hERG, and enhanced pharmacokinetic and physicochemical properties.

Key words

Type 5 17β-hydroxysteroid dehydrogenase (17β-HSD5); Aldo-keto reductase 1C3 (AKR1C3);

Castration-resistant prostate cancer (CRPC); Testosterone

1. Introduction

Prostate cancer (PC) was the most frequently diagnosed form of cancer and the second-most frequent cause of mortality reported in male patients between 2005-2009.[1] The advancement of prostate cancer is dependent on androgen, testosterone (T) and 5α-dihydrotestosterone (DHT). Therefore, hormone therapy such as surgical castration or administration of gonadotropin releasing hormone (GnRH) agonist is major therapeutic method. However, prostate cancer can develop into castration-resistant prostate cancer (CRPC), and the effect of hormone therapy declines progressively over time, positioning CRPC as the most significant obstacle at present in the effective treatment of prostate cancer. In this regard, the inhibition of the androgen biosynthesis route in the adrenal and prostate glands represents a potential therapeutic target.

Abiraterone acetate,[2] a 17α-hydroxylase/$C_{17,20}$-lyase inhibitor, has been approved for the treatment of CRPC in the US since April 2011. Abiraterone acetate works via the inhibition of dehydroepiandrosterone (DHEA) production, a precursor of testosterone, in the adrenal gland.[3] This approach has proven that inhibition of the androgen biosynthesis route involved with the adrenal gland is effective against CRPC. However, as abiraterone acetate is involved in biosynthesis of several steroids, such as 17OH-pregnenolone, and also acts as an inhibitor of upstream androgen biosynthesis,[4] this treatment could potentially cause side effects, such as increasing levels of mineralocorticoid, which requires concomitant use of prednisone (Figure 1).

Type 5 17β-hydroxysteroid dehydrogenase (17β-HSD5), also known as aldo-keto reductase 1C3 (AKR1C3), is one of the enzymes involved in androgen biosynthesis.[5] 17β-HSD5 is expressed in the human prostate, with elevated levels of expression reported in prostate cancer.[6] 17β-HSD5 plays an essential role in producing testosterone from DHEA secreted from the adrenal gland and in transforming androstenedione (AD), a weak androgen, to testosterone, a potent androgen (Figure 1).[5] As 17β-HSD5 is involved in downstream androgen biosynthesis, compared to 17α-hydroxylase/$C_{17,20}$-lyase, inhibition of 17β-HSD5 is expected to avoid putative side effects of abiraterone acetate, bypassing the need for combination therapy with prednisone. From this point of view, the inhibition of 17β-HSD5 is an attractive target for the treatment of CRPC, with 17β-HSD5 inhibitors having the potential to be effective and safe therapeutics for the treatment of CRPC.

Surgical or chemical castration has been reported to reduce serum testosterone levels by 97%.[7] In contrast, the concentration of intraprostatic DHT is only reduced by 61%, compared to healthy men. Since DHT is produced by 5α-reductase from testosterone in the prostate, testosterone should exist in the prostate despite incorporation of testosterone from blood scarcely contributes to intraprostatic testosterone concentration after castration. This is because testosterone can be synthesized through two steps from DHEA which is secreted from the adrenal gland and incorporated into the prostate, and 17β-HSD5 is involved in one of the two steps. These facts suggest that 17β-HSD5 would have about half of contribution to intraprostatic testosterone concentration and therefore would have a large impact on prostate cancer growth.

Two closely related isoforms of 17β-HSD5 have been identified that play a role in steroid biosynthesis: AKR1C1 and AKR1C2.[8] As both isoforms are involved in DHT metabolism, their inhibition may lead to the accumulation of DHT in the prostate and thereby induce the development or progression of prostate cancer.[9,10] Therefore, 17β-HSD5 inhibitors with no inhibitory activity against both AKR1C1 and AKR1C2 are desirable for the treatment of prostate cancer. We therefore conducted extensive research to identify novel, potent, isoform-selective, orally bioavailable, and nonsteroidal 17β-HSD5 inhibitors.

Previous reports have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs), such as fulfenamic acid (**1**), exert inhibitory activity against 17β-HSD5 (Figure 2).[11] In addition, some NSAIDs derivatives have also been reported to show selectivity for 17β-HSD5 versus COX-1,2, as well as AKR isoforms in their inhibitory activity.[12,13]

Here, to identify a compound that inhibits 17β-HSD5, we conducted high-throughput screening (HTS) using a 17β-HSD5 enzyme assay. We describe the discovery of novel structural class of 17β-HSD5 inhibitors.

2. Chemistry

Compounds **3**-**18a** were synthesized, except where noted, by an amidation reaction between indole-2-carboxylic acid **19** or **20** and the corresponding amine (Scheme 1). Compounds **8** and **10** were synthesized by an amidation reaction between, indole-2-carboxylic acid **19** and the corresponding amine,

followed by reduction of the ester moiety with $LiBH_4$ (Scheme 2). Compound **11,** an azetidine derivative, was synthesized by first reacting tert-butyl 3-(2-ethoxy-2-oxoethylidene)azetidine-1-carboxylate[14] (**11a**) under an acidic condition to remove the tert-butoxycarbonyl (Boc)-group and then conducting an amidation reaction with indole-2-carboxylic acid **19** to generate ester **11b,** which was reduced with $LiBH_4$, and hydrogenated (Scheme 3). Compound **15**, a secondary alcohol derivative, was synthesized by first reacting aldehyde **15a** with methyl magnesium bromide to generate secondary alcohol **15b**. The Boc-group on secondary alcohol **15b** was then deprotected and subjected to an amidation reaction with indole-2-carboxylic acid **19** (Scheme 4). Compound **18**, a carboxylic acid derivative, was synthesized by hydrolyzing ester **18a** with sodium hydroxide (Scheme 5). Amine **23** was synthesized by first reacting benzyl 4-(2-ethoxy-2-oxoethyl)piperidine-1-carboxylate[15] (**21**) with methyl magnesium bromide to generate tertiary alcohol **22**, followed by deprotecting the benzyloxycarbonyl group on tertiary alcohol **22** by hydrogenation (Scheme 6). Amine **26** was synthesized by first reacting ketone **24** with ethyl (diethoxyphosphoryl) acetate and then hydrogenated to generate the ester **25**, followed by deprotecting the Boc-group on ester **25** by hydrochloric acid (Scheme 6).

3. Results and Discussion

For the evaluation of the synthesized compounds, we conducted a cell assay that detects testosterone converted from androstenedione in 17β-HSD5 expressing HEK293 cells.[16] Using HTS approach based on a 17β-HSD5 enzyme assay for in-house chemical library, we identified compound **2** (Figure 3), which

exhibited both inhibitory activity against 17β-HSD5, and a unique structure from known 17β-HSD5 inhibitors. We evaluated the inhibitory activity of compound **2** against 17β-HSD5 in a HEK293 cell assay and observed an IC_{50} value of 2800 nM. Based on structure-activity relationship (SAR) data obtained from HTS results and subsequent our preliminary SAR study data, we introduced a primary alcohol group to compound **2**, which generated compound **3.** We then evaluated the inhibitory activity of compound **3**, against 17β-HSD5, and noted an IC_{50} value of 37 nM. The potent inhibitory activity and the structural novelty of compound **3** prompted us to carry out further structural modifications, in order to obtain compounds with an enhanced pharmacological profile.

We first modified the carbon chain length of the 2-hydroxyethyl moiety attached to the piperidine ring in compound **3** (Table 1), observing a significant reduction in inhibitory activity on either shortening or lengthening the carbon chain of compound **3**, which generated compounds **4** and **5**, respectively. This phenomenon suggests that carbon chain length may represent an important factor in exerting potent inhibitory activity. Substitution of the piperidine ring of compound **3** with a piperazine ring, which generated compound **6**, also resulted in a considerable decrease in inhibitory activity (IC_{50} = 770 nM), as did insertion of a basic nitrogen atom on the ring.

The removal of the methyl group on the indole ring of compound **3** yielded compound **7** and brought about an 8-fold improvement of the inhibitory activity with an IC_{50} value of 4.5 nM. We therefore conducted further modifications of the 4-(2-hydroxyethyl) piperidine moiety based on compound **7**. Compounds **8** and **9**, with 2-hydroxyethyl groups on the 3- or 2-position of the piperidine, respectively,

showed considerably weaker inhibitory activities than compound **7** (respective IC_{50} = 2100 and 230 nM). The pyrrolidine analogue **10** and azetidine analogue **11** also showed significant reductions in inhibitory activity compared with compound **7** (respective IC_{50} = 1100 and 140 nM). Taken together, these results indicate that modifying the position at which the 2-hydroxyethyl group is attached or reducing the ring size of the piperidine moiety leads to a significant attenuation of inhibitory activity. Further, compound **12**[17], a linear alcohol derivative with the same number of carbon atoms between the amide moiety and the hydroxyl group as compound **7**, showed weaker inhibitory activity than compound **7** (IC_{50} = 1200 nM), suggesting that a sterically-restricted structure, such as a piperidine ring, might be essential for the exertion of potent inhibitory activity.

As our modification of the 4-(2-hydroxyethyl)piperidine moiety showed, the 4-(2-hydroxyethyl)piperidine structure is optimal for exerting inhibitory activity. We therefore conducted further modifications on the terminal hydroxyl moiety in an attempt to improve its metabolic stability, as primary alcohols are known to be vulnerable to conjugative or oxidative metabolism (Table 2). Compound **13**, a methyl ether, showed relatively good inhibitory activity (IC_{50} = 33 nM), although the potency was less than that of compound **7**, possibly due to the proton of the hydroxyl moiety of **7** not acting as a hydrogen bond donor but rather as an acceptor when binding to the enzyme. Compound **14**, an acetyl derivative, showed rather potent inhibitory activity (IC_{50} = 13 nM), which seems to support our expectation that the existence of a hydrogen bond acceptor is preferable in attempting to achieve potent inhibitory activity. While compound **15**, a secondary alcohol derivative, displayed even more potent

inhibitory activity (IC_{50} = 12 nM), compound **16**, a tertiary alcohol derivative, showed 2-fold more potent inhibitory activity than compound **7** (IC_{50} = 2.1 nM). Similarly, compound **17**, the 5-methyl indole analogue of compound **16**, exerted 13-fold more potent inhibitory activity than compound **7** (IC_{50} = 0.34 nM). In contrast, Compound **18**, a carboxylic acid derivative, showed little inhibitory activity.

Both compounds **3** and **17** were tested for their inhibitory activity against testosterone conversion from androstenedione using the human PC cell line CWR22R, which highly expresses 17β-HSD5 (Table 3). Compound **17** showed more potent inhibitory activity (IC_{50} = 1.9 nM) than compound **3** (IC_{50} = 58 nM), which is consistent with the results from the HEK293 cell assay.

Since compounds **3**, **16**, and **17** showed good membrane permeability and good solubility, the plasma concentrations were evaluated after the oral administration of 30 mg/kg of each compound to mice (Table 3). Compound **3** was not detected in the plasma 1 h post-administration; however, the corresponding carboxylic acid derivative **18** was detected. As we expected, compounds **16** and **17**, which are both tertiary alcohol derivatives, were detected in the plasma at 1 h post-administration. Further the plasma concentration of compound **17** was 4-fold higher than that of compound **16** at 1 h post-administration and was detected in the plasma as late as 4 h post-administration, indicating that metabolic stability of compound **17** is improved by the presence of a methyl group at the 5-position of the indole ring.

Examination of the pharmacokinetic properties of compound **17** in rats showed the compound to have good oral bioavailability (F = 65%) at a dose of 1 mg/kg in rats (Table 4). The half-life of compound **17** was 0.46 h after intravenous administration. The maximum drug concentration was observed 0.5 h

after oral administration, demonstrating that compound **17** was promptly absorbed from the intestine and transported into the blood.

The inhibitory effect of compound **17** on AD-induced intratumoral testosterone production in prostate cancer was evaluated in castrated nude mice bearing the CWR22R xenograft (Figure 4).[18] Compound **17** orally administered at 1, 3, and 10 mg/kg inhibited testosterone production in a dose-dependent manner. In addition, compound **17** exerted a complete suppression of testosterone production when orally administered at 10 mg/kg.

Evaluation of the AKR1C2 inhibitory activity of compound **17** showed up to 800-fold selectivity against AKR1C2 (Table 5). Since AKR1C1 and AKR1C2 differ by only a single amino acid at their respective active sites, an inhibitor of AKR1C2 could potentially also act to inhibit AKR1C1.[11,12] Compound **17** was also found to be clean with regard to various CYP enzyme inhibitory activities up to 25 μM and demonstrated minimal hERG inhibition up to 100 μM (Table 5).

4. Conclusion

In summary, we conducted SAR studies to identify novel, potent, selective, and orally bioavailable 17β-HSD5 inhibitors. We started our SAR studies with compound **2**, an indole derivative discovered using HTS, and successfully enhanced its inhibitory activity against 17β-HSD5 by introducing a hydroxyl group. Further examination led to an improvement in both inhibitory activity and metabolic stability by substitution with a tertiary alcohol, generating compound **17**, which displayed potent inhibitory activity in

the PC cell line CWR22R and good isoform selectivity, and suppressed AD-induced intratumoral testosterone production when orally administered to castrated nude mice bearing the CWR22R xenograft. Further, compound **17** demonstrated minimal inhibitory activity against either CYP or hERG and therefore is expected not to have a high risk of drug-drug interactions or unfavorable cardiovascular effects. In addition, the pharmacokinetic properties and the physicochemical properties of compound **17** were preferable. The results of further studies on this compound will be reported in due course.

5. Experimental

Chemistry

1H NMR spectra were recorded on a JEOL JMN-EX-400, and the chemical shifts were expressed in δ (ppm) values with trimethylsilane as an internal reference (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). Mass spectra (MS) were recorded on a JEOL LX-200 or Thermo Electron LQC Advantage or Thermo Electron TRACE DSQ spectrometer. Elemental analyses were performed with Yanaco MT-6 (C, H, N) or Elementar Vario EL III (C, H, N) instruments, and results were within ±0.4% of theoretical values.

2-{1-[(5-Methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}ethanol (3)

To a solution of 5-methyl-1H-indole-2-carboxylic acid (**20**) (263 mg, 1.50 mmol) in THF (7 mL) were added 2-(piperidin-4-yl)ethanol (213 mg, 1.65 mmol), 1-hydroxybenzotriazole (HOBt, 101 mg, 0.750 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) hydrochloride (316 mg, 1.65 mmol), followed by stirring at room temperature for 14 h. The reaction mixture was partitioned between ethyl acetate and 0.5 M aqueous hydrochloric acid. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated in vacuo. The residue was recrystallized from ethyl acetate/acetonitrile (5 mL/2 mL) to give **3** (365 mg, 85.0%) as a beige powder. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.11-1.19 (2H, m), 1.38-1.43 (2H, m), 1.73-1.76 (3H, m), 2.36 (3H, s), 2.98 (2H, s), 3.45-3.49 (2H, m), 4.37-4.44 (3H, m), 6.63 (1H, m), 7.00 (1H, m), 7.29 (1H, m), 7.36 (1H, s), 11.38 (1H, s). EI-MS: *m/z* 286 $[M]^+$. Anal. Calcd for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.10, H, 7.87; N, 9.67.

1-[(5-Methyl-1H-indol-2-yl)carbonyl]piperidin-4-ylmethanol (4)

Compound **4** was prepared in 73% yield from piperidin-4-ylmethanol using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.08-1.20 (2H, m), 1.65-1.78 (3H, m), 2.36 (3H, s), 2.99 (2H, m), 3.29 (2H, m), 4.45 (2H, m), 4.52 (1H, m), 6.63 (1H, s), 7.00 (1H, d, J = 8.4 Hz), 7.29 (1H, d, J = 8.4 Hz), 7.36 (1H, s), 11.39 (1H, s). EI-MS: *m/z* 272 $[M]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.42; H, 7.41; N, 10.19.

3-{1-[(5-Methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}propanol (5)

Compound **5** was prepared in 73% yield from 3-(piperidin-4-yl)propan-1-ol using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.03-1.16 (2H, m), 1.22-1.29 (2H, m), 1.41-1.49 (2H, m), 1.54 (1H, m), 1.75 (2H, m), 2.36 (3H, s), 2.98 (2H, m), 3.39 (2H, m), 4.38 (1H, m), 4.43 (2H, m), 6.63 (1H, m), 7.00 (1H, m), 7.29 (1H, m), 7.36 (1H, s), 11.39 (1H, s). FAB-MS: *m/z* 301 $[M+H]^+$. Anal. Calcd for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.78; H, 8.08; N, 9.35.

2-{1-[(5-Methyl-1H-indol-2-yl)carbonyl]piperazin-4-yl}ethanol (6)

Compound **6** was prepared in 47% yield from 2-(piperazin-1-yl)ethanol using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 2.36 (3H, s), 2.43 (2H, m), 2.46-2.51 (4H, m), 3.53 (2H, m), 3.73 (4H, m), 4.45 (1H, m), 6.67 (1H, m), 7.01 (1H, m), 7.30 (1H, m), 7.36 (1H, s), 11.42 (1H, s). FAB-MS: *m/z* 288 $[M+H]^+$. Anal. Calcd for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.75; 7.47; N, 14.87.

2-[1-(1H-Indol-2-ylcarbonyl)piperidin-4-yl]ethanol (7)

Compound **7** was prepared in 85% yield from 1H-indole-2-carboxylic acid (**19**) using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.07-1.20 (2H, m), 1.41 (2H, m), 1.67-1.79 (3H, m), 2.99 (2H, m), 3.47 (2H, m), 4.39 (1H, m), 4.43 (2H, m), 6.73 (1H, m), 7.03 (1H, m), 7.17 (1H, m),

7.41 (1H, m), 7.59 (1H, m), 11.52 (1H, s). EI-MS: *m/z* 272 $[M]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.54; H, 7.48; H, 10.28.

2-[1-(1H-Indol-2-ylcarbonyl)piperidin-3-yl]ethanol (8)

To a solution of 1H-indole-2-carboxylic acid (**19**) (500 mg, 3.10 mmol) and ethyl piperidin-3-ylacetate (530 mg, 3.10 mmol) in DMF (8 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) hydrochloride (610 mg, 3.18 mmol) and 1-hydroxybenzotriazole (HOBt, 430 mg, 3.18 mmol), followed by stirring at room temperature for 2 h. 0.5 M aqueous hydrochloric acid was added, followed by extraction with ethyl acetate. The organic layer was washed with 0.5 M aqueous sodium hydroxide solution and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give ethyl [1-(1H-indol-2-ylcarbonyl)piperidin-3-yl]acetate (519 mg, 53%). To a solution of the resulting ethyl [1-(1H-indol-2-ylcarbonyl)piperidin-3-yl]acetate (392 mg, 1.25 mmol) in THF (7 mL) was added lithium borohydride (30 mg, 1.38 mmol), followed by stirring at room temperature for 24 h. Water was added to the reaction liquid, followed by extraction with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography [hexane:ethyl acetate = 1:0-2:1], and then crystallized from diisopropyl ether to give **8** (92 mg, 27%) as a white solid. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.24 (1H, m), 1.32-1.52 (3H, m), 1.63-1.76 (2H, m), 1.87 (1H, m), 2.70-3.21 (2H, m), 3.46 (2H, m), 4.31 (2H, m), 4.43 (1H, m), 6.75 (1H, s), 7.03 (1H, dd, J = 7.6,

7.6 Hz), 7.17 (1H, dd, J = 7.6, 7.6 Hz), 7.41 (1H, d, J = 7.6 Hz), 7.60 (1H, d, J = 7.6 Hz), 11.52 (1H, s). FAB-MS: *m/z* 273 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.54; H, 7.33; N, 10.14.

2-[1-(1H-Indol-2-ylcarbonyl)piperidin-2-yl]ethanol (9)

Compound **9** was prepared in 19% yield from 1H-indole-2-carboxylic acid (**19**) and 2-(piperidin-2-yl)ethanol using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.44 (1H, m), 1.55-1.84 (6H, m), 1.96 (1H, m), 3.07 (1H, m), 3.43 (2H, m), 4.30 (1H, m), 4.51 (1H, m), 4.78 (1H, m), 6.79 (1H, s), 7.03 (1H, dd, J = 7.6, 7.6 Hz), 7.16 (1H, dd, J = 7.6, 7.6 Hz), 7.40 (1H, d, J = 7.6 Hz), 7.58 (1H, d, J = 7.6 Hz), 11.52 (1H, s). FAB-MS: *m/z* 273 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.59; H, 7.37 ; N, 10.14.

2-[1-(1H-Indol-2-ylcarbonyl)pyrrolidin-3-yl]ethanol (10)

Compound **10** was prepared in 43% yield from ethyl pyrrolidin-3-ylacetate (**26**) using a similar procedure to that of **8**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.47-1.71 (3H, m), 1.99-2.39 (2H, m), 3.08-4.07 (6H, m), 4.50 (1H, s), 6.95 (1H, s), 7.03 (1H, m), 7.18 (1H, m), 7.44 (1H, m), 7,63 (1H, m), 11.51 (1H, s). FAB-MS: *m/z* 259 $[M+H]^+$. Anal. Calcd for $C_{15}H_{18}N_2O_2.0.1H_2O$: C, 69.26; H, 7.05; N, 10.77. Found: C, 69.13; 6.92; N, 10.67.

Ethyl [1-(1H-indol-2-ylcarbonyl)azetidin-3-ylidene]acetate (11b)

To a solution of tert-butyl 3-(2-ethoxy-2-oxoethylidene)azetidine-1-carboxylate[14] (**11a**, 237 mg, 0.98 mmol) in $CHCl_3$ (4 mL) was added trifluoroacetic acid (0.1 mL, 1.30 mmol), followed by stirring at room temperature for 1 h. 4 M hydrochloric acid/ethyl acetate (2 mL, 8.00 mmol) was added to the mixture, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced

pressure to give the residue. To a solution of the resulting residue in DMF (5 mL) was added 1H-indole-2-carboxylic acid (**19**, 180 mg, 1.12 mmol), 1-hydroxybenzotriazole (HOBt, 160 mg, 1.18 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) hydrochloride (230 mg, 1.20 mmol), and triethylamine (410 uL, 2.94 mmol) at 0 °C, followed by stirring at room temperature for 2 h. The reaction mixture was partitioned between ethyl acetate and 0.5 M aqueous hydrochloric acid. The organic layer was washed with 1 M aqueous sodium hydroxide solution and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give **11b** (96 mg, 34%) as a colorless oil. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.24 (3H, m), 4.15 (2H, m), 4.89 (2H, m), 5.33 (2H, m), 5.98 (1H, s), 6.89 (1H, m), 7.06 (1H, m), 7.21 (1H, m), 7.45 (1H, m), 7.64 (1H, m), 11.67 (1H, s). ESI-MS: *m/z* 285 $[M+H]^+$.

2-[1-(1H-Indol-2-ylcarbonyl)azetidin-3-yl]ethanol (11)

To a solution of ethyl [1-(1H-indol-2-ylcarbonyl)azetidin-3-ylidene]acetate (**11b**, 94 mg, 0.33 mmol) in THF (2 mL) was added lithium borohydride (20 mg, 0.92 mmol), followed by stirring at room temperature for 24 h. Water was added to the reaction liquid, followed by extraction with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give the residue. To a solution of the residue in methanol (2 mL) was added 10% palladium on carbon (wet, contains 50% water, 10 mg), followed by stirring under a hydrogen atmosphere at room temperature for 24 h. Insoluble material was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [chloroform:methanol = 1:0-10:1], and then solidified from diisopropyl ether to give **11** (25 mg, 31%) as a white powder. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.78 (2H, m), 2.80 (1H, m), 3.45 (2H, m), 3.74 (1H, m), 4.17 (2H, m), 4.48 (1H, m), 4.60 (1H, m), 6.79 (1H, m), 7.04 (1H, m), 7.19 (1H, m), 7.43 (1H, m), 7.61 (1H, m), 11.55 (1H, s). ESI-MS: *m/z*

245 $[M+H]^+$. Anal. Calcd for $C_{14}H_{16}N_2O_2.0.1H_2O$: C, 68.33; H, 6.64; N, 11.38. Found: C, 68.28; H, 6.60 ; N, 11.27.

2-[1-(1H-Indol-2-ylcarbonyl)piperidin-4-yl]-1-methoxyethane (13)

Compound **13** was prepared in 5% yield from 1H-indole-2-carboxylic acid (**19**) and 4-(2-methoxyethyl)piperidine using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.15 (2H, m), 1.48 (2H, m), 1.64-1.80 (3H, m), 2.99 (2H, m), 3.23 (3H, s), 3.38 (2H, m), 4.43 (2H, m), 6.73 (1H, m), 7.03 (1H, m), 7.17 (1H, m), 7.40 (1H, m), 7.60 (1H, m), 11.52 (1H, s). FAB-MS: *m/z* 287 $[M+H]^+$. Anal. Calcd for $C_{17}H_{22}N_2O_2.0.1H_2O$: C, 70.85; H, 7.76; N, 9.72. Found: C, 70.77; H, 7.65; N, 9.63.

1-[1-(1H-Indol-2-ylcarbonyl)piperidin-4-yl]acetone (14)

Compound **14** was prepared in 47% yield from 1H-indole-2-carboxylic acid (**19**) and 1-(piperidin-4-yl)acetone[19] using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.15 (2H, m), 1.71 (2H, m), 2.01-2.12 (4H, m), 2.43 (2H, m), 3.03 (2H, m), 4.40 (2H, m), 6.73 (1H, m), 7.03 (1H, m), 7.17 (1H, m), 7.41 (1H, m), 7.59 (1H, m), 11.52 (1H, s). FAB-MS: *m/z* 285 $[M+H]^+$. Anal. Calcd for $C_{17}H_{20}N_2O_2.0.1H_2O$: C, 71.35; H, 7.12; N, 9.79. Found: C, 71.20; H, 7.10; N, 9.80.

tert-Butyl 4-(2-hydroxypropyl)piperidine-1-carboxylate (15b)

To a solution of tert-butyl 4-(2-oxoethyl)piperidine-1-carboxylate (**15a**, 250 mg, 1.10 mmol) in THF (5 mL) was added 1.4 M methyl magnesium bromide in toluene-THF (1.2 mL, 1.68 mmol), followed by stirring at room temperature for 2 h. Water was added to the reaction liquid, followed by extraction with ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography [chloroform:methanol = 1:0-10:1] to give **15b** (236 mg, 88%) as a

colorless oily substance. ^{1}H NMR-$CDCl_3$ (400 MHz) δ 0.99-1.78 (20H, m), 2.70 (2H, m), 3.93 (1H, m), 4.08 (2H, m). FAB-MS: *m/z* 244 $[M+H]^+$.

1-[1-(1H-Indol-2-ylcarbonyl)piperidin-4-yl]propan-2-ol (15)

To a solution of **15b** (233 mg, 0.96 mmol) in ethyl acetate (4 mL) was added 4 M hydrochloric acid/ethyl acetate (3 mL, 12.0 mmol), followed by stirring at room temperature for 2 h. The reaction liquid was concentrated under reduced pressure to give 4-(2-hydroxypropyl)piperidine hydrochloride. To a solution of the 4-(2-hydroxypropyl)piperidine hydrochloride and 1H-indole-2-carboxylic acid (**19**, 155 mg, 0.96 mmol) in DMF (5 mL) were added triethylamine (0.15 ml, 1.08 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) hydrochloride (190 mg, 0.99 mmol) and 1-hydroxybenzotriazole (HOBt, 130 mg, 0.96 mmol), followed by stirring at room temperature for 24 h. 0.5 M aqueous hydrochloric acid was added to the reaction liquid, followed by extraction with ethyl acetate. The organic layer was washed with 0.5 M aqueous sodium hydroxide solution and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography [chloroform:methanol = 1:0-10:1], and then solidified from diisopropyl ether to give **15** (49 mg, 18%) as a white solid. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.02-1.24 (6H, m), 1.34 (1H, m), 1.69-1.83 (3H, m), 2.81-3.18 (2H, m), 3.72 (1H, m), 4.36 (1H, d, J = 4.8 Hz), 4.42 (2H, m), 6.73 (1H, m), 7.03 (1H, m), 7.17 (1H, m), 7.40 (1H, m), 7.59 (1H, m), 11.52 (1H, s). FAB-MS; *m/z* 287 $[M+H]^+$. Anal. Calcd for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.10; H, 7.76; N, 9.71.

1-[1-(1H-Indol-2-ylcarbonyl)piperidin-4-yl]-2-methyl-propan-2-ol (16)

Compound **16** was prepared in 3% yield from 1H-indole-2-carboxylic acid (**19**) and 2-methyl-1-(piperidin-4-yl)-2-propanol (**23**) using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.11-1.21 (8H, m), 1.34 (2H, d, J = 5.6 Hz), 1.76-1.88 (3H, m), 3.03 (2H, m), 4.11 (1H, s), 4.37 (2H, m), 6.72 (1H, m), 7.03 (1H, m), 7.17 (1H, m), 7.40 (1H, m), 7.59 (1H, m), 11.51 (1H, s). FAB-MS: *m/z* 301 $[M+H]^+$. Anal. Calcd for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.83; H, 8.08; N, 9.23.

2-Methyl-1-{1-[(5-methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}propan-2-ol (17)

Compound **17** was prepared in 71% yield from 2-methyl-1-(piperidin-4-yl)-2-propanol (**23**) using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.08-1.21 (8H, m), 1.33 (2H, d, J = 5.6 Hz), 1.75-1.88 (3H, m), 2.36 (3H, s), 3.01 (2H, m), 4.11 (1H, s), 4.37 (2H, m), 6.62 (1H, m), 6.99 (1H, m), 7.29 (1H, m), 7.36 (1H, m), 11.37 (1H, s). FAB-MS: *m/z* 315 $[M+H]^+$. Anal. Calcd for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.46; H, 8.23; N, 8.81.

Ethyl {1-[(5-methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}acetate (18a)

Compound **18a** was prepared in 94% yield from ethyl piperidin-4-ylacetate using a similar procedure to that of **3**. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.12-1.28 (5H, m), 1.75 (2H, m), 2.02 (1H, m), 2.29 (2H, m), 2.36 (3H, s), 3.02 (2H, m), 4.07 (2H, m), 4.42 (2H, m), 6.64 (1H, m), 7.00 (1H, m), 7.29 (1H, m), 7.36 (1H, m), 11.40 (1H, s). FAB-MS: *m/z* 329 $[M+H]^+$.

{1-[(5-Methyl-1H-indol-2-yl)carbonyl]piperidin-4-yl}acetic acid (18)

To a solution of **18a** (600 mg, 1.83 mmol) in ethanol/THF (6 mL/3 mL) was added 1 M aqueous

sodium hydroxide solution (2.74 mL, 2.74 mmol), followed by stirring at room teperature for 14 h. The reaction mixture was concentrated in vacuo. To the residue was added 1 M aqueous hydrochloric acid (2.74 mL) and water, followed by stirring at 0 °C for 10 min. The resulting precipitate was filtered and washed with water to give **18** as a beige powder. The suspension of **18** (251 mg, 0.836 mmol) in acetonitrile /water/ethanol (1:1:1, 12 mL) was stirred under reflux for 10 min, then at room temperature for 1 h. The precipitate was filtered and washed with EtOH to give **18** (173 mg, 69%) as a beige powder. ^{1}H NMR-DMSO-d_6 (400 MHz) δ 1.19 (2H, m), 1.77 (2H, m), 1.99 (1H, m), 2.21 (2H, m), 2.36 (3H, s), 3.02 (2H, m), 4.42 (2H, m), 6.64 (1H, m), 7.00 (1H, m), 7.29 (1H, m), 7.36 (1H, m), 11.39 (1H, s), 12.13 (1H, s). ESI-MS: *m/z* 301 $[M+H]^+$. Anal. Calcd for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N,9.33. Found: C, 67.83; H, 6.83; N, 9.31.

Benzyl 4-(2-hydroxy-2-methylpropyl)piperidine-1-carboxylate (22)

To a solution of benzyl 4-(2-ethoxy-2-oxoethyl)piperidine-1-carboxylate[15] (**21**, 8.9 g, 29.145 mmol) in THF (100 mL) was added 1.4 M methyl magnesium bromide in toluene-THF (46 mL, 64.4 mmol) at 0 °C, followed by stirring at room temperature for 3 h. 1 M aqueous ammonium chloride solution was added to the reaction liquid, followed by extraction with ethyl acetate. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give **22** (8.5 g, 100%) as a colorless oily substance. ^{1}H NMR-$CDCl_3$ (400 MHz) δ 1.11-1.31 (7H, m), 1.35-1.58 (4H, m), 1.66 (1H, m), 1.78 (2H, m), 2.81 (2H, m), 4.13 (2H, m), 5.12 (2H, s), 7.27-7.43 (5H, m). CI-MS: *m/z* 292 $[M+H]^+$.

2-Methyl-1-(piperidin-4-yl)-propan-2-ol (23)

To a solution of **22** (8.5 g 29.171 mmol) in methanol (120 mL) was added 10% palladium on carbon (wet, contains 50% water, 500 mg), followed by stirring under a hydrogen atmosphere at room temperature for 24 h. Insoluble material was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure to give **23** (5.6 g, quantitative) as a white solid. ^{1}H NMR-$CDCl_3$ (400 MHz) δ 1.15-1.31 (8H, m), 1.42 (2H, m), 1.61 (1H, m), 1.78 (2H, m), 2.02 (1H, m), 2.64 (2H, m), 3.06 (2H, m), 3.48 (1H, s). EI-MS: *m/z* 157 $[M]^+$.

tert-Butyl 3-(2-ethoxy-2-oxoethyl)pyrrolidine-1-carboxylate (25)

To a suspension of sodium hydride (60%, 420 mg, 10.5 mmol) in DMF (15 mL) was added dropwise a solution of ethyl (diethoxyphosphoryl)acetate (2470 mg, 11.0 mmol) in DMF (5 mL) at 0 °C, and the mixture was stirred at room temperature for 0.5 h. To the reaction mixture was added dropwise a solution of tert-butyl 3-oxopyrrolidine-1-carboxylate (**24**, 1850 mg, 10.0 mmol) in DMF (5 mL) at 0 °C, and the mixture was stirred at room temperature for 1 h. To the reaction mixture was added 1 M aqueous ammonium chloride solution at 0 °C, and the mixture was partitioned between ethyl acetate and water. The organic layer was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give an orange oil. To a solution of the oil obtained above in ethanol (30 mL) was added 10% palladium on carbon (wet, contains 50% water, 255 mg), followed by stirring under a hydrogen atmosphere at room temperature for 2 h. Insoluble

material was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [n-hexane:ethyl acetate = 2:1] to give **25** (2210 mg, 86%) as a colorless oil. ^{1}H NMR-$CDCl_3$ (400 MHz) δ 1.26 (3H, t, J = 9.6 Hz), 1.46 (9H, s), 1.54 (1H, m), 2.06 (1H, m), 2.38 (2H, m), 2.56 (1H, m), 2.94 (1H, m), 3.29 (1H, m), 3.45 (1H, m), 3.60 (1H, m), 4.14 (2H, q, J = 9.6 Hz). FAB-MS: *m/z* 258 $[M+H]^+$.

Ethyl pyrrolidin-3-ylacetate (26)

To a solution of **25** (2200 mg, 8.55 mmol) in ethyl acetate (10 mL) was added 4 M hydrochloric acid/ethyl acetate (10.7 mL, 42.8 mmol), followed by stirring at room temperature for 24 h. The reaction mixture was concentrated in vacuo and the residue was made alkaline with 1 M NaOH at 0 °C (pH 10). The mixture was partitioned between $CHCl_3$ and saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give **26** (1170 mg, 87%) as a slightly yellow oil. ^{1}H NMR-$CDCl_3$ (400 MHz) δ 1.26 (3H, t, J = 9.6 Hz), 1.38 (1H, m), 1.99 (1H, m), 2.34-2.55 (4H, m), 2.89-2.99 (2H, m), 3.15 (1H, m), 4.14 (2H, q, J = 9.6 Hz). EI-MS: *m/z* 157 $[M]^+$.

AKR1C enzyme assay by monitoring NADPH oxidation

All assays were performed on SAFIRE spectrophotometer by monitoring the change in absorbance of NADPH at 340 nm. The potency of the compounds was determined by measuring their ability to inhibit NADPH-dependent reduction of 9,10-phenanthrenequinone (9,10-PQ) catalyzed by human AKR1C as

previously described.[20] Purified enzyme (a final concentration of 10 μg/mL), 9,10-PQ (4 μmol/L), NADPH (200 μmol/L), and a test compound were mixed in 100 mM potassium phosphate buffer (pH 6.0) to give a total volume of 80 μL, and then react at room temperature for 10 to 20 min. Subsequently, the change in absorbance of NADPH at 340 nm was measured. The amount of oxidation of NADPH in the presence of the compound was obtained as a relative value with respect to the amount of NADPH in the absence of the enzyme set at 0% and the amount of NADPH in the absence of the compound set at 100%. The IC_{50} values were calculated using Sigmoid-Emax model non-linear regression.

Cell-based 17β-HSD5 enzyme assay

CWR22R cells and HEK293 cells stably expressing human 17β-HSD5 were seeded in 96-well plates at 2×10^4 cells/100 μL/well in RPMI-1640 medium supplemented with 10% FBS and in D-MEM medium supplemented with 10% FBS, respectively. After overnight incubation at 37°C in 5% CO_2, androstenedione (a final concentration of 300 nmol/mL) and a test compound were added to each well. Four hours after incubation, the cell supernatants were removed to measure testosterone concentration using DELFIA Testosterone reagents® (PerkinElmer Life Sciences Inc. MA, USA) according to manufacturer's instructions. The inhibitory effect was expressed as the concentration inducing 50% inhibition (IC_{50}) of conversion from AD to testosterone. The IC_{50} values were calculated using Sigmoid-Emax model non-linear regression.

In vivo model.

Male BALB/c athymic nude mice (Charles River Japan, Inc) aged 4-6 weeks old were used for the CWR22R xenograft model and were castrated before tumor implantation. An established tumor from a host mouse was minced into small fragments, and digested with protease (0.1%). The digested cells were dispersed in RPMI 1640 medium with 20% FBS and then mixed with Matrigel® (1:1 v/v). About 100 μL of the mixture was subcutaneously injected into each flank of castrated mice. About 3 weeks after transplantation, mice carrying CWR22R tumors with similar sizes were selected and randomly divided into five groups (n = 4 for each group). In this study, compound **17** was administered orally. Androstenedione was injected into xenografts (1 ng/100 mm^3 tumor) 1 h after administration of compound **17**. The tumor tissues were removed and weighed for measurement of intratumoral testosterone 2 h after administration of compound **17**.

Detection of intratumoral testosterone concentration in CWR22R xenograft

The removed tumor tissues were homogenated with 200mmol/L phosphate buffer (pH 7.4), and testosterone were extracted by *tert*-Butyl Methyl Ether. Then, testosterone concentrations of the reconstituted extracts were determined using DELFIA Testosterone reagents® (PerkinElmer Life Sciences Inc. MA, USA) according to manufacturer's instructions.

Concentration of test compounds in mice plasma

Test compounds were administered in ICR mice by oral administration. Blood samples were collected 1 or 4 h after administration, and then the test compounds were extracted from mice plasma by acetonitrile and measured by HPLC.

Rat pharmacokinetic study

The pharmacokinetic properties of compound **17** were evaluated in male Sprague-Dawley rats (aged 8 weeks). Compound **17** was administered at 1 mg/kg intravenously in a solution of saline containing 15% DMF and 20% polyethylene glycol 400 and orally in a solution of water containing 10% DMF and 10% propylene glycol. Blood samples were taken at multiple points up to 24 h after a single administration of compound **17** and centrifuged at 16,000 *g* for 2 min at 4 °C to obtain plasma. The concentrations of compound **17** in rat plasma were determined using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) after sample preparation.

PAMPA

The PAMPA Evolution instrument from *p*ION Inc. was used in this study. In PAMPA, a "sandwich" is formed from a 96-well microtiter plate (*p*ION Inc., PN 110163) and a 96-well filter plate (Millipore, IPVH), such that each composite well is divided into two chambers: donor at the bottom and acceptor at the top, separated by a 125 μm-thick microfilter disc (0.45 μm pores) and coated with a 20%(w/v) dodecane solution of a lecithin mixture (*p*ION Inc., PN 110669). Drug samples were introduced as 10mM DMSO stock solutions in a 96-well polypropylene microtiter plate. The robotic liquid handling system draws a 5 μL aliquot of the DMSO stock solution and mixes it into an aqueous buffer solution including 10%(v/v) of DMSO so that the final typical sample concentration is 50 μM. The drug solutions were

filtered using a 96-well filter plate (Corning, PVDF), and added to the donor compartments. The donor solutions were adjusted in pH 6.5 (NaOH-treated universal buffer, *p*ION Inc., PN 110151), while the acceptor solution had the same pH 7.4 (*p*ION Inc., PN 110139). The plate sandwich was formed and allowed to incubate at 25 °C for 2 h in a humidity-saturated atmosphere. On completion of the prescribed incubation time, the sandwich plates were separated and both the donor and acceptor compartments were assayed for the amount of material present by comparison with the UV spectrum (270–400 nm) obtained from reference standards. Mass balance was used to determine the amount of material remaining in the membrane barrier and permeability (*Pe*) was calculated using PAMPA Evolution software (*p*ION Inc.).

Solubility test in pH 6.8 buffer solution with the precipitation method

To 13 μL of a 10 mM DMSO solution of a test compound that had been prepared in advance was added exactly 1 mL of a second liquid (pH 6.8) for a disintegration test of Japanese Pharmacopoeia, followed by shaking at 25 °C for 20 h, thereby giving a sample stock solution. Next, using a filter impregnated with 200 μL of the sample stock solution, 200 μL of a fresh sample stock solution was added for filtration to obtain a liquid, which was taken as a sample solution. Separately to this, to 10 μL of the 10 mM DMSO solution of the test compound was added accurately 1 mL of methanol, followed by stirring, thereby giving a standard solution. 10 μL portions each of the sample solution and standard solution were tested by liquid chromatography, and the ratio of the peak area of the sample solution to the peak area of the standard solution was determined, thereby calculating the solubility.

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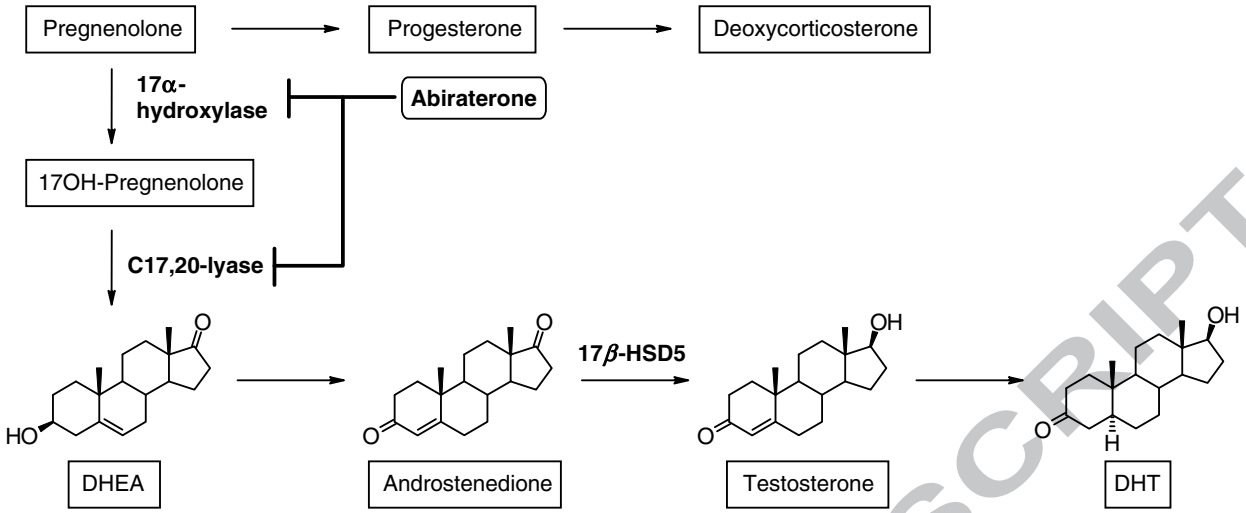
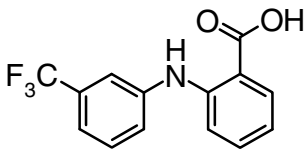
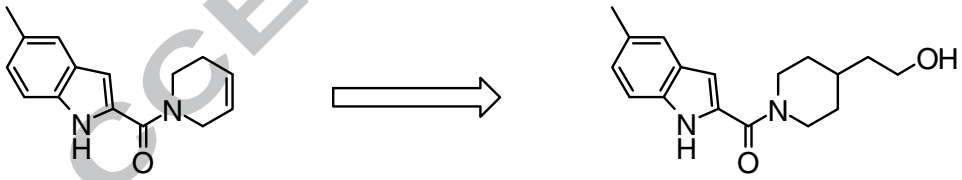


Figure 1. Function of 17β-HSD5 in androgen biosynthesis.



Flufenamic acid (**1**)

Figure 2. Flufenamic acid: a reported 17β-HSD5 inhibitor.

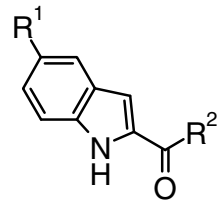


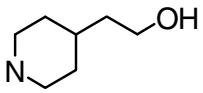
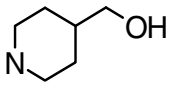
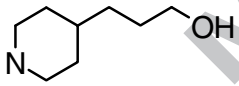
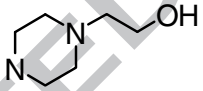
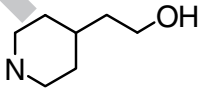
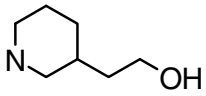
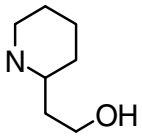
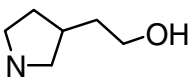
HTS hit**2**
IC50: 2800 nM

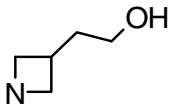
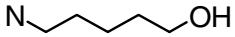
3
IC50: 37 nM

Figure 3. HTS hit and lead compounds.

Table 1. Inhibitory activity of testosterone production in HEK293 cell assay

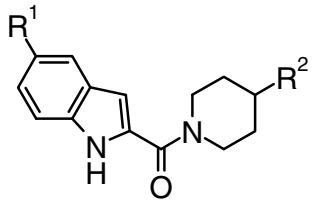


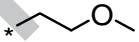
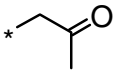
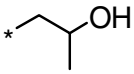
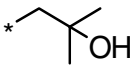
Compound	R^1	R^2	h17β-HSD5-HEK293 (AD→T) IC_{50} (nM)
3	Me		37
4	Me		790
5	Me		160
6	Me		770
7	H		4.5
8	H		2100
9	H		230
10	H		1100

11	H		140
12[a]	H		1200

[a]Reference 17

Table 2. Inhibitory activity of testosterone production in HEK293 cell assay



Compound	R^1	R^2	h17β-HSD5-HEK293 (AD→T) IC_{50} (nM)
13	H		33
14	H		13
15	H		12
16	H		2.1

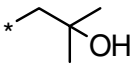
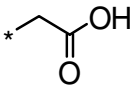
17	Me		0.34
18	Me		>10000

Table 3. Inhibitory activity of testosterone production on CWR22R and plasma concentration

Compound	CWR22R (AD→T) IC_{50}(nM)	Plasma concentration 30 mg/kg, po (μM) 1 h	4 h	PAMPA P_e (×10^{-6}cm/sec) at pH 6.5[a]	Solubility[b] (μM) at pH 6.8
3	58	<0.1	<0.1	>30	>100
16	NT	2.61	<0.1	26	>100
17	1.9	10.9	3.1	>30	>100

[a]pH of donor buffer.

[b]Solubility of tested compounds in the buffer solution of pH 6.8.

Table 4. Pharmacokinetic parameters for compound **17** in rats

Route	Dose (mg/kg)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng·h/mL)	$t_{1/2}$ (h)	T_{max} (h)	Vdss (L/kg)	CLtot (mL/min/kg)	F (%)
iv	1	-	395	0.46	-	1.7	42	-

po	1	180	255	-	0.5	-	-	65

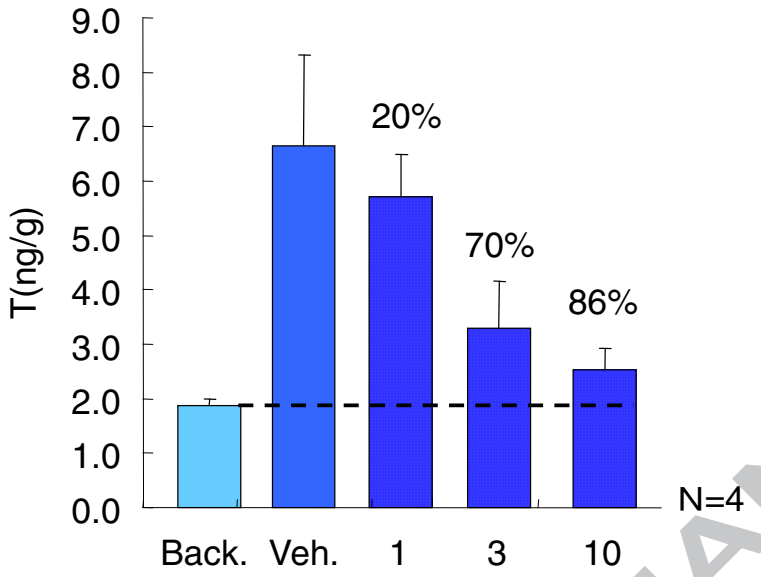


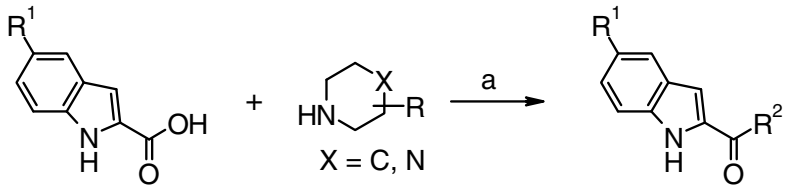
Figure 4. Dose-dependent inhibitory effect of oral administration of compound **17** on AD (1 ng/100 mm^3 tumor)-intratumoral testosterone (T) production in castrated nude mice bearing the CWR22R xenograft.

Table 5. *In vitro* selectivity of compound **17**

Compound	NADPH assay IC_{50} (nM)		CYP inhibition IC_{50} (μM)		hERG inhibition[b]
	17β-HSD5	AKR1C2	1A2/2C9/2C19/2D6	3A4[a]	IC_{50} (μM)
17	24	>20000	>25	>100	>100

[a]Nifedipine was the substrate in CYP 3A4 inhibition assay

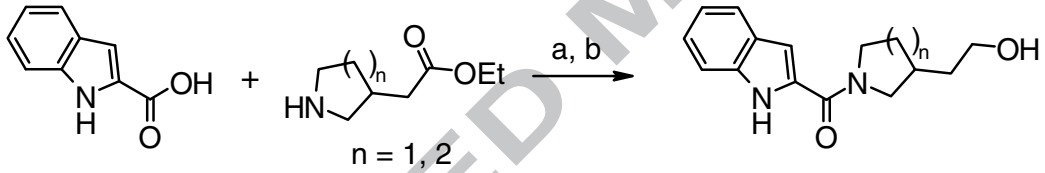
[b]Rb effux assay



19 R^1 = H
20 R^1 = Me

3 R^1 = Me, R^2 = 4-(2-hydroxyethyl)piperidinyl
4 R^1 = Me, R^2 = 4-hydroxymethylpiperidinyl
5 R^1 = Me, R^2 = 4-(3-hydroxypropyl)piperidinyl
6 R^1 = Me, R^2 = N-(2-hydroxyethyl)piperazinyl
7 R^1 = H, R^2 = 4-(2-hydroxyethyl)piperidinyl
9 R^1 = H, R^2 = 2-(2-hydroxyethyl)piperidinyl
13 R^1 = H, R^2 = 4-(2-methoxyethyl)piperidinyl
14 R^1 = H, R^2 = 4-acetonylpiperidinyl
16 R^1 = H, R^2 = 4-(2-hydroxy-2-methylpropyl)piperidinyl
17 R^1 = Me, R^2 = 4-(2-hydroxy-2-methylpropyl)piperidiny
18a R^1 = Me, R^2 = 4-(2-ethoxy-2-oxoethyl)piperidinyl

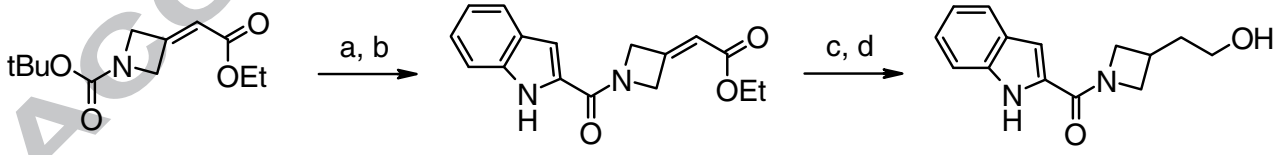
Scheme 1. Reagents and conditions: (a) EDAC·HCl, HOBt, THF, rt.



19

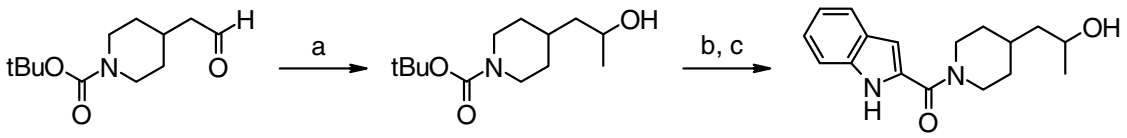
8 n = 2
10 n = 1

Scheme 2. Reagents and conditions: (a) EDAC·HCl, HOBt, DMF, rt; (b) $LiBH_4$, THF, rt.



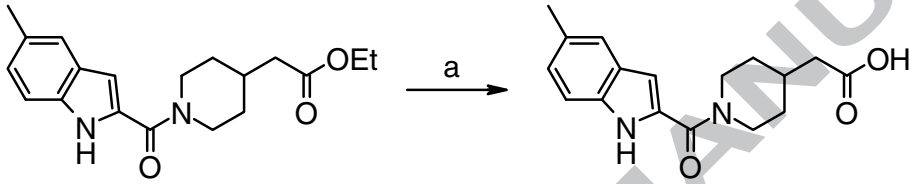
11a **11b** **11**

Scheme 3. Reagents and conditions: (a) TFA, 4N-HCl/EtOAc, $CHCl_3$, rt; (b) **19**, EDAC·HCl, HOBt, DMF, rt; (c) $LiBH_4$, THF, rt; (d) H_2, 10% Pd/C, MeOH, rt.



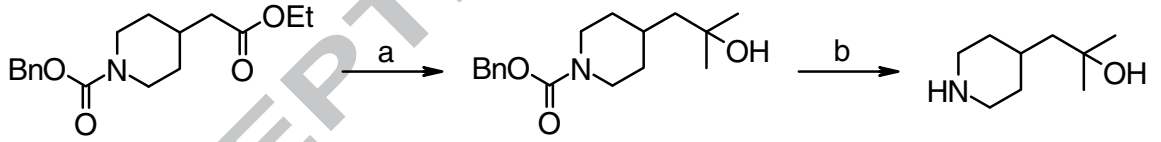
15a **15b** **15**

Scheme 4. Reagents and conditions: (a) 1.4 M MeMgBr, THF, rt; (b) 4 M HCl/EtOAc, EtOAc, rt; (c) **19**, EDAC·HCl, HOBt, DMF, rt.

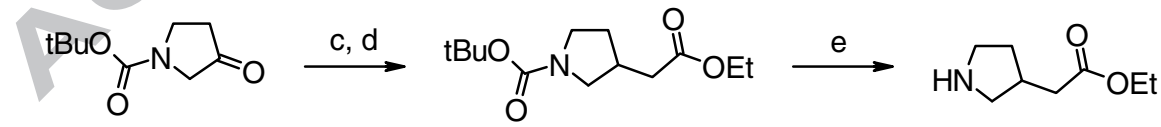


18a **18**

Scheme 5. Reagents and conditions: (a) 1 M NaOH aq., EtOH/THF, rt.



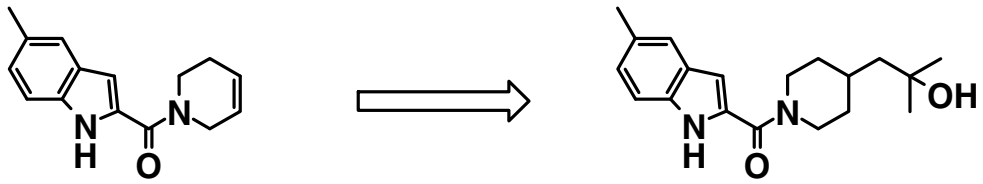
21 **22** **23**



24 **25** **26**

Scheme 6. Reagents and conditions: (a) 1.4 M MeMgBr, THF, rt; (b) H_2, 10% Pd/C, MeOH, rt; (c) $(EtO)_2P(O)CH_2CO_2Et$, NaH, DMF, rt; (d) H_2, 10% Pd/C, EtOH, rt; (e) 4 M HCl/EtOAc, EtOAc, rt.

Graphical Abstract



HTS hit **2**
17β-HSD5 IC50: 2800 nM

17
17β-HSD5 IC50: 0.34 nM